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TESTS OF ORGANIC COMPOUNDS

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Abstract

In this paper, several methods for identification, characterization, tests of organic compounds, determination of molecular weight, solubility of unknown compounds, TLC technique .Each of you will receive two vials: one containing a liquid unknown and one a solid unknown. You will perform a number of experimental procedures on these compounds to gather data. The compounds are part of a finite number of compounds that are listed for you in order of increasing mp and bp. You will determine the actual structure of your unknowns by applying your experimental data to these lists, obtaining a shorter list of possible compounds and performing further experiments to make the final determination.

INTRODUCTION

Since each of you has a different compound, you will have to work more independently than before and you will have to evaluate your data and the reliability of your data.

Solubility tests:

For these tests, you should use approx. 30 mg of your compound in 1 mL of solution to give you a 3% solution. The words soluble and insoluble are qualitative, not quantitative. We will use 3% as a determination of solubility. If your compound is not completely soluble as a 3% solution, you can call it insoluble or partially soluble. Moreover, some compounds which are not soluble at room temperature, may be soluble at higher temperature. Therefore, after obtaining data at room temperature, heat your solution in a water bath at 70°C or higher and observe any further changes. The results you record may have more meaning later when you know more about your unknown compound. You will test the solubility/reactivity of your compound in 4 different solutions.

EXPERIMENTAL

Procedure: Into 4 labeled test tubes, add 1 mL of : water; 5% aq HCl; 5% aq NaHCO3; and 5% aq. NaOH. To each tube, add approx. 30 mg of the unknown. Shake or stir for a few seconds. Record your observations. It may take several minutes for the unknown to appear to dissolve or react. After suitable time, heat the four test tubes in a water bath and observe. Look for color changes, evolution of gas, any evidence of reaction such as precipitates, and enhanced solubility. Record your observations.

Repeat the solubility test for the other unknown.

Most organic compounds are not water soluble. Only low molecular weight and/or highly polar compounds will be water soluble to 3%. Salts of acids or bases will often be soluble.

If the compound is not soluble in water, solubility in acid suggests that it is basic (eg, amines), solubility in strong base suggests that it is at least weakly acidic (eg, phenols) and solubility in weak base (bicarbonate) suggests that it is a stronger acid (eg, carboxylic acids).

Whatever determination you make from this solubility test, you will corroborate that suggestion with other data including class tests and analysis of the compound's infrared spectrum.

Compounds which are insoluble in all of the above liquids can be tested for solubility and color change in concentrated sulfuric acid. Compounds giving a color change or showing solubility include neutral compounds such

as alkenes, alkynes, alcohols, ketones, aldehydes, esters, ethers, amides and nitro compounds. Compounds that appear inert include alkanes, alkyl halides, and simple aromatic hydrocarbons. Dispose of the sulfuric acid solution carefully by pouring it into a beaker of cold water, carefully rinsing the test tube with water, and washing both down the sink.

Class tests

All class tests must be done in triplicate: known, unknown, and blank. The known gives you a positive test for reference and tells you if the reagents or procedure are faulty. The blank gives you the other extreme, a negative test. The results you obtain from your unknown can then be put into perspective. The triplicate tests should be done simultaneously thereby taking little extra time. For each test, use clean test tubes. For certain tests, the presence of even trace amounts of acetone can give false positives.

Do only those tests that you think will be useful based upon solubility results and your analysis of the infrared spectrum of the compound. If that analysis has not been done and you wish to do some class tests, do them in the order shown below.

Beilstein test for halogenated compounds: This test is very sensitive. Take a piece of copper wire with a loop on the end and heat it in the flame of a Bunsen burner until it glows red. Allow the wire to cool but avoid contaminating it especially if you put it down on the hood surface. Dip the cool loop into the known compound, and place it in the flame. You should observe a green flame after the first few seconds when your known compound burns (yellow flame). Burn off all remaining halogenated known compound, heat the loop until it glows red, then let the loop cool and run the test on you unknown solid or liquid. For knowns use bromobenzene or chlorbenzene for the liquid and a halogenated benzoic acid for the solid. This test should be done in a hood.

Ignition Test for Aromaticity: Place a small amount of compound on the end of a spatula or on a porcelain lid and apply the flame from a Bunsen burner. Highly unsaturated compounds such as aromatic compounds burn with a yellow, sooty flame. Use naphthalene for the solid unknown and toluene or xylene for the liquid known. This test should be done in a hood.

Bromine Test for Unsaturation: Alkenes and alkynes will readily add bromine across the multiple bond unless there are electron withdrawing groups on the multiple bond. One observes the rapid disappearance of the red-brown bromine color. Aromatic compounds can react with bromine more slowly to give bromine substitution and the formation of HBr, which can sometimes be observed by placing a piece of wet litmus paper over the mouth of the test tube. Warning: the reagent deteriorates with the formation of HBr so compare the results with a blank.

Into a dry, clean test tube, dissolve 0.1 mL of a liquid (or 50 mg of a solid) in 1 mL of methylene chloride. Add a 2% solution of bromine in a dichloromethane dropwise with agitation until the bromine color persists. A positive test requires five or more drops of bromine solution to reach a persistent red-brown color. Use cyclohexene, octene, or another simple alkene as the known. Use toluene as a known to test for aromaticity.

Permanganate Test for Unsaturation (Baeyer Test): Aqueous permanganate rapidly oxidizes double and triple bonds while being reduced to MnO2, a brown precipitate. Therefore, disappearance of the purple color and formation of a brown precipitate in minutes is a positive test. However, other compounds react slowly with the reagent including alcohols, aldehydes, phenols, and aromatic amines so interpret your results carefully and look for corroboration from the other tests.

Into a clean test tube, dissolve 0.1 mL of a liquid (or 50 mg of a solid) in 1 mL of 95% ethanol or 1,2-dimethyoxyethane. Add a 1% solution of aqueous potassium permanganate dropwise with agitation.

Use cyclohexene, octene or another simple alkene as the known.

Aldehydes and Ketones--2,4-DNP. Hydrazines such as 2,4-dinitrophenylhydrazine react with the carbonyl group of aldehydes and ketones to give colored precipitates. Normally the reaction is fast but heating may be necessary. The test solution is prepared using sulfuric acid and 95% ethanol. Later, if you wish to make a derivative of your compound, you can use a different 2,4-DNP solution prepared with HCl and methanol. This usually gives a slower forming precipitate which often provides a derivative of higher purity (and higher mp). However, the slow formation of the precipitate is not desirable when looking for a qualitative test signal. The 2,4-DNPs are usually yellow, orange, or red with the deeper color often signifying higher conjugation via double bonds or aromatic rings.

Dissolve only 1 drop of your liquid compound (or 10 mg of your solid) in a minimum number of drops of 95% ethanol in a test tube. Add 1 mL of the 2,4-DNP *test* solution and agitate. If a precipitate does not form in 10 minutes, heat on a water or steam bath for a few minutes. For knowns, use cyclohexanone and benzaldehyde. Warning: a trace of acetone will give a positive test.

Tollens Test for Aldehydes and other easily oxidized functional groups: In this test, a stabilized silver ion is reduced to elemental silver by an easily oxidized compound, such as an aldehyde. The aldehyde is oxidized to a carboxylic acid. A positive test is the formation of a silver mirror as the elemental silver adheres to the wall of the glass tube. Prepare the Tollens reagent immediately before you plan to use it. Mix 3 mL of Tollens solution A (aqueous silver nitrate) with 3 mL of Tollens solution B (10% aq. NaOH) resulting in the formation of solid silver oxide. Add 10% ammonium hydroxide solution dropwise, with agitation, until the silver oxide just dissolves. This produces a silver ammonium complex and is the Tollens solution you will use for the test.
Into each of 3 clean, dry test tubes, add 2 mL of the Tollens reagent which is freshly prepared as above. Dissolve 10 mg of a solid (or 1 drop of a liquid) unknown in the minimum amount of bis(2-ethoxyethyl)ether required to give a clear solution (less than 1 mL). Add the unknown solution dropwise, with agitation, to the first test tube. Mix vigorously and allow the solution to stand. Do the same for the known compound. Do not use benzaldehyde for the known. For the blank, simply add 0.5 mL of bis(2-ethoxyethyl)ether. **Warning**: Wash any minor amounts of residual Tollens reagent into a sink and flush with water. The reagent forms silver fulminate which is very explosive. The test solutions can be disposed of in a jar labeled for that purpose. The silver mirror can usually be washed clean with soapy water and a scrub brush. If not, see your instructor.

Chromic Acid Test for Aldehydes and Alcohols: Dissolve 10 mg of a solid (or 1 drop of a liquid) unknown in reagent grade acetone in a clean, dry test tube. Add a few drops of chromic acid solution one drop at a time with shaking. Aldehydes and primary and secondary alcohols are oxidized very quickly. Tertiary alcohols are not oxidized. The orange color of the Cr(VI) ion is replace by the green color of Cr(III) as the chromium is reduced. Aliphatic aldehydes are oxidized in less than a minute, aromatic aldehydes take a bit longer. Since the condition of the acetone is critical, it is wise to carefully run the blank to be certain that the acetone itself is not giving a false positive. Use benzaldehyde and an aliphatic aldehyde for aldehyde knowns and 1- and 2-butanol for alcohol knowns. The chromic acid solution is prepared by dissolving 1.0 g of CrO_3 in 1.0 mL of concentrated sulfuric acid and then carefully diluting with 3 mL of water. **Warning**: Cr(VI) compounds are considered suspect carcinogens and should be handled carefully.

Ferric Hydroxamate Test for Esters. If you have a carbonyl compound which is not an aldehyde or ketone or carboxylic acid, it could be an ester. One test for esters is the ferric hydroxamate test whereby the ester is converted to a hydroxamic acid (HOHN-C=O) which will give a positive ferric chloride test.
Since enols can give a positive ferric chloride test, first test your compound with ferric chloride solution as follows: dissolve 10 mg of solid (or 1 drop of liquid) unknown in 1 mL of 95% ethanol, add 1 mL of 1 N HCl, and then a 1-2 drops of 5% ferric chloride solution. If you obtain a color other than yellow, the test cannot be used. Otherwise, the test is conducted as follows: dissolve 50 mg of solid or 2 drops of liquid unknown in 1 mL of 0.5N hydroxylamine hydrochloride in 95% ethanol and 0.2 mL 6N NaOH. Heat to boiling for 2-3 minutes, then cool and add 2 mL 1N HCl. If the solution becomes cloudy, add 1-2 mL of 95% ethanol to clarify. Add 1 drop of 5% ferric chloride solution. If a color forms and then fades, add additional drops of 5% ferric chloride until the color persists. The color is due to a complex between the hydroxamic acid and the ferric ion. A deep burgundy color is positive. Use banana oil or methyl benzoate as knowns.

Ferric Chloride test for Phenol's: Just as enols can form colored complexes with ferric ion, phenolate ions can as well. Therefore, this test is designed to convert the weakly acidic phenols to their conjugate base which can then complex with ferric ion. If the phenol is water soluble, add a few drops of 2.5% aqueous ferric chloride solution to a 3% aqueous solution of the phenol. A deep red, green, or blue color is positive. If the phenol is not water soluble, dissolve 20 mg of the solid (or 1 drop of the liquid) in 1 mL of methylene chloride and add 1 drop of pyridine. Add 3 drops of 1% ferric chloride in methylene chloride. An intense color is a positive test. Use phenol as a known. Not all phenol's will give a positive test.

Iodoform test for methyl ketones: In this test you will convert the methyl ketone to a triidomethyl ketone which is then cleaved to form iodoform, HCI3, a yellow solid. Acetone gives a nice positive test so be certain that no traces of acetone are in your glassware. In a large clean test tube or a vial, place 100 mg of a solid or 5 drops of a liquid unknown. Add 2 mL bis(2-ethoxyethyl)ether, 5 mL of water, and 1 mL of 10% NaOH, and mix well. Add a total of 3 mL of iodine-potassium iodide solution in six equal portions, stopper and shake well after each addition. **Caution**: seal the tube carefully and avoid skin contact with the iodine solution. The color of the iodine will disappear more slowly in the later additions. The solution should be slightly yellow. Heat if necessary and shake again to force the iodine to react. When the color is slightly yellow, add water to nearly fill the test tube or container, stopper, and shake vigorously. After standing for 15 minutes, a pale yellow precipitate of iodoform (mp 119-121°C) is a positive test for a methyl ketone. Acetone can be used for the known.
The iodine-potassium iodide solution is prepared from 10 g of iodine and 20 g of potassium iodide in 100 mL of water.

Hinsburg Test for Amines: If you have a basic compound which you believe to be an amine, you can corroborate your suspicion and determine if you have a primary, secondary, or tertiary amine using the Hinsberg test. You will react the amine with a sulfonyl chloride forming an insoluble sulfonamide of a primary or secondary amine or the soluble salt of a tertiary amine. The insoluble sulfonamide of a primary amine will be made soluble in base (via removal of the slightly acidic proton on N) but that of a secondary amine will not (no proton on N to remove).
Add 100 mg of a solid or 0.1 mL of a liquid unknown, 200 mg of *p*-toleuenesulfonyl chloride, and 5 mL of 10% KOH solution to a clean test tube. Stopper the tube and shake it for several minutes. Remove the stopper and heat the mixture on a steam bath for 1 minute. Cool the solution and if it is not basic to pH paper, add additional KOH solution. If a precipitate has formed, add 5 mL of water and shake vigorously. If the precipitate does not redissolve in the basic solution, it is indicative of a sulfonamide of a secondary amine. If there is no precipitate, add 5% HCl until the solution is just acidic when tested by pH paper. Formation of a precipitate under acidic conditions suggests that the previously soluble sulfonamide was of a primary amine. If no precipitate has formed, the initial amine could have been tertiary.
Use aniline, N-methylaniline and N,N-dimethylaniline for knowns.

UNKNOWN REPORTS:

You should submit an Unknown Preliminary Report as soon as you have completed the experiments described below:

For the **solid unknown**, you will take an accurate mp, an IR spectrum using the KBr procedure, solubility tests and, based upon an analysis of this information, do specific functionality tests as described in the manual. If you suspect that you have a carboxylic acid, we will ask you to do a neutralization equivalent. Then submit a preliminary analysis form. This form is not graded. It gives us a chance to verify that you have not been misled. We will either OK your data or suggest that you repeat certain procedures. At this stage, you should have determined the class of your compound (eg, aldehyde, ketone, ...) and possibly some additional functionality such as unsaturation, presence of a halogen, etc.

For the **liquid unknown**, you will take a micro bp, you will take an IR spectrum using the sandwich cell NaCl method, do solubility tests and, based upon an analysis of this information, do specific functionality tests as described in the manual. Once again, submit a preliminary analysis form.
After the preliminary analysis has been confirmed, try to determine the actual structure of your unknown. You will need to make a solid derivative of each unknown to verify your identification of the compounds. For carboxylic acids, the neutralization equivalent may substitute for the derivative.

Possible compounds and their derivatives are listed in the manual and in other texts which are available to you. This semester, all of your unknowns are listed in the Manual but you can consult other texts such as Shriner, Fuson, Curtin, "The Systematic Identification of Organic Compounds", Vogel, "Elementary Practical Organic Chemistry, Part 2, Qualitative Organic Analysis", "CRC Handbook of Tables for Organic Compound Identification"--all of these are in the lab and available to you. All tables are listed first by class of compound (alcohol, acid, ether, etc). Some headings are more specific such as aliphatic acid, aromatic acid, carboxylic acid, dicarboxylic acid,

halogenated acid, , etc. Be certain that you have checked all the tables that pertain to your search. The compounds are then listed by escalating mp and bp. Therefore, it is critical that you determine the major functional group or groups in your compound (IR analysis, solubility and classification tests) so that you know the CLASS of compound. Also, your mp and bp must be accurate in order to narrow the possible choices within that class. Consider all possibilities
within at least plus or minus 5 ^{o}C of your mp or bp up to 175 degrees and a wider range for mp and bp above 175^{o}C.

Final report of the unknowns: report the unknown number, your name, appropriate properties, the results of the solubility tests, the results of the class tests (list the test and whether it was + or - or could not determine), the neutralization equivalent if taken (must be reported for all acids and bases using correct significant figures), all possible compounds within that class and close to that mp or bp (plus or minus 5 degrees if near 150 ^{o}C, plus or minus 7 degrees if above 160 ^{o}C), how you distinguished between these possibilities, a thorough analysis of your infrared spectrum that includes as many features of your compound as you can determine (eg, the major functional group, other functional groups, aromatic units, double or triple bonds, etc.) List the pages in your notebook where we can find information related to this particular unknkown.
If you made a **derivative**, you should report the name of the derivative, its structure, its literature mp, and the mp you observed. If there is a large difference between lit mp and obs mp, comment on it (eg, did you recrystallize the derivative). List the mp of the corresponding derivative of the other possible compounds.
Submit the derivative in a properly labelled vial which includes your name, name of product, mp of product, lit mp of product, date prepared. Also return unused unknown compound.
When you submit this final report you should attach your properly labelled infrared spectrum; eg, your name, the compound name and/or unknown number, and the method you used (KBr, Nujol, neat).
Having the correct structure is insufficient! **You must list other possibilities** and explain how you differentiated between the "correct" compound and those other possibilities.

Determination of Equivalent Weight (or Neutralization Equivalent)
Molecular Weight Determination
Generally, any acid (or base) can be titrated using standard solutions of base (or acid). The neutralization equivalent obtained is usually a simple fraction of the molecular weight (1, 1/2, 1/3, etc). In the titration of an acid with standard base, the endpoint is reached when all the acid is neutralized and a drop of excess base is added. If phenolphthalein is used as the indicator, it will turn red at this instant. For a dicarboxylic acid such as malonic acid, the endpoint is reached when the last of the acid is converted to the carboxylate anion. The neutralization equivalent will be one-half its molecular weight.

Equivalent weights must be done in duplicate and the values obtained should agree within a few percent. If not, do a third determination.
Procedure for acids. Into a 125 ml Erlenmeyer flask place approx. 150 mg* of the unknown, accurately weighed to the nearest 1 mg or 0.1 mg for at least three place accuracy. Add approx. 5-10 ml of 95% ethanol to dissolve the unknown and add an equal amount of water. Add a drop or two of phenolphthalein solution, a magnetic stirring bar, and titrate with standardized aqueous base to the first permanent pink color that lasts about 60 seconds.
The equivalent weight (neutralization equivalent) can be calculated as follows:
V (ml) x **M (mmol/ml)= wt (mg)/equivalent weight (mg/mequiv)**; **V** = volume of standard base used measured accurately to at least 3 significant figures; **M**= molarity of standard base as long as the base is monobasic, probably listed as 0.100M NaOH or KOH (note three significant figures); **wt**= weight of unknown used. An equivalent weight of 120 mg/mequiv means that the molecular weight is some whole number multiple of 120; for example, 120 (if monoacid there is 1 mequiv/mmol) or 240 (if diacid there are 2 mequiv/mmol) or 360 (if triacid), etc.
Procedure for Base. Into a 125 ml Erlenmeyer flask place approx. 150 mg* of the unknown, accurately weighed to the nearest 1 mg or 0.1 mg for at least three place accuracy. Add approx. 5-10 ml of 95% ethanol to dissolve the unknown and add an equal amount of water. Add a drop or two of an appropriate indicator solution, a magnetic stirring bar, and titrate with standardized aqueous acid to the first permanent color change that lasts about 60 seconds.

The calculation for equivalent weight is the same except wherever it says *acid*, substitute the word *base*, and where it says *base*, substitute the word *acid*. Usually 0.100 M standardized HCl is used for the titration.
* larger quantities will be required if 150 mg is less than 1.0 meq since at least 10.0 mL of 0.100 N of solution is required for three place accuracy.

Thin Layer Chromatography (TLC)
You will be using prepared tlc plates which are research quality ($1.50/sheet). Each large TLC sheet will be carefully cut to provide mini tlc plates. Do not touch the adsorbent with your fingers, hold the plates at the sides. Do not use ink on the plates, write in pencil *lightly*.

For a developing chamber, you will use your 4 oz. wide-mouth jar with a piece of filter paper *ca*. 3 inches in diameter (trim if necessary). The half TLC plate you receive can be cut width-wise into thirds to make three mini-tlc plates about 4 cm wide. Cut evenly and carefully using sharp scissors. These mini tlc plates will fit into the 4oz. jars. 2.5-3 mL of ethyl acetate should be used as the developing solvent to obtain the proper depth with a baseline 0.5 cm above the bottom of the mini tlc plate. The solvent front should be allowed to run as close to the top of the plate as possible but not to the top, stop at least 1 mm from the top. These plates take about 5 min. to develop.
1. Prepare at least 5 micropipettes for sample spotting.
2. Prepare samples of your aspirin, acetaminophen, and caffeine by dissolving ca. 10 mg of compound in 1.0 mL of 1:1 methylene chloride/ethanol in a stoppered vial.
3. Practice your spotting technique on a sheet of filter paper (or towelling) until you are satisfied that you can apply narrow diameter samples and that your pipettes are reliable.
4. Draw a very light **pencil** line at 0.5 cm from the bottom of your mini tlc plate (it is very important that the pencil does not gouge out the adsorbent).
5. Spot samples on the line in a predetermined order or, even better, code the samples in pencil at the top of the tlc plate. Include a sample of the unknown you have chosen.
6. Observe your spotted samples under ultraviolet light to see if they are in appropriate positions on the starting line of the tlc plate.
7. Carefully place the tlc plate into the developing chamber so that the bottom of the plate is flat in the ethyl acetate developing solvent and the plate does not touch the filter paper. Close the developing chamber carefully without disturbing your tlc plate. The solvent front should be rising along the tlc plate evenly.
8. When the solvent front is 0.5 cm from the top, remove the tlc plate from the developing tank and immediately mark the solvent front on the plate with a pencil. Let the tlc plate dry in the hood then observe it under UV light. Circle the spots with a pencil. Record the Rf values.
9. Visualize the tlc plate in an iodine chamber. Record any differences you observe.
The number of variables affecting absolute chromatographic values is large. To the obvious variables of solvent, medium, and sample, can be added**:**
A. activation of adsorbent; **B**. amount of sample applied; **C**. solvent composition; **D**. amount of adsorbent; **E**. solvent developing distance; **F**. chamber saturation (actually an error if not saturated, not a variable); **G**. impurities in both phases; **H**. temperature; **I**. room humidity; **J**. distance of the sample spot from the chamber solvent level;...
With so many variables, qualitative information is usually obtained by separating known solutions (standards) on the same sheet as the sample.
TLC is a simple, quick, and inexpensive procedure that gives the chemist a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC plate).
A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.
As the solvent moves past the spot that was applied, an equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualization is straightforward. Usually the compounds are not colored, so a UV lamp

is used to visualize the plates. (The plate itself contains a fluorescent dye which glows everywhere *except* where an organic compound is on the plate.)

How To Run a TLC Plate

Step 1: Prepare the developing container
The developing container for TLC can be a specially designed chamber, a jar with a lid, or a beaker with a watch glass on the top (the latter is used in the undergrad labs at CU). Pour solvent into the chamber to a depth of just less than 0.5 cm. To aid in the saturation of the TLC chamber with solvent vapors, you can line part of the inside of the beaker with filter paper. Cover the beaker with a watch glass, swirl it gently, and allow it to stand while you prepare your TLC plate.

Step 2: Prepare the TLC plate
TLC plates used in the organic chem teaching labs are purchased as 5 cm x 20 cm sheets. Each large sheet is cut horizontally into plates which are 5 cm tall by various widths; the more samples you plan to run on a plate, the wider it needs to be. Shown in the photo to the left is a box of TLC plates, a large un-cut TLC sheet, and a small TLC plate which has been cut to a convenient size. Handle the plates carefully so that you do not disturb the coating of adsorbent or get them dirty.

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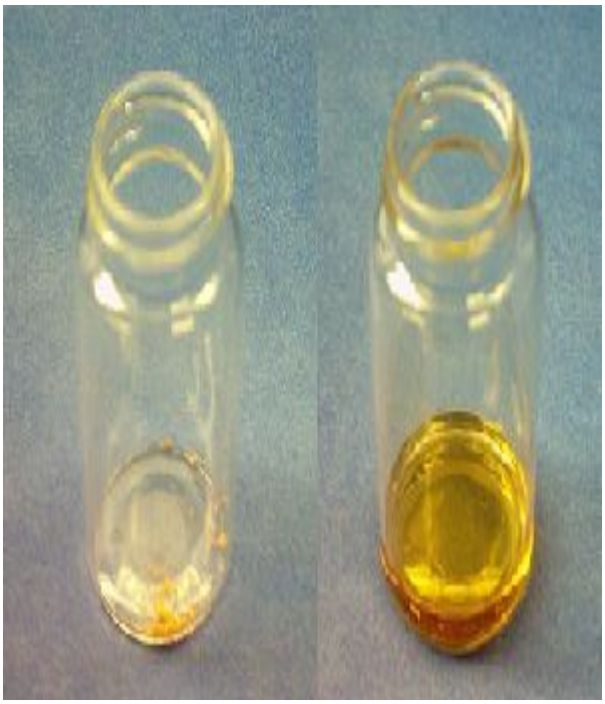
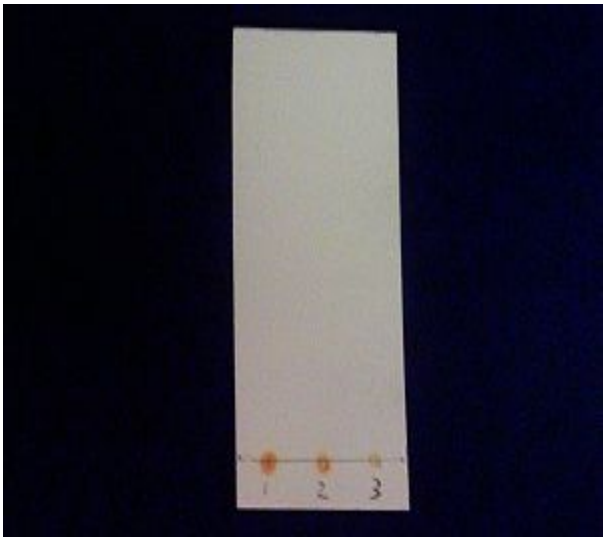
Measure 0.5 cm from the bottom of the plate. Using a pencil, draw a line across the plate at the 0.5 cm mark. This is the *origin*: the line on which you will spot the plate. Take care not to press so hard with the pencil that you disturb the adsorbent. Under the line, mark lightly the name of the samples you will spot on the plate, or mark numbers for time points. Leave enough space between the samples so that they do not run together; about 4 samples on a 5 cm wide plate is advised.



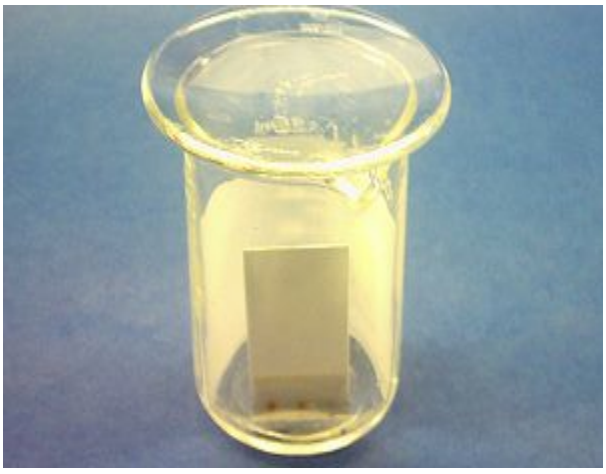
Step 3: Spot the TLC plate

If the sample is not already in solution, dissolve about 1 mg in 1 mL of a volatile solvent such as hexanes, ethyl acetate, or methylene chloride. As a rule of thumb, a concentration of 1% usually works well for TLC analysis. If the sample is too concentrated, it will run as a smear or streak (see troubleshooting section below); if it is not concentrated enough, you will see nothing on the plate. Sometimes you will need to use trial and error to get well-sized, easy to read spots.

Obtain a a microcapillary. In the organic teaching labs, we use 10µL microcaps - they are easier to handle than the smaller ones used in research labs. Dip the microcap into the solution and then **gently** touch the end of it onto the proper location on the TLC plate. Don't allow the spot to become too large - if necessary, you can touch it to the plate, lift it off and blow on the spot. If you repeat these steps, the wet area on the plate will stay small.

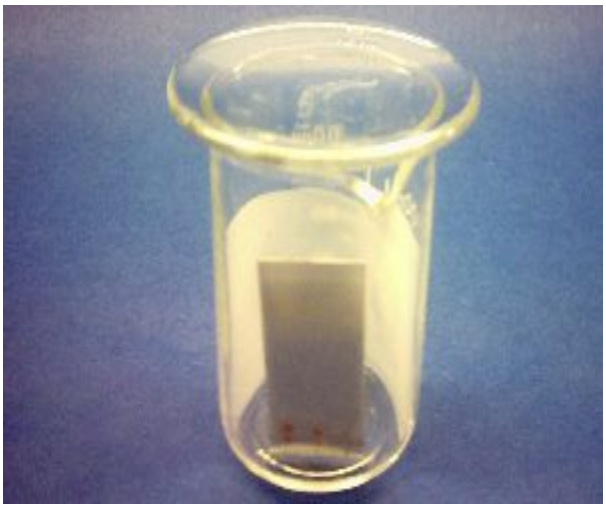


This example plate has been spotted with three different quantities of the same solution and is ready to develop. If you are unsure of how much sample to spot, you can always spot multiple quantities and see which looks best.

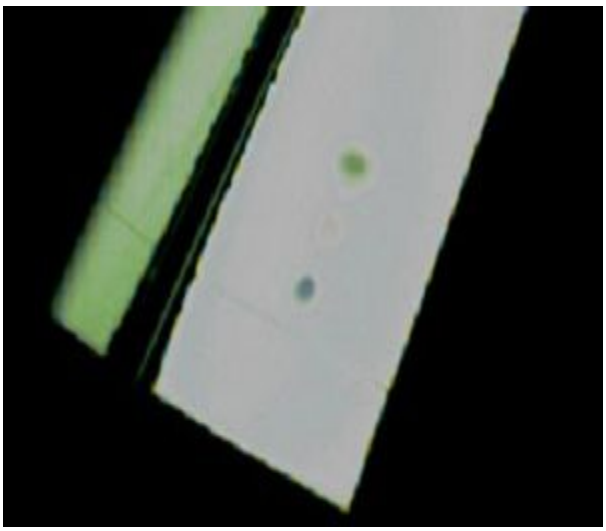


Step 4: Develop the plate

Place the prepared TLC plate in the developing beaker, cover the beaker with the watch glass, and leave it undisturbed on your bench top. The solvent will rise up the TLC plate by capillary action. Make sure the solvent does not cover the spot.

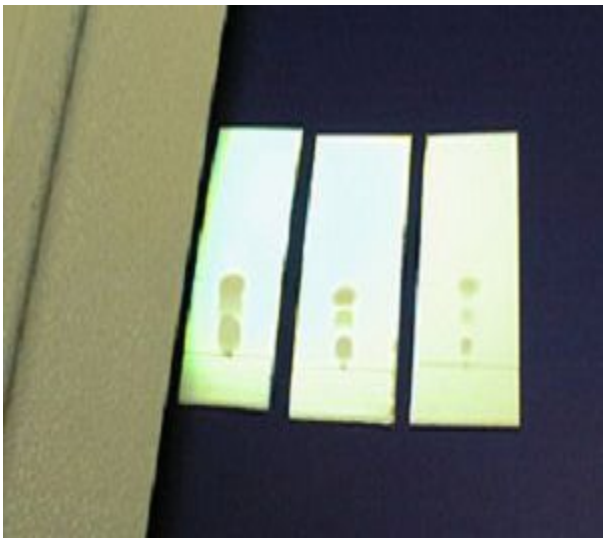


Allow the plate to develop until the solvent is about half a centimeter below the top of the plate. Remove the plate from the beaker and immediately mark the solvent front with a pencil. Allow the plate to dry.

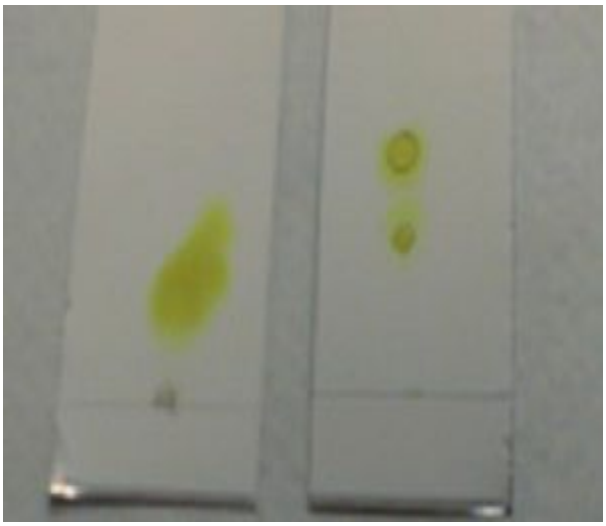


Step 5: Visualize the spots

If there are any colored spots, circle them lightly with a pencil. Most samples are not colored and need to be visualized with a UV lamp. Hold a UV lamp over the plate and circle any spots you see. Beware! UV light is damaging both to your eyes and to your skin! Make sure you are wearing your goggles and do not look directly into the lamp. Protect your skin by wearing gloves.



If the TLC plate runs samples which are too concentrated, the spots will be streaked and/or run together. If this happens, you will have to start over with a more dilute sample to spot and run on a TLC plate.



Here's what overloaded plates look like compared to well-spotted plates. The plate on the left has a large yellow smear; this smear contains the same two compounds which are nicely resolved on the plate next to it.

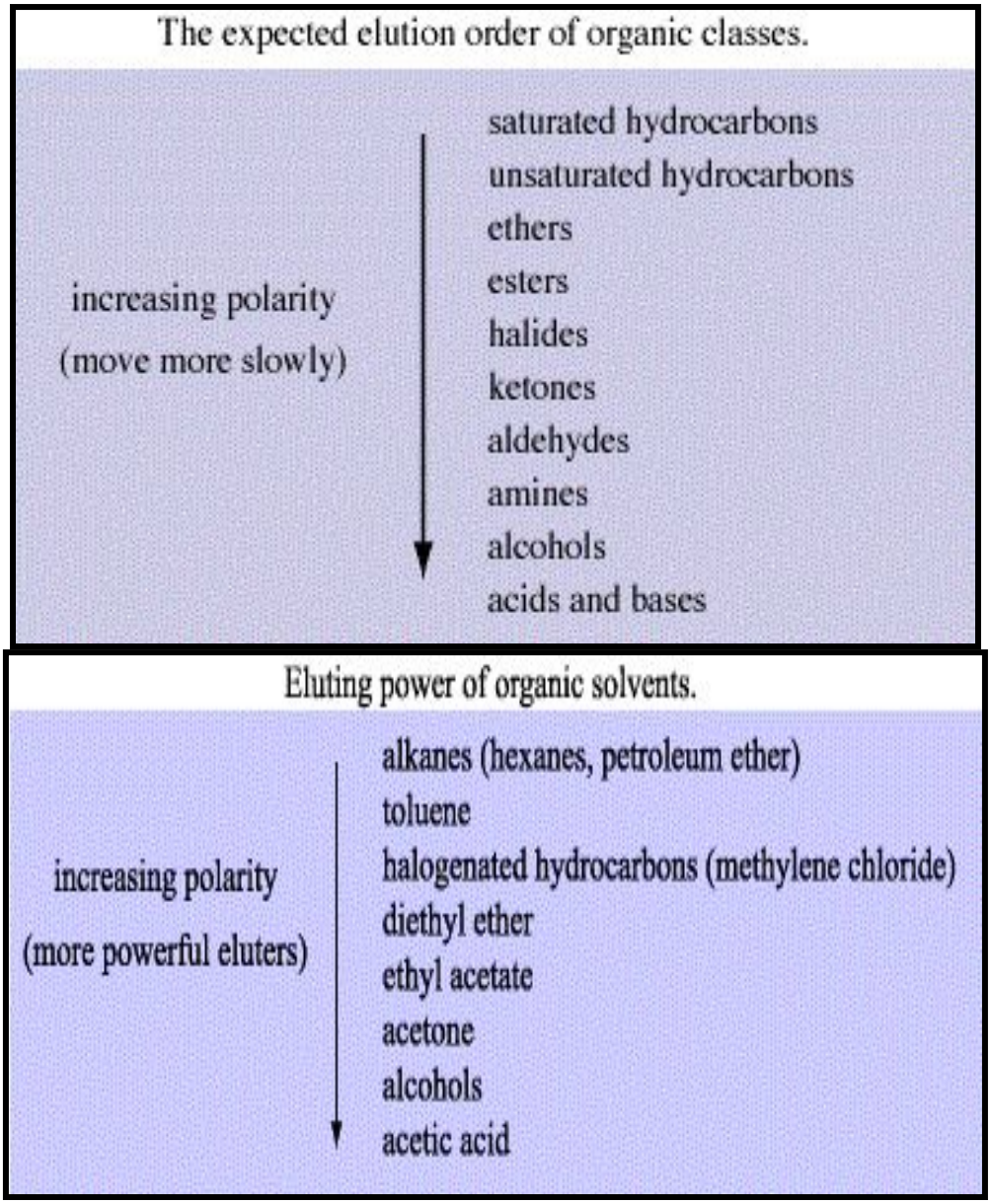
TLC Solvents Choice

When you need to determine the best solvent or mixture of solvents (a "solvent system") to develop a TLC plate or chromatography column loaded with an unknown mixture, vary the polarity of the solvent in several trial runs: a process of trial and error. Carefully observe and record the results of the chromatography in each solvent system. You will find that as you increase the polarity of the solvent system, all the components of the mixture move faster (and vice versa with lowering the polarity). The ideal solvent system is simply the system that gives the best separation.

TLC elution patterns usually carry over to column chromatography elution patterns. Since TLC is a much faster procedure than column chromatography, TLC is often used to determine the best solvent system for column chromatography. For instance, in determining the solvent system for a flash chromatography procedure, the ideal system is the one that moves the desired component of the mixture to a TLC R_f of 0.25-0.35 and will separate this component from its nearest neighbor by difference in TLC R_f values of at least 0.20. Therefore a mixture is analyzed by TLC to determine the ideal solvent(s) for a flash chromatography procedure.

Beginners often do not know where to start: What solvents should they pull off the shelf to use to elute a TLC plate? Because of toxicity, cost, and flammability concerns, the common solvents are hexanes (or petroleum ethers/ligroin) and ethyl acetate (an ester). Diethyl ether can be used, but it is very flammable and volatile. Alcohols (methanol, ethanol) can be used. Acetic acid (a carboxylic acid) can be used, usually as a small percentage component of the system, since it is corrosive, non-volatile, very polar, and has irritating vapors. Acetone (a ketone) can be used. Methylene chloride or and chloroform (halogenated hydrocarbons) are good solvents, but are toxic and should be avoided whenever possible. If two solvents are equal in performance and toxicity, the more volatile solvent is preferred in chromatography because it will be easier to remove from the desired compound after isolation from a column chromatography procedure.

Ask the lab instructor what solvents are available and advisable. Then, mix a non-polar solvent (hexanes, a mixture of 6-carbon alkanes) with a polar solvent (ethyl acetate or acetone) in varying percent combinations to make solvent systems of greater and lesser polarity. The charts below should help you in your solvent selection. You can also download this pdf chart of elution order.



Interactions Between the Compound and
Interactions Between the Compound and the Adsorbent

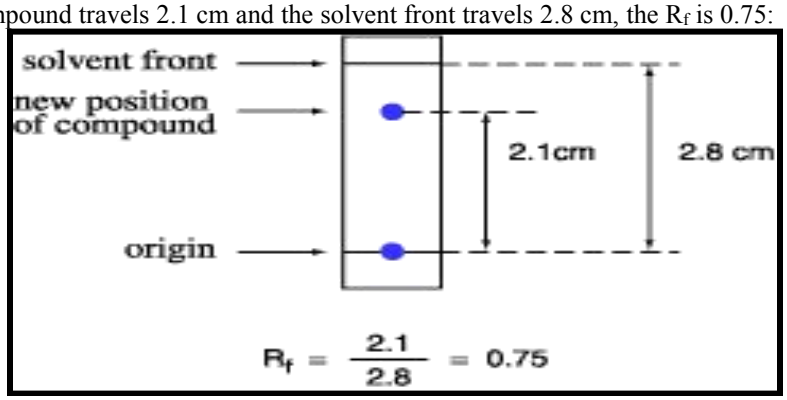
The strength with which an organic compound binds to an adsorbent depends on the strength of the following types of interactions: ion-dipole, dipole-dipole, hydrogen bonding, dipole induced dipole, and van der Waals forces. With silica gel, the dominant interactive forces between the adsorbent and the materials to be separated are of the dipole-dipole type. Highly polar molecules interact fairly strongly with the polar SiOH groups at the surface of these adsorbents, and will tend to stick or adsorb onto the fine particles of the adsorbent while weakly polar molecules are held less tightly. Weakly polar molecules generally tend to move through the adsorbent more rapidly than the polar species. Roughly, the compounds follow the elution order given above.

The R_f value

The retention factor, or R_f, is defined as the distance traveled by the compound divided by the distance traveled by the solvent.

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

For example, if a compound travels 2.1 cm and the solvent front travels 2.8 cm, the R_f is 0.75:



The R_f for a compound is a constant from one experiment to the next only if the chromatography conditions below are also constant:

- solvent system
- adsorbent
- thickness of the adsorbent
- amount of material spotted
- temperature

Since these factors are difficult to keep constant from experiment to experiment, relative R_f values are generally considered. "Relative R_f" means that the values are reported relative to a standard, or it means that you compare the R_f values of compounds run on the same plate at the same time.

The larger an R_f of a compound, the larger the distance it travels on the TLC plate. When comparing two different compounds run under identical chromatography conditions, the compound with the larger R_f is less polar because it interacts less strongly with the polar adsorbent on the TLC plate. Conversely, if you know the structures of the compounds in a mixture, you can predict that a compound of low polarity will have a larger R_f value than a polar compound run on the same plate.

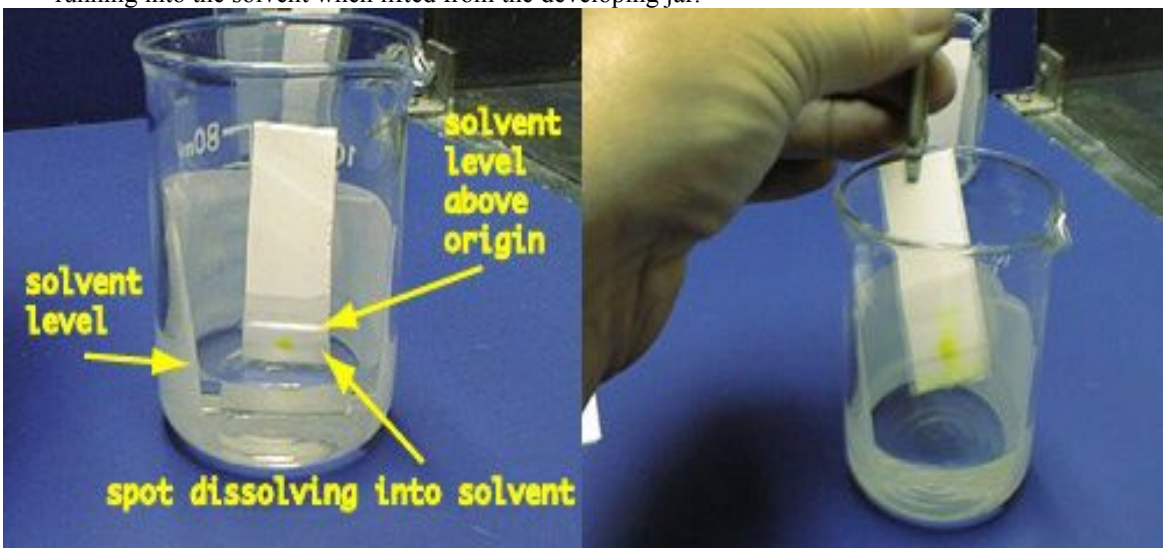
The R_f can provide corroborative evidence as to the identity of a compound. If the identity of a compound is suspected but not yet proven, an authentic sample of the compound, or standard, is spotted and run on a TLC plate side by side (or on top of each other) with the compound in question. If two substances have the same R_f value, they are likely (but not necessarily) the same compound. If they have different R_f values, they are definitely different compounds. Note that this identity check must be performed on a single plate, because it is difficult to duplicate all the factors which influence R_f exactly from experiment to experiment.

Troubleshooting TLC

All of the above (including the procedure page) might sound like TLC is quite an easy procedure. But what about the first time you run a TLC, and see spots everywhere and blurred, streaked spots? As with any technique, with practice you get better. Examples of common problems encountered in TLC:

- **The compound runs as a streak rather than a spot:** The sample was overloaded. Run the TLC again after diluting your sample. Or, your sample might just contain many components, creating many spots which run together and appear as a streak. Perhaps, the experiment did not go as well as expected.
- **The sample runs as a smear or a upward crescent:** Compounds which possess strongly acidic or basic groups (amines or carboxylic acids) sometimes show up on a TLC plate with this behavior. Add a few drops of ammonium hydroxide (amines) or acetic acid (carboxylic acids) to the eluting solvent to obtain clearer plates.
- **The sample runs as a downward crescent:** Likely, the adsorbent was disturbed during the spotting, causing the crescent shape.
- **The plate solvent front runs crookedly:** Either the adsorbent has flaked off the sides of the plate or the sides of the plate are touching the sides of the container (or the paper used to saturate the container) as the plate develops. Crooked plates make it harder to measure R_f values accurately.

- **Many random spots are seen on the plate:** Make sure that you do not accidentally drop any organic compound on the plate. If get a TLC plate and leave it laying on your workbench as you do the experiment, you might drop or splash an organic compound on the plate.
- **You see a blur of blue spots on the plate as it develops:** Perhaps you used an ink pen instead of a pencil to mark the origin?
- **No spots are seen on the plate:** You might not have spotted enough compound, perhaps because the solution of the compound is too dilute. Try concentrating the solution, or spot it several times in one place, allowing the solvent to dry between applications. Some compounds do not show up under UV light; try another method of visualizing the plate (such as staining or exposing to iodine vapor). Or, perhaps you do not have any compound because your experiment did not go as well as planned. If the solvent level in the developing jar is deeper than the origin (spotting line) of the TLC plate, the solvent will dissolve the compounds into the solvent reservoir instead of allowing them to move up the plate by capillary action. Thus, you will not see spots after the plate is developed. These photos show how the yellow compound is running into the solvent when lifted from the developing jar.



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