Solid state approach in biophoton research

Francesco Musumeci*, Giuseppe Privitera, Agata Scordino, Maurizio Tedesco, Antonio Triglia & Salvo Tudisco
Dipartimento di Metodologie Fisiche e Chimiche per l’Ingegneria-Catania University- v.le Andrea Doria, 6 - 195125 Catania, Italy

Main characteristics of the delayed luminescence (DL) emitted in the seconds range from biological systems is analyzed. The correlation between change in DL and cell’s organization, and similarity with some characteristics of DL from solid state system suggest to connect DL in biological system to decay of collective electron states formed during energy and charge transport along the macromolecular ordered structures which form the cell. Results of a proposed soliton model are discussed, together with some phenomenological evidence which emphasize the possibility of using DL measurements as an intrinsic probe in biophysical investigations.

Keywords : Charge transport, Collective electron states, Delayed luminescence, Optical photon, Solid state system, Soliton, Ultraweak intensity.

Most of the biological and other materials emit, on being illuminated, optical photons characterized by an ultra-weak intensity and long duration. This low level emission is termed delayed luminescence (DL) and is something like $10^3$-$10^5$ times lower in intensity than fluorescence.

In biological system, for the first time the DL was observed as a totally unexpected light emission from green plants, seconds after illumination\(^1\). DL is typical not only of green plants, but has been measured in seeds, yeast cells and mammalian cells as well\(^2,3\).

Interest in studying such a phenomenon has been increased after a long experimental work that has established that DL is a sensitive indicator of the biological state of the emitting system. Moreover, in some cases it has been possible to express this connection through an analytical relationship between a biological parameter and one of DL\(^4,5\).

This fact has produced notable interest for the application potentialities of DL measurements in different sectors, as for example the control in real time of pollution in water, the checking of the quality of food, or clinical diagnosis. Nevertheless it is necessary to understand properly the mechanisms that underlie the phenomenon to obtain further applicative developments. Therefore, at first it is necessary to study the problem of its origins.

Biological cells contain nanoscale machineries that exhibit a wonderful combination of high efficiency, high adaptability and high reliability. Their molecules are hierarchically organized. The challenge for physicists is to achieve a description of the excitation transfer through application of quantum physics\(^10\).

From this point of view Fröhlich\(^11\) underlined that biological materials are extremely complex and complicated systems that, once they are activated, may be characterized by the following physical properties:

(i) relatively stable but far from equilibrium;
(ii) exhibit a non-trivial order, and
(iii) have extraordinary dielectric properties, considering that biological membranes sustain electrical fields of the order of $10^5$ V/cm.

These characteristic require that various excitations are stabilized, pointing to the excitation of metastable states, and also require the establishment of a motional order, whose generalization leads to coherence.

The idea of Fröhlich was that coherent polar vibrations are strongly, over-thermally excited during biological activity causing particular long-range interactions which lead to collective properties of the whole biological systems.

The experience shows that some general characteristics of DL, like the hyperbolic trend of the temporal decay, the non-linearity of the response with the intensity of the stimulating light and the behaviour of the emission spectrum components, are present in...
all the biological systems analyzed\textsuperscript{12}. In addition, similar characteristics have been evidenced from the analysis of DL emitted by some solid state systems. Thus, in order to study the analogy between the two systems, existing in cell’s organisation and the order of the solid state systems, a series of measurements of DL from biological and solid state samples has been made and described in the present study.

This communication is based on the idea that, due to the long time involvement, from measurements of DL, one may gather information about energy transfer pathways from more distant molecules, thus taking into account for long-range interactions along the biological ordered structure.

Materials and Methods
The experimental apparatus able to measure the low level decay dynamics has been previously described\textsuperscript{13,14}.

Experiments consisted illuminating the sample with light of different wavelengths and different durations, and measuring the time dependence of the number of photons re-emitted. A cooled low-noise photomultiplier, working in single photon counting mode, was used; its spectral sensitivity ranged from 200-850 nm. Samples to be analysed were placed in a light-tight chamber that can be maintained at constant temperature. The background emission was measured in the same experimental conditions for each set of measurements and subtracted from each sample measurement. As light sources LEDs, flash lamp and lasers were used.

The spectral analysis of the emitted photons were done performed by broadband filters (40 nm FWHM) ranging from 400-900 nm.

As a biological sample the unicellular alga, \textit{Acetabularia acetabulum} (L.) was used. Dr. Sigrid Berger of (MPI fuer Zellbiologie, Ladenburg) and Dr. G. Thiel (Pflanzenphysiologisches Institut der Universität Göttingen) kindly provided \textit{A. acetabulum} cultures. They were maintained in artificial sea water SIGMA Provasoli’s (ASP12) marine medium, with a D/L cycle of 12:12 hr at a light intensity of 8 W/m\textsuperscript{2} provided by Nathura Tropiclite lamps, in an incubator maintained at 20° ± 2°C.

Thiopental sodium (commercial name: pentothal sodium), supplied by Abbot was dissolved directly into artificial sea water according to the concentration used in the experiments (from 0.2-4 mM). Each solution, was tested using a different alga. In order to normalise data, relative to different algae, preliminarily for each sample, a reference measure in standard artificial sea water was performed. Freezing of \textit{A. acetabulum} cells was performed by placing a cell on a piece of metal, free of adherent fluid, and dipping it into liquid nitrogen for 10 sec. The cell was then thawed and re-incubated at 20°C. The procedure was completed within 1 min. This process in general does not alter the chloroplasts functionality; as a matter of fact it is used as a preliminary operation for their isolation in the study of the function of the photosynthetic chain. Nevertheless, it causes heavy damage to the cytoskeleton and the death of the algae\textsuperscript{15}.

For the experiments with cadmium sulphide (CdS) a powder manufactured by the Aldrich Chem. Co., with purity greater than 99.995% and sizes less than 5 mm was used. Latter powder was selected in size by suspending it in a suitable solution of water and acetone and then collecting the sediment at different times. For each sample so determined the average size of the powders was calculated by using the microscope observations of several regions of the sample. In such a way different samples of powders, characterised by the same chemical composition and with average sizes of the crystals varying from 400-1000 nm, were obtained. Then the seven samples used for the measurement were made putting the same quantity of each powder on identical metal brackets in order to have samples characterised by the same mass and exposed surface.

The nematic liquid crystal 4-methoxybenzylidene-4’-n-butylaniline (MBBA) obtained from Aldrich Chem. Co., was characterised by purity greater than 98%. Molecular crystal, phenyl salicylate from Sigma was selected for its low point of fusion and for its tendency to crystallise easily into large transparent crystals. During the experiments about 10 g crystal was employed for each sample of these substances. The data were obtained during experiments performed in air. Nevertheless, in case of measurements relative to inorganic substances, they have been repeated under an argon atmosphere in order to monitor the eventual presence of the quenching phenomenon due to oxygen. Such measurements, however, gave results indistinguishable from those performed in air.

Results

Hyperbolic trend

For all the sample examined, the experimental data of the decay of DL, in the time range of present
measurements fits to the hyperbolic trend described by the equation:

\[ I(t) = \frac{I_0}{(1 + t/t_0)^n} \quad \ldots (1) \]

Typical trend of DL from different samples in standard condition is presented in Fig. 1. The markers refer to points obtained processing the experimental raw data with a smoothing procedure in order to reduce random noise; solid line refers to fit according to Eq. (1). The parameters \( I_0, t_0 \) and \( n \) which characterize Eq. (1) were calculated adapting to the specific problem the general mathematical method of nonlinear least-squares fitting. Usually the agreement between the experimental data and the kinetics described in Eq. (1) is very good (reduced \( \chi^2 \) less than 2).

DL from different solid state systems that exhibit different physical mechanisms were at the basis of the phenomenon: cadmium sulphide luminescence is described by the recombination of Wannier-Mott excitons, phenyl salicylate luminescence was described by the recombination of Frenkel excitons; and ruby luminescence was due to impurity centers and was almost independent on temperature. As a matter of fact, while for the first two systems the trend was still hyperbolic, in the case of Ruby crystal DL trend better accords an exponential trend (Fig. 2). The above results confirm that, for certain materials, the afterglow intensity diminished following a non exponential time trend, typical of independent excitation levels, but a hyperbolic one, according the Becquerel empirical law:

\[ I(t) = (a + bt)^w \quad \ldots (2) \]

Some attempts for a theoretical substantiation of this dependence were made\(^{14-16}\).

**Non-linearity**

The effects on DL kinetics from the unicellular alga *A. acutabulum* when the intensity of the impinging light changes by using neutral density filters, show that a more or less remarkable non linearity with the intensity was observed both as it regards the total number of emitted photons and the slope of the decay curves; it appears that on decreasing the intensity the slope of the curve decreases, i.e. the curve were not parallel.

**Spectral analysis**

The spectral analysis of the ultra weak photon fluxes emitted by biological samples was measured using broad-band filter; the decays obtained illuminating a sample of the unicellular alga *A. acutabulum* with white light are presented in Fig. 3.

As a result DL decay of various components of the emission spectrum have the same time trend, i.e. they are parallel curves, showing that, in a first approximation, the probability of decay, which is connected to the slope of the decay trend, is independent of the frequency. This phenomenon seem to exclude that such a luminescence could be generated by a mixture of independent excited levels, whose time constants are distributed in such a way as to give origin to a hyperbolic decay. The same behavior has been observed for all the biological systems examined.

Figure 4 shows the time trends of the DL intensity \( I_0(t) \) emitted at a wavelength \( \lambda \), inside a wavelength \( \Delta \lambda \), from a polycrystalline sample of cadmium sulfide.
The different spectral components show also in this case the same time trend.

Cell's organisation

Chloroplasts in alga *A. acetabulum* move in 'trains' connected with tubules which actively take part in the motile mechanism, interact with the cytoskeleton and effectively modify chloroplast behavior.

To investigate if action which affect the dynamic chloroplast network integrity can be revealed by means of DL measurements, tests which could give both reversible and irreversible effects in the chloroplast organization were performed. In particular there were tested the effects of freezing in liquid nitrogen and subsequent thawing were tested. These gave an irreversible loss of the organelles motility and the effects of addition of chloroform, which allowed to obtain a reversible effect if neither strong concentration nor longer time of incubation were used.

The sample of *A. acetabulum* cell was first tested in normal condition; they were placed in a Petri dish containing artificial seawater (standard culture medium) at 20°C. Then the sample, free of adherent fluid, was dipped in liquid nitrogen for about 15 sec. thawed and re-incubated at 20°C in normal artificial seawater.

Freezing procedures were routinely used in order to isolate chloroplasts from algae and to study their fluorescence characteristics. As a matter of fact fluorescence measurement performed on the sample before and after the freezing-thawing procedure did not give significant differences.

After the freezing-thawing procedure, the algae remained intact, but no evidence of streaming of chloroplasts, as observed in standard condition, was revealed. The organelles appeared to be frozen and only some of them, which had more room nearby, showed a vibrational motion round a fixed position. No indication of any recovery within 1 hr was observed. The corresponding DL decay trends are reported in Fig. 5.

It appears that after the freezing-thawing procedure the trend is about parallel to the initial unperturbed trend, but considerably lower (only 3% of DL remains). This trend do not change in the first hours even if the sample appears "green" colored. After a few days, in any case, the algae becomes completely empty and white colored, while the signal disappears.

It has been suggested that general anaesthetics may act through the agency of their van der Waals interactions which interfere with the normal switching
actions of tubulin interrupting the actions of microtubules. Therefore a test was been performed by adding chloroform to the artificial seawater where the alga lives.

It has been proved that suitable concentration of chloroform for not long time of incubation give reversible effects. After the addition of chloroform organelles moved progressively slower and in about 5 min the streaming was frozen. When the alga was replaced from seawater with chloroform solution and placed in a new cuvette filled with fresh seawater at 20°C, in about 3 min the streaming was recovered. The viability of the alga was checked for some weeks after the treatment, and no significant change was observed.

The measurements of DL performed at the initial standard condition, 5 min after the addition of chloroform solution (1 mM) and after re-incubation in standard fresh medium at 20°C show first a decrease and then a progressive recovery of the DL decay; even if the total intensity, i.e. the total number of emitted photons, becomes much lower than the initial one after 5 min of incubation in chloroform, the DL after re-incubation show a restored trend.

**Ordered system**

The influence that the order parameters of the structure have on DL features have been studied by performing DL measurements on samples constituted by microcrystals of cadmium sulfide. Different samples were of the same origin and present a more or less organized structure according the different average dimensions of the microcrystals. Figure 6 reports DL kinetics of cadmium sulfide polycrystalline samples of different grain dimensions.

Figure 7 reports the total number of photons emitted by such samples as a function of their average grain dimensions determined by microscopic observations. It appears that the total intensity decreases, roughly linearly, with the reduction of the dimension of the grains.

However the signal is even well measurable for grains having dimension of the order of hundreds nanometers, comparable to the dimension of the ordered structures existing inside the cells.

It is not easy to analyse the dependence of DL on the dimensions of the ordered structures present in the biological systems. It has been observed that chloroform altered the integrity of the dynamic chloroplast network. Taking into account such results it is assumed that addition of anaesthetic in the culture medium of the alga can artificially provoke a “fragmentation” of the ordered structure in *A. acetabulum*. Thiopental sodium has been used in this case. In presence of low concentrations of such anaesthetic, it has been assumed that a parameter $D_{\text{in}}$ proportional to inverse of the cubic root of the concentration (number of molecules per unit of volume) could have for the biological system the same meaning of the average dimension of grains for the solid state system. Figure 8 shows that in this case also a linear increase with “size” is obtained, even if data are more scattered around a linear trend.

**Temperature phase transition**

To study with an aim the influence of topologic coherence on DL in another way both in biological and solid state systems, an experiment was performed to measure how the DL varies with change in the temperature when this change has a notable influence on long-range order of the system.

![Fig. 6 - DL temporal decays of cadmium sulfide polycrystalline samples of different grain sizes: (●) less than 1 μm, (▲) of the order of 10 μm, (●) larger than 100 μm.](image)

![Fig. 7 - Total number of photons emitted from samples of cadmium sulfide microcrystals of different grain sizes as a function of their average grain size.](image)
Delayed luminescence measurements have been performed on liquid crystalline system MBBA (4-methoxybenzyliden-4'-a-butyramine) in the range of temperature where it passes from the solid state to the nematic liquid crystal state.

Figure 9 shows DL total counts emitted by a MBBA sample on changing the temperature, MBBA at about 22°C passes from the solid phase to the nematic liquid crystal phase, and at higher temperatures becomes an isotropic liquid. Results show that, as the order of the structure declines, the DL signal diminishes until it vanishes in the isotropic liquid state.

The figure also shows the trend of DL total counts as a function of temperature for an A. acetabulum sample, in the range of the physiological temperature of the biological sample. The similarity of the two trends let us to think to a kind of “phase transition” which occurs also in the biological system. It can be connected, on increasing the temperature, to the increase of the rate of the polymerization process, which produces a more ordered structure.

It must be noted that such effect does not appear when we measure DL from algae that previously underwent a freezing-thawing procedure, which provokes the breaking of the integrity of the dynamic chloroplast network.

Discussion

Decay of DL is known to take two forms, corresponding to the Becquerel law Eq. (2), with \( \alpha = 1 \) or 2. The case \( \alpha = 2 \) is explained by assuming the existence of recombination processes between reactants of the same concentration (biquimolecular reaction), while the case \( \alpha = 1 \) is explained by assuming the existence of many equilibrium states with different decay constants exponentially distributed. On the other hand, the existence of a continuance of equilibrium states, with different decay constants suitably distributed, can explain values of \( \alpha \neq 1 \). However, these equilibrium states have not been identified and what control their distribution has not been determined. Another hypothesis asserts that a hyperbolic relaxation under ergodic conditions has been registered, the decay products can originate only from a fully coherent field, which plays an important role in the promotion and control of living processes.

Such theoretical models are not concerned with other features of DL and predictions have not been extracted. Moreover, experimental results above described show that non linear phenomena characterize the emission mechanism, and it depends on the organized structure of the system. Therefore, the idea that this phenomenon in biological systems can be connected with the collective electron states has been expressed.

The idea is that in a biological cell there is a large variety of low-dimensional macromolecules (as, for instance, alpha-helical polypeptide proteins, actin filaments, etc. whose structure is represented by the arrays of parallel quasi-one-dimensional (quasi-1D) polypeptide chains formed by periodically placed peptide groups along the chain of hydrogen bonds. From the point of view of electronic structure, these macromolecules are semiconductor-like quasi-1D systems with the filled valence band and empty
conduction band, separated by the gap of finite width\(^2\). These characteristic properties favour the existence of coherent collective electron and exciton states, in general, and solitons in particular.

The concept of molecular solitons or 1D polaron-type states, which participate in charge and energy transport during the metabolic processes, was first suggested by Davydov and Kislukha\(^23\). It has been useful to explain numerous phenomena in biological systems\(^24,25\) and some experimental evidence for soliton existence in biological systems has been found\(^26\).

When in the electron transfer chain an extra electron is transferred from a donor molecule to one of the sub-units of the macromolecule, it affects the chain structure due to the exchange interaction with the nearest neighbours and electron-phonon interaction with hydrogen bonds along a chain. This results in the creation of the local distortion of the chain, which, in turn, plays the role of potential well for the electron itself and leads to the self-trapping (auto-localisation) of an electron.

The suggested model\(^27,28\) assumes that the DL is connected with the formation and dissociation of non-linear coherent self-trapped (localized) electron states (solitons and electrosolitons) which are much more stable, in view of long duration of DL, than the corresponding delocalised states, and can be created, in low-dimensional macromolecular structures in the cell during the charge and energy transfer processes, by the pre-illumination of the sample.

In a model whose energy scheme is given in Fig. 10, and where \(N\) is the number of free electrons in the conduction band, \(n\) is the number of hole in the valence band, \(v\) is the number of electrons in localized soliton state and \(p\) is the rates of the different processes, the intensity of DL is determined by the electron-hole recombination processes according to the equation:

\[
I = -\frac{dn}{dt} \quad \ldots (3)
\]

where \(n(t)\) satisfies the following system of equations, which is similar to the system of equations obtained for crystallophosphors in presence of localised trap levels\(^14\).

\[
\frac{dn}{dt} = -p_{rec} N n
\]

\[
\frac{dN}{dt} = p_{exc} /v - n_{exc} N n - p_{ma} N (v_{n} - v)
\]

\[
\frac{dv}{dt} = -p_{ma} v + p_{ls} N (v_{n} - v)
\]

Solution of Eqs (4) can be obtained in implicit form; it appears that DL kinetics depend on two parameters, the excitation level \(n_0 = n(0)\), where \(n_0 = n(0)\), and the ratio \(f = p_{rec}/p_{exc}\).

As a result the soliton model allows to explain: (i) the large variability of the DL kinetics, including simple exponential, hyperbolic and more complicated time decays; (ii) the same law of the time decay of various components of the emission spectrum; and (iii) the non-linear relation between the initial intensity of DL and the intensity of the stimulating light and the DL kinetics dependence on the intensity of the stimulating light. In addition, depending on the number of extra electron in a chain, the existence of correlated many-soliton states is possible, characterized by localization energy which depend on (bi)soliton concentration. Results show that such correlated model gives high precision of the theoretical fit of DL experimental data from \(A. acetabulum\) in a wide interval of intensities of the stimulating light and in the whole time interval of the experimental measurements\(^28\).

As an example how the model works Figure 11 shows the time trends of the DL emission from \(A. acetabulum\) at different intensities of the stimulating light, \(I_S\), obtained by reducing a maximum intensity \(I_{max}\) by means of metallic neutral density filter. Symbols show experimental data, solid lines correspond to theoretical fit within the correlated soliton model.

Reasonably best fit (reduced \(\chi^2\) values ranging from 1.6 to 3.8) of the experimental data at the different intensities of the impinging light in the

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Fig. 10 — The energetic scheme for the theoretical model of delayed luminescence. DL is determined by the electron-hole recombination process in presence of a self-trapped state whose energy level is \(E_s\).

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Fig. 11.—Temporal decays of DL emitted from a sample of A. acetabulum cell at different intensities of the stimulating light $I_s$. [Markers refer to the experimental data, solid line correspond to the theoretical predictions in the framework of the correlated many-soliton states model. (o) $I_s = I_{max} = 2.8 \times 10^6$ photons/(cm$^2$-sec). (x) $I_s = \frac{I_{max}}{5}$, (e) $I_s = I_{max}$]. The wavelength of the stimulating light was 450 nm. Parameters of the fit: the rate ratio $\gamma = p_{ds}/p_{ds} = 10$ and the excitation level $x_0 = 0.85$, 0.62 and 0.22 respectively].

whole time interval of measurements was obtained at $\gamma = 10$, with $p_{ds} = 5.2$ s$^{-1}$.

The strong dependence of DL on the ordered structures of both living and solid state systems has allowed to develop, using the non-linear approaches applied in solid state physics, a model able to explain some of the physical characteristics of DL.

The experimental and theoretical study on DL reported in this communication opens new horizons in the interpretation of the energy and information transfer mechanisms in biological systems, and let to think to the possibility of using DL measurements in order to closely investigate the biophysics of a living organism as a whole complex system.

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References


