Melting Point Experiment

Carry out the procedure described in the Lab Guide and in addition have each person in your group do one additional melting point of one of the following: brown sugar, pure sugar, and gelatin. Make sure your group has at least one melting point determination for all three of these compounds.

Also in Procedure 5, instead of using a 0.01% solution of methylene blue each of you will decolorize a solution of colored sugar. On the common shelf you will find containers of red and green colored sugar. Place enough colored sugar in 1 mL of water to produce a colored solution. Follow the instruction for decolorization using this solution.

In your final report, give the structure of table sugar (sucrose) and a segment of gelatin and discuss the mp behavior of each.

The Technique of Distillation

Isolation of Volatile Oils from Spices by Steam Distillation and Bioassay for Biological Activity

Introduction
The spices cloves, ginger, nutmeg, and tumeric will be provided. There are many other possible spices such as caraway, cumin, or thyme. Feel free to bring in ~10 grams of a spice of your choice. There is no guarantee that a particular spice will yield much volatile oil or that the components of this oil will have biological activity, but it will be fun to see if they do. If you don’t get anything from your spice, you can continue the procedure with some clove oil that is available.

Activities:
✓ In the first lab, you will use steam distillation to separate any volatile oils from your spice of choice. In preparation for this, read the theory of steam distillation presented below. Also read the introductory information in the Procedure section on page 65 and 66. You will be using a variation of the distillation set-up as in Procedure 1A and 1B. In place of the liquid mixtures toluene/cyclohexane or ethanol/water, you will use water and your solid spice to carry out steam distillation. However, typical distillation data for simple and fractional distillation is provided below and you will plot these for your Final Report. You are responsible for understanding how simple and fractional distillations are done and the advantages and disadvantages of these types of distillations as well as steam distillation.
In the second lab, you will be carrying out Procedure 3, a microscale fractional distillation of an unknown mixture which will give you experience with the fractional distillation technique. Read Procedure 3.

You will prepare sterile agar plates and inoculate them with Bacillus Cereus bacteria and incubate them to test biological activity. Samples of the components you isolated will be tested for antibacterial activity.

Read the introduction to the Liquid/Liquid Extraction laboratory (Introduction through Drying Agents), because the essential oil will be isolated using liquid/liquid extraction.

You will use GC and GC/MS to characterize your volatile oils. In preparation for product analysis using these methods, read Sections 11.7 and 11.7.1 on gas chromatography, Sections 11.2.1 – 11.2.4 on mass spectral interpretation, and section 11.8 on gas chromatography/mass spectrometry of Chapter 11 in the Chem 36 Lab Guide.

PreLab:
Your chemical data table should include the chemicals mentioned in on p 61 of your Lab Guide and also eugenol, thymol, and quinine. Hand in a copy of the completed Common Shelf Chemical Date Table with your PreLab.

Prelab Questions:

Answer the prelab questions asked in the lab guide for the Distillation experiment.

After reviewing the assay, your essential oil shows antibiotic activity, how would you determine which compound(s) in the oil is (are) the active ingredient?

Modeling the Drug Discovery Process
Adapted from William H. Miles, (Department of Chemistry, Lafayette College, Easton, PA 18042; milesw@mail.lafayette.edu) and Patricia M. Smiley (Southern Lehigh High School, 5800 Main Street, Center Valley, PA 18034; smileyp@solehi.k12.pa.us), J. Chem. Ed. 2002 79, 90

Chemical Relevance: Chemistry and Medicine
Since ancient times, humankind has sought medicines from nature. Quinine, the antimalarial from the bark of the cinchona tree, and reserpine, the antidepressant from the Indian plant Rauwolfia serpentina, represent two of the more famous natural products used in modern medicine.
Today, pharmaceutical companies use several approaches for the discovery of new compounds to treat human illness, including the screening of natural products derived from bacteria, fungi, plants, or animals (No, not puppies or even mice, but animals like slugs or sponges, simple multi-cellular, bottom-dwelling animals called “Porifera”). For example, extraction of a marine sponge with an organic solvent can yield a complex mixture of organic compounds, which can then be tested for biological activity or “bioassayed”. If the mixture shows promising biological activity, the components of the complex mixture may be separated and purified. When further testing identifies the active component, its chemical structure can be determined using modern spectroscopic techniques (IR, NMR, Mass Spectrometry, X-ray crystallography). Even if the new compound has side effects that render it unsuitable as a drug candidate, the pharmaceutical chemists will use the compound as a guide for the synthesis of new drug candidates with similar structures.

The synthesis of organic compounds, whether of natural products or of "unnatural products" (compounds that are not found in nature), has been a primary goal of organic chemists. Some of the first synthetic compounds produced industrially were dyes, fragrances and flavorings. It was not until the twentieth century that synthetic drugs were successfully developed. The synthesis of the antisyphilitic drug arsphenamine, Compound 606, by Ehrlich in 1910, was a dramatic demonstration of the power of chemistry to attack a medical problem. (Yes, arsphenamine contains arsenic. Not good stuff, but remember, you’re dying of syphilis, so you can’t be fussy.) Since then, many new compounds, including biologically active compounds found in nature, have been synthesized in the laboratory and have become useful drugs. For example, Ritalin is used for the treatment of attention deficit disorder (ADD), 5-fluorouracil is a potent anti-cancer drug, and L-DOPA is used to treat Parkinson's disease.
Essential oils, which are isolated by steam distillation of volatile organic compounds present in plants and animals, have a long and rich history in medicine. Typically complex mixtures of organic compounds, essential oils have been used to treat a variety of illnesses for thousands of years. In some cases these essential oils do have therapeutic utility by modern medical standards. The essential oils of cloves and thyme contain phenolic compounds (organic molecules that have an OH group attached to an aromatic ring), which are effective antiseptics (germ-killers). Even before the establishment of the germ theory, the antiseptic power of both thyme oil and clove oil was recognized. Joseph Lister, whose name is immortalized by the mouthwash Listerine, used synthetic phenol (carbolic acid) to prevent infection during surgical procedures in the mid-nineteenth century, revolutionizing the medical treatment of wounds. He recognized that antiseptic chemicals can prevent infection and proved the value of some of the old herbal remedies. Phenolic compounds, both synthetic phenol and thyme oil, were used as antiseptics until World War I.

The clove tree produces flower buds which, when harvested and dried, become the familiar spice of the same name. It has long been known that cloves contain an ingredient with an anesthetic property that is particularly effective against dental pain. Before modern medicine developed better remedies, people often chewed on cloves to dull the pain of a toothache. The active compound responsible for this effect is found in the essential oil of cloves which makes up approximately 16-18% of the clove by weight. The major component of this oil is the compound eugenol. Eugenol has a boiling point of 254° C. It would be difficult to isolate this oil by simple distillation since this high temperature could lead to its decomposition as well as the decomposition of other components of the cloves. However, eugenol can be isolated relatively easily from cloves using the technique of steam distillation. Once the aqueous distillate containing the clove oil has been obtained, the oil can be separated from the water by extraction into the organic solvent dichloromethane.
The dried fruit of cumin (Cuminum cyminum L.), a small shrub cultivated in eastern Europe and India, is an important seasoning in curries, goulashes, and sausages. It is also a major component of chili powder used in Mexican food. The major volatile constituent of cumin is \( p \)-isopropylbenzaldehyde (cuminaldehyde). Cumin oil also contains limonene and other compounds which contribute to the aroma of this condiment.

The Isolation of Organic Compounds

As you know, functional groups give the molecules distinctive chemical reactivity, as well as play a role in the physical properties compounds have. Different physical properties allow the separation of one organic molecule from another. In some cases, separations can be based on the solubility of the compound in a given organic solvent, so that a compound can be recrystallized, as you learned in the recrystallization technique experiment. As you will learn in this techniques experiment, organic compounds with appreciable vapor pressure can be separated by distillation at atmospheric pressure, or if their vapor pressure is low, steam distillation or vacuum distillation at reduced pressure can be used.

Biological Testing

The testing of compounds for biological activity is the first step in the determination of their potential as a drug in medicine. In most cases, the compound is tested against an organism (bacteria, fungi, or virus) \textit{in vitro}, that is, outside a living organism in a controlled environment such as a Petri dish. One method for testing antibiotic activity is to apply the compound onto a sterile paper disk and place the disk on an agar plate containing a culture of bacteria. If the compound is active, then a zone of inhibition will appear around the disk; other areas of the Petri dish will show evidence of bacterial growth. The size of the zone indicates the potency of the drug. Adjusting the amount of compound applied to the disk allows a crude dosage effect of the compound to be seen.

If the pharmaceutical companies discover a promising lead, the next step is to investigate the potency of the drug \textit{in vivo}. Animal studies determine the effectiveness of the drug as well as provide information about the toxicity, metabolic pathway of decomposition, and side effects of the drug. After exhaustive animal studies, the drug will then undergo human trials. Human trials start with Phase I clinical trials, in which the toxicity and dosage response of the drug is determined with healthy volunteers. In Phase II clinical trials, the drug is tested on people (100-300) who have the disease. In Phase III clinical trials, the compatibility of the new drug with other medications is determined in several clinics and hospitals with as many as a thousand patients. If no complications or serious side effects are found, then the company can apply for Food and Drug Administration (FDA) approval for this drug in the treatment of a given disease. If FDA approval is
granted, then the drug is available for physicians to prescribe. Since problems may appear even after such extensive testing, the pharmaceutical companies continue to monitor the use of their drugs. This process is long and expensive. The average cost has been estimated at 250 million dollars, with at least a ten-year span from the initial testing of the drug to final FDA approval. It has been estimated that only one compound out of ten thousand compounds prepared by a pharmaceutical company receives final FDA approval. Although the research and development costs are considerable in the pharmaceutical industry, the rewards are significant: a blockbuster drug like Prozac has sales in the range of two billion dollars a year.

**Modeling the Drug Discovery Process**

The separation of a biologically active compound such as eugenol from a mixture of organic compounds obtained from a natural source (cloves in the case of eugenol), and biological testing of this compound (as well as the other compounds) using a simple bioassay for antibiotic behavior, models many aspects of the drug discovery process. The separation of essential oils from plant materials and spices is readily accomplished by a combination of steam distillation and acid/base liquid/liquid extraction methods described earlier. For example, eugenol, a phenolic compound, is easily separated from the neutral components in clove oil, eugenol acetate and β-caryophyllene. In this laboratory experiment we will use the whole oil without further separation. If you were to identify which component(s) in the oil exhibited antibiotic behavior you would need to carry out a separation that would probably include acid/base liquid/liquid extraction methods and extensive column chromatography.

![Chemical structures of Eugenol, Eugenol acetate, and β-Caryophyllene](image)

You will test the antibiotic activity of the following samples using *Bacillus cereus* growing on agar plates which you will prepare.

1. A sample of the initial organic extract of the steam distillate containing. Dichloromethane, CH$_2$Cl$_2$, will be used for this extraction.
2. A control sample that does not contain the extracted oil.

**Steam Distillation**

Distillation is a common method for the separation and purification of organic compounds. These are usually liquids at room temperature although numerous lower melting solids can be distilled at higher temperatures, say above 150°C. However, many organic substances decompose at these higher temperatures. By distilling at low pressure (~1 torr or 1/760 of an atmospheric pressure), the material boils at lower temperatures.
and decomposition is minimized. The vacuum pump carts you see in the lab are used for this purpose. Steam distillation is another way to distill high boiling substances and is useful for the isolation of oils, waxes, and some complex fats. Any organic liquid that is immiscible with water can be distilled at a temperature around 100°C, the boiling point of water. For example, eugenol boils at 254°C and would decompose extensively if we tried to distill it at this temperature. We could use vacuum distillation or steam distillation. How does steam distillation allow the vaporization of such a high boiling substance as eugenol at ~100°C? Unlike the normal distillation of two miscible liquids, in steam distillation, each component of an immiscible liquid mixture contributes to the total vapor pressure as if the other component were not present. Another words, the total vapor pressure of the mixture is the sum of the vapor pressure of the water plus the vapor pressures of each of the organic components in the mixture.

$$P_{\text{total}} = P^0_{\text{component 1}} + P^0_{\text{component 2}} + P^0_{\text{component 3}} + \ldots + P^0_{\text{component n}} + P^0_{\text{water}}$$

A liquid or liquid mixture boils when the total vapor pressure of the liquid is equal to atmospheric pressure. The vapor pressure of water is 760 torr at 100°C. The presence of any immiscible organic material contributes to the total vapor pressure and thus causes the boiling point of water to drop below 100°C. While eugenol only has a vapor pressure of about 20 Torr at 100°C, the eugenol vapor is swept out of the boiling flask by the boiling water vapor or steam into the condenser where everything condenses, separating into liquid water with small amounts (~20/760’s) of immiscible eugenol condensing out also. This yields a cloudy distillate of water and eugenol (plus other volatile organic substances). Since it takes a lot of water vapor to sweep out the higher boiling organic oils, it is often necessary to add water to the distilling flask at frequent intervals throughout the distillation until the organic compounds have been removed from the mixture. The liquid in the condenser will become clear when the organic compounds have all be isolated from the starting mixture. The water is added via separatory funnel, burette or pipet. The addition rate should be about equal to the collection rate of drops.

**Procedure for Steam Distillation**

(Procedure adapted from Introduction to Organic Laboratory Techniques A Microscale Approach by Pavia, Lampman, Kris, and Engel, Saunders College Publishing, 1990, pg. 91 and 688.)

**Spices Used in this experiment:** Cloves, Ginger, Nutmeg, Tumeric or a spice of your choice.

**Distillation Set-up:** Clamp your 100-mL heating mantle (You should NOT fill the heating mantle with sand) clamped to a ring stand above a magnetic stirrer and clamp a 100-mL round bottom flask to the ring stand and sitting in the heating mantle. Weigh out 5 to 6 g of ground spice and transfer to the flask using the weighing paper as a funnel. Fill the flask half full of distilled water and drop in a 1 in. magnetic stir bar. Complete the distillation set-up as shown in Figure 5.2 of the Lab Guide except replace the thermometer with Pasteur pipet or a separatory funnel or a burette to allow water addition. Also, put a 50-mL round bottom flask on the connecting tube or vacuum adapter to collect the distillate. The thermometer is unnecessary as the temperature will always equal approximately the boiling point of water. Make sure you securely clamp the round bottom flask and the condenser. Also, make sure to use a rubber band to hold
the vacuum adapter on the end of the condenser. Start stirring and heating the mixture with a varistat setting of 60. Lower the heating voltage if foaming is a problem. Also, if material starts to foam into the condenser, you can put a Claisen adapter into the apparatus as shown below.

Steam Distillation: Turn on the cooling water. Make sure that water is flowing through the condenser. You only need to turn the water valve slightly. No matter how much you turn the valve the water flow will remain close to a trickle, which is fine. You do not want to heat the mixture too intensely or it will bump over. Wrap the top of the 100-mL round bottom flask and the 3-wat connector with some glass wool to insulate it so that the steam vapors don’t condense there very much. Once the distillation begins, record the distillation temperature (it should be just below 100 °C). You should try to adjust the heating so that distillate drips at a rate of one drop every 2-5 seconds. Begin adding the water in small amounts through the thermometer opening so that it roughly matches the rate of distillate collecting in your 50-mL round bottom collection flask. Replenish the ice in the beaker that is used to cool your collection flask as necessary. Use a Pasteur pipet to remove some of the water before adding more ice. The vapor should be cloudy when the natural products are co-distilling with the water. When the vapor becomes clear you can remove the heat from the round bottom flask.

Liquid/Liquid Extraction
Separation of the mixture of essential oils from water: Remove the 50-mL round bottom flask containing your essential oil and water. Use your 125-mL separatory funnel to extract the essential oils in your distillate into dichloromethane. Making sure the stopcock is closed, transfer the contents of the 50-mL round bottom flask to the empty 125-mL separatory funnel. Remember a stopcock can leak so always double check your
stopcock to make sure it is tight and always have an Erlenmeyer flask or beaker under
the funnel to catch any possible leaks. Add 10-mL of dichloromethane (CH₂Cl₂) to the
separatory funnel and follow the extraction procedure you learned in the liquid/liquid
extraction technique experiment. Repeat this process two more times with 10-mL
additions of dichloromethane and combine all the organic layers into a 125-mL
Erlenmeyer flask labeled “TOTAL OILS”.

Isolation of the “TOTAL OILS” mixture: After drying over anhydrous sodium sulfate
for at least 10 min, a small amount the CH₂Cl₂ extract can be decanted into a labeled
shorty vial and a labeled and tared 20-mL vial, both labeled “TOTAL OILS”. Cap the
shorty vial, but leave the 20-mL vial with the cap off in your locker to allow the
dichloromethane to evaporate until the next lab period. (Alternatively, if time permits,
one could blow off the dichloromethane in the 20-mL vial with a stream of nitrogen
while immersing the flask in a beaker of warm tap water.)

Gas Chromatography (GC) and GC-Mass Spectrometry (GC-MS) Analysis:
Each member of your research group will analyze your “total oils” mixture using the HP
5890 GCs. You will need to take your small vial containing the dichloromethane and the
oil mixture to the instrument room for analysis. You should have signed up for time on
the instrument and you will need to do the CALIOPE GC tutorial on the computer
adjacent to the GC. The tutorial is interactive so it will show you how to do the
analysis, while you go through it. The tutorial will not provide you with a temperature
program so use the following: column temperature program from 40 to 280°C at 10°/min.
Inject 1 uL of solution. After obtaining your chromatogram show it to your TA before
running the GC-MS.

Antibiotic Behavior of Spice Essential Oils:
As stated earlier, essential oils extracted from natural sources may exhibit antibiotic
behavior. You will be bioassaying your extracts with *Bacillus cereus*¹ to test for its
antibiotic behavior. Agar culture plates are prepared in sterile petri dishes as
demonstrated by the instructor or your TAs. These plates are stored in the refrigerator in
Room 216 Whitmore. Whenever you handle the sterile agar petri dishes try to minimize
any exposure to random forms of bacteria or dirt in the laboratory. Always wear gloves
that have been sprayed with ethanol when handling the plates and the *Bacillus cereus*,
because your hands can easily contaminate the agar. Open the cover of the dish a few
inches vertically so that you have just enough room to swab the plate with a known strain
of bacteria or insert a testing disk and immediately cover the agar to reduce the risk of
outside contamination. You will use a pair of forceps to handle the test disk and sterile
copper loops to streak the plates with the *Bacillus cereus*. After preparing your assay you
will observe the growth of bacteria on the plate. If the bacteria do not grow in the area

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¹ There are many bacterial cultures commercially available, but we chose *Bacillus cereus* because it is non-pathogenic, requires
no special medium for growth, and grows optimally at room temperature in a short period. Cultures of *E. coli* not only have to be
handled with care due to their potential pathogenicity, but their optimal growth is at 37°C, requiring an incubator for optimal growth.
surrounding the disk treated with your essential oil then one or more of the compounds in this isolated mixture is exhibiting antibiotic behavior.

**Bioassay using agar medium bacterial growth plates**

Sterilize a copper wire loop by heating in a flame. Once cool, use the loop to transfer the bacteria from the starter culture plate to a previously prepared sterile agar culture plate as demonstrated.

Add the required volume of acetone to your 4 samples to make 5 to 10% (wt/vol) solutions of your isolated oil in acetone. Using a pair of forceps cleaned by wiping with an ethanol soaked tissue, pick up a sterile disk and dip it into the acetone solution. Allow the disk to soak for 10 minutes then remove the disk and lean it against a clean glass-stirring rod on a paper towel to air dry. Lift the lid on the agar plate just enough to insert the disk into one quadrant of the plate using clean tweezers. Label the cover of the culture plate with your name and the each disks identity so you will know which disk is which and tape the cover to the bottom. Place in one of the incubator ovens in 216 Whitmore. The results of the essay can be assessed after twenty-four hours or longer.

![Image of test disks](image)

Test disks (clockwise from top left) of: control, 2 mg of clove oil, 2 mg of neutral compounds from clove oil, and 2 mg of eugenol from clove oil.

**Cleaning Up**

It is important to properly dispose of the chemical and biological wastes. The aqueous acid and base solutions can be poured down the drain with plenty of running water. The acetone solutions of clove oil, eugenol, and the neutral components can go in the NHO container. The dichloromethane solutions used for GC/GC-MS should go in the HO container. If there is an autoclave available, then the agar plates and contaminated materials should be autoclaved for at least 1 h. If an autoclave is not available, then the agar plates and contaminated materials should be soaked in bleach for several days.
Final Report

Record data and observations in your notebook as you work (see the section The Laboratory Notebook: Observations and Data section). For the Results and Discussion section of your final lab report, follow the point distribution on the grading sheet for this experiment and be sure to include:

1. Distillation curves of simple and fractional distillation data supplied to you below for the distillation of ethanol/water. Plot both simple and fractional distillation curves so that you have two graphs that look like Fig 5.1 from the data below. Adjust observed boiling point temperatures to what they would be if corrected to standard atmospheric pressure of 760 Torr, then plot boiling point versus volume of distillate (drops or mL) of the type shown in the curves in the Introduction. Tape the Data Table below in your Notebook.

2. The distillation curve for your Procedure 3 unknown with boiling points adjusted to 760 Torr.

3. Give the identities of your two liquids in your distillation unknown.

4. Discussion of simple versus fractional distillation. Do the results support predictions?

5. Interpret the mass spectra for two compounds detected by you or others from GC-MS.

6. Discuss the antibacterial activity you observed for each of your four spice essential oil extracts.

7. Postlab Questions:

   1. The liquid boiling in a simple distillation flask consists of 80 mole percent of hexane and 20 mole percent of n-propylbenzene. At 79°C a small amount of distillate is collected. The standard vapor pressures of pure hexane is 812 mm and pure n-propylbenzene is 560 mm Hg, respectively, at 79°C. Calculate the percentage of each of the two components in this fraction of distillate. Show your calculations. Hint: Use the formulae in the introduction.

   2. Liquids that have boiling points greater than 200°C are usually distilled under vacuum (<20 torr). What effect would this have on the distillation, and give a reason why this might be advantageous.

   3. What criteria would you use to decide which type of distillation technique is appropriate for the purification of a reaction mixture.

References:

Distillation Data for Ethanol/Water

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Technique of Thin-Layer Chromatography

Experiment Title: Applying TLC As A Method to Monitor the Multistep Synthesis of Aspirin

Introduction
Aspirin will be synthesized from methyl salicylate in two steps. Thin layer chromatography will be used to monitor the reaction for both steps of the synthesis. The first step requires the saponification of methyl salicylate to salicylic acid. The second step is the acetylation of salicylic acid with acetic anhydride to produce acetylsalicylic acid (aspirin).

Activities:
✓ Read the Introduction, Theory, Spotting the TLC Plate, Development, Visualization, and Rf Values sections of Experiment 7 in the Lab Guide.
✓ In the first part of the experiment you will determine an appropriate solvent system to separate methyl salicylate, salicylic acid and acetyl salicylic acid using standard solutions.
✓ Then you will saponify methyl salicylate with NaOH to produce salicylic acid. You will follow the progress of this experiment by TLC.
✓ You will isolate the crude salicylic acid and purify it by recrystallization before proceeding to the next step.
✓ You will then acylate the salicylic acid with acetic anhydride to produce acetylsalicylic acid. This reaction will be monitored by TLC.
✓ You will purify the acetylsalicylic acid and report a final melting point and % yield.
✓ You will obtain a 400 MHz 1H NMR spectrum of the acetylsalicylic acid.

PreLab Exercise
Do questions 1 and 2 from the PreLab exercises of the Thin-Layer Chromatography Experiment 7 in your lab guide.

3. What is the white solid that is formed when the NaOH is added to the methyl salicylate before the reaction mixture is heated?

4. What is the purpose of adding the concentrated phosphoric acid to the reaction mixture in the synthesis of aspirin?

5. Reaction mechanisms for both steps of the synthesis must be written out with arrows showing the direction of electron flow.
Read the introductory material from Chapter 7 of the Lab Guide on thin-layer chromatography. You will be doing a variation of the TLC experiment in this chapter as a means of monitoring the two-step synthesis of aspirin.

Analgescics are substances that relieve pain. The most common of these is aspirin, a component in more than 100 nonprescription drugs. Aspirin has wide spread use in medicine and over 30 million pounds of it are consumed each year in the United States. Willow leaves and bark have been used for centuries for their pain relieving and fever-reducing properties, however the active ingredient in these home remedies is salicylic acid, which over time irritates the stomach lining. In 1893 a German chemist Felix Hofmann synthesized acetyl salicylic acid that offered similar medicinal properties without the stomach irritation. Since the contents of the stomach are acidic, aspirin passes through unchanged and does not get absorbed until it reaches the basic environment of the intestines. Salicylic acid is a white crystalline compound that is commonly used in ointments and plasters for the removal of warts.

Aspirin is synthesized by acetylating salicylic acid to produce the corresponding acylated carboxylic acid. You will begin with methyl salicylate a component of oil of wintergreen that is used in flavoring candies. In this two-step synthesis of aspirin a methyl ester (methyl salicylate) is saponified to produce the corresponding carboxylic acid (salicylic acid). In this second step the phenolic functional group of salicylic acid is acetylated with acetic anhydride to produce a new ester (acetyl salicylic acid). Keep in mind that anhydrides decompose readily with the moisture in the air, so open and close the bottle quickly and cover the containers used to transfer this reagent. Use all anhydrides in the hood and use gloves when handling them. You will be monitoring the progress of both reactions by TLC and identifying an appropriate solvent system for the separation of the starting material, intermediate product and final product of this multi-step synthesis.

To identify an unknown by TLC, the usual strategy is to find a stationary phase/mobile phase combination that will separate all the compounds you are analyzing. In this experiment, you will not vary the stationary phase or the silica gel, but will vary the polarity of the mobile phase by using differing ratios of the solvents hexane (nonpolar) and ethyl acetate (polar) to develop TLC plates spotted with standard solutions of the starting material and the two products in the two-step synthesis of aspirin. Once you and your lab group determine the optimal mobile phase that will give the best separation of these, you will use this composition to run a chromatogram of the reaction mixture to monitor the progress of both reactions. From the chromatogram, you should be able to determine whether or not the products have been produced by matching \( R_f \)’s of the standards to the compounds in the reaction mixture. You will need at least a 50% reaction yield after isolation and purification of the salicylic acid to carry out the second step in the synthesis.

**Procedure for TLC.**

You will be working in your research groups of 4 or 5 so choose the solvent composition(s) found below for the appropriate number of members in your group. Each group member will prepare two solvent compositions to be used as mobile phases for the separation of the methyl salicylate, salicylic acid, and acetylsalicylic acid.
Table 1. Mobile phase compositions for 5 group members

<table>
<thead>
<tr>
<th>Mobile Phase Mixture Composition Number</th>
<th>%Ethyl Acetate</th>
<th>%Hexane</th>
<th>R_f Methyl salicylate</th>
<th>R_f Salicylic Acid</th>
<th>R_f Acetyl Salicylic Acid</th>
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Table 2. Mobile phase compositions for 4 group members

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<th>Mobile Phase Mixture Composition Number</th>
<th>%Ethyl Acetate</th>
<th>%Hexane</th>
<th>R_f Methyl salicylate</th>
<th>R_f Salicylic Acid</th>
<th>R_f Acetyl Salicylic Acid</th>
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Label the green lids of your two 4 oz. TLC jars with mixture composition numbers assigned in the diagram and table above. Use a 10 mL graduated cylinder to prepare 10 mL of each of the two compositions assigned. Don’t try to pour out the correct amount from the supply bottles, but rather use the plastic pipet attached to the supply bottle to transfer the solvent to better control the exact volume to be measured out. Working in the hood, pour each 10 mL mixture into the corresponding wide-mouth jar. Swirl to mix and then pour about half out so that the solvent level won’t be so high that it will wash compound spots off the TLC plate. (Dispose of the excess in the non-halogenated waste bottle.) Insert a piece of 4 cm filter paper into each jar so that it wraps around the inside wall of the jar and dips into the liquid. This creates a saturated vapor atmosphere that improves spot shape and reproducibility. Cap both jars. Using a lead pencil (not a pen) and a ruler, mark two plates as shown in Figure 7.13 in the lab guide. First, draw a light
pencil line across the plate about 1 cm from the bottom of two TLC plates. Make four equally spaced vertical dashes on this line. The dashes should be about 5 mm from the edge and 5 mm apart. Then label the lanes at the top of the plate.

In a shorty vial, obtain a small amount of dichloromethane from the common shelf. Using a TLC spotting capillary tube from your desk, practice spotting on a paper towel using pure dichloromethane. After filling the capillary by dipping it in the liquid, touch it quickly to the towel so that the spot is no larger than 1 to 2 mm diameter. The smaller the spot, the better the final TLC analysis. After the solvent evaporates, you can apply more material in the same spot by again quickly touching the capillary to the surface at the same place.

Three people in your group of four or five should obtain just a few drops of one of the 1% or 2% solutions of the three standards: methyl salicylate, salicylic acid, and acetyl salicylic acid in shorty vials, properly labeled. Spot both of your plates with each of these three standards, placing the spot at the origin mark corresponding to each. Examine the plate under the UV light to see that enough of the compound has been applied by observing a visible dark purple dot, if it is not visible spot more. Using forceps, gently place one in each TLC bottle, being careful not to splash solvent up the plate (see Figure 7.9 in lab guide). Be sure that the spots are not below the solvent level or they will wash away into the solvent. Allow each plate to develop until the solvent front is approximately 1 cm from the top of the plate. Using forceps, remove the TLC plate and quickly mark the solvent front with a pencil. Allow the plates to dry in the hood.

Examine the plate under the UV lamp to see the components as dark spots against a bright orange or green-blue background. Outline the spots with a pencil. [The spots can also be visualized by putting the plate in an iodine chamber that can be found on the side shelf. After a few minutes sitting inside the closed bottle, compound spots turn brown.] Calculate the $R_f$ value for the center of each spot as shown in Figure 7.11 in the lab guide and enter the value in Table 1 or Table 2 above. Tape the properly labeled TLC plates in your notebook using the wide sticky tape available on the side shelf. Cover the whole plate with tape.

Obtain $R_f$ values for the other mobile phase compositions from the other students in your group and enter them in Table 1 or 2 also. As a group, decide which composition gives the best separation, in other words: (1) it does not allow any of the compounds to remain on the baseline, (2) it does not allow any of the compounds to travel with the solvent front, and (3) it provides the greatest differences in the $R_f$ values for the compounds to be separated.

Cleaning Up. Do not dispose of the spotting capillaries; they are reusable!! They may be cleaned by dipping the ends into acetone and blotting the ends with a paper towel. Store them safely in the test tube labeled for TLC spotting capillary storage in your drawer for use in other experiments.

Used mixed solvents should be placed in the appropriate organics waste container in your hood. Return iodine chambers to the side-shelf.
Saponification of Methylsalicylate

You will saponify this ester to produce salicylic acid, a precursor to acetylsalicylic acid. Methyl salicylate has two functional groups: an ester and a phenol. Esters are easily hydrolyzed with base to the corresponding carboxylic acid. When a strong base such as NaOH is added to methyl salicylate, three reactions occur: 1) ester hydrolysis, 2) phenoxide ion is formed, and 3) the carboxylic acid product is converted to its conjugate base. Addition of a strong acid in the reaction workup protonates both the phenolate and the carboxylate anions. Salicylic acid is insoluble in cold water so it can easily be isolated via filtration.

The hydrolysis reaction is very slow at room temperature but the reaction time can be shortened when the mixture is refluxed for a period of time. Refluxing occurs when the solvent in the reaction mixture is heated to boiling and resulting vapor is condensed and drops back into the reaction flask as a liquid after coming in contact with a condenser. You have already observed this behavior when you carried out the distillation experiment, however in that case you were interested in transferring the vapor to a second round bottom flask. In this case, you will be returning the vapor to the reaction flask as liquid solvent.

After allowing the reaction mixture to reflux, you will obtain a sample for TLC to monitor the progress of the reaction. You can stop the reaction when the spot corresponding to the starting material is only slightly visible. You will begin the workup of the reaction by cooling and acidifying the reaction mixture and isolating the resulting solid product. Salicylic acid is only slightly soluble in cold water but is soluble in hot water so you will do your filtration while the mixture is cold. After isolating the product, you can take advantage of salicylic acid’s solubility in hot water and use this solvent for recrystallization.

**Experimental Procedure:** Have one member in the group make a 20 mL of a 4 M sodium hydroxide solution in a 50 mL Erlenmeyer flask. When weighing out the NaOH pellets work quickly and do not touch them with your hands. The group will share this sodium hydroxide solution for their saponifications. Weigh out 230 mg of methyl salicylate using the 100 – 1000 µL syringe into a large test tube 20 X 150 mm. Lay the test tube on the pan of the balance and have it rest on the two little dents designed to keep the tube from rolling. Add 3.5 mL of the 4 M sodium hydroxide solution to the tube containing the methyl salicylate and swirl to ensure that the two compounds mix. A white solid will quickly form. Add a 1/2 inch stir bar and clamp the tube and heat the water solution to reflux using your heating mantle. The tube is large compared to the volume of the solution so the walls of the tube will serve as the condenser. Reflux for 15 minutes, cool and check the reaction mixture by TLC. If the reaction has nearly gone to completion you can proceed to the work up section, if not reflux for an additional 5 – 10
minutes depending on amount of methyl salicylate remaining. Cool and check reaction mixture again by TLC.

Workup:
Place the reaction tube in a 250 mL beaker containing ice to keep the tube cool. Add 3 M sulfuric acid solution to the tube in 0.5 mL increments, (about 10 drops from a disposable pipet) until a heavy white precipitate forms and remains when the tube is stirred. Add an additional 10 drops of acid to ensure complete precipitation of the salicylic acid. Using a clean glass stirring rod test the pH of the salicylic acid solution to be sure it is acid using pH paper. Filter the cold solution using a Hirsh funnel and isolate the white solid.

The crude solid will be purified by recrystallization from water. Transfer the solid to a 10 mL Erlenmeyer flask and add 2 mL of water. Heat to boiling using a boiling stick to control bumping. Add water in small increments until all the solid is dissolved and then remove from the heat. Allow the solid to recrystallize with placing it in an ice bath. Once a significant amount of crystals have formed, cool the flask in an ice bath and then isolate the solid using vacuum filtration with a Hirsh funnel. Wash the crystals once with ice-cold water and remove the solvent using a vacuum. Transfer the crystals to a watch glass to dry until the next lab period.

Yield and Characterization:
Weigh the solid and obtain a melting point.

**Acylation of Salicylic Acid**

Now you will carry out the second step of the synthesis and produce acetyl salicylic acid.

**Experimental Procedure**
Carry out this procedure in the Hood. Add 100 mg of salicylic acid produced in the TLC experiment to a clean 13 X 100 mm test tube. Add 250 µL of acetic anhydride using a disposable syringe without the needle to the test tube containing the salicylic acid. (Have everyone in the your team use the same syringe for the addition of the acetic anhydride). Add one drop of 85% phosphoric acid from a Pasteur pipet and then loosely cork the top of the test tube. Shake the tube gently to dissolve the salicylic acid and then place the tube in a beaker with hot tap water. Leave the tube in the hot water for 15 minutes. Check temperature of water and if significant cooling has occurred replace with more hot water. Monitor the reaction by TLC. When the reaction has gone almost to completion remove the test tube from the water bath and add 700 µL of water from an adjustable pipet. Allow the solution to cool to room temperature. Crystallization of the crude product should begin. Now place the sample in an ice bath and force out the remaining crystals. Vacuum filter the crude product with a Hirsh funnel and allow it to dry on a piece of filter paper until the next lab period.
PostLab Questions:

1) When making the measurements for the Rf values, it is conceivable to measure the distance the spot travels from the top, middle or bottom of the spot. In practice, however, it is best to measure only from the middle of the spot. Why?

2) Tell why it is important that the solvent level in the TLC developing jar be below the level of the sample spots.

3) In each pair, pick the molecule which would have the HIGHER Rf value. Explain your answer.
   a) phenyl chloride or phenol  
   b) N,N-dimethylformamide or 1,3-butadiene  
   c) acetonitrile or benzene  
   c) decanol or propanol

4) Assuming that 1g of aspirin dissolves in 450 mL of water at 10°C, how much aspirin would be lost in the 16 mL of water or 1.4 mL of water added to the reaction mixture if the mixture were at 10°C during the filtration?

5) How much difference would the amount lost in the water make in your percent yield?

References:

Final Report
Tape Tables 1 and 2 into your In-Lab Data and Observations section. In your RESULTS AND DISCUSSION section, give the composition of the mobile phase your group chose and tell why, show explicitly how you calculated the Rf values. Describe the reproducibility of TLC Rf values. Discuss how your observed relative Rf’s corresponded to relative Rf’s predicted by you in the PreLab. Also include the % yield and weight of each purified product. You must also have an NMR spectrum for both products. Label the peaks and integrate all peaks. Include a discussion the interpretation of these spectra.
Technique of Column Chromatography

Experiment Title: Applying Column Chromatography As A Method to Isolate An Optically Pure Alcohol.