CHAPTER II

EXPERIMENTAL AND SAMPLE PREPARATION
II.1.1. Introduction:

A number of instruments have been described in the literature for recording TL glow curves. Most of them are meant for radiation dosimetric purposes or studying TL arising above room temperature (Schulman et al., 1960; Bonfiglioli et al., 1962; 1968; Kenney et al., 1963; Bhasin, 1975; Sunta, 1975). A few of them are for studying TL at low temperatures (Weissbluth et al., 1968; Kukushkin and Kuznetsov, 1966; Tatake, 1970; Fleming et al., 1971; Prakash, 1975). The low temperature TL studies have unique advantage over high temperature studies. A series of electronic rearrangements take place at a fast rate (Augenstein et al., 1961; Claude Helene, 1974) due to interaction of ionising radiation with matter. In the course of this process certain metastable states may get excited or occupied. At room and higher temperatures these states decay fast. As a result they can not be studied. However, the decay of these states can be decreased or arrested by lowering the temperature of the material. These states and their interactions can then be studied utilizing suitable experimental procedures. Low temperature TL technique happens to be one of the simplest yet sensitive technique employed to study these and similar low temperature post-irradiation effects. The set up for TL studies normally consists of
1. Cryostat with sample chamber
2. Heater for sample
3. Photomultiplier
4. Power supply for photomultiplier and an amplifier
5. Recorder
6. Integrator to integrate the area under the glow curve.

Generally, it is observed that a number of difficulties crop up while studying TL at low temperature. Few amongst them are: fogging by ice on photomultiplier, formation of ice on the sample stage and need for heating the sample at a linear rate. Many of the designs described in literature have these and other problems. In addition they have complicated designs which make them inconvenient for smooth operation. Low temperature TL study has also an advantage. The photomultiplier tube gets cooled making the dark current low. Hence signal to noise ratio is high. This enables one to record signals at higher gain when necessary.

A cryostat, simple in design and operation and overcoming most of the difficulties mentioned above, was therefore fabricated and is described later in the Chapter. Some of the salient features of a good cryostat are given below.
II.1.2. Requirements of a good cryostat:

(a) One of the important requirements that most cryostats lack is warming of the sample at predetermined controlled rates. This is very essential for obtaining well resolved glow peaks. In the absence of controlled slow linear heating, there is a possibility of mixing of adjoining glow peaks. In addition, sudden changes in the heating rate during the course of warming of the sample leads to appearance of fake glow peaks. It is therefore necessary that during warming of the sample, there should not be abrupt fast or slow heating. Uniform heating rate alone is not enough for obtaining good resolution of glow curves. To obtain well separated glow peaks, which is required for TL studies, the rate of heating should be sufficiently slow. Fast heating, though linear mix up adjoining glow peaks. Consequently peaks appear as 'spikes' (Arnold and Azzi, 1971). We believe this is due to inertia of the recording system. If the inertia of the system is large and does not match with the signal then adjoining peaks mix up. On the other hand very slow heating would lead to better resolution of glow peaks. However, too slow heating will lead to fading of TL and consequently loss of TL and some glow peaks appearing near to liquid nitrogen temperature. In addition, it will be time consuming. An optimum rate of heating is therefore required for obtaining well resolved glow peaks. In our set up a heating rate of
10 K/min has given a good resolution of TL bands. This has enabled to make accurate measurements of the glow peak temperature.

(b) With a good cryostat it should be possible to hold the temperature of the sample stage at any desired level. This is necessary when it is required to study the quality of TL emission which requires the sample to be held at the desired temperature for several minutes. The sample in this case is held at a temperature slightly lower than glow peak temperature and the TL emission spectrum is recorded with a monochromator if emission is strong enough. In case of weak emission, interference filters are used for obtaining the spectrum. For those compounds with more than one glow peak, TL emission is recorded one after another by holding the sample near peak temperature of each TL band, sequentially from the lowest glow peak up. This facility has an additional advantage of studying the decay kinetics of TL bands at peak temperature.

(c) The TL set up should be sensitive enough to record the weakest possible signal. Ability of the equipment to record signals of low intensity depends upon a number of factors such as those mentioned below. (1) Suitable photomultiplier: This needs prior information about the quality of TL emission. Photomultiplier with S-I spectral response is suitable for studying TL emission in the far red region. Most of the
biochemicals show TL emission in ultraviolet and visible region. This could be studied with RCA-IP28 (spectral response S-13) or EMI 9558QB (spectral response S-20 extended) photomultiplier. The EMI tube has additional advantage of being also sensitive in the red region. Therefore this photomultiplier is most suitable for studying TL emission of chlorophylls which is predominantly in the red region. (2) Suitable distance between the sample and the photomultiplier: This distance should be as small as possible so as to enable the photomultiplier to collect most of the light emitted from the sample. Slight increase in the distance reduces TL yield drastically. This is expected since the solid angle made by the sample with the photomultiplier is decreased. However, keeping the photomultiplier tube very close to the sample is not advisable specially when working at low temperature, because of frosting of the window of the tube. All these factors are to be considered while deciding the distance between the sample and the photomultiplier.

(d) It should be possible to transfer the sample to the stage of cryostat quickly and conveniently. This is necessary particularly with those compounds having fast decaying luminescence at liquid nitrogen temperature. Generally the glow peaks appearing near 77 K decay fast. In order to record these glow peaks, the time interval between the end of irradiation and the readout should be as small as possible. With longer duration,
these peaks fade away. As a result many a time these peaks are not observed in the glow curve profile. Another facility that a good cryostat should have is that after warm up the sample stage should attain 77 K in a short time for the next readout. With this arrangement the wastage of time between successive readouts is reduced so that more glow curves could be recorded.

A cryostat satisfying most of these requirements was designed and fabricated by us. This is described below.

The cryostat

II.1.3. Construction:

The cryostat (Fig. 3) consists of a cylindrical stainless steel twin container assembly. The container with higher capacity (2) (14 cm dia, 17 cm ht) is connected to the container of lower capacity (8 cm dia, 5 cm ht) with a stainless steel tube. A stainless steel lid (3) is provided to the bigger container which serves as a storage tank for liquid nitrogen. The two containers are joined with another stainless steel pipe (4) (1 cm dia, 15 cm length). The entire assembly is thermally insulated with 6 cm thick polystyrene foam (1) with smooth surface and finer grains and then encased in an aluminium cabinet.

Two copper blocks (0.8 x 0.8 x 2.6 cm) 2.7 cm apart are welded on the top surface of the smaller container. A
Fig. 3. Schematic diagram of the cryostat (not to scale) and associated equipment.
copper plate (0.3 x 4.5 x 2.5 cm) serving as a sample stage is firmly screwed to the copper blocks. At the centre of the copper plate a well (1.4 cm dia, 0.1 cm depth) is carved to accommodate the sample pellet. Whenever it is desired to study samples of different sizes the plate is replaced with another having a well of suitable dimension. A fine hole (0.1 cm dia, 0.5 cm long) is drilled to the side of the copper plate for inserting the thermocouple junction (9-9'). The output from the thermocouple is fed to one pen of a three pen Nikadenki recorder (10).

II.1.4. Sample heater:

A nichrome ribbon (21 cm long, 0.05 mm thick, 0.6 mm width) is wound on a mica sheet (4 x 2.5 cm) serving as a heater element (5). It is electrically insulated on either side with additional mica sheets. This is then placed beneath the copper plate and firmly screwed to the copper blocks.

The input to the heater is given from a step-down transformer (6) delivering at full power 4 Amps of current at 16 Volts. Different heating rates are obtained by varying the input energy to the heater with a motor controlled variable transformer system (7) described later in detail.

The photomultiplier housing (11) described later is hinged to the bakelite plate (8) and thus thermally insulated from the aluminium cabinet. Output from photomultiplier is
amplified with an electrometer amplifier (12) (BA 812, Electronics Corporation of India, Limited) and fed to the recorder (10).

Quantitative measurement of the luminescence is simultaneously carried out by feeding the output from the amplifier to a voltage-to-frequency converter (Hewlett-Packard model 2211 BR Dymec) and scaler (Electronics Corporation of India Limited model DS 326) not shown in the diagram.

II.1.5. Motor controlled variable transformer system

The system (Fig. 4) consists of two variable transformers (variacs), a DC motor and two rectifiers. Output from the variac A is rectified before feeding to the motor. Input energy to the motor is thus controlled by the variac A. By setting the variac at precalibrated position any desired energy can be given to the motor. The variac B is driven by the motor. Depending upon the power supplied to the motor, the variac B is driven at different rates. In this system it takes from 30 seconds to 30 minutes to complete one rotation scanning through 0 to 230 V. When variac B attains full voltage a microswitch operates switching on the red lamp and disconnecting the motor. The continuity of motor circuit is restored by manually operating a reset switch shown in the circuit diagram (Fig. 4). The variac B then completes the rotation with the same speed, and returns to its starting point. To obtain desired rates of heating output from the variac B is fed to the primary of the heater transformer described earlier.
Fig. 4. Circuit diagram of motor controlled variable transformer system.
II.1.6. **Operational characteristics of heater element**

The cryostat is capable of warming the sample from 77 to 340 K. Iron-constantan thermocouple is used to record the temperature of the sample. It has an advantage over other thermocouples. Due to large thermo-electromotive force (emf) developed at the junction accurate estimate of glow peak temperature is possible. However, due to rusting of iron, the thermocouple junction breaks often causing inconvenience. We have been using iron-constantan thermocouple for quite sometime. Recently we switched over to chromel-alumel thermocouple to avoid the difficulties that arise due to breaking of the junction.

With motor controlled transformer system it was possible to heat the sample at a predetermined rate over the temperature range of 77 to 300 K. Some of the heating rates obtained by changing sweeping rate of variac B are given in the Fig. 5. The curves presented in the figure indicate that the rates of heating are not uniform throughout the temperature range. Nevertheless they appeared quite linear in the initial part of the curves particularly when the heating rate is slow (Fig. 5 curve E). As stated earlier, in our experiments heating rate of 10 K per minute gives best glow curves. It is observed that the initial part of the curve for this rate of heating is linear over quite large range of temperature (Fig. 5
Fig. 5. Performance of the cryostat at different heater input sweep rates (V s\(^{-1}\)). The heater input sweep rate refers to the rate at which the voltage input to the primary of the heater transformer increases.
curve E) in which most of the glow peaks of biochemicals appear.

In recent years using a temperature controller and programmer any desired linear heatings are obtained over the entire temperature range of 77 to 340 K.

II.1.7. Holding of sample at steady temperature:

Sample stage would reach steady state only when there is thermal equilibrium. In such a case the rate of heating of the sample stage is compensated by the rate of cooling by the liquid nitrogen. This is achieved in the following manner.

The stainless steel cryostat is filled to capacity with liquid nitrogen. A desired level of power is supplied to the primary of the heater transformer directly from a variac. After an initial fast rise of about 5 to 10 minutes, there is a gradual increase in temperature for 10-15 minutes before the sample stage finally attains a steady temperature appropriate with the level of power supplied. After applying the power to the heater, it takes about 25 to 30 minutes to reach such steady temperature. Once the steady state is reached the temperature is maintained for several hours so long as there is liquid nitrogen in the cryostat. Various steady temperatures in the temperature range of 77 to 250 K can be obtained by applying different voltages to the primary of the heater transformer (Fig. 6). A graph of voltages applied to the heater transformer against steady temperatures attained by the sample stage is shown in the Fig. 7.
Fig. 6. Performance of the cryostat at steady input power applied to the primary of the heater transformer.

Fig. 7. Steady temperature attained by the cryostat thirty minutes after the application of a constant voltage to the primary.
It is interesting to observe that a linear relationship is obtained between them. This enables one to calculate the voltage to be applied to heater transformer to obtain a desired steady temperature.

II.1.8. **Operation of the cryostat:**

A little over a litre of liquid nitrogen is generally consumed before the cryostat cools and stabilises at 77 K. By filling the reservoir to its full capacity with about three litres of liquid nitrogen, it is possible to use the system for about four hours without any need of refilling. This enables one to record glow curves of up to 30 samples/pellets within this time depending on the width and peak temperatures of the glow curves.

II.1.9. **Photomultiplier housing:**

It is essential that the photomultiplier housing should be perfectly light proof. Backelte is chosen for housing as it is sturdy and does not get spoiled whenever it came in contact with water. It is a reasonably good thermal insulator also. The photomultiplier housing consisted of a rectangular backelte box (12 x 12 x 20 cm) open at the top and bottom. On a separate backelte base plate (22 x 25 cm) a 6 mm deep groove is carved to the size of rectangular box so as to accommodate the bottom. The rectangular box with its bottom
sitting into the groove of the base plate, prevents light from outside to enter into the box when its top is closed. The photomultiplier housing assembly is hinged to the baseplate which is provided with a circular hole (8.6 cm dia) to accommodate the sample stage. A thick aluminium sheet (12 x 12 cm) covered the top of the rectangular box. A circular hole (8.6 cm dia) is made at the centre of the plate and provided with threads. The hole is covered with a metal disc with matching threads. A thin annular metal ring is screwed with the disc. The photomultiplier socket is screwed to the metal ring. Spacers are used between the disc and the ring to change the distance between sample and photomultiplier whenever desired.

II.1.10. The cold finger:

Thermoluminescence yield of most of the biochemicals is normally low. Some of the compounds have a number of TL bands with different emission maxima. The TL emission set up should be sensitive enough to record the weakest TL emission. In addition it should have the facility of holding the temperature of the sample at desired level. Special efforts are made to fabricate a cold finger possessing all the facilities mentioned above. This is described below.

A thick copper strip (6 mm x 2.5 cm x 15 cm) bent twice at right angles (2) is used to fabricate the cold finger.
A T-shaped slot (1) is made at one end of the strip to accommodate the sample pellet. A rectangular (4.8 x 5.8 x 18 cm) non-porous thermocole block (3) is carved to the desired shape so as to provide a cavity (4) and accommodate part of the copper strip. During the experiment the cavity is filled with liquid nitrogen cooling the copper strip to 77 K. A narrow hole is drilled at the side of T-shaped slot to insert one junction of chromel-alumel thermocouple to record the sample temperature. A nichrome ribbon (15 cm long, 0.05 mm thick, 0.6 mm width) is wound on a mica sheet serving as the heater element. After insulating the heater element on either side electrically with additional mica sheets, it is firmly screwed at the back side of T-shaped slot.

Method similar to that described earlier (II.1.7) is followed to hold sample at any desired temperature. Once the steady temperature is reached it is possible to maintain sample at that temperature for a couple of minutes. This, then enables one to record the TL emission spectrum normally at temperature slightly lower than glow peak temperature. During recording of the emission spectrum, it is necessary that the intensity remains constant. To some extent this is achieved by holding the sample at a temperature slightly below the glow peak temperature.
Fig. 9. Top and side view of the cold finger (not to scale).
Due to small size of the cold finger, it can be handled conveniently during experiment. Normally it is adjusted such that the sample is close to the slit of the monochromator so that most of the light emitted by it is passed through the monochromator. Emission spectra of TL of the compounds are recorded as described later in the Chapter.

SAMPLE PREPARATION

II.2.1. Pellet making:

Dry amorphous powders of analytical grade chemicals obtained from different make (Table I) is used for making pellets of the compound. Powder weighing 100 mg is pressed under vacuum into standard size pellets (1.3 cm dia, 0.1 cm thick). Pressure applied for making pellet under vacuum is about 13 tons/cm² and the duration for which the pressure is maintained is about two minutes.

II.2.2. Sources of irradiation:

Cobalt-60 gamma ray source is used for irradiating samples at liquid nitrogen temperature. Sample pellets are immersed in liquid nitrogen. Dewar containing sample pellets is filled with liquid nitrogen and irradiated with gamma rays. The dose delivered to the sample was from few rads to several
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Material</th>
<th>Make</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adenine</td>
<td>Mann Research Laboratories</td>
</tr>
<tr>
<td>2</td>
<td>Adenosine</td>
<td>Koch-Light Laboratories Ltd.</td>
</tr>
<tr>
<td>3</td>
<td>Cytosine</td>
<td>Mann Research Laboratories</td>
</tr>
<tr>
<td>4</td>
<td>Cytidine</td>
<td>&quot;</td>
</tr>
<tr>
<td>5</td>
<td>Guanine</td>
<td>&quot;</td>
</tr>
<tr>
<td>6</td>
<td>6-Aza guanine</td>
<td>Sigma Chemical Company</td>
</tr>
<tr>
<td>7</td>
<td>Hypoxanthine</td>
<td>Mann Research Laboratories</td>
</tr>
<tr>
<td>8</td>
<td>Thymine</td>
<td>Koch-Light Laboratories Ltd.</td>
</tr>
<tr>
<td>9</td>
<td>Uracil</td>
<td>Sigma Chemical Company</td>
</tr>
<tr>
<td>10</td>
<td>6-Azauracil</td>
<td>&quot;</td>
</tr>
<tr>
<td>11</td>
<td>5-bromouracil</td>
<td>&quot;</td>
</tr>
<tr>
<td>12</td>
<td>1,3-dimethyluracil</td>
<td>Koch-Light Laboratories Ltd.</td>
</tr>
<tr>
<td>13</td>
<td>Xanthine</td>
<td>Mann Research Laboratories</td>
</tr>
</tbody>
</table>
kilorads depending upon the experimental requirement. Using led blocks of different thickness intensity of gamma-rays was reduced to half, one third and one fourth of its original value when required. This is described in Chapter III.

Xenon arc lamp (XBO-150W, operating on stabilized DC source 22V and 7 Amps) of Aminco-Bowman Spectrophotofluorometer is used to irradiate sample pellets at 77 K with ultraviolet light or to expose to visible light. The light from the lamp is passed through the exciting monochromator of the spectrophotofluorometer. Sample pellet cut to the size (8 mm x 10 mm) of sample holder of quartz dewar is irradiated at 77 K with desired wavelength from excitation monochromator. Intensity of excitation is cut down to the required value by introducing suitable neutral density filters. Tungsten lamp (200W or 500W) working on mains is used to photobleach the gamma-ray irradiated pellets at liquid nitrogen temperature. It is also used to freeze chlorophylls at 77 K in the presence of white light and for subsequent illumination.

II.2.3. Recording of glow curves:

The sample pellet irradiated with ultraviolet light or gamma-rays at 77 K is placed on the precooled sample stage of the cryostat and warmed in dark at a slow rate of about 10 K/min. Luminescence emitted by the sample during the warm up
is collected by the photomultiplier (IP 28 or 9558 QB) placed close to the sample. Output from the photomultiplier is amplified with electrometer amplifier and fed to one pen of three pen recorder. Simultaneously output from the thermocouple is fed to another pen of the recorder. Speed of the recorder chart drive and gain of the amplifier are adjusted to obtain well resolved good glow curves. Time interval between irradiation and readout is about 15 minutes.

II.2.4. Recording of emission spectra of TL:

Special efforts are made to record emission spectra of TL of biochemicals. Due to small size (4.8 x 5.8 x 18 cm) of cold finger it is possible to adjust its position conveniently even in the limited space available in front of monochromator of spectrophotofluorometer. The cavity of cold finger is initially filled with liquid nitrogen. Sample pellet irradiated with gamma-rays is inserted into the slot made to the copper strip of cold finger. The sample is warmed and then maintained at a temperature slightly lower than glow peak temperature. Position of the cold finger is adjusted such that maximum light emitted by the sample pellet illuminated the monochromator entrance slit of spectrophotofluorometer. Output from the monochromator is collected by IP 28 or R 446 photomultiplier. The signal from the photomultiplier is amplified and fed to Y axis of the X-Y recorder. To obtain emission spectrum input proportional to
the wavelength is fed to the X axis. With repeated slow scanning, a shift in emission peak if any is confirmed. With those compounds having more than one glow peak, TL emission spectra for each glow peak are recorded by holding the sample slightly below glow peak temperature, starting from the lowest temperature glow peak.