CHAPTER I

INTRODUCTION
1.1. The thermoluminescence phenomena:

Thermoluminescence (TL) is defined as thermally stimulated release of stored energy in the form of visible light (sometimes accompanied by ultraviolet and infrared) of previously excited material. The substance is observed to emit light on heating to a temperature below that required for incandescence. It is a physical phenomenon releasing stored energy and not due to any chemical change in molecules. The luminescence usually lies in the spectral region where the material is non-absorbing, whereas the incandescence emissivity is strongest where absorptivity of the material is also maximum. There is also a wide gap in the temperature at which these two emissions occur. Thermoluminescence involves absorption of energy by the substance from external source such as ionizing radiation, ultraviolet light (UV) and sometimes also due to mechanical pressure, storage of the absorbed energy in the material for long periods and finally its re-emission as luminescence on warm-up. It differs from other luminescence phenomena in the sense that the re-emission is thermally stimulated. Normally, the luminescence is categorised by its causative agent. It is therefore common to speak of Photo-, Cathode-, Tribe-, Chemi-, or Bio-luminescence which means luminescence excited by photons, cathode rays, mechanical pressure, chemical energy and biological processes respectively.
In this context the word "thermoluminescence" is misleading as it means luminescence excited by heat. On the contrary it is the thermal stimulation given to material which causes the luminescence, the excitation being caused previously by one of the processes mentioned above. It would therefore be appropriate to use the phrase "thermally stimulated luminescence" to denote this phenomenon. Nevertheless, the term "thermoluminescence" is in general use and it is therefore decided to continue to use it in the present thesis.

Thermoluminescence is exhibited by large number of minerals, inorganic crystals and soils (Pringsheim and Vogel, 1943; Garlick, 1949; Leverenz, 1950; Hill and Schwed, 1955; Bonfiglioli et al., 1959; Curie, 1960; Modougall, 1968; Nishita and Hamilton, 1968; 1970; Nambi, 1977). It is also observed with organic compounds, polymers and biochemicals (Augenstein et al., 1960, 1961, 1964, 1967; Carter et al., 1960, 1961; Weinberg et al., 1962; Lehman and Wallace, 1964; Soldatsev and Kukushkin, 1967; Thomas et al., 1968; Partridge, 1972; Augenstein and Williams, 1973; Tatsaka, 1975). In recent years TL is shown by biological materials including seeds (Arnold and Sherwood, 1957; Cervigni et al., 1966; Rubin and Venedictov, 1969; Sanadi and Facker, 1973; Govindjee, 1975; Ishikawa et al., 1975; Sane, 1975; Yamaguchi et al., 1975; Inoue et al., 1976). During the last two decades the phenomenon
is studied extensively. In spite of previous studies, the mechanism of TL is still not fully understood. The TL is a highly complicated phenomenon and needs further extensive systematic investigation for its understanding. It is normally studied by irradiating material with ionising radiation, preferably at low temperatures, and then recording light emitted by the sample during subsequent warming. The record of intensity of luminescence as a function of temperature is called a "glow curve". The curve so recorded may show one or more peaks over the temperature range of investigation. In a compound with a number of glow peaks, also called TL bands, overlap of the bands is observed many a time. The shape and overlapping of the glow peaks depend on many factors. Amongst them, the rate of heating is one of the major factors. The spectral quality of luminescence and the temperatures at which glow peaks appear are characteristic for the material. In addition they are influenced by the defects in the crystal structure, chemical composition of the material, impurities present in the compound and previous history of the material (Augenstein et al., 1961; Solntsev and Kukushkin, 1967; Jelinek and Pospisil, 1969, 1973; Nummedal and Steen, 1969; Knappe et al., 1972; Nambi, 1977).

The technique employed for investigating the phenomenon of TL is a versatile one and has been employed for
(a) The mechanism of energy absorption, storage and re-emission
(Randall and Wilkins, 1945; Augenstein et al., 1960; 1967;
McDougall, 1968);
(b) Post-irradiation radiation effects such as intermolecular
energy transfer (Dexter, 1953; 1972; Tataka and Gopal-
Ayengar, 1971; Feofilov, 1972; Piquett, 1973; Solntsev et al.,
1973; Tataka, 1975);
(c) Locating and studying molecular motion (Partridge, 1965;
Ranicar and Fleming, 1972);
(d) Structural defects such as imperfections in the crystal
lattice namely vacancies, dislocations, impurities etc.
(Solntsev and Kukushkin, 1967; McDougall, 1968; Knappe et al., 1972);
(e) Charge diffusion and trapping (Nikol'skii and Buben, 1960);
(f) Role of impurities in materials (Augenstein et al., 1960;
Solntsev and Kukushkin, 1967; Altekar et al., 1975);
(g) Physical and chemical structures of the compounds
(Augenstein et al., 1960; Singh and Charlesby, 1966);
(h) Electron transport in photosynthetic material and molecular
interactions (Arnold and Sherwood, 1957; Shuvalov and Litvin,
1969; Arnold and Azzl, 1971; War and Govindjee, 1971;
Jursinic and Govindjee, 1972; Fleischman and Wayne, 1973;
Malkin and Harf, 1973; Lurie and Bertsch, 1974; Ichikawa
et al., 1976; Sane, 1975; Inoue et al., 1976). In this thesis
an attempt is made to study some of these post-irradiation
phenomena in biomolecules by the method of TL.
1.2. Cell mass:

The dry mass of cells of organisms primarily consists of several organic molecules. Most important amongst them are carbohydrates, proteins, fats and nucleic acids. A great deal of work has been done to understand the phenomenon of TL in inorganic materials (Hill and Schwed, 1955; Bonfiglioli et al., 1959; McDougall, 1968). In comparison organic compounds and biomolecules which possess more complex structures have not attracted the same degree of interest. Among the important biomolecules that have been studied are nucleic acids and their constituents, proteins, amino acids, polymers, pigments and cell membrane. In these investigations very little attention has been paid to understand the nature of electron traps, intermolecular energy transfer and TL emission and excitation mechanisms.

Among the well known biological materials, nucleic acids play very important role as carriers of genetic information. We have therefore chosen nucleic acid constituents for TL study.

There are two types of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). There are in all five bases commonly observed in nucleic acids. They are heterocyclic ring compounds containing nitrogen. The bases are adenine, guanine, cytosine, thymine and uracil. The first two compounds
are purines, the rest of them are pyrimidines. Individuality of the bases is known by the chemical groups attached in the C₂, C₆ and N₇ positions (Fig. 1). They are either amino or keto groups. The bases that are commonly present in RNA are adenine, uracil, guanine and cytosine. In DNA uracil is replaced by thymine.

A compound with a base and pentose sugar but without the phosphate group is called nucleoside.

Nucleotide has three constituents. They are: (1) a base, (2) a five carbon sugar or pentose and (3) a phosphate group. In ribonucleotide the sugar is ribose. In the deoxyribonucleotide the sugar is deoxyribose which differs from ribose in that the hydroxyl of latter is replaced by a hydrogen (Fig. 2). There are four nucleotides that are commonly observed for each of the two types of nucleic acids.

Among the biochemicals that have interested workers in the field of TL are polymers, proteins, amino acids, nucleic acids and their constituents. Ultraviolet (UV) light, X-rays, gamma rays from cobalt-60 and fast electrons from accelerators were used as sources of irradiation. Experiments were carried out at 77 K and liquid helium temperature (Riehl and Thoma, 1966; Aulov et al., 1968) with samples in dry amorphous forms and in glassy matrix under various pressures of different gases.
Fig. 1. Chemical structure of nucleic acid bases.
A DNA nucleotide
(deoxyadenosine-3'-phosphate)

An RNA nucleotide
(adenosine-3'-phosphate)

Fig. 2. Chemical structure of ribonucleotide and deoxyribonucleotide.
The TL characteristics of a compound are represented by parameters such as activation energy or trap depth, frequency factor, order of decay kinetics. Knowledge of the nature of these parameters, the effect of gases on the glow curves, photobleaching of TL, intermolecular energy transfer and TL excitation and emission studies is necessary for understanding the phenomenon of TL. In the following section a survey of work carried out to study these aspects is presented.

I.3. Trapping centres:

Excellent work related with the factors determining the TL properties of amino acids was reported by Augenstein and his group (Augenstein et al., 1960; 1961; Carter et al., 1961; Weinberg et al., 1962; Augenstein and William, 1973). They carried out extensive work on amino acids and proteins in dry form. Ultraviolet (UV) light and gamma rays were employed as sources of irradiation. An attempt was made to answer some of the basic questions such as, (1) is TL from crystals composed of relatively complex molecules dependent upon the chemical composition of the individual molecules or upon the structure of the crystals?, (2) do the radiation displaced charge carriers responsible for TL become localized at random in protein?
Some of their important findings are: (1) Amino acids having ring structure exhibit TL intensity about three orders of magnitude greater than those which do not possess such structure; (2) In hydrogen bonded crystals chemical structure is more important in determining the nature of the TL than intermolecular arrangements; (3) Glow curves obtained from proteins are not the sum of contributions from the constituent amino acids but seem to be characteristics of proteins.

Supporting evidence came later from the studies of DNA. Soltsev and Kulushkin (1967) observed that the TL intensity of DNA was substantially less than the sum of the intensities of the components. The glow peak appeared at a temperature different from those obtained with the constituents.

Alkali-halide crystals with simple structure showed numerous peaks in glow curve profile (Bonfiglioli et al., 1959; Fieschi and Scaramelli, 1966). Macromolecules have numerous inhomogeneities in charge distribution which were expected to provide potential sites for trapping displaced electrons or binding holes, in addition to those sites associated with lattice imperfections. In contrast proteins with complex structure showed simple glow curves. Singh and Charlesby (1966) observed simple glow curves from complex molecules with maximum light coming from the bases. Addition of sugar and sugar phosphate to the bases decreased the intensity of light.
studies provide further evidence that radiation induced electronic rearrangements become localized not at random but only at limited number of sites.

In the case of very simple crystals TL is determined primarily by the structure of the crystal. In crystals made of molecules of complex structures trapping and binding sites might be expected to depend not only upon lattice imperfection but also upon the chemical make up of the constituent molecules (see Weinberg et al., 1962; Crittenden, 1974). Hence TL should depend on both chemical composition and crystal structure. To some extent this was found to be true. Charlesby and Partridge (1963) observed glow curves of polyethylene less dependent on type of ionizing radiation than its crystallinity. In contrast to this Jelinek and Pospisil (1969) reported that TL peak depends on number of factors such as method of preparation of the sample, the mode of excitation, number of excitations, rate of heating etc. Fairly close relation was established by them between TL peak and rate of evaporation of water from solution of sample prepared by evaporating water at room temperature or at 70°C. They attributed this to the properties of crystalline structure as a whole rather than the structure of the molecules forming the crystal.
I.4. **Role of impurities in TL:**

The role that the impurities play in TL emission has been delineated by number of workers. Augenstein *et al.* (1960, 1961) observed that impurities serve as activation sites. With increasing concentration of impurities such as Cu⁺, Fe⁺⁺⁺ and KI reduction in the TL intensity of amino acids and nucleic acid bases was observed (Kukushkin and Kuznetsov, 1966; Solntsev and Kukushkin, 1967; Altekar *et al.*, 1975). Further, they noticed that KI not only reduce the intensity of TL but also shifted the glow peak temperature. More recently, Altekar *et al.* (1975) reported quenching of TL of aqueous solution of bovine serum albumin (BSA) in the presence of salts such as KI and KSCN. The extent of quenching was shown to be a function of the concentrations of the interacting molecules. They attributed quenching of TL to interaction of salts with aromatic amino acid residues in the proteins and resultant changes in protein conformation.

I.5. **Dependence of TL on the source of irradiation:**

Nummedal and Steen (1969) investigated glow curves of trypsin, trypsinogen, ribonuclease and tryptophan in the form of a film as well as the ethylene-glycol-water glass irradiated with X-rays at 77 K. Kukushkin and Kuznetsov (1966) noticed that the TL curves of tyrosine, tryptophan, phenylalanine
and serum albumin do not differ significantly whereas in the powder form tyrosine and tryptophan gave TL curves differing in form. Solntsev and Kukushkin (1967) observed little difference in TL yield of nucleic acid bases irradiated with UV as against large difference reported by Singh and Charlesby (1966) when irradiated with gamma-rays. However, no shift in glow peak temperature was noticed. Charlesby and Partridge (1965) compared TL of polyethylene induced by UV at low temperature with that obtained with gamma-rays and noticed considerable similarity between them.

I.6. Activation energies:

Activation energies associated with the glow peaks of amino acids and proteins have occupied the attention of many investigators. Different methods were employed for calculating these values. Augenstein et al. (1961) obtained activation energies of proteins to be less than 0.1 ev as compared to 0.1 to 0.45 ev reported to amino acids. On the basis of a monomolecular model Gill and Weissbluth (1964) computed smaller activation energies for proteins. Caserta et al. (1969) felt that the low values of frequency factors obtained for the glow curves of some plant seeds cannot be attached the same significance as in Randall-Wilkin theory (1945). Activation energies for protein and amino acids have been computed also by others (see Weinberg et al., 1962;
Kulushkin and Kuznetsov, 1966; Thomas et al., 1968). Charlesby and Partridge (1972) reported activation energies for different glow peaks of polyethylene. Jelinek and Pospisil (1969) calculated activation energies of nucleic acid bases. In spite of number of reports on activation energies, the significance of these values and their role in TL is not yet clear.

1.7. Effect of gases on TL:

Effect of dissolved gases and those present during recording was studied by many workers. Partridge (1972) had shown that dissolved oxygen had a dramatic effect on the glow curves of polyethylene. It eliminated two glow peaks designated as $\beta$ and $\gamma$ from the four glow peaks $\alpha_1$, $\alpha_2$, $\beta$ and $\gamma$ observed in oxygen-free polyethylene. In addition, a new peak was produced at 145°K designated as $\epsilon$. Two alternative mechanisms were proposed to explain production of $\epsilon$ peak. Oxygen acts as an efficient electron trap in one mechanism (Charlesby and Partridge, 1965a). According to second mechanism (Nikol'skii et al., 1964), the mobile oxygen at higher temperature, combines with electron traps assigned to alkyl radicals and in doing so releases trapped electrons. The support to this hypothesis came from the observation that peak appears at the temperature when $O_2$ starts becoming mobile and combines with alkyl radicals to form peroxide radicals. Further they observed that maximum peroxide formation coincides
with peak temperature of the glow curve. Argument going against this mechanism is that irradiated sample containing O$_2$ and kept at 77 K for several days showed very little change in the intensity of $\xi$ peak, whereas $\kappa$ and $\gamma$ peaks decayed by a factor of three (see Partridge, 1964) suggesting the presence of a new and "deep" electron trap, presumably O$_2$ itself which can effectively capture electrons at 77 K. Appearance of $\xi$ peak in the region of O$_2$ mobility suggested that the reaction site could be alkyl radical and the electron is released during formation of peroxide radical (see Nikolskii et al., 1964) except that initial trapping is on O$_2$ rather than an alkyl radical.

Complete quenching of $\beta$ and $\gamma$ peaks by O$_2$ was explained by proposing mobile O$_2$ molecules capturing electrons from other traps and delivering them to the various cations and that the instantaneous complex formed during this exchange promoted radiolysisless dissipation of the energy.

Reschukhin (1966) observed decrease in the intensity of TL in amino acids by two orders of magnitude when irradiated in vacuum than in air. However, drastic effects such as those observed in polyethylene are not observed with amino acids and nucleic acid bases. Thermoluminescent characteristics were similar when argon was used in place of air indicating that the formation of the solvated electron and cation radical was not dependent on the presence of O$_2$. 
I.8. ITL and TL decay kinetic studies:

Biochemicals irradiated at 77 K with ultraviolet light or ionizing radiation emit luminescence even at liquid nitrogen temperature for several hours. This luminescence observed by others and called "afterglow" is referred to as isothermal luminescence (ITL). The reason for calling it ITL is given in Chapter IV. Post-irradiation long afterglow of adenine in solid state was studied by Jelinek and Tals (1975). Hyperbolic decay law of afterglow was interpreted as due to tunneling recombination luminescence between pairs of localized charge species. It was assumed that both tunneling and temperature dependent processes occur at the same trapping sites which were believed to be structural defects. In contrast to this Moan and Steen (1971) proposed that afterglow was associated with a small fraction of the trapped electrons which were fairly close to the cations and were guided by the coulomb field of the cation for their recombination. From the studies of aromatic hydrocarbon dissolved in boric acid glass at 77 K, Robert and Jacques (1968) reported two types of traps for the electrons. Kulushkin and Kuznetsov (1966) analysed kinetics of TL of amino acids and proteins. They observed that the glow curves from the amino acids can be fit with curves derived on the basis of a monomolecular model in which the molecules absorb, hold and remit energy independently. However, they noticed that TL curves from proteins did not follow monomolecular kinetics.
It was felt that these studies are not sufficient to establish a relationship between afterglow (ITL) and TL. A detailed qualitative and quantitative study of both afterglow and TL is necessary for arriving at a conclusion.

I.9. **TL emission studies**

Thermoluminescence emission spectra of trypsin and trypsinogen both in dry state and in glassy solution were reported (Nummedal and Steen, 1969). Using sensitive spectrographs (Weinberg *et al.*, 1962; Frydz and Rogeberg, 1964; Weissbluth *et al.*, 1965; Moan and Steen, 1971; Hola *et al.*, 1973; Altekar *et al.*, 1975) reported a detailed study of TL emission of tyrosine, tryptophan, phenylalanine and other compounds in solution as well as in solid form. Skvortsov *et al.* (1975) reported TL emission spectrum of DNA consisting of two bands appearing at 410 and 470 nm corresponding to phosphorescence emission peaks of thymine and adenine respectively. Fleming and Kerr (1965) and Singh and Charlesby (1966) observed that the TL emission spectra of nucleic acid bases were identical to the phosphorescence spectra of the compounds. This led these workers to believe in the identity of TL emission with phosphorescence. Yet, Charlesby and Partridge (1965) reported for polyethylene two broad TL emission bands around 350 and 470 nm corresponding to fluorescence and phosphorescence of the compound.
It is noticed that in some cases recording of TL emission spectra of the compounds was not reliable because of poor TL intensity. Special efforts are therefore made to record accurately the TL emission spectra of nucleic acid bases. In contrast to earlier reports, these results showed ultraviolet component in TL emission of pyrimidines. This is discussed in detail in Chapter III.

I.10. Photobleaching studies

Nikol'skil et al. (1963b) and Alfimov et al. (1964) reported reduction in TL yield of polyethylene irradiated with gamma-rays at liquid nitrogen temperature when exposed to visible light (photobleached) prior to heating in dark. Further, it was shown (Nikol'skil et al., 1963b; Partridge, 1970c) that photobleaching of TL simultaneously bleaches a broad absorption spectrum probably due to trapped charges. On the basis of these studies it was suggested that bulk of the thermal untrapping occurs from all types of traps at all temperatures and not just from shallow traps at low temperatures and deep ones at higher temperatures. It was concluded that untrapping is predominantly due to molecular chain motion and not due to thermal escape from fixed traps of different depth. Weissbluth et al. (1965) and Thomas et al. (1968) reported photobleaching studies of amino acids and polymers. Although number of reports have appeared on photobleaching it appears that no attempt was made to understand the mechanism of photobleaching.
I.11. TL and TSC studies

A good correlation is observed between TL and thermally stimulated current (TSC) (Ranicar and Fleming, 1972; Blake et al., 1974; Lawangar and Narlikar, 1975; Mathur, 1975; Yoshino et al., 1975). A sharp increase in TSC was correlated with TL peaks and were attributed to the release of electrons from traps. Ranicar and Fleming (1972) compared TSC and TL glow curves for number of polymers.

I.12. Mechanism of TL

Various models were proposed by different workers to explain the mechanism of TL. Prominent amongst them are

1. Biphotonic interaction leading to TL;
2. Electron-hole or Ion-electron recombination model;
3. Radical-radical interaction;
4. Weissbluth model.

It is evident from the following discussion that none of these models are capable of explaining all the results.

Evidence for the photoionisation of aromatic compounds in hydrocarbon glass at 77 K was reported by Moan and Steen (1971). It was observed that TL emission was very nearly proportional to the square of the intensity of photolyzing light. It was argued that under certain conditions photochemical changes may
result from the absorption of light by metastable excited triplet states in solution of aromatic compounds in rigid aliphatic hydrocarbon glasses. In aromatic amino acids some photochemical reactions proceed according to a biophotonic mechanism (Fesenko et al., 1967). The TL of these compounds was directly proportional to the square of the light intensity. Double beam method was used for this investigation. It is believed that following UV irradiation photo products are formed within 5-10 seconds after the start of irradiation. The intermediate product which absorbs the second photon accumulates slowly. The disappearance of metastable product absorbing second photon proceeds according to the first order equation. It is reported that the life span of intermediate product of tyrosine is 1.3 and tryptophan is 2.8 seconds. Photoionisation occurs as a result of the absorption of the second photon by the molecule in the triplet state. Equal life span of triplet state and photoproduct absorbing second quantum favours the theory that the triplet state is the intermediate product. Yet this is in contrast to the fact that the spectral range of photoionisation by second photon is 290-360 m/\mu while triplet-triplet transition is in the range 400-500 m/\mu (Fesenko et al., 1967). It is uncertain whether there is a triplet-triplet absorption by amino acids at 280-400 m/\mu. However, the possibility of absorption of second active photon by the radical-electron complex cannot be
excluded. It is believed that the ionisation occurs exclusively by triplet absorption. From the study of tryptophan in ethylene-glycol-water glass both afterglow and TL are attributed to recombination between tryptophan cations and electrons formed by ionisation of tryptophan by triplet absorption (Nolen and Steen, 1971). The spectra of TL and afterglow are observed to be identical with phosphorescence spectrum of tryptophan demonstrating that the only radiative transition of both processes is \[ T \rightarrow S_0 \] transition. Supporting evidence came from the studies of TL on UV irradiated purines and pyrimidines in ethylene-glycol-water matrices. Dependence of TL on the square of excitation intensity was observed (Charlesby and Partridge, 1965; Gibbons et al., 1965; Brynda, 1971). It is noticed that the lifetime of the intermediate absorbing the second photon is the same as the phosphorescence lifetime (Fesenko et al., 1967). Emission spectrum of TL was in the same wavelength region as phosphorescence spectrum. Square law relationship of TL with excitation intensity was also reported with solutions (Charlesby and Partridge, 1965; Gibbons et al., 1965; Brynda, 1971). However these results are not observed when dry powders are used (see Weissbluth et al., 1965). What is responsible for the difference observed in dry powders and solutions is yet to be explained. This aspect is dealt with in Chapter III.

Shioda et al. (1963) measured intensity of TL glow and the concentration of surviving free radicals at different
temperatures of the sample. Except for $\gamma$ peak, the other peaks of polyethylene did not show correlation of TL with disappearance of free radicals. Though remarkable dependence of $\gamma$ peak is observed with disappearance of free radicals they reported failure to understand early saturation of the TL peak at much lower doses as against saturation of free radicals at higher doses (Alfimov et al., 1964; Nikol'skii et al., 1969) and noticed visible light exposure on the irradiated sample prior to warming reduces TL yield considerably (photobleaching) whereas this has no effect on the radical concentration. However, Crittenden (1971) felt that the light emission accompanying radical reactions cannot be ruled out.

In the models involving recombination of electron with hole or electron with ion, it is expected that the TL emission spectrum should contain fluorescence and phosphorescence of the compound. However this has not been observed. Purines show TL emission corresponding to phosphorescence alone. Weissbluth's model (1965) with electron traps located below the triplet state of the energy level diagram can explain TL emission corresponding to phosphorescence, but it cannot explain fluorescence component observed in TL emission of some class of compound. A detailed analysis of all the models is given in Chapter III. A new model is proposed which overcomes the limitations of other models and explains all the results satisfactorily.