

MOHAWK

COLLEGE OF APPLIED ARTS AND TECHNOLOGY

**CHEMICAL, ENVIRONMENTAL, AND BIOTECHNOLOGY
DEPARTMENT**

Thin Layer Chromatography (TLC) of Food Colour Dyes

by Professor David Cash

September, 2008

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This Experiment is a 3 hour Organic Chemistry laboratory exercise. It is designed for students in the fourth term of a 3-year diploma program in Chemical or Environmental Technology.

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Experiment 5

Thin-Layer Chromatography

Summary

The technique of thin-layer chromatography (TLC) will be applied to investigate water soluble food dyes. The TLC plates to be used consist of a thin layer of solid silica gel coated onto a flexible plastic material:

- Some food dye knowns will be investigated.
- Some food dye unknowns will be investigated using the same procedure.

References

Zubrick

Topic	5 th Edition	6 th Edition	7 th Edition
Chromatography: Some Generalities	pages 185-187	pages 218-220	pages 220-223
Thin-Layer Chromatography	pages 188-198	pages 221-233	pages 224-237

Shriner

Topic	7 th Edition	8 th Edition
Thin-Layer Chromatography	pages 59-63	pages 86-90

Skoog, Douglas A., **West**, Donald M., **Holler**, F. James, and **Crouch**, Stanley R., Fundamentals of Analytical Chemistry, 8th Edition, Thomson-Brooks/Cole, 2004; or

Skoog, Douglas A., **West**, Donald M., and **Holler**, F. James, Fundamentals of Analytical Chemistry, 7th Edition, Saunders, 1996.

Topic	7 th Edition	8 th Edition
Thin-Layer Chromatography	pages 721-723	pages 1000-1003

Table of Dye Substances

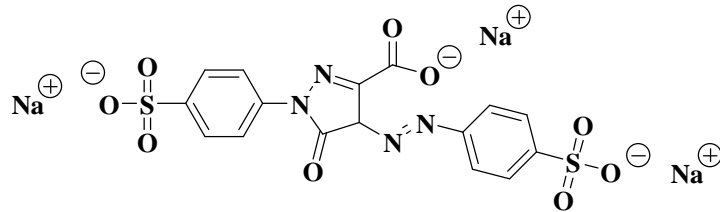
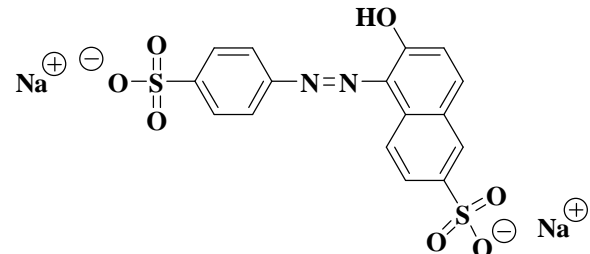
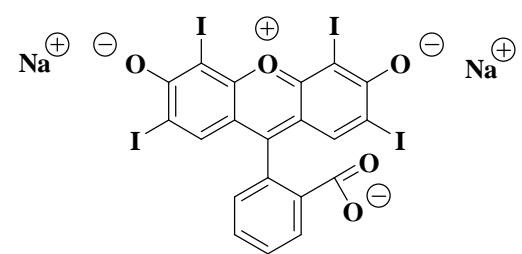
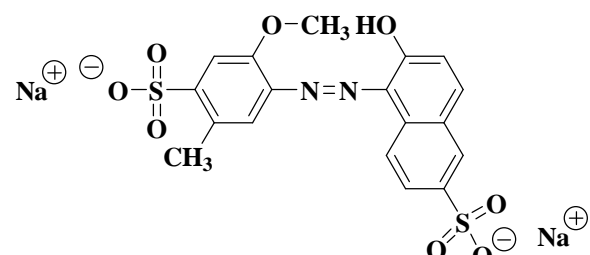
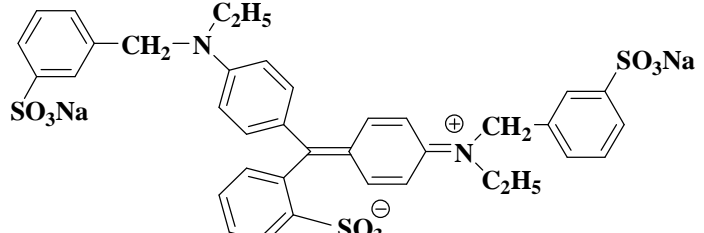
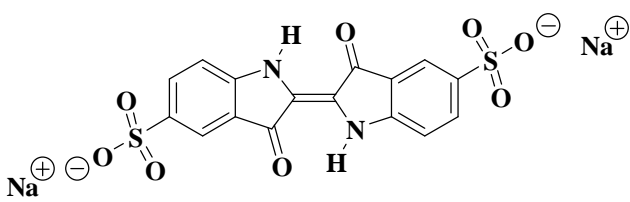
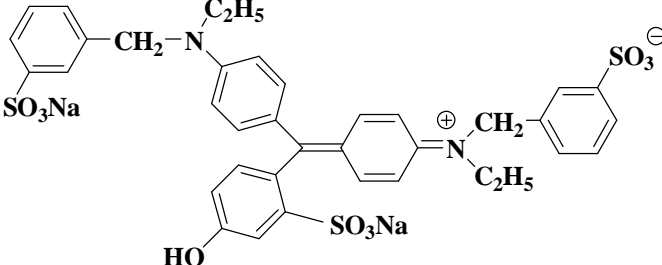
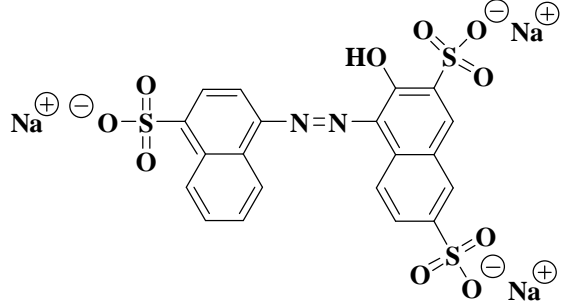
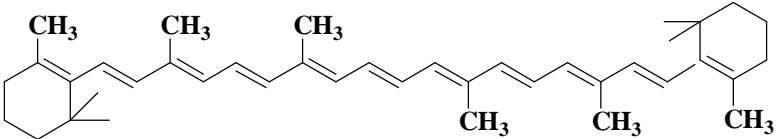
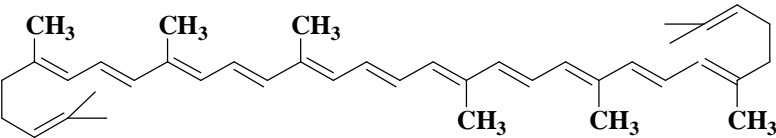
No.	Name (Colour)	Chemical Structure
1	<p align="center">tartrazine or FD&C Yellow 5 (yellow)</p>	
2	<p align="center">sunset yellow or FD&C Yellow 6 (red-yellow)</p>	
3	<p align="center">erythrosine B or FD&C Red 3 (red)</p>	
4	<p align="center">allura red or FD&C Red 40 (red)</p>	
5	<p align="center">brilliant blue FCF or FD&C Blue 1 (blue)</p>	

Table of Dye Substances (Cont.)

No.	Name (Colour)	Chemical Structure
6	indigo carmine or FD&C Blue 2 (blue)	
7	fast green FCF or FD&C Green 3 (blue-green)	
8	amaranth or FD&C Red 2 (purple) (Allowed in Canada but not allowed in the US)	
9	beta-carotene (orange)	
0	lycopene (red-orange)	

Background Information

Thin-Layer Chromatography

This technique is well described in **Zubrick**, in **Shriner**, and in **Skoog** as listed in the **Reference Section**. Thin-layer chromatography is an improvement on paper chromatography because the more uniform particles used generally make the method more reproducible. It is a very fast and convenient method of performing separations for quality control and for screening unknown mixtures for their composition.

In the textile industry, both paper chromatography and thin-layer chromatography are employed to make rapid determinations of the nature of dyes. It is very important to know if a given dye-stuff is a single substance or a mixture. This information is required particularly when the colour of the fabric has to be corrected.

Silica Gel

This substance is commonly used as the stationary solid phase in thin-layer chromatography. Silica gel is an amorphous form of silica with a very porous structure. It is formed by acidification of aqueous sodium silicate. The initial gelatinous precipitate is washed free of all impurities and then roasted or spray dried. The material has typical pore diameters of 2200-2600 pm and a surface area of 750-800 m² / g.

Food Dyes

According to **Pavia**, **Lampman**, and **Kriz**, there were more than 90 dyes regularly used in foods prior to 1906, many of them also used as textile dyes. As scientific knowledge of the hazards has become more precise and government safety regulation more stringent, the number of allowed food dyes has been gradually decreased. In 1938, the number of food dyes allowed in the U.S. was 15, and in 1950 it was 19. At the present time (2006), there are 7 FD&C (food, drug, and cosmetic) dyes allowed for food use in the U.S. The same 7 and one other dye are allowed for food use in Canada. Some other dyes are also allowed in some other countries around the world.

Supermarket Food Dyes

The soluble food dyes available in Hamilton supermarkets are sold by **McCormick Canada**, which is a division or subsidiary of a U. S. company, using the brand name **Club House™**. Four colours are available, sold individually or in sets – yellow, red, blue and green. The labels of the **Club House™** products do not specify the dyes contained; however, the web site of the parent company (www.mccormick.com) does list the names of the dyes in each of the similar products. See the **Table** below. In this experiment you will determine if the **Club House™** dyes have the same content as the **McCormick** dyes.

Table: Supermarket Food Dyes

Food Dye Colour	Club House™ Label	McCormick Label
Yellow	Water, Propylene Glycol, Colours and Propylparaben as preservative	Water, Propylene Glycol, FD&C <u>Yellow 5</u> , Propylparaben as preservative and FD&C <u>Red 40</u>
Red	Water, Propylene Glycol, Colours and Propylparaben as preservative	Water, Propylene Glycol, FD&C <u>Red 40</u> , FD&C <u>Red 3</u> and Propylparaben as preservative
Blue	Water, Propylene Glycol, Colours and Propylparaben as preservative	Water, Propylene Glycol, FD&C <u>Blue 1</u> , FD&C <u>Red 40</u> and Propylparaben as preservative
Green	Water, Propylene Glycol, Colours and Propylparaben as preservative	Water, Propylene Glycol, <u>Yellow 5</u> , FD&C <u>Blue 1</u> and Propylparaben as preservative

The following sections are adapted from Experiment 13: Paper Chromatography, CHEM PH209 General Chemistry 2 Laboratory Manual.

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 then because most substances are not 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 courses.

Technical Terms in Paper Chromatography

Water is not often used 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 \times 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.

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, for the same reason that water forms a meniscus in a pipet stem.

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.

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 \times is marked on the line. A small spot of ink is placed on the \times -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 water 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. See the **Illustration: Paper Chromatography of an Ink** on page 7.

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 \times -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.

Difficulties in Paper Chromatography

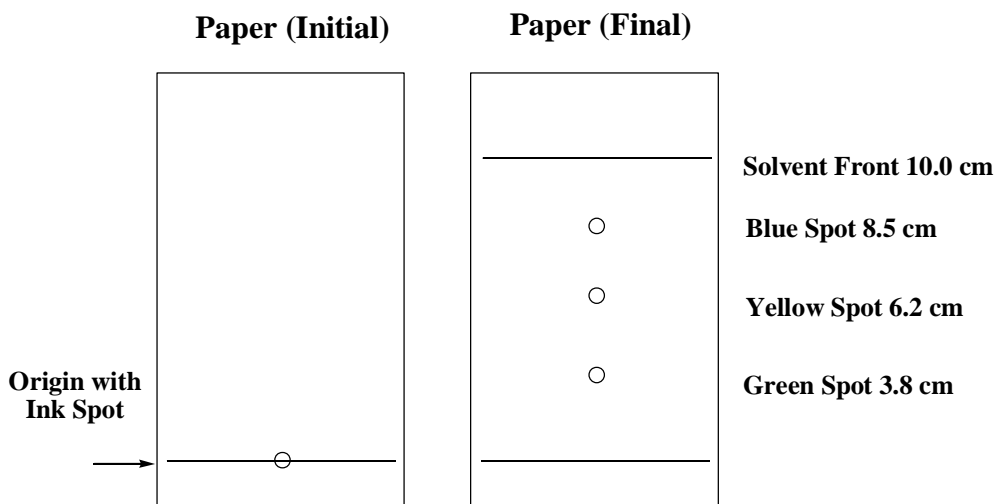
For several reasons, paper chromatography has been replaced by GC and HPLC in modern laboratories, although it has a place in some uses and it does have the advantage of simplicity and low cost.

First, it is difficult to locate the components of the mixture if they are not intensely coloured. This can be solved by exposing the paper to a gas or liquid spray containing a reagent that 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.

Illustration: Paper Chromatography of an Ink



Cash / ChemDraw / 2001

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. These must then be identified by further analysis.

Measurement of R_f Values

The behaviour of any component is referred to as its R_f 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. The R_f values are calculated from observed distances in an experiment.

$$R_f = (\text{distance to centre of spot}) / (\text{distance to solvent front})$$

Example

Calculate the R_f values of the three dyes in the ink experiment described on pages 6 and 7.

Answer

$$R_f (\text{Blue}) = (8.5 \text{ cm} / 10.0 \text{ cm}) = \mathbf{0.85};$$

$$R_f (\text{Yellow}) = (6.2 \text{ cm} / 10.0 \text{ cm}) = \mathbf{0.62};$$

$$R_f (\text{Green}) = (3.8 \text{ cm} / 10.0 \text{ cm}) = \mathbf{0.38}.$$

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.

Name		Lab Day / Time	
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Pre-Laboratory Preparation **Instructor's Initials:** _____

Questions: Answer in the space provided or on an attached page.
Your Mohawk College ID number is **nnnnnnXYZ**.

This work must be completed before the laboratory period. It is your responsibility to have the Pre-Laboratory Preparation ready for initialing by the instructor at the beginning of the laboratory period. The completed Pre-Laboratory Preparation must be attached to your report in order to receive credit.

Before beginning the laboratory, form a group of **4 (± 1)** students as directed by the instructor. Each group should have available in the laboratory a small ruler graduated in mm units, a sharpened soft-lead pencil, and if possible a watch.

Each group should appoint or select a recorder or recorders, who will be responsible for ensuring that all observations are properly recorded into the **Data Tables and Report Section**.

Q-1. You are assigned substance **X** in the **Table of Dye Substances** on pages 2 and 3. The Table contains the names and the chemical structures of ten dye substance which are used as food additive dyes. Seven of these substances (1 to 7) are allowed for food additive use in Canada and the United States. One substance (8) is allowed in Canada but not in the United States. The last two substances (9 and 0) are natural products that are allowed as additives to food in Canada and the United States.

The colours of many of these substances may be viewed at the site:
wwwchem.uwimona.edu.jw:1104/lecture/ecode.html

If this site is unavailable, a search on the name of any of the dyestuffs will probably lead to a site that shows the colours.

Task: Obtain an MSDS of substance your assigned substance **X** and report on any hazards associated with its use. If you cannot obtain an MSDS, report on your search method.

Hazards and Safe Handling of: _____

The Pre-Laboratory Preparation Continues on the Next Page →

Pre-Laboratory Preparation (Cont.)

- Q-2. Draw out in full, by hand or using chemical drawing software, the structural formula of your assigned substance **X**. If you use a drawing program, attach the structure here and state the name of the program.

Identify, name and label on the structural formula those functional groups or structural regions of substance **X**:

- a. that are polar or ionic, and thereby promote solubility in water;
- b. that can hydrogen-bond to water, and thereby promote solubility in water;
- c. that are non-polar, and thereby promote solubility in fat or oil.

Predict whether substance **X** will tend overall to be **water soluble** or **fat soluble**. **Explain** your answer.

Name		Lab Day / Time	
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Handbook Exercise This Task is Mandatory and will have Staggered Due Dates
Satisfactory Completion = 50 points (Included in Laboratory Mark)

Due Date : _____

This exercise refers to one of the twenty alcohols in the **Table of Alcohols** on pages **Error! Bookmark not defined.** and **Error! Bookmark not defined.**. You are assigned one of the alcohols in the table by using your number on the class list for the assignments. Use your number if it is between **1** and **20**; if it is between **21** and **40**, subtract **20**; if it is between **41** and **60**, subtract **40**; and so on.

Task

Consult all of the references in the **List of Handbooks and Sources** below to find if values for the **melting point temperature**, the **boiling point temperature**, the **density**, and the **refractive index** of your assigned alcohol are listed. Fill in the **Table** on the next page.

Reference: **Zubrick**, Interpreting a Handbook.

Zubrick (5th)	pages 19-31	Zubrick (6th)	pages 22-35	Zubrick (7th)	pages 25-36
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The purpose of this exercise is to allow you to become familiar with the variety of handbooks and other sources of physical data available in the Library Resource Centre and elsewhere.

To avoid crowding, the due date will be staggered over several weeks (consult your instructor). Your objective is to visit all of these references.

Please quote the name, edition and date of the reference, and the entry number or page as appropriate. **If the alcohol you are seeking is not listed in a particular handbook or source**, report that the compound was not listed; give the page consulted. **The search is the task, not the data.**

List of Handbooks and Sources

1. Hawley's Condensed Chemical Dictionary (Library Reference Section QD5.H38 2001)
2. CRC Handbook of Chemistry and Physics (Library Reference Section, or Library Stacks, or Lab E304)
3. Lange's Handbook of Chemistry (Library Reference Section QD65.L36)
4. Merck Index (Library Reference Section RS51.M4)
5. Patty's Industrial Hygiene (Library Reference Section RC967.P37)
6. Aldrich Catalogue On-Line (www.sigma-aldrich.com)

The Data Table for this Task is on the Next Page →

Data Table for the Handbook Search Task

Alcohol Name: _____

Handbook	Edition, Date, Etc.	M. Pt., B. Pt., Density, RI	Page or Entry No.
Hawley's Chemical Dictionary			
CRC Handbook			
Lange's Handbook			
Merck Index			
Patty's Industrial Hygiene			
Aldrich Catalogue			

? Acceptable (= 50 Points)

? Unacceptable (see Instructor)

Procedure

Ventilation System Verify that all fume hood and bench fans are **ON**.

The experiment will be carried out in groups as directed by the instructor. Each group will hand in a single joint report. One member of the group should act as the data recorder.

Each person must submit Pre-Laboratory Preparation Questions 1 and 2 individually.

TLC Analysis of Soluble Food Dyes

Reference: Pavia, Donald L., Lampman, Gary M., and Kriz, George S., Introduction to Organic Laboratory Techniques A Contemporary Approach, 3rd Edition, Saunders, 1988, pages 276-277.

The food dye separation used in this experiment is adapted from **Pavia, Lampman, and Kriz**. The method was investigated by **Donna Burton** of class 3CH15, working for the Chemical and Environmental Technology Department as a student intern during the Winter 2000 term.

A. Preparation of the Chambers and the Solvents

Each group will require **4 to 5** clean, dry **100 mL** beakers, each of which will be used as a chromatography chamber. Clean, and then oven-dry the beakers at **110 °C** for **15 minutes**.

Alternatively, special **tall-form spout-less beakers** will be used as chambers.

Caution: fume hood only. To ensure uniformity for all chromatograms the solvent will be prepared in a single batch in advance. Keep the prepared chromatography solvent in closed containers in the fume hood until it is needed.

Food Dye Chromatography Solvent

The chromatography solvent is a **4:1** mixture by volume of **isopropyl alcohol** and **concentrated ammonia**.

Alternative Solvent: a **50:25:25:10** mixture of **1-butanol, ethanol, water** and **concentrated ammonia**.

B. Preparation of the Spotting Capillary Tubes

Follow the instructions in **Zubrick**, The Plate Spotter. The instructor will demonstrate how to use a burner flame to draw a capillary tube down to a suitable, narrow bore.

- Although **Zubrick** says that the capillary tube must not be rotated while heating, **Pavia**, **Lampman**, and **Kriz** say you should rotate the tube.

Zubrick (5th)	page 190	Zubrick (6th)	pages 223-224	Zubrick (7th)	pages 226-227
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Class exercise. Make sufficient good quality narrow-bore spotters so that each container of solution to be investigated has several dedicated spotting capillary tubes.

Each known and unknown solution will have a dedicated station or location.

Move from station to station as you are preparing your plates.

Do not move the known or unknown solution containers or the spotting capillary tubes from their stations. In this way, cross-contamination of the substances will be avoided.

C. Preparation of the Thin-Layer Plates

- C-1. Each group will be responsible for cutting out sufficient small pieces of TLC plate¹ for their chromatograms. Each small plate will allow the separation of three samples simultaneously, as **illustrated** on page 19.
- C-2. Make sure that each of your cut-out TLC plates will fit inside the beaker that you are going to use. Also check that the plate will stand up in the beaker and does not stick out of the top of the beaker.

¹ **Macherey-Nagel**-silica gel plastic-backed TLC plates; cost approximately \$5 per 20 × 20 cm plate.

D. Spotting of the Thin-Layer Plates

- D-1. Each group will make its own plan to spot and develop a set of chromatograms. Each of the solutions listed in the **Table** below must be done at least once. **Bonus marks** will be given for duplicated chromatograms and for **Bonus Unknowns**.

The unknown food dyes in powdered drinks or on the surface of candies may be extracted into water or ethyl alcohol in the well of a porcelain spot-plate.

Samples for Chromatography

Food Dye Knowns		Food Dye Unknowns	Bonus Unknowns
FD&C Yellow 5	FD&C Blue 1	Club House™ Yellow	Kool-Aid Powders
FD&C Yellow 6	FD&C Blue 2	Club House™ Red	No-Name Powders
FD&C Red 3	FD&C Green 3	Club House™ Blue	M & Ms
FD&C Red 40		Club House™ Green	Smarties
			Etc.

- D-2. Describe the colour of each of the food dye solutions in **Table 1** in the recorder's **Data Tables and Report Section**.
- D-3. On a scrap piece of TLC plate, practice spotting with a small-bore capillary tube until you can reproducibly place a **2 mm** or smaller diameter spot of solution.
- D-4. If using the line method, practice drawing a pencil line on a plate until you can draw the line without any damage to the chromatography layer. If the layer is broken, the solvent cannot cross the break, and chromatography cannot occur.

Alternatively, prepare a template as suggested by **Zubrick**.

- **Pavia, Lampman**, and **Kriz** suggests that you draw a line on the plate very lightly and carefully with a soft-lead pencil to mark the origin line of the spots on each plate. **Zubrick** suggests the alternative method of using a template page.

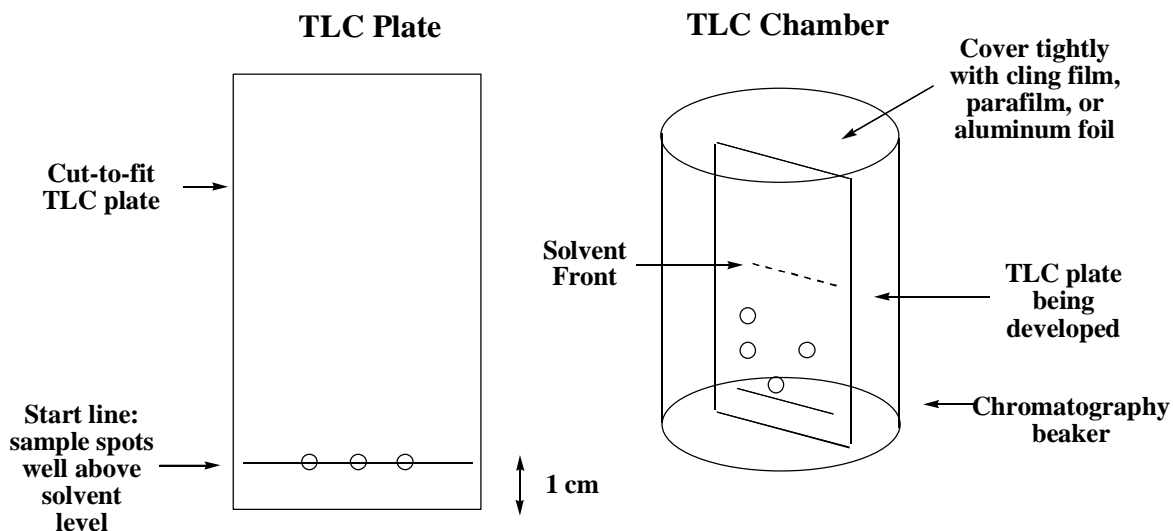
Zubrick (5th)	Not in this edition	Zubrick (6th)	page 225	Zubrick (7th)	pages 227-228
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This Section of the Procedure Continues on the Next Page →

D. Spotting of the Thin-Layer Plates (Cont.)

- D-5. If using the line method, draw an origin line using your soft-lead pencil, 1 cm from what will be the bottom edge of each TLC plate. Alternatively, use a template. Spot each of the three samples on the plate at an equal spacing of about **0.8 cm (8 mm)** apart. Make each spot no more than **2 mm** in diameter.
- Although **Zubrick** suggests that the spots may be larger, **Pavia, Lampman, and Kriz** suggest that the TLC spots should be no more than **2 mm** across. Our trials suggest that a **2 mm** spot diameter is adequate; this will allow you to place three samples on each plate.
 - This plate is very small. Spots too near to the edges will suffer from undesirable edge effects, which occur as the solvent moves towards the edges of the plate.
- D-6. Dry the solvent (water) from the spots with a hot-air blower. If the instructor suggests it, apply a second layer of the same solution on top of the first, again no more than **2 mm** across. Dry the second layer as before. **The sample spots must be completely dry or the chromatography will not be successful.**
- D-7. Label each plate that you prepare in pencil (e.g. – **A, B**, etc.) or be sure you can identify it. Record a description of the colour of each of the dried food dye spots in **Table 1**.
- D-8. Keep a complete record, of which sample is in each position, on each plate. Record the details in **Table 1** of the **Data Tables and Report Section**.
- D-9. Draw a simple labeled sketch of each food dye chromatogram plate before development, showing the location, size and colour of all spots on each plate. The sketches will be required for the report.
- D-10. Each plate requires a clean, dry beaker to be used as a chromatography chamber. Prepare a cover for each beaker. The instructor will suggest the cover material; cling film, parafilm, or aluminum foil.
- D-11. **In the fume hood.** Place a shallow layer of the chromatography solvent into each beaker to a depth of no more than **0.5 cm**. Add just enough solvent to cover the bottom of the beaker with no gaps. Check that no part of any of the sample spots will be below the surface of the solvent when the plate is inserted.

Illustration: Chromatography Plates and Chambers



Cash / ChemDraw / 2006

E. Development of the Chromatograms

- E-1. **In the fume hood.** Place a TLC plate into each beaker. Immediately cover each beaker tightly to prevent loss of any solvent vapours. Make a note of the start time for each chamber. You may remove the beaker from the fume hood after it has been tightly covered.
- E-2. Watch the progress of the development of each chromatogram as the eluting solvent front travels up the plate by capillary action. Do not move or disturb the chambers during the development process.
- E-3. Allow the development of each plate to proceed until the solvent is at least three quarters to the top of the plate, or for at least **30 minutes** (consult the instructor). Do not allow the solvent front to move within **1 cm** from the top edge of the plate.
- E-4. **In the fume hood.** When each TLC plate development is complete, take off the cover and remove the developed chromatogram from the beaker. **Immediately** mark the solvent front position.

F. Drying of the Chromatograms

- F-1. If this has not already been done, **immediately** mark the position of the solvent front on every plate with your pencil at the edges of the TLC plate.
- F-2. Unless the beaker and solvent are to be reused, dispose of the solvent as instructed.
- F-3. Allow the solvent to evaporate from the TLC plates, if possible by using the hot-air blower.

G. Measurement of the Food Dye Chromatograms

- G-1. Record a description of the number and colour of all of the food dye solution spots seen on each developed TLC plate in **Table 2** in the **Data Tables and Report Section**.
- G-2. Draw a simple labeled sketch of each developed food dye chromatogram, showing the location, size and colour of all spots on each plate. The sketches will be required for the report.
- G-3. Mark the positions of the centres of all coloured spots on the TLC plates.
- G-4. Measure the distance (in mm units) from the origin line to the solvent front and to the **centre** of all coloured spots. Record all of the measured distances in **Table 2** in the **Data Tables and Report Section** and on the sketches.
 - According to **Pavia, Lampman, and Kriz** it is conventional or usual to measure the distance from the origin to the front edge of spots in paper chromatography, but to the centre of spots in TLC. This is because TLC spots tend to be much smaller and more regular in shape than paper chromatography spots.
- G-5. Calculate the **R_f** value of every spot seen. Record the values of **R_f** in **Table 2** and on the sketches.

Report

Each team will submit a group report, to which each student in the group must attach the answers to her or his own Pre-Laboratory Preparation.

- R-1. List the names of all of the group members.
- R-2. List the names of and describe the colour and appearance of each of the food dye known and unknown solutions in **Table 1** of the **Data Tables and Report Section**.
- R-3. Describe the colour and the appearance **after drying but before development** of each of the spots of the food dye knowns and unknowns on the TLC plates in **Table 1**.
- R-4. Report the colour and the appearance **after development and drying** of every one of the spots on the TLC plates in **Table 2** of the **Data Tables and Report Section**. Add additional pages of **Table 2** if more space is necessary.
- R-5. Attach one or more pages with simple labeled sketches of all of the plates before development, and of the developed and dried chromatograms that include all of the measured distances and observed colours.
- R-6. Tabulate in **Table 2** descriptions of all of the spots and colours seen and all of the measured distances to the centre of each spot and to the solvent fronts.
- R-7. Calculate the R_f value of each dye or dye component observed in the experiment and report these values in **Table 2**.

Show one sample of the calculation method.

- R-8. Attach the labeled, developed chromatograms to the report, in the form of a display, such that all of the chromatograms are easily seen.

Post-Laboratory Questions

1. For each of the four **Club House**TM food dye unknowns that the group investigated, report and **explain** any conclusions or inferences that may be made concerning:
 - a. The number of components present in each food dye solution.
 - b. The possible presence in any of the unknown food dye solutions of any of the food dye knowns.

2. Refer to the **Table: Supermarket Food Dyes** on page 5:

According to the results of your experiments on the four **Club House** brand food colour solutions, do they contain the same food dye ingredients as are listed on the labels of the four **McCormick** brand food colour solutions? **Explain** your answer.

Bonus Questions

1. The food dye **Amaranth** (Structure 7 on page 3) is allowed for use in Canada, but not in the U.S. Research this substance. Why is it not allowed in the U.S.? Why is it allowed in Canada? Give your reference source(s).
2. The **McCormick** company uses a mixture of an allowed blue and an allowed yellow dye to produce a green colour, rather than using the one allowed green dye. This may be due to considerations of **consumer safety**, **cost**, **convenience**, **esthetics**, or **solubility**.

Write a brief assessment of each of these factors, and any others you can think of, using appropriate researched facts, with references.

Mark Scheme for Experiment 5**Total Marks = 100**

	Mark	
Pre-Laboratory Preparation (15) (must be attached to the report for credit) completed prior to the laboratory session (individually initialed) MSDS obtained and interpreted (individually) structure, polarities and solubility predictions (individually)	/6 /3 /6	/15
Experiment Performance (35) co-operativeness, general behaviour, clean-up, etc. quality of observations	/10 /25	/35
Report (40) initialed data tables and report section must be attached to the report clarity and organization of report sketches display of TLC plates calculation of R_f values	/5 /10 /10 /10 /5	/40
Post-Laboratory Questions (10) question 1 question 2	/5 /5	/10
Bonus Experiments (10) Bonus Questions (10) question 1 question 2	/10 /5 /5	/20
Total = /100		

Name		Lab Day / Time	
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Data Tables and Report Section

Instructor's Initials: _____

The following Tables are to be used for taking data **IN INK**. Leave this page open on your bench during the experiment period. Have it initialed by the instructor before leaving the laboratory. The completed and initialed **Data Tables and Report Section** must be attached to your report.

Table 1: Food Dye Descriptions		
Plate / Dyestuff	Solution Colour	Dried Spot Colour

The Data Tables and Report Section Continues on the Next Page →

Data Tables and Report Section (Cont.)

Table 2: Food Dye Observations and R _f Calculations				
Plate / Dyestuff	Spot(s) Observed after Development and Drying (Colours)	Distance to Solvent Front (mm)	Distance(s) to Spot Centre(s) (mm)	Calculated R _f Value(s)

Add Additional Pages of Table 2 if Necessary

Names of the Group Members:

1. _____

2. _____

3. _____

4. _____

5. _____

6. _____