**EXPERIMENT 1 - Determination of the purity and identity of organic compounds by melting point and/or analytical thin layer chromatography**

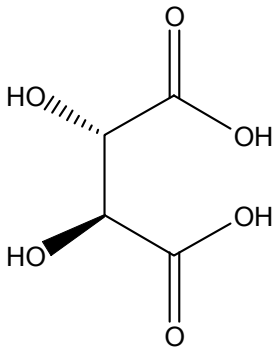
PART A Melting points and mixed melting points.

As discussed in the introductory handout, a sharp melting point is generally a reliable indicator of the purity of an organic compound. Solid mixtures tend to show broad melting point ranges. The identity or non-identity of 2 solids, which have similar melting points, can easily be established by carrying out a mixed melting point experiment. Yani, if the melting point does not change upon mixing, then the 2 solids are identical. If the 2 samples have different compositions, a significant lowering and broadening of the melting temperature range will be observed.

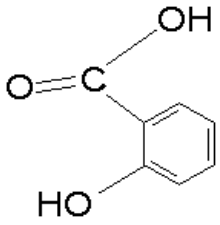
**The Experiment**.

You will be supplied with two standards:

- **A**: L-tartaric acid [m.p. 160°C]




- **B**: Salicylic acid [157-159°C]



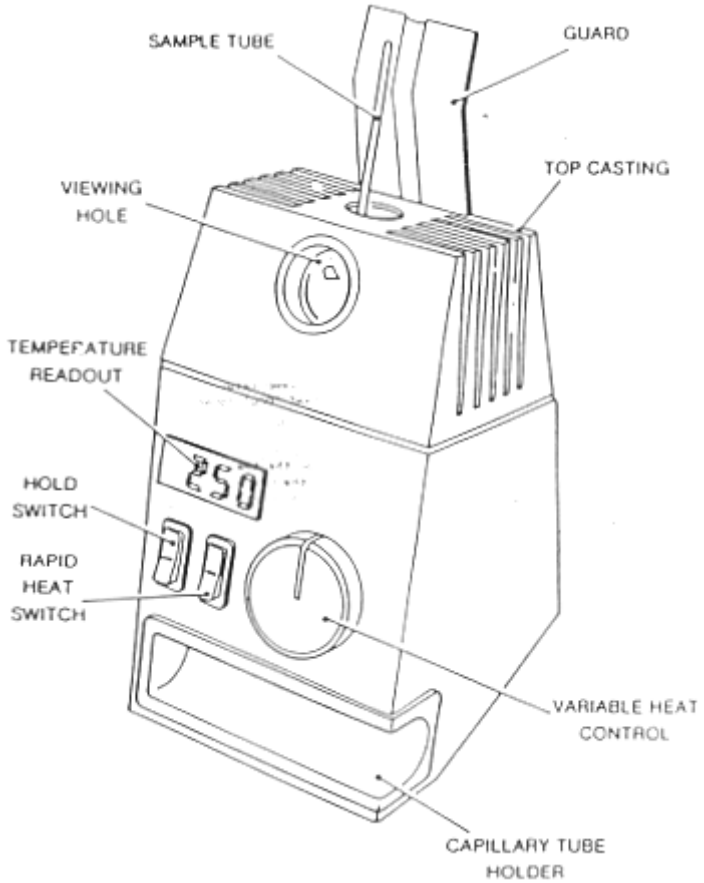

You will also be supplied with an **unlabeled** sample **C**, that is one of the above two compounds.

Clearly, the melting points of the standard compounds are similar. Thus, a simple melting point determination of the unknown sample C will not allow you to conclusively distinguish whether it is of the type A or the type B.

To determine whether the unknown sample C is A or B, perform the following steps:
(i) Determine the melting points of samples A, B and C.
(ii) Grind together a small amount (several mg at most) of your unknown sample C with an equal amount of sample A or sample B to afford two binary mixtures. Determine the melting points of the two mixtures. Draw the appropriate conclusion regarding the identity of your unknown compound. Perform the 3 melting point determinations at the same time.

**Melting point apparatus**.
Please share the two melting point apparatus that are provided. Take turns using the instruments. A schematic drawing of the apparatus and a brief set of instructions may assist your analysis (see below). Please familiarize yourself with the operation of this instrument and if you have any doubts, please consult your demonstrator.

DETERMINATION OF A MELTING POINT
Place a few mg of solid material onto a clean surface and grind it to a fine powder with a clean spatula. Note that grinding will not affect the melting point of the sample unless impurities are introduced. The grinding makes it easier to introduce the sample into the capillary tubes. Insert the sample into a capillary tube and pack it firmly by tapping the capillary on the bench. The height of the sample in the capillary should be about 2-3 mm. Insert the sample in the apparatus and determine the melting point. The speed of heating should be adjusted so that near the melting point the rate does not exceed 1-2 degrees per minute. Be patient! To save time, heat the melting apparatus quickly to 80-90°C by setting the heating rate accordingly. Then reduce the rate of heating to that suggested above and insert your samples.




Basic Procedure:
1. Load sample crystals into a capillary tube to a depth of about 2mm and tap the bottom of the tube on the bench a fixed number of times. Try tapping five times, for example. The sample depth and packing can affect the result and should be kept as consistent as possible.

2. Insert the sample tubes(s) into the top of the heating block and insert empty sample tubes into any holes not being used.

3. If the approximate melting point of the sample is not known, a quick initial result can be obtained by heating the block rapidly. Yani, turn the control to medium-high initially and note the temperature range at which the sample melts. Remember that at high heating rates, a temperature difference will exist between the thermometer and the sample of up to 15°C. If you were to take the reading under these circumstances, you would be in error, as the apparent melting temperature would be artificially elevated compared to the true value. To obtain a proper reading, please repeat the experiment using a proper temperature gradient.

**CHROMATOGRAPHY BACKGROUND**

Chromatography is a term applied to any separation technique that is based on the principle of distributing a sample between a stationary phase and a mobile phase. Simply put, a compound will partition between the mobile phase and stationary phase. In other words, there are moments when a compound is adsorbed/immobilized to the stationary phase and other moments when the same molecule is desorbed/dissolved in a mobile phase. Since the mobile phase is made to flow along a stationary phase, any solutes (eg., the compound) physically move with respect to the stationary phase. Thus, a compound delivered to one end of a chromatography column will adsorb-desorb from the stationary phase and eventually emerge at the other end of the column under the influence of the moving mobile phase. The relative amount of time spent adsorbed in comparison to dissolved is a characteristic function of the compound's partition coefficient under the given experimental conditions. It follows that a separation of two or more components can be achieved whenever their partition coefficients differ.

If one component of the mixture interacts favorably with the stationary phase, a high pentage of molecules of that component will be held immobile. A second component, held weakly, will display a high percentage of molecules in the mobile phase. On average, the molecules of the component that are held less strongly will chromatography (move over the stationary phase in the direction of the flow) at a higher rate than the molecules of the tightly adsorbing compound. In this way, different components will define separate regions or bands along the stationary phase. This separation of the bands is related linearly to the distance travelled on the stationary phase. In other words, easy separations require only a short stationary phase and difficult separations, in which components have very similar distribution coefficients, require a long stationary phase.

<u>Types of Chromatography</u>

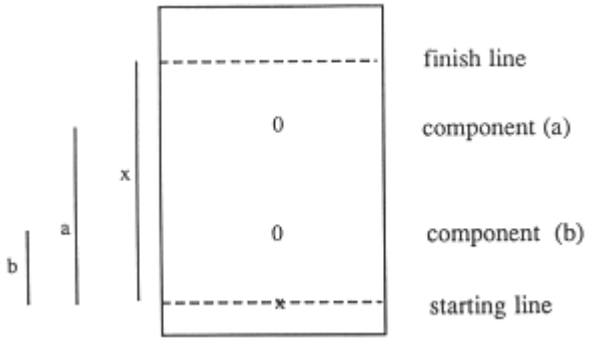
| **Stationary Phase** | **Mobile Phase** |
|---|---|
| a)*Vapour Phase Chromatography (gas-liquid)* | |
| Liquid film adsorbed on a solid support | Helium or nitrogen gas |
| b)*Column Chromatography (solid-liquid)* | |
| Silica gel, alumina | Liquid eg. hexanes, ether, ethyl acetate |
| c)*Thin Layer Chromatography (solid-liquid)* | |
| Silica gel, alumina | Liquid eg. hexanes, ether, ethyl acetate, methanol |

**PART B - Thin Layer Chromatography**

In thin layer chromatography, the solvent (mobile phase) will move up a plate coated with silica gel (stationary phase) under the influence of capillary action. The plate is allowed to develop until the solvent has moved a desired distance (finish line). The plate is then removed from the developing chamber and the solvent on the plate is allowed to dry. By this method, components of a mixture are spatially separated.

The material separated on a tlc plate can be visualized in one of several ways:
(a) Place the plate into an iodine chamber (The iodine usually stains the plate a brown color wherever there is a concentration of organic material).
(b) Use ultraviolet light (Commercial plates generally contain a fluorescence indicator. Wherever the concentration of a component is sufficient, the fluorescence is extinguished and a spot is seen).
(c) Char the surface with sulfuric acid or molybdic acid preparations. These reveal dark spots where organic components are concentrated.

The ratio of the rate of migration of a particular component compared to that of the solvent is defined as its $R_f$ value for that system. In the above diagram the $R_f$ for component "a"is a/x and for component "b" is b/x. The $R_f$ for a particular substance varies with its structure, the nature of the developing solvent and the type of adsorbant used.




The typical adsorbant of a tlc plate is silica. The surface of the tlc plate is polar because silica is composed of -Si-O-Si-O- and -Si-OH bonds. Since like prefers like, a natural conclusion is that organic molecules containing polar groups will be adsorbed more strongly than organic molecules not containing polar groups. This adsorption process is mainly governed via dipole-dipole interactions and hydrogen bond interactions. Polar molecules, especially those capable of forming hydrogen bonds with the surface, will migrate more slowly than non-polar molecules (assuming that an appropriate developing solvent has been chosen).

Commonly used developing solvents, in increasing order of polarity, include:
(l) saturated hydrocarbons (hexanes, petroleum ether)
(2) aromatic hydrocarbons (toluene)
(3) halogenated compounds ($CH_2C1_2$, $CHCl_3$)
(4) ethers, usually diethyl ether
(5) esters, usually ethyl acetate ($CH_3CO_2C_2H_5$)
(6) alcohols such as methanol, ethanol

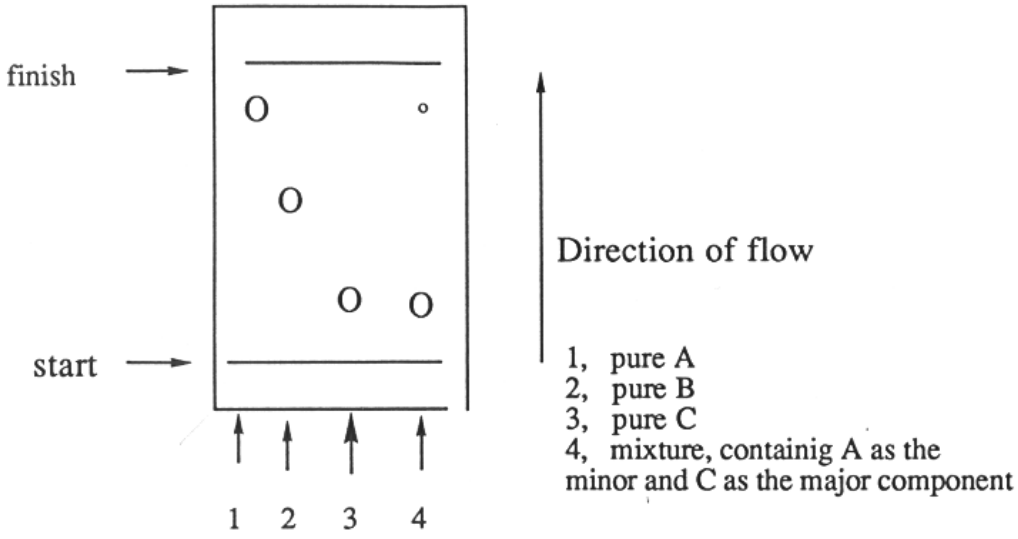
The first goal is to choose a developing system that can separate your components. If the system used is too polar, all the components of the mixture will move very fast ($R_f$

= 0.9 - 1.0). If the developing solvent is very non-polar and the components are polar, then the $R_f$ values of the components will be very small (<0.1) and again no satisfactory separation will be achieved. Often it is necessary to test many solvent systems in order to realize the one that gives a satisfactory separation. A good starting point for many typical organic mixtures is a solvent system composed of about 2 parts hexanes (a mixture of C-6 hydrocarbons) and 1 part ethyl acetate (relatively polar). Alcohols, which are hydrogen bond acceptors and hydrogen bond donors, are typically more strongly adsorbed onto silica than are comparably sized ketones or ethers which can only accept hydrogen bonds. Hydrocarbons, yani, compounds which contain only carbon and hydrogen, are quite non-polar and tend to have large $R_f$ values even when non-polar solvents are used as developing solvents.

| **POLAR** | **LESS POLAR** | **LEAST POLAR** |
|---|---|---|
| R'RCHOH (alcohols) | R"RC=O (ketones)<br>R'RCH-O-$CH_3$ (ether) | Hydrocarbons |

Based on the discussion above, a pure substance when spotted onto a silica-gel plate and properly developed and visualized, will yield a single spot on the chromatogram. The $R_f$ value of that spot will vary depending on the polarity of the developing solvent. Similarly, a mixture of two components, when spotted and developed, should afford two spots after development and visualization of the chromatogram. The $R_f$ values noted will vary depending on the structure of the two components and the choice of the developing solvent. **Conclusion: Only by properly choosing the developing solvent/mixture can two components of an analyte be seen as two distinct spots.**

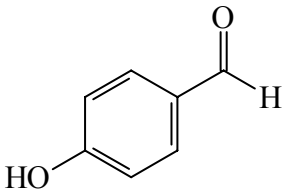
To analyze and identify individual components of a mixture, you should acquire all possible components in pure form (these will serve as standard markers). The analysis is carried out as follows: A small drop of a dilute solution of the mixture and a small drop of each pure component is placed in a separate lane on the silica gel plate. The plate is then developed with an appropriate solvent and the results are visualized. Ideally, the results will be as follows:



## The Experiement

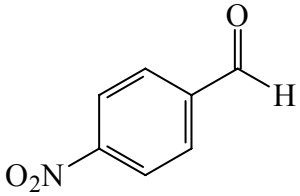
The Problem.

You will be supplied with a mixture which may contain a combination of compounds I and II, I and III, or II and III. You also have pure samples of I, II and III. Your goal is to determine the components of your mixture using analytical tlc.



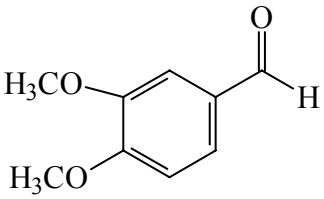

I

4-hydroxybenzaldehyde




II

4-nitrobenzaldehyde



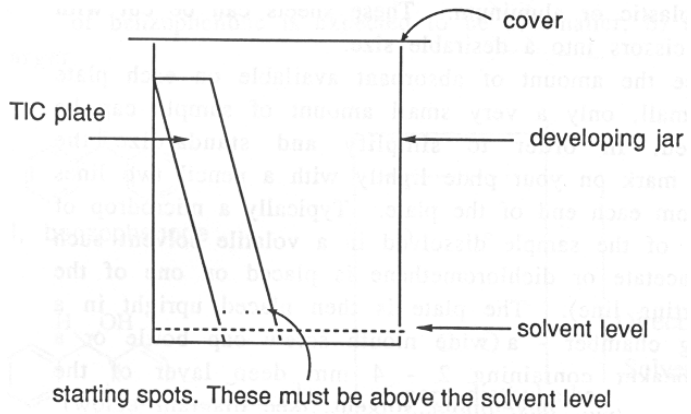

III

3,4-dimethoxybenzaldehyde

Thin layer plates suitable for analytical techniques can be prepared or purchased. Tlc plates are commercially available in 20x20cm sheets consisting of a 0.1mm layer of adsorbant bound to a 0.2mm thick sheet of plastic or aluminum metal. The sheet can be cut with ordinary scissors into a desirable size. Prepare solutions by dissolving about 5mg (several crystals) of I, II, III, and your unknown mixture. Use approximately 1ml of dichloromethane in four separate test tubes or Eppendorf tubes. Using the micro-pipette tips supplied by the demonstrator, spot each solution carefully onto a tlc plate (described below). Check the results with the ultraviolet lamp (use 254nm) to make certain that a small dark spot is visible at each starting point. Do not use too much material! If the spot is too large, the plate will be overloaded and separation may be poor. It may be useful to practice your spotting technique on a strip of 'waste' TLC plate.

In order to simplify and standardize the spotting/developing procedure, lightly mark on your plate with a pencil two lines 0.8cm from each end of the plate. Typically, a microdrop of a solution of the sample dissolved in a volatile solvent such as ethyl acetate or dichloromethane is placed on one of the lines (starting line). The plate is then placed upright in a developing chamber - a small jar or a covered beaker. The vessel should contain a very shallow layer (eg., 3ml total volume) of the developing solvent and its walls can be lined with some filter paper.

Try a 4:1 mixture of hexane/ethyl acetate as an initial condition. You can see a typical set-up below.



Allow the solvent to migrate to the pre-determined point [marked with a pencil] near the top of the plate. Remove the plate and allow it to dry. Visualize the results with a UV lamp. Record the results in your notebook by drawing a sketch of the tlc plate. Determine the $R_f$ values of each of the three pure compounds and those of the components of your mixture (please use a ruler!). Discuss the composition of your mixture in light of your results.

Repeat the above procedure but develop the plate in pure hexane. Run a third chromatogram using a 1:1 mixture of hexane and ethyl acetate. Again record the visual results and determine the $R_f$ values of the components. Each partner should tape at least one TLC plate in his/her notebook as part of their experimental results.

Give a reasonable explanation for the relative $R_f$ values for I, II, and III. Hint – think of the probable interaction of each compound with silica. If the $R_f$ values varied in different developing solvents, try to explain why. Which of the three developing solvents were most useful in allowing you to determine the composition of your mixture?

**Questions**

1. You have synthesized a compound, which you suspect is aspirin. One evidence in particular was the observed melting point of 133-135°C which is near the literature melting point of aspirin, 135°C. You have access to an authentic sample of aspirin. Describe, in less than three lines, a method based on the melting point technique which would enable you to determine whether your suspicion is true.

2. Benzophenone (I) can be reduced to afford benzhydrol (IV). Ibrahim Talıses carried out the experiment and analyzed the starting material and product using tlc. His results are shown below. The tlc shown was obtained using 6 parts of diethyl ether and 1 part of ethyl acetate as developer.

i) What conclusion can be drawn about the purity of the product?
ii) What is the $R_f$ of benzophenone under these conditions?
iii) What is the $R_f$ of benzhydrol?
iv) Give an explanation of the qualitative difference in the $R_f$ of the two compounds.
v) When the solvent is changed to 1 part ethyl ether and 1 part ethyl acetate, the $R_f$ of

- benzhydrol is expected to be a) smaller, b) the same, c) larger?
- benzophenone is expected to be a) smaller, b) the same , c) larger?