

V.V.VANNIAPERUMAL COLLEGE FOR WOMEN

An Autonomous Institution Affiliated to Madurai Kamaraj University

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DEPARTMENT OF CHEMISTRY

STANDARD OPERATING PROCEDURE for the EQUIPMENTS



सत्यमेव जयते

DEPARTMENT OF BIOTECHNOLOGY

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ANALYTICAL BALANCE



ANALYTICAL BALANCE

- The digital balance is very sensitive instrument used for weighing substances to the milligram (0.001 g) level at room temperature.

OPERATING PROCEDURE :

I) BEFORE START :LEVEL AND ZERO THE ANALYTICAL BALANCE

- **Level the balance:** look at the leveling bubble on the base of the unit. If it is not centered, center it by turning the leveling screws on the bottom toward the back of the balance.
- **Zero the balance:** after the leveling is completed. Close all the balance doors and press the button labeled '0' on the front of the balance. Wait until the unit has zeroed showing a value with all zeros. This indicates that the balance is zeroed and ready for use.

II) DIRECT WEIGHING

- The samples are weighed using an appropriate weighing container, weighing paper/beaker
- Ensure the analytical balance is set to the proper units grams(g), milligrams(mg)
- Place the weighing container on the balance pan and close the doors.
- Tare the container (Press '**T**'). The readout will read zero with the container sitting on the pan. This allows the mass of your sample to be read directly.
- Add the sample to the container; avoid spilling on to the balance.
- With the sample inside the vessel, close the balance doors and read the display when the mass stabilizes.

CARE AND MAINTENANCE

- Once weighing is completed, ensure you have properly cleaned up any chemicals that may have spilled on or around the balance.
- Take care not to press down on the pan.
- Use a brush provided to remove particulates from the pan area.

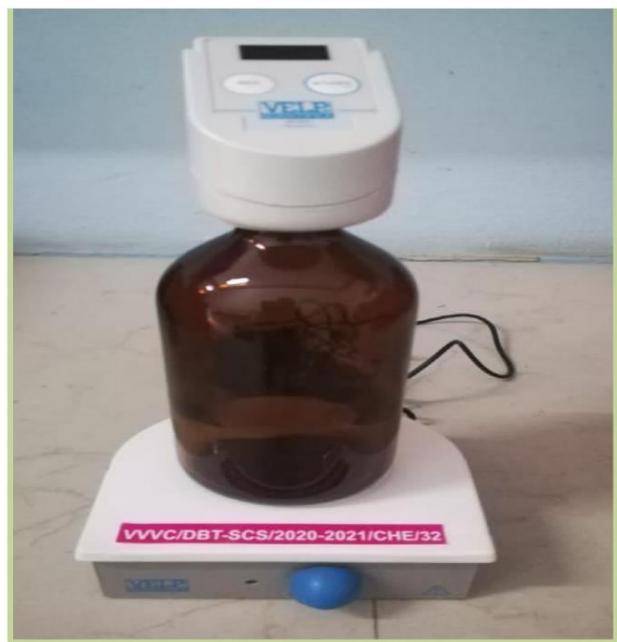
AUTOCLAVE



AUTOCLAVE

- Autoclave provides a physical method for disinfection and sterilization.
- It works with a combination of steam, pressure and time.
- It operates at high temperature and pressure in order to kill microorganisms and spores.
- Switch power breaker "ON". Power switch to "ON".
- Swing door close clockwise as tight you can open it when complete.
- Loading appropriate amount of content in the tray or chamber.
- Set Sterilizing temperature with appropriate timer.
- Press the mode switch which you like to sterile (Unwrapped, Wrapped, and Solutions)
- Set the dry timer.
- Press the mode switch, the sterilizer will automatically progress through cycle if the pressure of steam generator has reached the setting pressure.
- There are 2 cycles that the autoclave can be run on. The sterilize cycle should be used for liquids, the sterilize and dry cycle should be used for glassware.

BOD ANALYSER



BOD ANALYSER

- BOD (Biochemical Oxygen Demand) analyser is used to determine the presence of microorganisms in a water sample containing biodegradable organic substance, consume oxygen for their metabolic activity and produce a corresponding volume of carbon dioxide.
- The principle is that the carbon dioxide will get absorbed by a strong alkali positioned in the neck of the bottle. The result of this process is a decrease in gas pressure. The BOD will measure this change in gas pressure between the beginning and the end of the analysis and give a result expressed in mg/l. Water sample whose BOD has to be determined is taken in the bottle along with necessary reagents for oxidation
- Fits directly the bottle containing the sample to the BOD analyser
- It is to be kept undisturbed for 5 days for complete oxidation.
- The BOD Sensor is a mercury-free and reliable solution for BOD determination. It automatically stores 5 BOD measurements at 24-hour intervals meaning that analysis can continue over the weekend.
- A microprocessor-controlled pressure transducer transfers the BOD value directly to the display.
- Results are displayed directly in mg/l with no need for further calculation. Direct readout in mg/l (ppm)
- This equipment measures in the range of 90, 250, 600, 999 and 4000 ppm BOD.
- Higher values can be measured by diluting the sample.

CARE AND MAINTENANCE

As it is manufactured with premium materials, it should be handled with much care.

CODDIGESTER



CODDIGESTER

- COD digester is used to measure Chemical Oxygen Demand in water and wastewater samples by potassium dichromate - sulphuric acid digestion under acidic condition.
- It consists of an electrically heated (220 ± 10 volts/50 Hz AC power) metal block thermostat having 6 holes to take the reaction vessels.
- The capacity of these reaction vessels are 100 ml each. The heating with thermostatic control should provide a temperature range of upto 400°C , and built in timer with signal.
- Connect the instrument and switch on by means of Toggle Switch which is at the right side of the instrument.
- Set the temperature at 150°C & Time (2 hrs.) with the help of SET button by adjusting COURSE and FINE knob. When the temperature reaches 150°C , insert the reaction vessels containing sample and reagents into the digestion block and keep the air condensers on each vessel.
- Set the timer for the desired time. For COD Analysis, set time for 2 hours. Switch on the Toggle Switch. When Toggle Switch is ON, Red L.E.D. will glow.
- The Buzzer will sound after the set time and Red L.E.D. will be off. Then switch OFF the Toggle Switch.
- Remove the glassware and carry out analysis.
- If no further samples are to be digested, simply off the Instrument.

CARE AND MAINTENANCE

- Ensure proper earthing and proper supply of Voltage.
- Always use correct rating fuse. (5 A)
- No chemicals should be split on the block.
- The glassware must be free from any water droplets on the outer surface as it may cause crack.
- In case of accidental breakage, switch off the power immediately.

DIGITAL COLORIMETER



DIGITAL COLORIMETER

- Digital Colorimeter is used to measure the absorbance of light passing through a solution.
- Check that the area surrounding colorimeter is clean and keep it safely on the working table
- Switch on the instrument atleast 10minutes before use and allow it to stabilize.
- Move the filter wheel and select the desired wavelength (Range 400nm-700nm)
- Place the mode selector at %T and adjust the transmittance to 100 using ADJUST100% knob.
- Press the mode selector again and switch to absorbance mode.
- Adjust absorbance to 0 using the knob.
- Fill the cuvette with distilled water or a solution used as a blank.
- Clean the outer surface of the cuvette using filter paper.
- Insert the cuvette filled with a blank solution in the colorimeter.
- Adjust the absorbance to 0 using knob.
- Remove the cuvette and place it in cuvette holder.
- Fill the cuvette with the sample solution.
- Clean the outer surface of cuvette using filter paper.
- Insert the cuvette filled with a test sample in colorimeter and record the results.
- After obtaining results, discard the cuvettes containing blank and sample solutions and rinse with water.
- Switch off the power button after use.

- The calibration of a colorimeter is done by the use of a standard solution of known molar extinction coefficient(ϵ)
- Molar extinction coefficient value of the standard solution is determined using a colorimeter. Difference between the expected and the obtained value is calculated.

CARE AND MAINTENANCE

- Keep the space around colorimeter neat and clean
- Handle the cuvettes with much care

DIGITAL pH METER



DIGITAL pH METER

- A digital potential of hydrogen (pH) meter is an electronic gadget used to measure the pH value of liquids accurately. Measuring pH allows one to determine the acidity or alkalinity of a substance in a liquid state.
- After attaching the pH electrode to the BNC socket, wash the pH electrode with distilled water. Put the electrode in first buffer solution, say 7 pH buffer, together with temperature probe if Automatic Temperature Compensation (ATC) is desired. The display shows the pH value of the solution.
- Press 'Cal 7 pH' key. The display starts blinking. The blinking indicates that the system is in calibration mode. Let the pH reading stabilize (Note that the display shall still be blinking indicating calibration mode, but the variation in pH readings should stabilize).
- Press 'Cal 7 pH' key again to complete 7 pH calibration.
- Remove electrode from 7 pH buffer, wash with distilled water and put it in 4/9.2 pH' buffer, say in 4 pH buffers.
- Now press 'Cal 4/9.2 pH' key The display again starts blinking. Let the reading stabilize. Press 'Cal 4/9.2 pH' key again. The instrument is now calibrated for 4 pH.
- If 3-point calibration is desired, take out the electrode, wash with distilled water and put it in 9.2 pH buffer.
- Now press 'Cal 4/9.2 pH' key. The display again starts blinking, let the reading stabilize. Again press 'Cal 4/9.2 pH' key to complete the calibration process. The instrument is now ready to take any pH measurements.

CARE AND MAINTENANCE

- Always keep the pH electrode in 4M KCl solution.
- Do not store the electrodes in distilled water, this will cause ions to leak out of the glass bulb and render the electrode useless
- The electrodes are shipped with a protective rubber cap over the glass bulb to protect cracking.

DIGITAL FLAME PHOTOMETER



DIGITAL FLAME PHOTOMETER

Digital Flame Photometer is used to determine the concentration of alkali metals quantitatively.

The fluid under analysis is sprayed as a fine mist into a non-luminous flame, which becomes colored according to the characteristic emissions of the elements. The flame color is simultaneously monitored for both the channels. Each channel consists of a detector, which views the flame through a narrow band optical filter that only passes the wavelengths centered on the characteristics emissions of the elements (Na:589nm, K:768nm, Ca:622nm, Li:671nm). The output of the detector is connected to an electronic metering unit, which provides digital readouts. Before analyzing the unknown fluids, the system is standardized with solutions of known concentrations of elements of interest.

OPERATING PROCEDURE

(i) Pneumatics setting

After the system is properly installed and leak free connections are ensured, proceed as under.

- Ensure that the Air-Tube, Gas-Tube, and Drain-Tube are properly connected.
- Switch on the Compressor, Ensure that the output pressure is close to 0.50 Kg/cm² and is stable (No fluctuations or drift is seen in output pressure gauge).
- Dip the Atomizer capillary tube (plastic) in distilled water. Ensure that regular droplets fall in drain-cup and drains out.
- Open the fuel gas fine ADJ (*Fine Adjustment Valves*) valve by approximately half turn.
- Switch on the fuel supply from the fuel source/LPG cylinder and immediately ignite the flame through the IGNITION window.
- Watch the flame through the Flame view Window. Do fine adjustment of fuel flow with the help of the Gas control valve to get a stable flame having well defined cones.

(ii) Electronic Setting

- Connect the Unit to the mains through its Main Cord.
- Turn on mains POWER switch. Digital Display should turn on.
- Turn the SET F.S (Full scale) both COARSE & FINE; Set REF both COARSE & FINE controls in maximum clockwise position.
- Select appropriate filter(s) with the help of filter selector of the burner unit.
- Feed distilled water to atomizer (in burner unit). Wait at least for 30 seconds.
- Set SET FS both COARSE slowly, till the readout of the digital display approaches zero. (Appearance of – sign on the readout indicated that the SET ZERO control should now be turned slowly towards “+”).
- Adjust SET REF FINE to achieve precise zero setting. (Appearance of – sign on the readout indicates that the SET ZERO control should now be turned slowly towards “+”).

CARE AND MAINTENANCE

- Do not drain out Air-Filter of the Compressor when the flame is on.
- Before feeding next sample, feed distilled water to the atomizer, say for 5 sec, to avoid interference of the previous sample.
- Never keep gas supply on when the flame is not ignited.
- Do not leave the FPM unattended if the flame is on.
- Replace the gas cylinder well before it gets empty. Otherwise reproducibility will significantly deteriorate.
- Do not disturb OUTPUT REGULATOR setting unless it is required.

ELECTRIC WATER BATH



ELECTRIC WATER BATH

- Electric water bath is used to heat samples in water at a constant temperature over a long period of time.
- Fill three-fourth of the water bath with distilled water.
- Connect the power supply.
- Switch “ON” the main power supply and instrument mains.
- For temperature settings, Press SET key to set the required temperature. Press “↑” to increase the temperature and “↓” to reduce the temperature
- The temperature sensor will maintain the set temperature during use of water bath.
- Switch “OFF” the instrument mains and main power supply after use.

PRECAUTIONS:

- Set up the water bath on a steady surface away from flammable materials.
- Fill the sample holder with distilled water only. This is required to prevent salts depositing on the heater.
- Monitor the water level regularly.
- Raise the temperature to 90 °C or higher for once a week half an hour for the purpose of decontamination.
- If application involves liquids that give off fumes, operate the water bath in fume hood or in a well-ventilated area.
- Close the cover to prevent evaporation and to help reaching high temperatures.

HOT AIR OVEN

(THERMOSTATIC MODEL KOA/KOS)



HOT AIR OVEN

(THERMOSTATIC MODEL KOA/KOS)

- Hot air oven is an electrical device which uses hot air to dry and sterilize.
- It has a thermostat to control the temperature.
- Connect the power cord to the power socket (15 Amps capacity), connect the instrument to suitable 3pin, 230V Ac power having properly earth.
- Close the door completely. Turn the power switch to ON position.
- ON the fan motor switch (if it is present) Setting the temperature.
- Set required temperature by turning the knob. Temperature values in 0°C given on the knob is approximate.
- Turn the rotary switch to either one of three modes.
 - Low - upto 60°C
 - Medium - upto 100°C
 - High - upto 250°C
- When the equipment is switched on, the controller displays the current temperature and the set point is shown when the SET button is pressed.
- Press PGM Read the work CODE. Enter code 50 by pressing \wedge button.
- Press SET and read SP (set point).
- Set the required temperature by pressing either \wedge (or) \vee button.
- Press SET button. Then displays (if the program is successfully completed), shows the normal running temperature (chamber temperature) which gradually increases till reach the setting temperature.
- After setting appropriate temperature, turn the rotatory switch to either one of three modes.

CARE AND MAINTENANCE

- Unplug the equipment before cleaning or servicing. Do not wash the equipment with a water jet or high pressure water.
- Do not spray or pour water into the control enclosure. To clean the chamber, wipe with a damp cloth and dry with a towel. Use only cleaning agents approved for stainless steel or aluminium (depending on your cabinet construction).
- Do not use cleaners with chlorides or phosphates as they may cause damage to stainless steel. Do not use strong alkalis on aluminium as they may discolour it.

MAGENTIC STIRRER (IMLH)



MAGENTIC STIRRER (IMLH)

- Magnetic stirrer is a device used to agitate the liquid for speeding up the reactions or improving mixtures.
- Place the glass beaker containing the solution to be stirred on the top plate in the center and immerse stirring paddle into the solution.
- Connect the magnetic stirrer to the supply (230VAC, 50 Hz).
- Illuminate the pilot lamp
- Stir the paddle to regulate the temperature slowly to the required speed
- If the speed is increased too quickly, it may occur that the stirring paddle cannot follow the rotation of the magnet and consequently stops rotation.
- Stop the stirring operation and to start again beginning with the lowest speed.
- Use small Teflon paddles especially for partial loads and/or/low stirring.
- Under the partial loading conditions of the magnetic stirrer, the rotation of speed knob makes the motor speed-up beyond rated maximum speed
- Avoid over speed of running the paddle.
- Switch on the heater
- Adjust the heat knob position to set the desired temperature.

CARE AND MAINTENANCE

- Ensure that stirrer speed knob and heat control knob are set to 'O' position and the corresponding switches are in "off" position before the magnetic stirrer is connected to the power supply.
- Place the magnetic stirrer on a rigid level table or bench which is free from vibration.
- Connect the magnetic stirrer to 230 volts A.C. mains having proper earthing.
- All the bearing of the driving motor should lubricated with oil after about 1000 hours of operation.

MICROWAVE OVEN



MICROWAVE OVEN

- Microwave ovens can be used in laboratories for the rapid heating of material – either to dry them completely or to subject a workpiece to sudden thermal stress or electric field stress
- Microwave ovens work on the principle of conversion of electromagnetic energy into thermal energy.
- Keep the reaction mixture in a suitable container inside the microwave oven
- Reaction time can be select by pressing CLOCK pad with 1-12 hours.
- During reaction, the current time can be seen by touching CLOCK pad.
- Use the number pads to enter the correct time.
- After reaction time, the system beeps to remind the user every two minute until the user press STOP/CANCEL pad or opens the door.
- Power save can be used to set power save program

CARE AND MAINTENANCE

- The microwave oven must be placed on a flat, stable surface to hold its weight.
- Do not place the oven where heat, moisture, or high humidity are generated, or near combustible materials.
- For correct operation, the oven must have sufficient airflow. Allow 20cm of space above the oven, 10cm at back and 5cm at both sides. Do not cover or block any openings on the appliance. Do not remove feet.
- Do not operate the oven without a glass tray, roller support, and shaft in their proper positions.
- Make sure that the power supply cord is undamaged.
- The socket must be readily accessible so that it can be easily unplugged in an emergency.
- Do not use the oven outdoors

MUFFLE FURNACE



MUFFLE FURNACE

- Muffle furnace is used to test the characteristics of materials at extremely high and accurate temperatures.
- Keep the Muffle Furnace on a levelled and firm platform.
- Ensure that the instrument is connected with the main power supply.
- Both the ON/OFF power switch and digital display illuminate.
- When the door is opened, the door safety switch removes power from the heating elements.
- The set point controller provides single display to indicate the current chamber temperature or set point temperature.
- When the controller knob is turned ON it will perform a short self-test and then display a current temperature inside the chamber.
- Then set the required temperature by pressing (▲) or (▼) arrow key until the desired set point value is displayed and then release the button.

Down Button Allows to decrease temperature

UP Button Allows to increase temperature

- A few seconds after the button is released the controller will accept the new value and raise its temperature gradually.
- The working temperature range is 100 to 1000°C.

CARE AND MAINTENANCE

- Make sure the desired temperature is not higher than the melting point of any of the materials put inside the furnace.
- After the muffle furnace is used, the power should be cut off to allow it to cool down naturally.
- The furnace door should not be opened immediately to prevent cracking of the furnace.

PHOTOFLUROMETER



PHOTOFLUROMETER

- Photofluorometer is used to analyse fluorophores like metal complexes, steroids, Vitamins and quinine.
- Systronics Photofluorometer consists of a strong light source, three digits seven segment LEDs, Sensitivity control, Standard control and Read shutter Sample tube.
- Connect the main plug of the instrument to the 230 V AC mains.
- Set the sensitivity control in low position STANDARD and BLNK controls to minimum
- Insert appropriate primary and secondary filters.
- Switch on the instruments. Check the working of the cooling fan.
- Set sensitivity switch control in high precision
- Insert test tube of blank . Depressing the SHUTTER, adjust the display to zero by BLANK. Insert standard and unknown.
- Return sensitivity switch control in low position
- To make the instrument direct reading, select the numerical values.

CARE AND MAINTENANCE

- The cuvette may come out with the exhaust fitting. When this happens, remove it with the exhaust fitting. Remove the lower intake fitting in the same manner as the upper fitting.
- If the cuvette did not come out with the Exhaust fitting, it should come out with the intake fitting.
- Wipe up any spilled liquids in the instrument and dry thoroughly to remove any saltwater that is highly corrosive to metal components

ULTRACENTRIFUGE



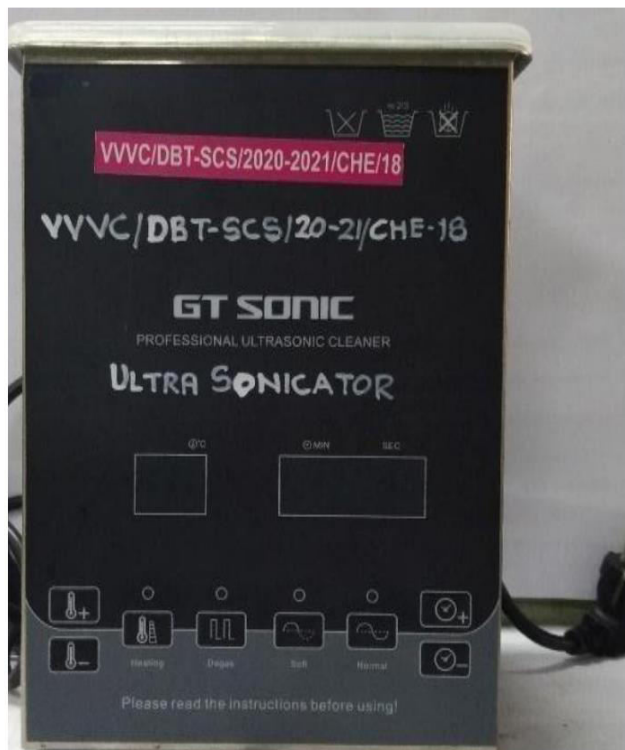
ULTRACENTRIFUGE

- Ultracentrifuge is used to **separate smaller biological molecules with extremely high speed.**
- **First ensure a sturdy, level worksurface.**
- Fill all sample tubes evenly.
- If additional tubes are required for balancing, fill them with water or a liquid of similar density.
- Close the centrifuge lid.
- Move the speed control knob **CLOCKWISE** from the 'O' position.
- To stop centrifuge correctly, turn the speed control to 'O'; the centrifuge will decelerate and eventually stop.
- When the rotor has come to a standstill open the centrifuge lid and remove the tubes.

CARE AND MAINTENANCE

- **The total mass of each tube should be as close as possible- this becomes increasingly important at very high rotor speeds.**
- Balance the masses to the nearest 0.1 gram and it is important to balance tubes by mass and not volume.
- Open the centrifuge lid only when the rotor has come to a standstill.

ULTRASONICATOR



ULTRASONICATOR

- Ultrasonicator is used to synthesis nanoparticles, extract phytochemical compounds and ultrasonic cleaning by speed dissolution. In ultra-sonication, high energy ultrasonic waves propagated into the reactants to agitate it.
- Connect the Ultrasonic Water Bath to the main power supply with the connecting plug.
- Press (+) Button adjustable for timer to increase 1 minute then hold the key till the time raised by 10 minutes continuously
- Press (-) Button adjustable for the timer to reduce 1 minute then hold the key till the time is reduced by 00:00.
- Press Button for the heating key for temperature setting with the range 0°C-80°C.
- Press (+) Button adjustable for heating a time temperature will rise 1°C holding the key temperature will rise continuously with 10°C.
- Press (-) Button for temperature to be reduced to 1°C then holding the key and temperature reduced continuously with 10°C.
- After time and temperature setting press Button Degas, Soft and Normal.
- Machine starts to work, the indicator lights up if needed to stop heating or ultrasonic press corresponding key again then corresponding indicator lights turn off.
- Machine stops working after 8hours working time (power saving mode) in this mode machine restored to the standby state once you press any key.
Three ultrasonic working modes:
- Degas mode: Press Degas Button intermittent operation of ultrasonic power for rapid removal of entrained air from liquids ultrasonic work 6 seconds and stop 2 seconds same cycle to proceed & press Degas key again to stop it.
- Normal mode: Press Normal Button to start the normal faction strong ultrasonic power with large current press again to stop it.
- Soft mode: Press Soft Button to start soft function continuous slight variation of ultrasonic frequency to eliminate hot spots dead zones and standing waves press again to stop it.
- While working, if "sizzling" voice arises, it indicates that the sonication is running properly.

CARE & MAINTENANCE

- Ensure the system is off and disconnect the power cable from the main socket, clean the water bath and replace the water on a daily basis.
- Fill the Water bath with water up to a height of minimum 7cm from the bottom of the tank. Whenever the Ultrasonic Water Bath is required with specific temperature then heater to be kept "ON".
- Ensure that there is no leakage of water from the water bath.
- Do not put any heavy objects on the bottom of the tank as it can damage the transducers. Always use a tray supplied with an ultrasonic bath.
- Ensure that liquid does not splash on the controls and switches.

UV PHOTOCHEMICAL REACTOR



UV PHOTOCHEMICAL REACTOR

- UV photochemical reactor is used to give safe and accurate heat management as well as the best possible reaction selectivity. It provides a temperature in controlled environment which can be set between -20°C and 80°C .
- The reactor and lamp are housed in separate, sealed quartz chambers. Air is circulated within each chamber to dissipate heat and then exhausted from the system.
- The high flow rate of gas gives effective temperature control. A dichroic mirror further removes heat from the system and reflects more than 90% of UV energy back into the reactor.
- Ensure all the components are fitted well before the start of experiment. If the photochemistry of the reactant is completely unknown, make up a solution in an appropriate solvent so that the absorbance at 254nm is about 1.0 (for 1 cm path length). Place the correct volume of solution in a reaction flask and deoxygenate by bubbling an inert gas through the solution for 30 minutes. While doing so, the reflux condenser and reaction flask must be placed in the immersion well.
- Attach the connecting tube of the immersion well to a water supply or a constant temperature bath and place the low pressure lamp inside the immersion well. Connect the lamp to the appropriate power supply.
- Aluminum foil may be wrapped around the reaction flask to minimize stray radiation.
- Monitor the extend of the reaction by taking aliquots of solution at 5-10 minute intervals and observe the changes in absorption/fluorescence spectra or any new products detected by GC or HPLC.
- It is wise to use low concentrated solutions in an immersion well reactor. Solutions should always be well agitated during photolysis to avoid the build-up of light absorbing polymer on the outside wall of the immersion well.
- The amount of light absorbed in the reaction mixture can be estimated using ferrioxalate actinometry. Estimation of the extend of photochemical reaction can only be made by a detailed study of the reaction products and their yields.
- It is a sound practice to maintain photochemical reactions at a constant temperature.

CARE AND MAINTENANCE

- The UV radiation emitted by the lamp is dangerous to the eyes and skin.
- Wear protective eye goggles at all times when the lamps are illuminated.
- Skin should be protected by protective clothing or cream.