

# **MOHAWK**

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**COLLEGE OF APPLIED ARTS AND TECHNOLOGY**

**CHEMICAL, ENVIRONMENTAL, AND BIOTECHNOLOGY  
DEPARTMENT**

## **Paper Chromatography of Food Colour Dyes**

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**This Experiment is a 2 ½ hour General Chemistry laboratory exercise. It is designed for students in the common first term of a 2-year diploma program (Biotechnology, Environmental, or Health Technician).**

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## Experiment 11

### Paper Chromatography

#### OBJECTIVE

A number of pure substances and mixtures of pure substances in solution will be analyzed by the method of paper chromatography. The retention factor ( $R_f$  value) of each individual substance will be measured. The  $R_f$  value of each component of a mixture will be measured and compared to the  $R_f$  values of the individual substances, in order to identify the substances present in the mixture.

- Substances will be separated using paper chromatography.
- Retention factor ( $R_f$ ) values will be determined.
- The component substances in mixtures will be identified by comparison of  $R_f$  values.

#### REFERENCES

| Wikipedia                                | URL   |
|--|---|
| ➤ Pencil                                 | <a href="http://en.wikipedia.org/wiki/Pencil">http://en.wikipedia.org/wiki/Pencil</a>   |
| ➤ Graphite                               | <a href="http://en.wikipedia.org/wiki/Graphite">http://en.wikipedia.org/wiki/Graphite</a>   |
| ➤ Chromatography                         | <a href="http://en.wikipedia.org/wiki/Chromatography">http://en.wikipedia.org/wiki/Chromatography</a>   |
| ➤ Gas Chromatography                     | <a href="http://en.wikipedia.org/wiki/Gas_chromatography">http://en.wikipedia.org/wiki/Gas_chromatography</a>   |
| ➤ High Performance Liquid Chromatography | <a href="http://en.wikipedia.org/wiki/High_performance_liquid_chromatography">http://en.wikipedia.org/wiki/High_performance_liquid_chromatography</a> |

## INTRODUCTION

### Chromatography

Some analytical methods can be applied to samples which contain mixtures of substances, but most methods require that the components of the sample be separated first. **Chromatography** is the general name of a very important group of closely related separation methods. These methods use the differing physical properties of the substances to separate them.

The method and name was invented around 1900 by a chemist (Tswett) who was able to separate the coloured pigments from plants, the yellow and red carotenes and the green chlorophylls, by passing an extract of pigments through a column packed with powdered chalk or sugar. The name chromatography means writing with colour. The method was not important at that time because most substances are not visibly coloured, and the only detector available at the time was the human eye.

### Modern Chromatography

The first modern use of chromatography came in the 1940's. The biochemists studying proteins recognized that all protein was made from only 20+ component amino acids. Hydrolysis of purified proteins gave mixtures of amino acids that were impossible to separate by the classical methods, such as distillation or fractional crystallization.

The chemists Martin and Synge developed **paper chromatography** as a method of amino acid separation, and were awarded the Nobel Prize in Chemistry (1952) for this and further work. Paper chromatography and its close relative thin layer chromatography (**TLC**) are still very important methods used today. Since 1950, new methods called gas chromatography (**GC**) and high pressure/performance liquid chromatography (**HPLC** or **LC**) have been introduced which are even more important. The theory and practice of GC and HPLC are introduced in Chemical Instrumentation and Sampling and Analysis courses.

### A Paper Chromatography Experiment

If you have ever put a drop of liquid ink on a piece of blotting paper or filter paper and seen the different colours of ink separate, you have done a chromatography experiment. The paper is a very open (porous) material composed of cellulose fibres. The ink is a solution of pigment (light absorbing) substances in water. The water of the ink moves into the paper by a surface action called **capillary action**.

Capillary action can move water or other liquids through porous paper in any direction, up, across or down, and for great distances. The dissolved ink pigments will tend to flow with the water, but the surface of the paper fibres also attracts them. The pigment molecules spend some of their time in the water, moving, and some of their time on the paper surface, not moving.

**This Section Continues on the Next Page →**

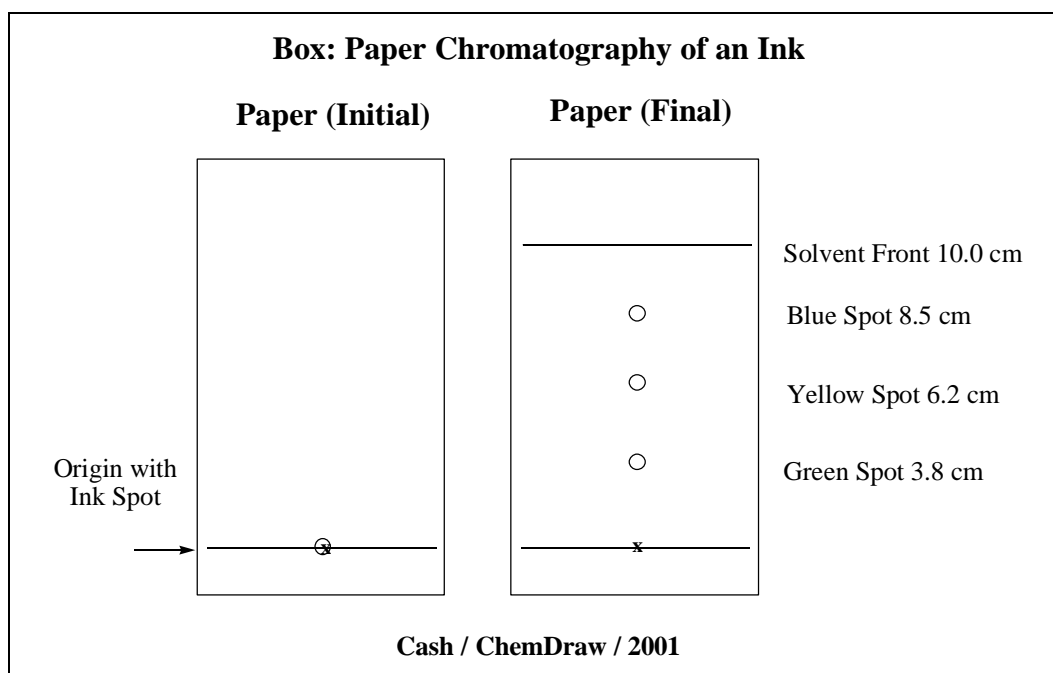
### A Paper Chromatography Experiment (Cont.)

Suppose that an ink has three pigments, one blue (B), one yellow (Y) and one green (G). A piece of porous paper (chromatography paper) is cut into a rectangle. A pencil line is drawn about 2 cm from the short end and an x is marked on the line. A small spot of ink is placed on the x-mark (origin) and allowed to dry. Some water is placed in a beaker to a depth of about 1 cm, and the paper is hung vertically into the water, but the pencil line is above the surface.

The solvent moves up the paper by capillary action, and soon crosses the ink spot, which looks green to the eye. As it moves up, each ink pigment molecule may at one time be dissolved in the water and move up the paper or may at another time stay on the paper surface and remain stationary.

Since each substance is different, often they will move different distances. Suppose the water front has moved 10 cm from the pencil line when the experiment is stopped, the front line is marked in pencil, and the paper is taken out and dried. The ideal situation is that the three pigments are now separated into a blue spot, a yellow spot and a green spot. The distance from the x-mark to the centre of each spot is measured: the distances are 8.5 cm (blue), 6.2 cm (yellow) and 3.8 cm (green).

The experiment has been successful in that it is obvious that there were three pigments in the ink, not one. See the **Box** below for a representation of the experiment.



### Technical Terms in Paper Chromatography

Water is not often used as a solvent for chromatography, since better separations are usually achieved with mixed solvents. The experiment is always carried out in a closed chamber or tank. The liquid is called the **travelling or mobile phase** and the paper surface is the **stationary phase**.

The components of the sample are **distributed** or **partitioned** between the two phases. The x mark or start position of a sample is called the **origin** point. The movement of the liquid is called **elution** or **development** and the position of the liquid front line at the completion of the experiment is called the **solvent front**.

### Measurement of Retention Factor ( $R_f$ ) Values

The behaviour of any component is referred to as its  $R_f$  or **Retention Factor** value, the ratio of component movement to solvent front movement. The  $R_f$  values are experimental, not theoretical, and due to the non-reproducibility of paper chromatography experiments they are used as a general guide only. All distances are measured from the origin spot.

$$R_f = \frac{\text{Distance to Centre of Spot}}{\text{Distance to Solvent Front}}$$

The  $R_f$  values are calculated from observed distances in an experiment. An  $R_f$  value must fall between **0.00** (no movement with the solvent) and **1.00** (no retardation by the paper).

### Difficulties in Paper Chromatography

There are many reasons why paper chromatography has been replaced by **Thin Layer Chromatography (TLC)** and by **Gas Chromatography (GC)** and **High Performance Liquid Chromatography (HPLC)** in modern laboratories, although it has a place in some uses and it does have the advantage of simplicity and low cost.

First, the location of the components of the mixture is difficult if they are not intensely coloured. This can be solved by exposing the paper to a gas or liquid spray containing a reagent which reacts with the components, forming an absorbing product to help **visualize** the sample. This additional step adds time and cost to the method.

Second, the solvent tends to rapidly evaporate off the chromatography paper, slowing down the movement of the solvent front, but not the movement of the sample components. This is solved by sealing the chromatography chamber and saturating it with the solvent vapour. This adds time and complexity to the process, and reduces the reproducibility of separations.

Third, the solvent evaporates unevenly at the edge of the paper, making movement of the components uneven. This is very hard to solve, except by using very large papers and tanks, which is costly and inconvenient. The need for reproducibility between experiments is reduced or eliminated by always running known samples of the expected components **at the same time and on the same paper** as the unknown sample mixture.

### **Sample Application**

In paper chromatography and its close relative, thin layer chromatography, the application of sample spots is difficult. There are three things to consider: the size of the spot on the paper or TLC plate; the amount of sample in the spot; the removal of the original solvent. The size of the spot must be kept as small as possible because there is no way to prevent the spot from spreading out as the chromatogram develops. If the spot is too large at the start, it will be difficult to observe separation into component spots if the sample is a mixture.

The concentration of sample in the spot must be high enough that each component of a mixture may be visualized at the end. Multiple applications of sample with removal of the solvent between applications may be used to increase sample concentration without increasing the size of the spot.

The original solvent must be removed completely before development, since it may mix with the developing solvent and cause error in the separation. In this experiment, you may be asked to prepare fine glass capillary tubing by glass working. Fine capillary tubing allows you to apply very small spots of sample. A heat lamp or a hair dryer may be used to evaporate the original solvent between sample applications, and before development of the chromatogram.

### **Development**

Development of the chromatogram occurs by placing the paper into a solvent chamber, and into physical contact with the solvent. The movement of the solvent up (or across or down) the paper by capillary action develops the chromatogram.

### **Visualization**

If the substances under study absorb visible light, and may be seen by the human eye, no further visualization is required.

For substances which are not visible, or are weakly coloured, a visualization aid is required. There are several possibilities, of which two are most common:

- UV light may cause the substances to fluoresce in the visible region of the spectrum;
- A chemical reagent may be sprayed onto the paper, reacting with the substances, causing a visible colour to form.

### **Unknown Identification**

Chromatography is a method of separation of mixtures, but it is not a method of identification. If an unknown behaves totally differently than a known substance in chromatography, it may confidently be said that they are probably not the same substance. However, it is not acceptable to conclude that similar behaviour in chromatography means that the unknown is the same substance as a known. This can happen due to coincidence. Other methods must be applied to prove the identity of an unknown.

Chromatography excels as a method of separation, or as a method of screening unknowns for suspicious components (e.g. – testing for suspicious drugs in the urine of race horses). These must then be identified by further analysis.

**The INTRODUCTION Section Continues on the Next Page →**

**Disposal**

There are no hazardous or toxic materials in this experiment. All chemical waste may be disposed of in the sink or the municipal garbage. Glass capillary tubes must be placed in the broken glass box after rinsing out the dyes into the sink.

**Example**

Calculate the  $R_f$  values of each of the three dyes (blue, yellow, and green) separated in the chromatography of an ink sample experiment described on page 3.

State each  $R_f$  value to 2 places after the decimal point.

**Answer**

$$R_f(\mathbf{B}) = (8.5 \text{ cm} / 10.0 \text{ cm}) = \underline{\mathbf{0.85}}$$

$$R_f(\mathbf{Y}) = (6.2 \text{ cm} / 10.0 \text{ cm}) = \underline{\mathbf{0.62}}$$

$$R_f(\mathbf{G}) = (3.8 \text{ cm} / 10.0 \text{ cm}) = \underline{\mathbf{0.38}}$$



## CH116 Experiment 11 Handout for Paper Chromatography

There are eight food dyes considered safe and allowed for use in Canada. The eight allowed dyes are listed in the table below. They are produced in quantity for distribution in bulk to the food industry. You can see the colours of the aqueous solutions of the food dyes by visiting the website of a distributing company (1, 2).

| Dyestuff                                       | Dyestuff                                       |
|--|--|
| Allura red or<br>FD&C Red 40 (red)             | Tartrazine or<br>FD&C Yellow 5 (yellow)        |
| Amaranth* or<br>FD&C Red 2 (purple)            | Fast green FCF or<br>FD&C Green 3 (blue-green) |
| Erythrosine B or<br>FD&C Red 3 (red)           | Brilliant blue FCF or<br>FD&C Blue 1 (blue)    |
| Sunset yellow or<br>FD&C Yellow 6 (red-yellow) | Indigo carmine or<br>FD&C Blue 2 (blue)        |

\* Amaranth is allowed for use in Canada but not in the United States.

In Canada (Toronto), aqueous food dye solutions sold under the brand name Club House<sup>®</sup> and powdered drink mixes sold under the brand name Kool-Aid<sup>®</sup> are convenient sources of food dyes for experiments.

Club House brand food dye solutions are available in the four colours red, yellow, green, and blue. Neither the Club House bottle labels nor the Club House company website (3) have any information about which dyestuffs are contained in the solutions. However, this company is a division or subsidiary of the U. S. M<sup>c</sup>Cormick company: the dye contents of the corresponding M<sup>c</sup>Cormick brand solutions are available from the M<sup>c</sup>Cormick company website (4). It seems logical to presume that the same dyes are present in the Club House brand solutions.

| Club House Food Colours / Presumed Dye Content |   |
|--|---|
| Red  | Allura red (Red 40) and Erythrosine B (Red 3)     |
| Yellow   | Tartrazine (Yellow 5) and Allura red (Red 40)     |
| Green  | Tartrazine (Yellow 5) and Brilliant blue (Blue 1) |
| Blue   | Brilliant blue (Blue 1) and Allura red (Red 40)   |

Continued on Page 2 →

Kool-Aid brand powdered drink mixes are available in a variety of formats, sizes, and flavours, with or without sugar and other sweeteners. The Canadian Kool-Aid labels have no information about which dyestuffs are contained in the powders but the dye contents of the corresponding products are available from the U.S. Kraft company website (5).

| <b>Kool-Aid Powdered Drink Flavours / Presumed Dye Content</b> |                            |
|--|----------------------------|
| <b>Cherry</b>  | <b>Red 40 and Blue 1</b>   |
| <b>Orange</b>  | <b>Yellow 5 and Red 40</b> |
| <b>Lime</b>  | <b>Yellow 5 and Blue 1</b> |
| <b>Grape</b>   | <b>Red 40 and Blue 1</b>   |

There is no information on any of these websites about dye amounts or concentrations in the various products.

We use the solvent suggested by Epp (6), 0.1 % w / v NaCl in distilled water and Whatman 1 CHR slotted sheet chromatography paper (7).

## References

1. Calico Food Ingredients Ltd. (2008 09 01),  
<http://www.calicofoods.com/colour/primary.html>
2. Emerald Hilton Davis LLC. (2008 09 01),  
[http://www.emeraldmaterials.com/epm/hd/page.html?p\\_name=FD%26C%20Dyes](http://www.emeraldmaterials.com/epm/hd/page.html?p_name=FD%26C%20Dyes)
3. Club House Brand, (2008 08 18),  
<http://www.clubhouse-canada.com/content.cfm?id=7060>
4. M<sup>c</sup>Cormick Inc., (2008 08 18),  
<http://www.mccormick.com/content.cfm?id=10869>
5. Kraft Foods Inc., (2008 08 18),  
<http://www.kraftfoods.com/kf/Products/ProductInfoSearchResults.htm?CatalogType=2&SearchText=Kool%20Aid&PageNo=1>
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<http://www.terrificscience.com/sciencestore/subcat.php?id=6>
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[http://www.fishersci.com/wps/portal/ITEMDETAIL?productId=3132643&catalogId=29104&catCode=RE\\_SC&keepSessionSearchOutPut=true&brCategoryId=null&hlpi=y&fromSearch=Y](http://www.fishersci.com/wps/portal/ITEMDETAIL?productId=3132643&catalogId=29104&catCode=RE_SC&keepSessionSearchOutPut=true&brCategoryId=null&hlpi=y&fromSearch=Y)

|      |  |     |  |            |  |
|------|--|-----|--|------------|--|
| Name |  | Day |  | Start Time |  |
|------|--|-----|--|------------|--|

**PRE-LABORATORY PREPARATION for Experiment 11**

**To be completed before the laboratory session.**

**To be submitted before beginning the experiment.**

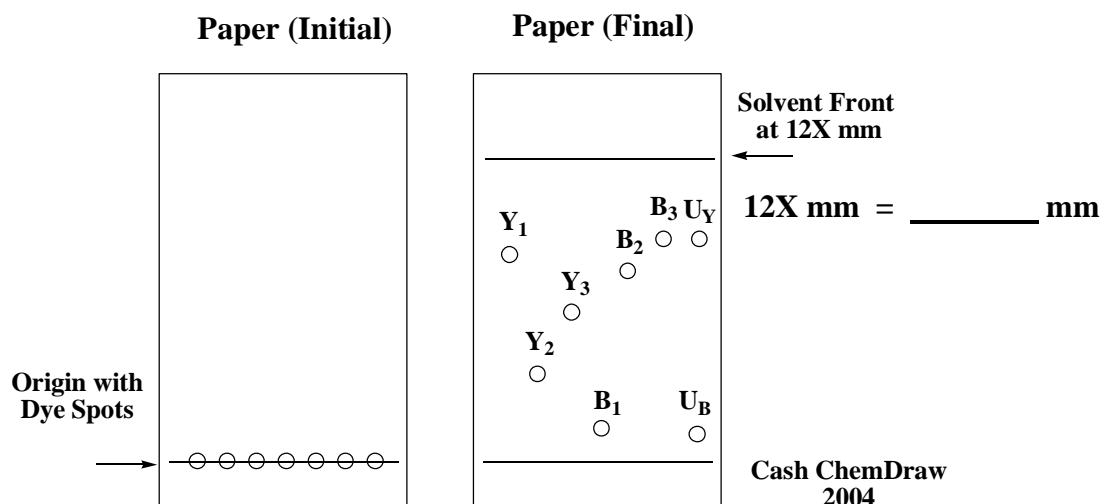
**Questions:** Answer in the space provided. **Show work.**  
Your Mohawk College ID Number is **nnnnnnXYZ**.

**Bring a small ruler (clear plastic if possible) with a mm scale and a pencil to the laboratory.**

A **GREEN** food dye of unknown composition was tested by the method of paper chromatography. Three known **YELLOW** dyes (**Y<sub>1</sub>**, **Y<sub>2</sub>** and **Y<sub>3</sub>**) and three known **BLUE** dyes (**B<sub>1</sub>**, **B<sub>2</sub>** and **B<sub>3</sub>**) and the unknown (**U**) were applied as separate spots on a pencil line on the chromatography paper, and a chromatogram of each was developed. The development was stopped and the solvent front marked at a distance of **12X** mm from the origin.

The known dyes all remained as single spots, but the **GREEN** unknown separated into two spots, one **BLUE** (**U<sub>B</sub>**) and the other **YELLOW** (**U<sub>Y</sub>**). The spot centres were measured from the origin line.

| Dye              | Y <sub>1</sub> | Y <sub>2</sub> | Y <sub>3</sub> | B <sub>1</sub> | B <sub>2</sub> | B <sub>3</sub> | U <sub>B</sub> / U <sub>Y</sub> |
|------------------|----------------|----------------|----------------|----------------|----------------|----------------|---------------------------------|
| Spot Centre (mm) | 9Y             | 3Z             | 6X             | 1Y             | 8Z             | 9X             | 1Y / 9Z                         |
| Your Values      |                |                |                |                |                |                |                                 |



**The PRE-LABORATORY PREPARATION Continues on the Next Page →**

**PRE-LABORATORY PREPARATION for Experiment 11 (Cont.)**

Q-1. Calculate the  $R_f$  values of each of the dye spots. State each value to 2 places after the decimal point. See the **Example** on page 6. Show your work for one spot.

12X mm = \_\_\_\_\_ mm          Calculation for Dye: \_\_\_\_\_

$R_f$  = \_\_\_\_\_

| Dye   | Y <sub>1</sub> | Y <sub>2</sub> | Y <sub>3</sub> | B <sub>1</sub> | B <sub>2</sub> | B <sub>3</sub> | U <sub>B</sub> | U <sub>Y</sub> |
|-------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| $R_f$ |                |                |                |                |                |                |                |                |

Q-2. What if the experiment had been stopped when the solvent front was only **90 mm** from the origin line. How far would the centre of the spot **Y<sub>1</sub>** have traveled? Answer in mm units to the nearest 1 mm. Show your calculation.

Q-3. Is the unknown **GREEN** dye **U** a single substance or a mixture? What proof do you have for your answer?

Q-4. What, if anything, can you say about the composition of the **GREEN** dye **U**, relative to the known dyes tested, from the experiment result?

## PROCEDURE

**The fume hoods must be operating. This Experiment may be performed in Groups.**

The instructor will inform you of:

- The nature of the samples to be tested;
- The type of chromatography paper to be used;
- The chromatography solvent;
- The chromatography chambers;
- The method of visualization of the chromatograms.

### A. Preparation of the Samples and the Chromatography Paper

- A-1. Handle the chromatography paper carefully. Hold it only by the edges, to avoid leaving finger marks on the surfaces. Place the paper on a paper towel or a cleaned surface to avoid contamination.
- Take the paper (or each piece, if more than one is to be used) and draw a pencil line using a ruler, about **20 mm** from the bottom of the paper.
  - The pencil line will be **above** the start of the slots on a slotted paper.
- A-2. Put a small × in pencil at each spot on the line where a known or unknown sample will be placed. Write a code or identifying number or letter in pencil below each × mark. Also write your name or initials on the paper.

The instructor may demonstrate the technique of drawing fine capillary tubes using a burner flame and soft glass tubing. If so, use a heat-proof pad to protect the surface of the bench. Take care to avoid burns from the hot glass.

The instructor will demonstrate the use of a glass capillary tube to apply a small spot of sample onto the chromatography paper.

- A-3. Use glass capillary tubes to put a very small spot (3 to 4 mm in diameter) of each sample at the proper position on the paper.
- If possible, allow the solvent to dry and apply a second spot of the same sample on top of the first spot.
  - Allow the solvent to dry completely before the development is started.
  - A hair dryer or heat lamp may be used to dry the solvent off the paper.

## **B. Development of the Chromatogram(s)**

- B-1. Clean the beaker or other vessel which is to be used as the chromatography chamber. Rinse it well with small portions of the chromatography solvent. Collect the rinse solvent in the fume hood for disposal.
- B-2. Stand or clamp each chamber to be used in an upright position and fill to a depth of no more than **5 mm** with the developing solvent.
- B-3. Cover each chamber with aluminum foil and rubber bands or plastic wrap, or otherwise as suggested by the instructor. Allow a few minutes for the solvent to fully saturate the volume of the chromatography chamber.

The instructor will demonstrate how the paper is to be placed into the chamber. Some special papers are made to be rolled into a cylinder and stapled with the edges almost, but not quite touching.

- B-4. Quickly place the paper into the chamber as directed. Cover the chamber again.
  - The paper should stand upright or be held upright.
  - The pencil line must be **horizontal** during the development of the chromatogram, and must be at least **10 mm** above the surface of the liquid moving phase.
  - The bottom edge of the paper must be under the surface of the solvent.
  - The sample spots must be entirely above the surface level of the solvent.
- B-5. When the solvent has travelled to within a few cm of the top of the paper, or if time is running short, remove the paper from the chamber.
  - Quickly mark the position of the solvent front with a pencil.
  - A hair dryer or heat lamp may be used to dry the solvent off the paper..

### C. Visualization of the Chromatogram (If Necessary)

The paper must be completely dry before this step begins. If necessary, a visualization reagent will be sprayed on the paper as an aerosol. The instructor will demonstrate this procedure.

**In the fumehood: Caution**, hazardous reagent spray and vapours. **Wear gloves**.

- C-1. Spray your chromatogram(s) evenly with the visualization reagent. The paper should be wet but not dripping with the spray.
- C-2. Allow the paper to dry in the fume hood if possible. Before the paper is totally dried, circle the spots in pencil.

### D. Measurement of Distances and Calculation of $R_f$ Values

- D-1. List all of the knowns by name or formula and the unknowns by code number in the **Chromatography Data and Conclusions Table** in the **REPORT PAGES** Section on page 15.

Use one (1) line in the **Table** for each known and three (3) lines for each unknown, since the unknowns may be mixtures with two or more components.

Every spot observed in the experiment must have its own line in the table.

- D-2. Measure the distance to the solvent front from the origin on each chromatogram in mm units, to the nearest mm.
- D-3. Mark the centre of each spot observed after visualization and measure the distance from the origin to the centre of each sample spot (or spots for mixtures). Record all of the measured distances in mm units, to the nearest mm in the **Table**.
- D-4. Calculate the  $R_f$  value for each known spot and all unknown spots. Record these values to 2 places after the decimal point in the **Table**.
- D-5. List observations of the colours of all observed spots.
- D-6. If the instructor confirms that the unknown mixture(s) contain one or more of the known substances, then identify the components of your unknown(s).
- D-7. Have the instructor initial your table.







**REPORT PAGES for Experiment 11 (Cont.)**

**Question 1:** What is pencil lead, and why is it used instead of an ink pen or a marker pen to write on the chromatography paper?

**Question 2:** What will happen if the sample spots are under the surface level of the solvent?

**Question 3:** Explain your reasoning for identifying the components of your unknown mixture(s).

**Attach the Labeled Chromatogram(s) to the Report  
of One Member of Your Group**

**Group Members:**