Hawker Research Group
Standard Operating Procedures
For Laboratory Equipment and
Common Synthetic Practices

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Computers

- Literature
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- Papers
- Endnote
- ChemDraw
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Guidelines for Common Reactions

- NMP
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Specific Compound Synthetic Procedures(?)

General Safety Guidelines

Appropriate Apparel

Safety glasses are required at all times when present in the lab.

Whenever chemistry involving toxic or hazardous materials is being done, it is important to wear appropriate clothing. Closed-toe shows are required, and shoes should be comfortable and practical anytime labwork is done. It is typically best to wear long sleeved shirts in order to cover your arms. Syntheitic fibers can be a considerable fire hazard, particularly when working with open flames or with highly combustable materials. In these situations, it is best to wear a lab coat which does not readily burn, and may be removed in the case of a fire.

Working Alone

There are sometimes instances in which a single person is working alone in the lab. This is not the ideal situation, as emergencies are best dealt with by multiple people. If you end up working alone for any reason, use your best judgement in deciding what chemicals to use, what procedures cannot wait until more people are present. Avoid any procedure that is excessively hazardous and can be postponed. Use your best judgement and extra caution.

Communication

When you conduct hazardous experiments, for example, $LiAlH_4$ reductions, you must keep in mind that everyone in the lab is exposed to the risks involved in the experiment. As such, it is only fair that they be aware of those risks. If you leave a reaction stirring overnight, and there are any special considerations, it is best to indicate those risks to other people in the lab. For example, during a reduction left to stir overnight, a roundbottom flask could be labeled with the following information:

- Notebook number
- Hazardous material (LAH, sodium azide, HMPA, etc.)
- Quantity
- Date/Time

The notebook containing the reaction scheme should also be left accessible on the benchtop near the reaction.

Emergency Information

Evacuation Procedure

In the case of a fire, earthquake, or other emergency requiring evacuation, stop working immediately. Turn off any equipment if unattended use is hazardous (such as rotovaps, stir plates, vacuum pumps, and water consenders). Exit the lab and exit the building using the closest set of stairs. All lab occupants should meet in front of the building to ensure the nobody is still in the lab.

First Aid Kits

Each lab has a first aid kit. Any time use of the kit is necessary, the items used must be replaced in a timely fashion. All kits should contain bandaids, bandages, antiseptic cream, adhesive tape, Tylenol, and burn gel. Locations are as follows
4606 PSBN: On table in entrance to lab, by printer.
_____MRL:
_____MRL:
_____Engineering II:
_____CNSI:

Spill Cleanup Materials

Each lab should be stocked with spill mats, typically stored by the sinks. Spill mats can be used to soak solvent off of the floor, and should be used immediately to prevent damage to the floor. Vermiculite can be used to clean up more hazardous materials than solvents, and should be swept up and disposed of in solid waste after use. In the case of an acid spill, weak bases such as bicarbonate may be used to neutralize before using spill pads.

Lab Monitors or Alarms

Should the fire alarm sound, turn off all equipment and quickly move to the assembly point. Fume hood alarms often sound if the pull is too low. Drop the hood sash to the suggested level as marked by arrows on the hood.

Per campus policy, all significant injuries must be documented via the UCSB Report of Injury to Employee/Student form as soon as possible – form available from Maureen Evans MRL 2068E. This is necessary for potential reimbursement for personal medical costs, or Worker's Compensation Claims.

Per SB County Fire and campus policy, all fires must be reported to 9-911 immediately – even if the fire is out. This is particularly true if there is use of an extinguisher (must be replaced); and injury; or property damage.

Health & Safety References

Web References for Health & Safety:

1. http://www.mrl.ucsb.edu/mrl/info/administration/mrlsafety.html

2. http://ehs.ucsb.edu/units/labsfty/labrsc/chemistry/lschemmsds.htm

3. http://siri.org/msds/

Material Safety Data Sheets (MSDS):
Per OSHA, all lab chemical users must know: a) what an MSDS is, b) MSDS relevance to their health and safety, c) how to readily access them.

Labs are encouraged to maintain their own MSDS for the hazardous chemicals they routinely use

General Lab Practices and Procedures

Lab Notebook

Your lab notebook is the primary document recording your experiments. Although it is ultimately your decision how to organize information in the lab notebook, please remember that others may rely on your notebook to repeat experiments in the future, with or without you present. For a typical reaction, the following information should be clearly presented:

- The date
- Reaction scheme – structures or formulas of all molecules in the reaction
- A reference if applicable – this can be anlother notebook page if you are scaling up or repeating a previous, or a lit reference
- Reaction stoichiometry – for each compound this typically involves MW, mass used, mols or mmols, equivalents, and, if liquid, density and volume
- Theoretical yield of the reaction
- A description of the reaction setup and conditions (i.e. in what size roundbottom, stir at what temperature for how long, order of addition, etc.)
- A description of the workup
    - For extractions, specify the volume of each layer for each wash in addition to the solvents or solution composition
    - For columns, specify the solvent conditions, and sketch the TLC, labeling spots when possible
    - For distillations, specify the temperature at which collection is started and ceased, as well as the pressure if you have a vacuum meter
    - For precipitations and crystallizations, specify the solvent as well as the volume
- The overall isolated yield should be clearly visible on the notebook page, and the isolated material should be described in terms of characterization methods used (i.e. 5.6g white powder isolated, 76% yield, pure by NMR)
- Related data should be easy to find, labeled with the compound notebook number or some other systematic labeling system.

Waste in the Lab

Organic Waste

Organic waste (primarily solvents) can be kept in either 20L drums, 4L glass or plastic solvent bottles, or waste containers provided by EH&S. If the drums or solvent bottles are used, be sure that the original label on the bottle is crossed out to prevent someone from accidentally trying to use the waste as solvent. Always be sure to tag the containers with the tags provided by EH&S available in the chem stockroom. Nonchlorinated and chlorinated solvents may be disposed of together by current EH&S policy.

Aqueous Waste

This is primarily for aqueous fractions from extractions, and typically contains water, sodium chloride, potassium carbonate, and sodium bicarbonate. If you use a large amount of another chemical, such as EDTA, phosphate buffers, etc, please add those compounds to the waste label. Refrain from adding strong concentrated acids or bases to an aqueous waste container, and although it is often aqueous, do not dispose of sodium azide here.

Solid Waste

Primarily silica gel, this is typically stored in either old silica drums or buckets lined with garbage bags. It is best to use such containers so that items like filter paper can be disposed of, which is more difficult with 20L drums. Please only put dry, solid, nonvolatile compounds in this waste, and add anything to the waste label if a significant amount is added to the waste container.

Glass Waste

This waste goes in the dumpster. As such, it must be clean. Pipettes, vials, test tubes, and broken glassware should all be rinsed with solvent and visually inspected before being thrown out. Plastic vial caps and other clean trash may be disposed of here, but please do not use it as a trash can for gloves, paper towels, etc, as this will only result in having to bring the waste to the dumpster more often.

Sharps Waste

Like the glass waste, needles that go here must also be clean. After using a needle and syringe, rinse it out with an appropriate solvent. When it is clean, throw the syringe out in the garbage and the needle in the sharps waste bin.

Special Considerations

Organic Azides

Organic azides, especially low molecular weight azides, can be dangerous. These should be diluted and disposed of in the regular organic waste unless they are of particularly low

molecular weight. If this is the case and detonation hazard exists, dilute the sample in a vial and label it for separate collection by EHS.

Sodium Azide

Inorganic azide is also extremely hazardous. Aside from being acutely toxic, it can form explosive compounds in the presence of organometallic compounds or certain metals. A separate aqueous waste should be labeled specifically for aqueous azide and only water and inert salts should be disposed of here.

Concentrated Acids and Bases

Be sure to dilute acids and bases before adding to the aqueous waste, as you cannot be sure of the pH of the waste present and need to avoid violent reaction. Particularly corrosive waste (such as aqua regia from cleanging glassware) can be labeled in a small glass bottle for sepaprat collection by EHS.

Old Chemicals and Samples

These can be either diluted and disposed of in the normal organic waste or labeled for separate pickup by EHS.

Glassware

Always remember that we use shared glassware, and the odds that you will be the next person to use a piece of glassware are always relatively low. This means you do not know how it will be used. As such, glassware should only be returned to its proper place in a clean, dry state.

Glassware should be cleaned, at minimum, by washing with soap, hot water, and a brush, followed by several DI water rinses and at least one actone rinse. Clean glassware should be colorless, clear, and should not have mineral deposits on the inside or outside.

Base Bath

If solvent rinses and soap and water can not clean the glassware, use a base bath. The base bath recipe is:

300g KOH, 4L isopropanol, 1L DI water

Glassware should be left in the base bath for no longer than 24 hours. It will be very slippery after any more than a couple of hours in the bath. Base bath will eat through most latex gloves given some time. Because the bath etches glass surfaces, glassware such as fritted funnels should never be cleaned in the base bath.

Aqua Regia

This is an extremely strong oxidizer, capable of dissolving pretty much any inorganic salt. The recipe is:

1 HNO_3 : 3 HCl

Where more nitric acid makes a stronger solution. This is highly reactive, very toxic, and should not be stored for any long amount of time. Great for cleaning frits and stirbars. Should be diluted with water and labeled for waste collection after use.

Piranha

Useful for removing very stubborn organics, this strong oxidizer is formed from concentrated sulfuric acid and hydrogen peroxide. The usual recipe is:

3 H_2SO_4 (conc.): 1 H_2O_2 (30%)

Flash Chromatography **(via http://chem.chem.rochester.edu/~nvd/howtoflash.html)**

Flash chromatography is performed according to the method of Still ("Rapid Chromatographic Techniques for Preparative Separation with Moderate Resolution." Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43* (14), 2923-5). All flash chromatography should be done with Silica 60.

1. **Choosing a Chromatography Solvent System**

    a. Identify a suitable solvent mixture for your compound or reaction mixture. As a general rule the desired compound should be at about rf=0.3 on the TLC plate. Two spots which are close or which co-spot in the regions of rf=0.7-1 or fr=0-0.2 may in fact be very easy to separate.
    b. For most application, mixtures of hexanes and ethyl acetate (100:0 - 0:100 hexanes/ethyl acetate) are best. Other useful solvent systems include methylene chloride/methanol (100:1 - 100:10); ethyl acetate/acetone (100:0 - 50:50); and toluene with acetone, ethyl acetate, or methylene chloride.
    c. For basic (i.e. nitrogen containing) compounds, it is sometimes useful or necessary to add a small amount of triethylamine or pyridine to the solvent mixture (about 0.1%).
    d. For acidic compounds, a small amount of acetic acid is sometimes useful. In this case, be very careful in concentrating the solvent as trace amounts of acids can be very dangerous when they are concentrated with a product. In these cases, the acetic acid can often be safely rotavaped away by adding portions of toluene and concentrating to a few mL volume and repeating this several times. As acetic acid boils at a lower bp than toluene, this will remove the acid without exposing the neat compound to it.

2. **Packing the Column**

    a. A chromatography column is plugged with a small piece of cotton wool, just enough to fill to stopcock hole.
    b. Sand, about 2 cm ,is added so that the diameter of the sand is approximately the same as the column.
    c. Silica gel is added dry. Usually, it is best if the silica is not too long, about 6 to 10 inches is best in most cases.
    d. Attach the house vacuum to the bottom of the column via the stopcock. Open the vacuum and the stopcock; this will compresses the silica gel and hold it tight for the next steps.
    e. Add sand to the top of the column, about 1-2 cm is enough. With the vacuum still applied, pour the solvent (premixed, i.e. 4:1 hexanes/ethyl

acetate). Allow the solvent to flow though the column until it is almost eluting. At this point, close the stopcock and remove the vacuum line.

f. Make sure enough solvent is in the column for 5-6 column volumes worth to flow though, to ensure complete packing. Now elute all of the solvent with air pressure, taking care not the let the column run dry. Stop with the solvent level parallel with the sand. A well-packed column should not have any cracks or patches. The solvent eluding from the stopcock should not be warm or hot.

3. **Loading the Column**

    a. Prepare a solution of your reaction or compound mixture in the minimal amount of methylene chloride possible. Using a pipette, add this carefully to the top of the silica, washing the flask 3-4 times with methylene chloride or the chromatography solvent. After each addition, allow the solvent level to descend into the very top of the silica gel (below the sand).
    b. Carefully added 2-3 pipettes of chromatography solvent and push this into the column (repeat 3-4x).
    c. Now, carefully fill the remaining column space with the chromatography solvent and elute using compressed air. A flow rate of about 2 inches/minute is ideal. This is measured by how fast the solvent column descends in the straight part of column, above the silica gel. It is most convenient to measure and adjust the flow rate before adding the compound!
    d. In cases where a reaction mixture or compound is not soluble in a suitable solvent for loading, it can be absorbed onto silica gel. This is done by dissolving the compound in acetone, adding silica gel and carefully concentrating the silica gel to dryness (careful: it bumps!). The dry silica is then added to the top of the packed silica column. In this case, sand should not be added to the column until after the silica-compound mixture is added. This method is recommend only as a last resort as separations are often inferior to solution loading.

4. **Running the column**

    a. Column fractions are collected in test tubes, of a size appropriate for the type of column and polarity. Use the 13 mm test tubes for small scale (ie 5-50 mg) and larger test tubes for bigger columns. Refer to the guidelines in Still's paper for choosing fraction sizes.
    b. Start collecting the fraction immediately after adding your compound; it does not take long for very non-polar compounds to elute from the column.
    c. Once you have loaded a column, it is best not to stop it for any length of time. This is due to slow diffusion of the compounds on the silica gel, resulting in poor separation and diminished yields.

d. To find your product, spot each fraction or so on a TLC plate and check which fractions contain compounds. Fractions containing the same compounds are combined, the test tubes washed with methylene chloride or (probably better for the environment), distilled ethyl acetate, and the solvent concentrated under reduced pressure.
e. Do not let a column run dry or elute the solvent until after you are sure all of the compounds have eluted! This is an easy mistake to make!

5. **After the column-cleaning up**

a. After you have finished, elute all of the solvent from the column using compressed air. Flowing air through the column for ~2 hours will give dry, free flowing silica gel.
b. Pour out the contents of the column into the silica waste container.
c. In most cases, washing the column with water and acetone is sufficient. If necessary, a small amount of liquid soap can be used. Try to avoid scratching the columns with abrasive brushes or soaps.

Using a Manifold/Air-Free Procedures

Extractions

Standard Aqueous Workup Protocol:

1) *Pick an organic solvent*. Ether is the most popular because it can be removed easily on the rotary evaporator, ethyl acetate also works well but is harder to remove, dichloromethane is a poor choice and should be avoided, if possible, since it often forms nasty emulsions and complicates matters because it is heavier than water.

2) *Pick the size of your separatory (sep.) funnel*. You will usually use 125 or 250-mL, large scale reactions (1–10 g) can require 500-mL or 1-L sizes. Remember that your sep. funnel will contain the solvent and wash liquid which must be thoroughly mixed.

3) *Dilute the crude reaction mixture with your solvent* of choice and transfer to your chosen sep. funnel. Large amounts of material require large amounts of solvent. Normal reactions (50–500 mg of product) can be diluted with between 25–100 mL of solvent.

4) *Wash the organic layer* to remove impurities. The volume of a wash phase is typically one tenth to one half the volume of the organic phase. It is sometimes best to repeat a wash two or three times. An acid wash (usually 10% HCl) is used to remove amines, while a basic wash (usually sat. NaHCO3 or 10% NaOH) is used to remove unwanted acids. In most cases, when neither acidic nor basic impurities are an issue, the solution is washed with distilled water to remove any non-organic compounds. (Note: When shaking mixtures in a sep. funnel be sure to vent it regularly by holding it upside-down, pointing it up and to the back of your hood, then opening the stopcock. This will release any pressure that has built up during mixing. Additionally, when draining liquids out of the sep. funnel, be sure to first remove the stopper.)

5) *Back-extract to recover lost product*. If your compound is somewhat water soluble (has several polar functional groups), you may need to back-extract the water layers with ether or ethyl acetate to avoid significant loss of compound in the aqueous phase. TLC can be used to determine when all of your compound has been removed from the water.

6) *Finish with a brine* (saturated NaCl solution) wash. This helps disrupt any emulsions and will "dry" the organic layer by extracting water that may have dissolved in the organic phase.

7) *Dry the organic layer*. After removing your solution from the aqueous phase, a drying agent is added to remove all traces of water. This is usually MgSO4 , more effective and faster, but slightly acidic; or Na_2SO_4 , less effective and slower, but neutral. These compounds bind to any water remaining in the organic solution, forming clumps when they react. A decent amount of drying agent should be added, but as long as some solid is not clumped, no more needs to be added. (This will make sense once you've done this a couple of times.)

8) While the compound is drying, it is time to flute the filter paper. Refer to Zubrick page 136-138 for directions. Some chemists prefer to use a Büchner funnel and unfluted filter paper (or a fritted funnel) under mild vacuum as their standard filtration method. Their motive is a slightly higher yield of product.

9) *Filter the solution* into a large round bottom flask using your expertly fluted filter paper and a large funnel (or the Büchner method). To guard against bumping on the rotavap, do not fill the flask more than half full.

10) *Concentrate the solution* on the rotavap, then dissolve the compound in a small

amount of solvent and transfer to a small pre-weighed (tared) flask.
11) *Concentrate the solution on the rotavap again*. Higher boiling solvents are more effectively removed by concentrating, adding dichloromethane then repeating once more.
12) *Use the vacuum pump to remove residual solvent*. For non-volatile compounds, residual solvent is most effectively removed by using the vacuum pump. One useful trick to speed up this process is the following: evacuate the flask and vent to N2 , repeat this again, then pump on the flask for 30 minutes. If your compound is volatile—low molecular weight and/or low boiling point—obtain a constant weight using the rotavap, not the vacuum pump.
13) *Obtain a constant weight*. Weigh the flask after leaving it on the vacuum pump (or rotavap), then return to the pump (or rotavap) for 15 to 30 minutes and weigh again. Once two weights in a row are the same, you're ready to take an NMR.

Useful Solutions

TLC Stains

As a general rule, permanganate and phosphomolybdic acid are great "first try" stains, which will clearly mark lots of functional groups. Other solutions might be useful for their specificity, such as ninhydrin.

Recipes (via http://orgprepdaily.wordpress.com/2006/09/27/tlc-staining-solutions/)

Cerium-ammonium-molybdate, CAM

40g of ammonium pentamolybdate + 1.6g of cerium(IV) sulfate + 800mL of diluted sulfuric acid (1:9, with water, v/v). On heating, blue-black spots on light background. Slowly fades over several days. Quite universal, often very sensitive. Some amines, amides and oxidation-resistant aromatics do not detect well.

Basic KMnO4

40g of K2CO3 + 6g of KMnO4 in 600mL of water, then 5mL of 10% NaOH added. (KMnO4 takes some time to dissolve completely. Lazy people like me substitute it with NaMnO4 concentrated aq. solution from Aldrich). No heating. Brown spots on pink background. Often very sensitive but staining very disproportionate to quantity, depending on the compound. Fades within hours. Oxidizes anything with diol, C=C, reactive methylene, phenol, thiol, phosphine etc. Particularly useful for detection of tertiary amines.

Phosphomolybdic acid

30-40g of phosphomolybdic acid in 100mL of ethanol (preferably non-denaturated). Good grade of phosphomolybdic acid should provide clear, bright yellow solution. (If there is cloudiness, let it settle and decant.) Light sensitive. On heating, blue-black spots on yellow-green. Good for lipids. Do not overheat or the background goes dark. Usefull for spraying but expensive as dipping-jar solution. I stopped using it, in favor of CAM.

Anisaldehyde

40mL of conc. H2SO4 is added (slowly!) into ethanol 800mL, followed by acetic acid 12mL and anisaldehyde 16mL. Light and oxidation sensitive. On heating, colorfull spots on pink background. Color varies on the compound. Good for all things with active methylene, and for distinguishing closely-spaced spots on TLC by their color difference.

Iodine

Iodine vapor chamber is made from a TLC jar by adding a mix of iodine crystals powdered with dry silicagel. Put dry TLC in the chamber and watch the spots to going. Works on variety compounds but often not very sensitive. Iodine stained TLC can be developed subsequently with other stains.

Functional group-selective stains

Ninhydrin

20g of ninhydrin in 600mL of ethanol. (Don't spill ninhydrin onto your fingers - they would go blue.) Primary amines produce blue spots at R.T., very sensitive detection. Boc-protected primary amines produce spots on heating (as the Boc falls off). Secondary amines sometimes detect but the stain is weak.

Dinitrophenylhydrazine

3g of dinitrophenylhydrazine in 750mL of 2M HCl. (If htere is some insoluble portion, decant it off.) Aldehydes and ketones produce yellow-orange spots at R.T., quite selective.

Dragendorff

Solution A: 1.7g of BiO(NO3) in AcOH 20mL + water (80mL) *Solution B:* 40g of KI in water 100mL

Before use, 20mL of solution A is mixed with 20mL of solution B and 40mL of acetic acid. This ready-made Dragendorff reagent A+B mixture must be stored in refrigerator, it is good for about 1 week. Amines and basic heterocycles like pyridine produce brown-orange spots at RT. Phosphines and crown ethers are also detected.

Stain dip station: 4 or 6 wide-mouth jars covered with aluminum foil (secured by tape) to protect from light, placed within a tray (to guard against spill).

Hawker Group Equipment

Rotary Evaporators

Rotary Evaporation is a commonly used instrument for the removal of volatile organic solvents. There are many components to a rotary evaporator of which one must be aware for proper and safe operation. As always, if unsure of proper procedure ask CJH or a senior member of the group.

Supplies for Rotavap

- Dry Ice
- Isopropyl Alcohol
- Solvent Waste Container

Turning on the Rotavap

1. Put your safety glasses on
2. Ensure that all lines to vacuum pump are closed
3. Turn on the Vacuum Pump
4. Add dry ice to cold finger of rotavap (see image). To begin, be sure to add dry ice in small portions.
5. Clip your sample onto the rotavap (ensure that your flask is no more than 50% full)
6. Turn on water bath to desired temperature and start flask rotation
7. Close the rotavap to atmosphere and open to vacuum (see image)

Operating Concerns

- Empty solvent reservoir regularly
- Keep dry ice (both cold finger and secondary trap) full
- Routinely check the solvent trap to see if it needs to emptied

Turning off the Rotavap

1. Prepare to leave rotavap in same condition you found it (CLEAN!)
2. Close the rotavap from the vacuum
3. Open air to the rotavap
4. Remove sample container
5. Empty solvent reservoir into labeled waste container and return lid
6. Clean cold finger and replace
7. Open vacuum
8. Turn off vacuum pump
9. NEVER leave dirty glassware (flasks, bump traps) in this area

High Vacuum/Manifolds

High vacuum (Hi-Vacs) systems are an integral part of everyday laboratory work. As such, one may encounter high-vacs when using a vacuum oven, drying compounds, sealing ampules for polymerization (freeze-pump-thaw cycles). One must know that a Hi-Vac will create extreme differences in pressure between the inside and outside of the system. This is an integral part of standard day-to-day laboratory practices but has many risks (explosive gas condensation and inherent implosion hazards). Therefore, we follow these procedures to ensure safety.

When operating a High VAC System ALWAYS use

1. Crack Free Glassware (to reduce risk of glass implosion)
2. Condensation Trap to collect solvents
3. As always, safety glasses are mandatory

Turning on High VAC System

1. Make sure all valves are closed
2. Turn on Vacuum Pump
3. Fill Dewar for Trap with Liquid N_2 or Isopropanol / Dry Ice (DO THIS AFTER TURNING PUMP ON to reduce risk of condensing oxygen/air)
4. Ensure that Trap Flask is submerged into the cold Dewar
5. Hi-VAC system is now operational

Turning off High VAC System

1. Remove all samples and experiments from Vacuum Line
2. Remove Trap Flask from Dewar, allow to warm to room temperature
3. Open Vacuum System to Atmosphere
4. Turn off Pump

FTIR (PSBN)

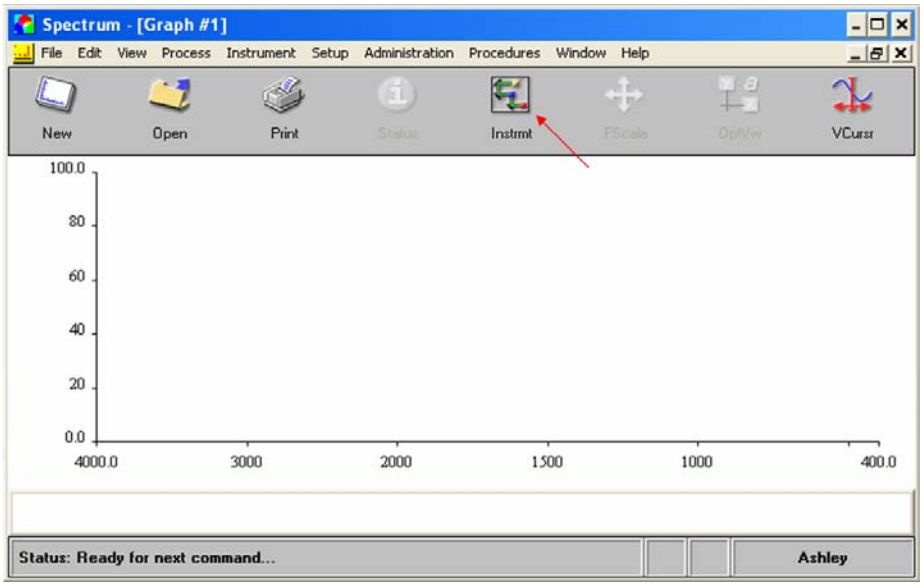
The Perkin Elmer FTIR is located in PSBN 4670. To use the FTIR, you must be trained by Ashley Piekarski. Email Ashley (apiekarski@chem.ucsb.edu) to schedule training. Do NOT use the machine without training!

General Procedure:

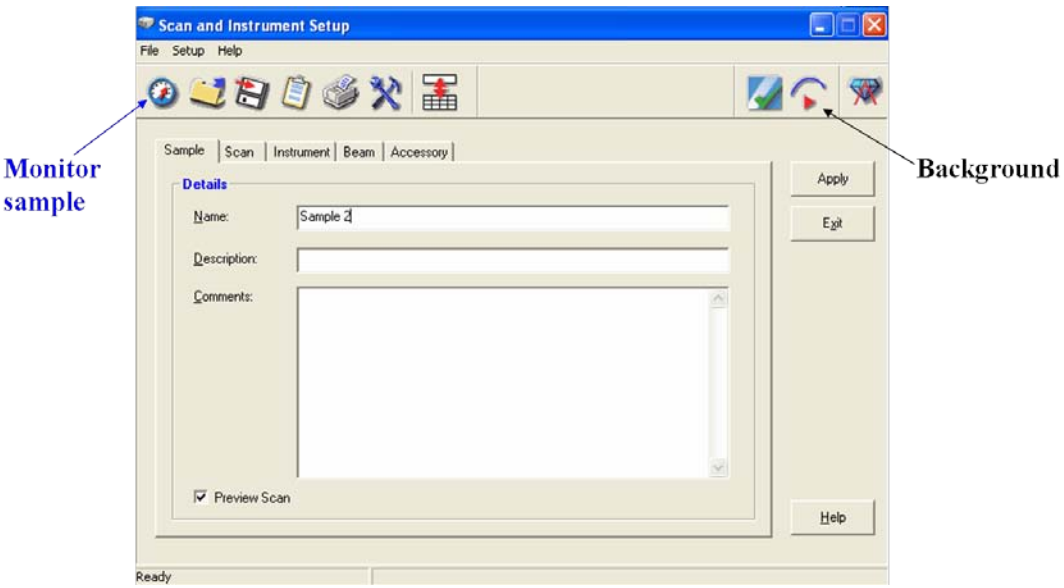
1) Login to FTIR User account (no password necessary).

2) Open Spectrum software by double-clicking on Spectrum icon. Login using your username and password.

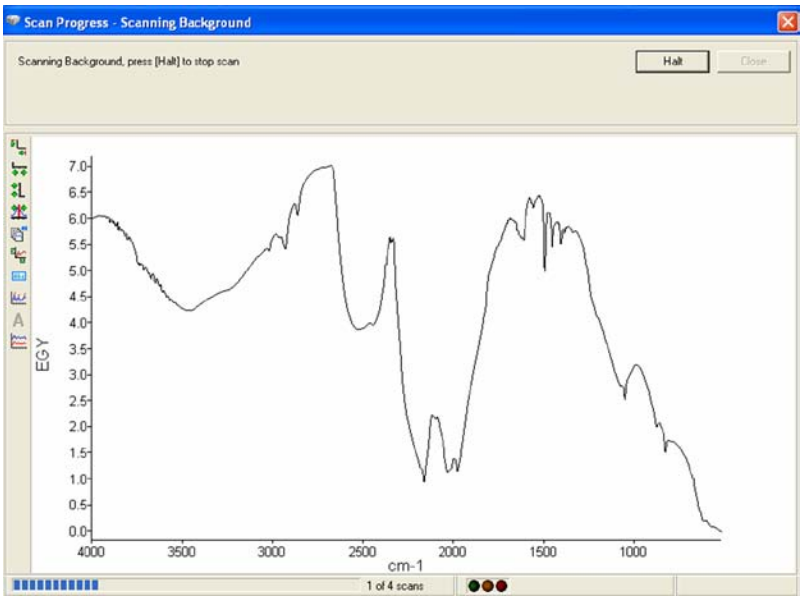
3) Open Instrument icon to set parameters for run.



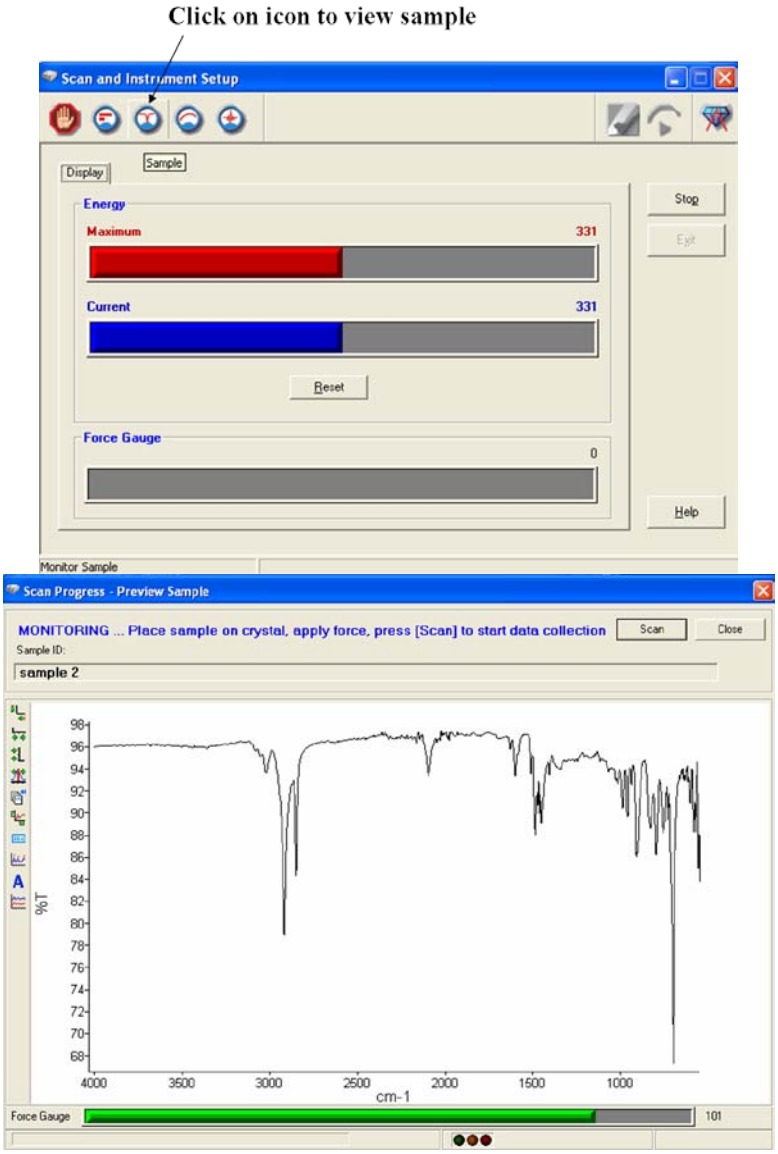
4) Change the sample name and click "Apply" to change the sample name for that particular run. You may change other parameters in this window under "Scan" such as wavelength range etc. Most users leave the default settings. If you need to change the Accessory then you need to contact the administrator.



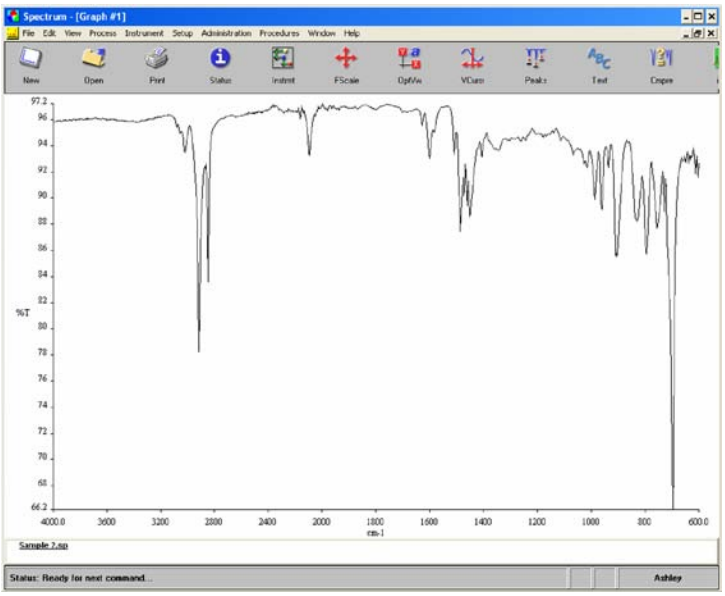
5) Run a background spectrum by clicking the icon (shown in picture above). Only one background is necessary if multiple runs are done consecutively.



6) Now click on the icon that allows you to monitor the sample (shown above) and click on the "Sample" icon (shown below) to view your spectrum. Place the sample on the crystal. Add appropriate attachment to the arm and apply force which will allow you to observe the spectrum prior to scanning. Do not apply a pressure more than 100-110. Stop the monitoring then start again to monitor your sample full screen as shown below.

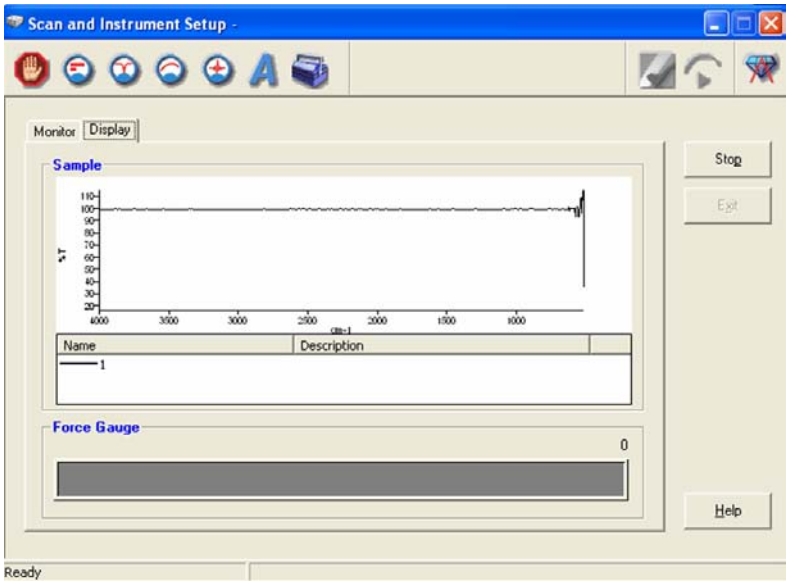


7) Once you are satisfied with the spectrum, start the scan of your sample.



8) Insert peaks by clicking on the "Peak" icon shown in toolbar. You can add additional peaks by clicking on "VCursr", aligning the cursor over the peak and double-clicking. Adjust parameters by navigating through "Process" and "View" menus.

9) After you are finished, go back to Instrument icon and Monitor your sample while cleaning. To clean use any volatile solvent, spray on a Kimwipe and clean the crystal and removal attachment to arm. Do NOT spray acetone or any other solvent directly on the crystal. Once the spectrum has disappeared (as shown below), then the crystal is clean.



10) Log in your name, date, time and if you cleaned the crystal in the log book. Do NOT leave the crystal dirty or you will lose all privileges to the IR machine.

Solvent Purification System (PSBN)

The Solvent Purification System from Innovative Technologies is designed to remove oxygen and water from flammable organic solvents. This instrument effectively supplants the need for solvent distillation and represents a significant upgrade in safety as the need for water-reactives such as sodium metal and calcium hydride is unnecessary. The following graphic is the basic system operations and specifications taken directly from the Innovative Technology, INC website.[1] As always, if unsure of proper procedure ask CJH or a senior member of the research group.

NOTE: The complete Manual for this equipment is located at the end of this guide.

Basic System Operation and Specifications:

A Purification Grade solvent is pushed from its storage container under low nitrogen pressure through two stainless steel columns containing activated alumina and copper. Trace amounts of water and oxygen are removed producing dry, deoxygenated solvent. The processed solvent is drained into in a storage flask where it can be dispensed, under nitrogen, using standard syringe techniques.

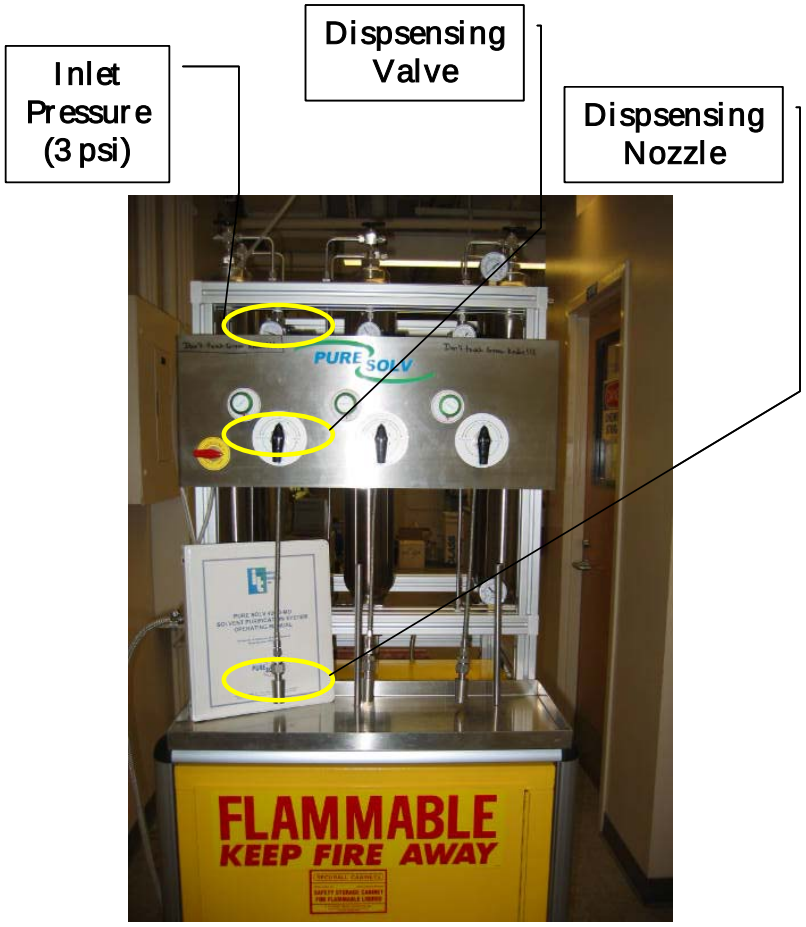
Dual solvent column design	Two pressure tested stainless steel columns per solvent, either 200L or 400L processing capacity until spent. Columns are supplied fully conditioned, ready for use. Spent columns are replaced with new columns.
Manifolds, vacuum and gas	Internal vacuum and gas manifolds are built into the extruded aluminum frame of the system. Includes a vacuum indicator and gas regulators and pressure relief valves dedicated to each of the solvent flasks to prevent over pressurization of glassware.
Gas connection	All solvents can be dispensed simultaneously using one main inert gas supply. A red colored 2-way Swagelok valve located on the main gas supply allows operator to shut off all pressure to system in an emergency.
Stainless steel tubing	All solvent tubing is ¼ inch stainless steel.
Check valves	Each solvent has it's own in-line check valve to prevent back flow, thus eliminating cross contamination of solvents.
Directional valves	Each solvent includes both 2-way and 3-way Swagelok directional valves to control flow of solvents and inert gas.
Dispensing	Innovative Technology now offers the safest most functional dispensing system available. A 5-Way Swagelok valve promotes correct dispensing procedures and prevents multiple functions from occurring simultaneously, thus causing problems like evacuation of solvent through the vacuum pump. A Swagelok metering valve allows a controlled flow of solvent from a braided stainless steel flex line connected to your desired dispensing joint (24/40 – 29/32 – 14/20 – Luer Lock Needle Valve) . A glass solvent storage flask mounts to the joint for dispensing solvents using standard syringe techniques.

Procedure to Dispense Solvents:

[1] www.gloveboxes.com

- Turn vacuum pump on and let run for 5 minutes before usage.

- Dry your 24/40 round bottom flask before dispensing solvent. If flame drying, do it in your hood. DO NOT FLAME DRY AT THE INSTRUMENT! If using the lab oven to dry, make sure the flask is at room temperature before dispensing. DO NOT BRING A HOT FLASK TO THE INSTRUMENT!



- Ensure that Argon pressure leading to inlet pressure is set to 3 psi (see image).

- To dispense dry solvent, you must do 3 successive evacuate-refill (dispensing valve) cycles.

- After the third cycle the valve should be pointed straight up. Turn the knob counter-clockwise (so that the flask receives a slight negative pressure from the vacuum) a total of 180^{o}. The knob should be pointed to dispense.

- Dispense the desired amount.

- When complete, continue turning the knob counter-clockwise so that a slight positive pressure is in the flask. If the flask does not come off the nozzle gently, turn clockwise 90^{o} to refill with argon.

- Congratulations, you now have dry solvent. Do not forget to turn off the pump AND record the amount of solvent you dispensed in the log book.

Accumet pH Meter

Note—This pH meter is equipped with a temperature probe for automatic temperature compensation. Both electrode and temperature probe should be immersed in calibration or measurement solutions for accurate readings.

To start:

1. Remove the electrode from storage bottle by unscrewing and completely removing cap from bottle (DON'T pull electrode directly out of bottle).
2. Slide storage bottle cap to the top of the electrode.
3. Rotate purple ring at the top of the electrode to open the fill hole.
4. Rinse the electrode with DI water and blot dry (DON'T wipe).

Standardization:

5. Immerse rinsed electrode and temperature probe into pH 4 buffer.
6. Press **std** to access Standardization mode.
7. Allow reading to stabilize
8. Press **std** again to initiate standardization.
9. Rinse electrode with DI water.
10. Repeat steps 5-9 with pH 7 and 10 buffers.

Measurement:

11. Immerse electrode and temperature probe into solution and allow reading to stabilize.
12. Rinse electrode with DI water.

To finish:

13. Rinse electrode with DI water.
14. Slide cap and o-ring back to end of the electrode and screw tightly back onto storage bottle.
15. Rotate purple ring to close fill hole.

Please contact Katie (feldman@mrl; x5770) or Nalini (ngupta@mrl; x5770) if you have any problems with the pH meter.

Labconco 1L Freeze-Dryer

Preparing your sample:

1. Make sure your sample is pre-frozen before attaching to the freeze-dryer. This requires the sample to be at least 10-20 degrees below the eutectic point, -40C.
2. Add your sample in the glass freeze-drying sample holder or a separate vial that can fit in the sample holder. For larger volumes, “pre-freeze” the sample with the sample holder slanted so that it is frozen mainly on one side.
3. After the sample is frozen, use a deep freeze method, such as placing the sample in liquid nitrogen to ensure that the sample is below -40C. Once cooled, the sample is ready to put on the freeze dryer. Alternatively, you can just place your sample in a deep freezer, such as a -80C freezer found in many of the bio labs on campus.

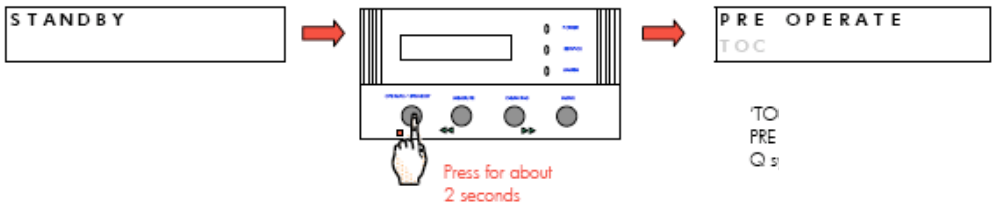
Freeze-dryer operation:

1. Before attaching the sample flask to the freeze dryer, make sure the manifold is placed on top of the condenser over the hole with the rubber gasket in between, and that freeze dryer has been turned on to “automatic” mode. Position all the sample holders on the manifold so the flat edge is pointed away from the attachment site for the sample flask. This is the “vent” position.
2. Turn the freeze-dryer “ON”. The freeze-dryer will automatically cool to -40C and once at that temperature, will start the vacuum itself (note, the vacuum pump is controlled by the freeze-dryer and should always be in the “ON” state).
3. Once the pressure drops to below ~0.1 Pa, and temperature below -40C, attach your sample flask to the freeze-dryer with the glass adapter. Turn the knob so the flat edge is pointed toward the flask. This is the “vac” position. Allow the sample to sit for a few hours to days, depending on the volume of water to be removed, until completely dried. Note, add one sample to the manifold at a time, allowing the vacuum pressure to go back down to < 0.1 Pa before adding the next one.
4. After your samples are dried, remove all samples from the sample holder and turn off the system. Release the vacuum through the sample holders on the manifold. Remove the manifold after the ice has melted and dry the condenser with paper towels.

Millipore Water Purification System

For most cases you can start at step 4; if a orange or red LED are light, contact Jasmine

1. Make sure the Milli-Q is in PRE OPERATE Mode. If the Milli-Q is in STANDBY Mode, then press the OPERATE/STANDBY Keypad Button for about 2 seconds. This will change the Milli-Q from STANDBY Mode to PRE OPERATE Mode. See the drawing below for more information.



2. Move the POU Dispenser Trigger forward and then back up. This will place the Milli-Q in PRODUCT Mode but water will not be dispensed at the moment. A very small amount of water (i.e. 5 ml) may be dispensed out.
3. It is recommended to dispense about 150 to 200 ml of Product Water to the drain (or into a sink) every morning. After this, use the Milli-Q Product Water for your specific application.
   NOTE: If you are using the Milli-Q frequently (i.e. a few times an hour), then you will not have to dispense 150 to 200 ml of Product Water to the drain.
4. When the green LED stops blinking, move the POU Dispenser Trigger forward to dispense water. It will stop blinking when the Product Water Resistivity (compensated to 25 °C) is greater than the Resistivity Setpoint. Normally, the Resistivity Setpoint is 16.0 MΩ.cm. It is recommended, but not necessary, to wait until the green LED stops blinking on the POU Dispenser and the screen reads: 18.2 MΩ.cm and 25 °C.
5. The Milli-Q will dispense water continuously for a maximum of 30 minutes. After 30 continuous minutes, the Milli-Q will stop dispensing water and will go into STANDBY Mode. Contact Millipore if you need to dispense water for more than 30 continuous minutes.
6. When you are finished dispensing water, move the POU Dispenser Trigger back to the upright position. The Milli-Q will continue to operate (but will not dispense water) for 90 seconds. After 90 seconds, the Milli-Q will automatically go to PRE OPERATE Mode.
7. Leave the Milli-Q in PRE OPERATE Mode. Do not leave it in STANDBY Mode unless you are going to perform some sort of maintenance (i.e. changing the Q-Gard and Quantum).

POU Dispenser: