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JSS College of Arts, Commerce and Science

(An Autonomous College of University of Mysore)

Ooty Road, Mysuru-570 025, Karnataka, India

PG DEPARTMENT OF CHEMISTRY

STANDARD OPERATING PROCEDURE FOR COLORIMETER

- Switch on the instrument at least 5 minutes before use to allow it to stabilize.
- Prepare a series of solutions as given in the table.
- Place water in the cuvette and zero the instrument. Make sure the clear faces of the cuvette are in the light path.
- Using different wavelengths (with 1 reference solution) identify the lamda max(wavelength with highest absorbance).
- With constant lamda max, place the prepared samples in the colorimeter and read the absorbance of the solutions.
- At intervals, recheck the reagent blank to ensure that there is no drift in the zero value.
- Absorbance is determined for unknown solution.
- Plot the graph of absorbance v/s concentration.

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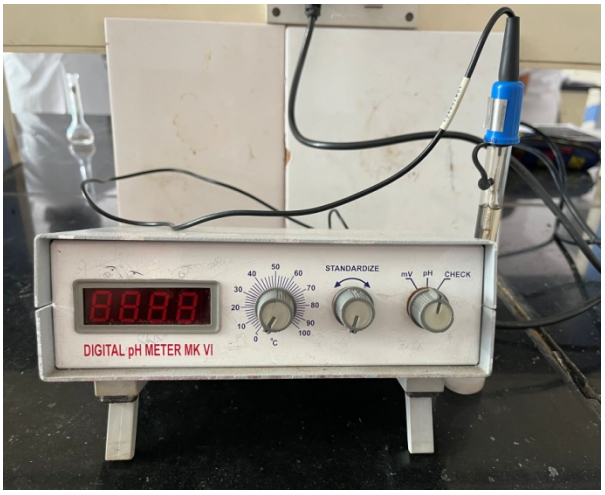
STANDARD OPERATING PROCEDURE FOR pH METER



- Switch on the instrument.
- Prepare buffer solutions of 4.01 pH and 9.14 pH (at 30 ^{o}C) by dissolving the respective buffer tablets separately in 100 ml of fresh distilled water in separate containers.
- Set the laboratory temperature.
- Immerse the electrodes properly in the solutions.
- Sufficient time is allowed for the electrodes to attain the temperature of the solutions.
- Push the pH/mV switch to pH position (pushed out) and push the STBY/READ switch to READ position and adjust CAL control to set 4.01 on the READOUT at the temperature and wait for 30 sec.
- Experiment is performed as given in the procedure
- Plot the graph of volume v/s pH

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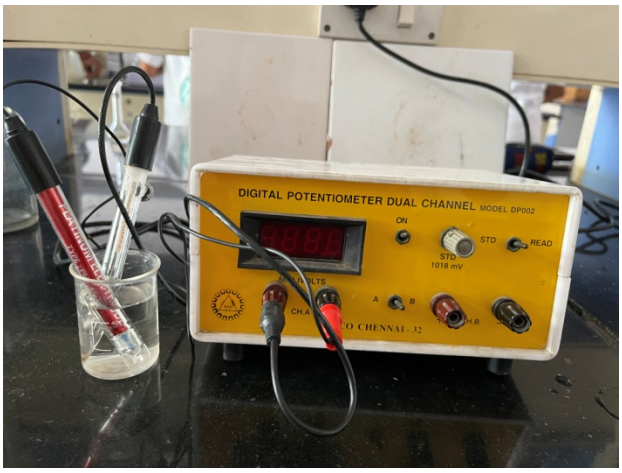
STANDARD OPERATING PROCEDURE FOR POTENTIO METER



- Switch on the instrument.
- Set the laboratory temperature.
- Immerse the electrodes properly in the solutions.
- Sufficient time is allowed for the electrodes to attain the temperature of the solutions.
- Introduce Pt and calomel electrodes to the instrument.
- Keep the instrument in read mode and adjust the meter to 1018 mV.
- Experiment is performed as given in the procedure.
- Plot the graph of Δe v/s emf.

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PG Department of Chemistry

STANDARD OPERATING PROCEDURE FOR CONDUCTIVITY METER

- Switch on the instrument.
- Set the laboratory temperature.
- Rinse the cell with de ionized water before use to remove any impurities adhering to the cell body.

Calibration of the conductivity meter

- Before starting calibration makes sure that the instrument is in the correct measurement mode.
- Change the standard when required, and record the details.
- Dip the cell into the sample.
- When dipping the cell into the sample, take care to ensure that the cell is completely immersed in the solution.
- Press the mode key to select conductivity mode.
- Allow time for the reading to stabilize. Note the reading on the display.
- Perform the calibration using a standard KCl (According to temperature).
- Experiment is performed as given in the procedure.
- Plot the graph of volume v/s conductance.

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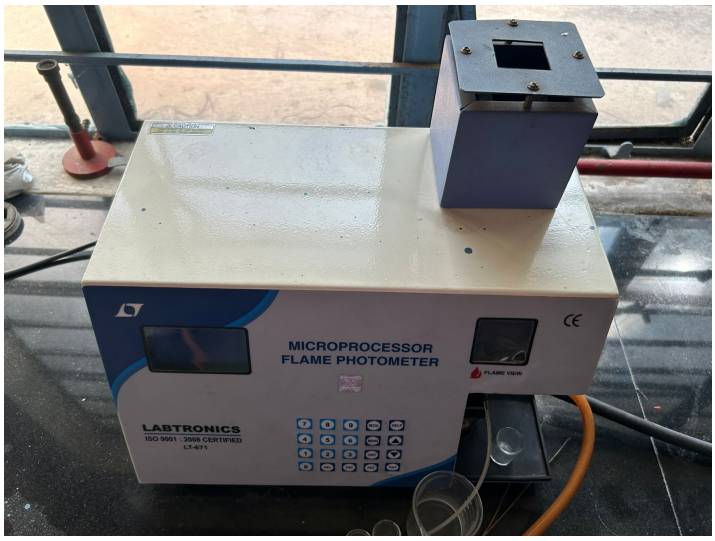
STANDARD OPERATING PROCEDURE

FLAME PHOTOMETER



Purpose:

The purpose of this Standard Operating Procedure (SOP) is to provide detailed instructions for the proper operation of a Flame Photometer. This SOP ensures accurate and reliable measurements of the concentration of specific elements in samples using the flame emission technique. Flame photometry is commonly used in various laboratory and research applications, such as clinical chemistry, environmental analysis, and pharmaceutical analysis.

Scope:

This SOP applies to all personnel involved in operating and maintaining the Flame Photometer in a laboratory or testing environment.

Responsibility:

Laboratory personnel: Responsible for performing flame photometry measurements as per this SOP.

Quality Assurance (QA): Responsible for reviewing and approving this SOP.

Procedure:

4.1. Equipment and Materials:

Flame Photometer

Appropriate standards or calibration solutions

Samples to be analyzed

Combustion gas (e.g., air, acetylene, or propane)

Relevant safety equipment (e.g., gloves, safety glasses)

Cleaning materials (lint-free cloth, laboratory-grade solvents, etc.)

4.2. Pre-Operational Preparations:

4.2.1. Ensure that the Flame Photometer is clean, free from dust or residues, and properly maintained.

4.2.2. Familiarize yourself with the specific operating instructions and technical specifications provided by the manufacturer for the particular model of the Flame Photometer being used.

4.2.3. Check the availability and condition of the required standards or calibration solutions.

4.2.4. Gather all necessary equipment and materials.

4.3. Instrument Calibration:

4.3.1. Perform the necessary calibration procedures as specified by the manufacturer or the laboratory's standard operating procedures. This involves preparing calibration solutions of known concentrations for the elements of interest and verifying the instrument's response.

4.3.2. Document the calibration process and results as per the laboratory's document control procedures.

4.4. Sample Measurement Procedure:

4.4.1. Prepare the samples to be analyzed, ensuring they are in a suitable form and concentration for flame photometry measurements.

4.4.2. Set up the Flame Photometer according to the manufacturer's instructions, including the proper selection of combustion gas and flame conditions.

4.4.3. Assemble the necessary safety equipment, such as gloves and safety glasses, to ensure safe handling of samples and chemicals.

4.4.4. Ensure that the instrument is warmed up and stabilized before starting the measurement.

4.4.5. Set the appropriate wavelength or filter for the specific element of interest.

4.4.6. Measure the blank or background signal by introducing the appropriate solvent or matrix without any sample.

4.4.7. Introduce the sample into the Flame Photometer using a suitable sample introduction technique (e.g., aspiration or nebulization).

4.4.8. Record the signal or emission intensity displayed by the Flame Photometer.

4.4.9. If multiple samples are to be analyzed, rinse the sample introduction system between measurements to prevent cross-contamination.

4.4.10. If necessary, calculate the concentration of the element in the sample based on the calibration curve or factors.

4.5. Post-Measurement Procedures:

4.5.1. Clean the sample introduction system and any contaminated parts of the Flame Photometer using appropriate cleaning materials as recommended by the manufacturer or the laboratory's standard operating procedures.

4.5.2. Store the Flame Photometer in a clean and secure location, following the manufacturer's guidelines for proper storage.

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PG DEPARTMENT OF CHEMISTRY

STANDARD PROCEDURE FOR OPERATION AND CALIBRATION OF FTIR

1.0 PURPOSE:

- The purpose of this Standard Operating Procedure (SOP) is to describe the start-up, operation, calibration, and maintenance procedure of the FTIR – Fourier Transform Infrared Spectrophotometer.

2.0 SCOPE:

- This SOP is applicable to the Shimadzu make and model IRAffinity-1 FTIR-Fourier Transform Infrared Spectrometer of the quality control department at the pharmaceutical drug manufacturing plant.

3.0 REFERENCES:

- Instrument manual of Fourier Transform Infrared Spectrophotometer (FTIR).
- SOP for Handling of out of Calibration results (OOC)
- SOP for Analytical Instrument Qualification.
- European Pharmacopoeia

1. SOP for -Instrument/Equipment usage log book.

- SOP for –Maintenance of Laboratory Instruments.

4.0 RESPONSIBILITY & **ABBREVIATIONS**

- The Analyst shall be responsible for the operate the instrument as per SOP,

- Calibrate the instrument as per SOP and Recording all the document related to the operation, calibration, and maintenance.
- Quality Control Head or Designee shall be responsible for give training to all the concerned persons before the implementation of SOP,
- To ensure proper documentation as per SOP and initiate the maintenance activity white breakdown.
- QA shall be responsible for check and ensure the implementation of the system as per SOP.
- Quality Head and Plant Head shall be responsible for the review, approve the SOP.
- **ABBREVIATIONS:**
- FTIR: Fourier Transform Infrared Spectrophotometer.
- KBr: Potassium Bromide
- NA: Not Applicable
- QA: Quality Assurance
- QC: Quality Control
- SOP: Standard Operating Procedure

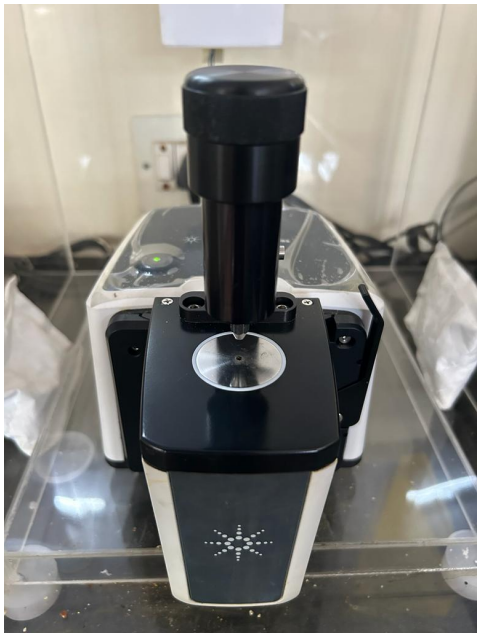
4.0 PROCEDURE FOR OPERATION AND CALIBRATION OF FTIR:

- **Startup of FTIR Software**
- Clean the Instrument and surroundings.
- Connect the instrument with the power supply.
- Switch on the instrument and Computer system.
- Double click on “IR Solution” Icon.
- After No. of internal operation.
- Select the “Measure” and select Measurement from the main menu, further select “initialize” from the measurement tab.
- Press “Yes” then following screen will appear:
- Select File to load standard method for analysis or fill the desired parameter of the method.
- Click on file,
- Select the above option to enter the main window and select the standard method for analysis or prepare new method as per STP.
- After selecting the standard method, double click on method or Open it from main window. A dialog box will appear and ask about downloading parameter of Method.
- Press “OK” to download the method parameters
- After completion of method downloading,
- Click on “BKG” for background correction. A dialog box will appear.
- Press “OK” if the Sample compartment is ready for Background.
- Select measure from the above screen window, fill all the required (sample/standards information) in comment/Date file and select sample for sample analysis.
- For the Sample Analysis click on the Data path file and give the appropriate file path for saving the data.
- Copy the file name and paste the data in the comment and click on the sample tab.

- After scanning the sample click on “Calculate” and then “OK” on the Peak Table.
- The number of peaks in the Peak Table must be between 15 to 25.
- To adjust the number of peaks, click on “Calculate” and then increase or decrease the “Min Area” and then click on “OK”.
- Click on “Window” from the main menu bar and select “Join Visible”.
- Then click on “Manipulation 2” and select Purity.
- Right click on the sample and select send to source similarly right click on the reference and select send to reference.
- Send to reference
- Click on “Calc”, then “OK”, select the print command from the main window.
- Click on “OK” and select the desired report template for the Peak purity Graph as shown bellow.
- Select the Report format and click on “Open” or double click on Report format. Press ‘OK’ for printing the document.
- For Peak table: select the Manipulation 1 and select Peak table.
- After selecting the peak table,
- Click on “Calc” then “OK”.
- Select “Print” from the above screen.
- Select the report format as ‘peak table’ and print. As per the above-described procedure.
- **Sample Preparation for FTIR Analysis:**
- **Sample Preparation for solid Sample for FTIR Spectrometer:**
- Dry the KBr (Potassium Bromide –IR Spectroscopy grade) at 105°c for about 1 hour, cool it in the desiccator before use.
- Crush the dried KBr in mortar.
- Clean the sample holder with carbon tetrachloride or acetone to remove grease.
- Take crushed KBr in the “Sample Cup” and plain the surface by using sample-pressing bar.
- Clean the excess powder that fall on the sides of the sample cup using a tissue paper and put the cup on “Sample Cup Holder” in the sample compartment of the instrument.
- Take the background IR.
- Weigh about 300 mg of previously dried KBr and transfer it to a clean mortar.
- Weigh about 2 to 4 mg of previously dried sample or as per the specified specification (to make sample concentration about 1.0% w/w) and transfer to the mortar, which contained the KBr.
- Mix the sample with KBr for homogeneous.
- Use this sample for the sample spectrum.
- **Sample preparation (For Liquid sample) for FTIR Spectrometer:**
- Select fixed thickness cell as per the requirement or use sodium chloride cell.
- Put the cell in cassette and take the background (Air background) or as per the requirement.
- Suck up the liquid sample by syringe.

- Inject the liquid sample into the cell through the one hole of cell until the liquid came out from another hole of cell and become a thin film and insert the plugin both the holes.
- Clean the cell with tissue paper.
- Use this sample for sample spectrum.
- Sample Preparations – Films.
- Use this technique for liquids or semisolids or low melting solids.
- Put the cell in cassette and take the background (Air background) or as per the
- A thin film can be made by dissolving a semisolid or a highly viscous liquid (such as polymers) in a minimum volume of a volatile solvent (such as chloroform, carbon tetrachloride) or the sample can be taken as such.
- Using a clean glass capillary, place a drop or a small portion of the sample onto a clean sodium chloride cell.
- Hold the other sodium chloride cell's edge on the top of the sample and slide horizontally applying slight pressure so as to form a thin film on the cell.
- In case film is to be made with a solution, evaporate the solvent to dryness using a hot air gun. Align the cell edges and assemble the two sodium chloride cells.
- Run a Background Scan as an air blank
- Cut the film cassette size and insert a cassette.
- Use this sample for the sample spectrum.
- If the sample preparation is specified in the STP/ATP or individuals monograph follow the same.
- **Calibration (Verification of the wave-number scale) of FTIR Spectrometer:**
- Follow the operational procedure and record the Spectrum of the **Polystyrene film** over the range of 3800 cm^{-1} to 650 cm^{-1}.
- Record the wave number in attachment no. 01.
- The spectrum should show the Transmission minima (absorption maxima) at the wave-number given in the attachment no. 01.
- **Frequency:** (Monthly± 3 days)
- **Validation Procedure of FTIR Spectrometer:**
- Ensure that, the instrument is ready for calibration and the start-up procedure is followed.
- Calibrate the instrument for power spectrum, resolution, wave number accuracy, repeatability of wave-number and repeatability of absorbance.
- Ensure that the Instrument is ready for the calibration and start-up the procedure is followed.
- Initialized the instrument.
- Click on the icon Measurement, select "EP5.0 validation".
- After selecting "EP 5.0 validation".
- Click on "Measurement".
- Verify the necessary information or change the details as per requirements.
- Click on "OK" to start the background.
- After background validation completion, "Set polystyrene film into the sample chamber" is displayed in the screen.
- Set the polystyrene film accordingly and click on "OK", scanning will start.

- After completion of the validation the report will be generated and printed automatically.
- Compare the results for its compliance against limits given in validation format and put remark regarding validation status.
- Make entry of the usage into the Instrument usage logbook.
- Calibration report shall come in form of validation format at the time of printing.
- File the validation report duly signed and checked.
- Affix calibration label on the instrument.
- **Frequency:** Monthly ± 3 days
- **Precautions/Maintenance Program of FTIR Spectrometer:**
- **Precautions during FTIR Calibration:**
- Use only spectroscopic grade KBr for the sample preparation and store a dry box.
- Clean and dry mortar and pestle immediately after the usage.
- Check silica bag of the dry box as well as sample compartment for its effectiveness.
- When not in use Polystyrene film should be kept in its accompanying protective cover.
- The exposed film surface should never be touched by fingers or any other objects and Dust should be removed by blowing with clean and dry air.
- **Breakdown Maintenance of FTIR Spectrometer:**
- Put “UNDER MAINTENANCE” label on the instrument when it is under breakdown maintenance / not functioning and intimate to Head quality or designer.
- Head quality or designee informs to the Instrument service engineer or Utility dept.
- Keep instrument UNDER MAINTENANCE between breakdown and service period.
- After maintenance, calibrate the instrument to check its satisfactory functioning and record the readings. (Note: Calibrate the instrument as per the calibration schedule, this calibration is additional)
- Retain service report copy attached in the Instrument Maintenance History File.



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PG Department of Chemistry

STANDARD OPERATING PROCEDURE

MUFFLE FURNACE

1. Connect the muffle furnace to the main supply.
2. Red indicator will begin to glow.
3. Set the required temperature of Muffle Furnace by pressing ‘set’ button of Digital temperature controller and set Increasing/Decreasing Temperature by adjusting coarse and fine button.
4. Start the furnace by turning energy regulator knob to ‘50’ position. The dial of the energy regulator is arbitrarily marked 0-100 position.
5. Green indicator will begin to glow indicating load on.
6. Press the set button of controller to view the set temperature.
7. Furnace will now start heating and load indicator will glow and after attaining the set temperature indicator will off and the indicator will again glow when temperature reduced below the set temperature.
8. Temperature of 900ºC is achieved in about 90-100 minutes.
9. Maximum temperature which muffle furnace attain is 925ºC.
10. Maximum load which muffle furnace attain is 3.5 K.W.

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STANDARD OPERATING PROCEDURE

ROTARY EVAPORATOR

1. **Purpose and Scope of Work/Activity:**

The process of removing a volatile solvent from a non-volatile sample is essential to many laboratories. A rotary evaporator (rotovap) is a fast and effective method of removing solvent from a flask. A rotovap removes solvent by reducing the pressure within the flask using a vacuum, rotating the sample to increase its effective surface area, and heating the solution.

The steps outlined below address general laboratory safety concerns while operating a rotovap. Modify this SOP with your laboratory's specific operational procedures with the help of the Principal Investigator and verify the SOP with EH&S prior to working with a rotovap. Ensure that the laboratory specific procedures outlined in this SOP are followed by laboratory workers at all times. This document is not designed to substitute hands-on training and supervision by experienced laboratory personnel.

BASIC OPERATING PROCEDURE

1. Read the SDS for all materials
2. Inspect the rotovap and all pieces of glassware to ensure that there are no cracks, chips, or defects.
3. Make sure that the solvent trap and bump trap are emptyand clean
4. Fill the hot water bath with DI water if not already filled.
5. Fill the cold trap (e.g., dry ice/acetone)
6. Cool the receiving flask
    a. It is a good idea to cool the receiving flask (e.x. solvent reservoir); it prevents any solvent from evaporating from the receiving flask, especially for low boiling solvents (e.g. pentane).

7. Connect the bump trap to the rotovap and secure with the metal clip or a keck clip depending on the model of rotovap (pictured below). The bump trap should be kept clean, it provides a receptacle in case the solution splashes orboils beyond the flask. A clean bump trap can allow for recovery of any solution that "bumps".



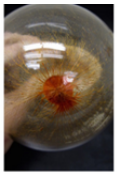
8. Pre-weight around bottom flask; fill it less than halfway with the solution to be evaporated.
9. Connect the round bottom flask containing the solvent to the bump trap and secure with a Keck clip.

10. Turn on the vacuum. You should hear a hissing sound if the stopcock on the evaporator is not closed. Slowly close the stopcock on the evaporator, once closed the sound should stop.

11. Begin rotating the flask. Ensure that the solution does not foam or boil too much.
12. Wait until the rotation and apparatus equilibrate to proper reduced pressure and speed. Keep a watchful eye on the evaporation (boiling) of the solvent (vent or change speed as needed).
13. Allow the solution to evaporate. The solvent should collect in the solvent reservoir.
14. Once the flask changes in temperature and becomes cool to the touch, lower the mechanism into the water bath or lift the water bath to the evaporation flask (depending on the rotovap you are using). Make sure that the solvent level is equal to the water bath level. Never put the flask so low that the Keck clip touches the water.

15. If needed you should start to heat the water bath. [Keep in mind that the sample is under vacuum, this will lower the boiling point]
    a. Never have the water bath temperature higher than the boiling point of the solvent
16. Keep a watchful eye on the evaporation of the solvent.
    a. If the sample is boiling too vigorously, remove it from the water bath, decrease the temperature, or change the rotation speed.
17. Allow the solution to evaporate until the product has been isolated or until the desired amount of volatiles have been removed.

    a. When the sample appears to be completely done evaporating allow the flask to remain at reduced pressure, while heating, and rotating for at least a few minutes to ensure that all of the volatiles are removed.
18. Once the evaporation is complete conduct the same steps in the reverse order: turn off the hot water bath, remove the flask from the water, stop the rotation, turn off the vacuum, slowly open the stopcock to vent the system (remove residual vacuum) while holding the flask (to avoid it falling and breaking), and remove the flask.



19. Make sure to empty the solvent reservoir into the appropriate waste container and clean the bump trap. Remove the water from the hot water bath when finished using the rotovap, this will prevent it from evaporating and leaving behind a residue.

Emergency procedure (if safe to do so)

1. Turn off the vacuum. Vent to remove the residual vacuum.
2. Turn off power.
3. Unplug the electrical cord.

Hazards and Controls

In general, the main hazards and controls associated with Rotary Evaporation are:

Risks and Hazards	Controls
Vacuum/Reduced pressure • Implosion	• Ensure that there are no star cracks, chips, or defects in the glassware • Always keep the rotovap in the fume hood with the sash down • More permanent exterior glass parts (e.g., solvent trap, condenser) should be Plexiglas coated, mesh encased, or taped with electrical tape (pictured below). 
Hot water bath • Burns • Electrical heating • Buildup of residues or bacteria colonies	• Do not touch the hot water bath or the flask that has been in the hot water bath • Make sure that the water bath never goes dry and do not leave it on for extended periods without supervision • Always fill the water bath with DI water to prevent the buildup of residues. ○ Residues are difficult to remove and decrease the efficiency of the water bath. • Change the water regularly to prevent the buildup of bacteria colonies.  water bath with a buildup of residues
Cold bath • Cryogenic burns or frost bite	• Only use vessels that are rated for extreme cold when using a cold bath. • Use cryogenic gloves when handling the cold bath • Filled dewars and cold fingers should be secured and clamped within a clutter-free fume hood with the sash down
Rotating equipment • Pinch hazard • Loose articles can be caught in the system	• Always secure all loose hair, articles of clothing, jewelry, etc. • Always turn the rotovapoff before adjusting the equipment
Health hazards • Chemical exposure/inhalation	• Keep the rotovap in the fume hood to prevent inhalation • Always wear the appropriate PPE
Flammable chemicals • Fire	• Remove any sources of ignition and combustible materials • Know the location of all emergency equipment (fire extinguisher, safety shower, etc.) • Always wear the appropriate PPE
Chemicals can become unstable or explosive when being concentrated • Explosion • Violent reaction	• Never isolate peroxides, azides, acetylides, nitro-containing compounds, molecules with strain energy, or thermally unstable compounds using a rotovap, this can lead to a fire or explosion • Ensure the sample is not reactive or explosive upon concentration • Never use a rotovap to isolate a solution which produces

	explosive impurities during evaporation (e.g., ethereal solvents can produce peroxides) • For peroxide forming solvents that might be exposed to air over an extended period of time - peroxide tests must be conducted
Condensed oxygen • Explosion	• Never use liquid nitrogen for the cold trap o Dry ice/acetone baths are sufficient for most applications • Do not leave the cold finger to condense overnight
Water cooled condenser • Flooding	• Use a water circulator (never use single-pass cooling) and properly secure all hoses
Air/water reactive reagents • Violent reaction	• Extra caution must also be applied to operations with air and/or water reactive materials. A leak can draw air into the apparatus and a violent reaction can occur.
Broken glassware • Laceration	• Make sure to clamp all flasks and joints, this will ensure that the glassware does not fall and break. • Glass parts (e.g., solvent trap) should be Plexiglas coated, mesh encased, or taped with electrical tape

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STANDARD OPERATING PROCEDURE

UV VISIBLE SPECTROPHOTOMETER

1. Switch on the power supply from power board.

2. Start the computer system and UV machine.

3. Now use password on comp system: uv123.

4. Put reference solvent in both curettes in UV machine.

5. Click on carry win UV (software on desktop). 6. Click on scan icon.

7. Set up - visible to UV up - baseline up - baseline correction up - ok up – baseline – yes.

8. Put reference inner portion and put sample front portion the UV chamber.

9. Click on start button (Green button).

10. After getting complete graph click on finish option.

11. Now click on trance reference baseline - removed by selection - new get single UV graph of sample without baseline on display.

12. Now click on print per view – print - select cute PDF writer - OK and saved.

13. For shut down the computer - click on close button in software. After shutting down computer close back switch of machine.

14. Switch on the power supply from power board.

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