Fluorescence Spectroscopy as an Interface of Engineering and Basic Science: Its Evolution and Principle

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Abstract The fluorescence spectroscopy is a very emerging research area. Its application is extended in the diverse arena of physical, chemical, analytical and medical sciences. Use of fluorescence for imaging is an important application in the field of biomedical sciences. It is multidisciplinary field of research area. Engineers can contribute a lot for its development. This review is aiming to cover its stages of development and underlying theories and principles that are very useful to interpret any experimental data in this field.

Keywords Fluorescence · Incandescent · Stokes' law · Fluorspar · Jablonski diagram

1 Introduction

The first known report of the phenomenon of the light emitting material without heating came in the literature in the long back in 1565 when Spanish botanist and physician Nicolus Monardes [1] talked about a slight blue colour of water originated when it had been kept in a special wood cup of a plant 'lignum nephriticum'. Afterwards, many greats (like Isaac Newton and Robert Boyle) of seventeenth and eighteenth centuries had interested in exploring this new arena. The cold light emitting materials (other types emit light in hot condition—known as incandescent material) had continued to be discovered during seventeenth and eighteenth centuries enriching the libraries of this new sort of materials. But the science behind this strange phenomenon had not been understood by them. Brewster [2] in 1833 again observed 'this light emission in cold' when he noticed a red light in an alcoholic solution of green leaves (chlorophyll) on exposing to white light. He also observed a blue light from a crystal of fluorspar and tried to explain the two phenomena on the basis of scattering of light. The research in this field then continued discovering many new facts centring basically in Europe.

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1.1 A New Scientific Look: Advent of Stokes

In nineteenth century, a huge advancement of this new field had occurred because of pioneering works of the investigators like J. Herschel, Stokes and many others. A very meaningful experiment was carried out by an English astronomer and mathematician John Herschel in 1845 [3]. He, with the help of a prism, used different part of a visible light to illuminate a solution of quinine sulphate and arrived at some important conclusions that—(i) only blue part of the light is responsible for the light emission from the 'surface' of the quinine solution and (ii) emitted light is made of a mixture of blue, green and yellow. But unfortunately, he could not understand that the emitted light had a higher wavelength than the absorbed light. Seven years later, an Anglo-Irish physicist and mathematician at the University of Cambridge, Stokes [4] at his young age of thirty-three published a monumental paper in *Philosophical Transaction* and coined the term 'fluorescence' in analogy with the mineral fluorspar (consisted of crystal of CaF_2 containing fluorescent impurities like Eu^{2+} and some other lanthanides). This mineral emits blue light. Stokes carried out some important investigations and reached to some significant decisions. He carefully observed that when sunlight had been split with a prism and various parts were exposed to the solution of quinine sulphate it was the invisible part of the spectrum beyond the ultraviolet region which causes the glow of the solution. He established that the glow was due to the *absorption of radiation* and not due to the *scattering*. Stokes next statement was emitted light which would always have the longer wavelength than the absorbed light. This is what that later becomes Stokes' law. Stokes had been continuing his research in this direction for many years and explained many important properties of 'fluorescence' such as its dependency on concentration and use as an analytical tool [5]. In the first half of twentieth century, a remarkable progress in this field had happened, many interesting aspects of the fluorescence disclosed, and people tried to apply it as a tool in diverse areas of physical, chemical and medical sciences. The design of *fluorescence microscopy* to study the living organism in the beginning of twentieth century by the companies like Carl Zeiss and Carl Reichert [6] put forward a thrust in research in the direction of fluorescence spectroscopy. Ellinger and Hirt also contributed a lot in the development of fluorescence microscopy where they treated living organism with fluorescent substances that can act as a source of light in that species. The discovery of green fluorescent protein (GFP) is another great finding which extends the use of fluorescence in the field of biomedical science. During the early second half of twentieth century, it was Davenport and Nicol who reported [7] the light emitting tissue in the eosinophils of a jelly fish called *Hydromedusae.* But the author had no idea about the origin of light. Seven years later Shimomura et al. [8] identified that the basic compound behind the origin of the light was a protein. This is what later popularly known as green fluorescent protein (GFP). The Nobel Prize in chemistry in 2008 went to Shimomura, Tsien and Chalfie for their outstanding contribution in the area of green fluorescent protein (Table 1).

Table 1 Chronological order in the evolution of fluorescence

Sl no.	Event	Scientist	Year
1	Bioluminescence of *lignum nephriticum*	N. Monardes	1665
2	Study of the green leaves solution in alcohol	D. Brewster	1833
3	Reporting of light emission by quinine sulphate solution	J. Herschel	1845
4	Publication of famous paper on 'refrangibility of light'	G. G. Stokes	1852
5	Theoretical distinction between fluorescence and phosphoresescence	F. Perrin	1929 [9]
6	An approach to the theoretical understanding of fluorescence	Aleksander Jablonski	1935
7	Reporting of luminescence in *hydromedusae*	D. Davenport and J. A. C Nicol	1955
8	Discovery of green fluorescent protein (GFP) in *hydromedusae*	Osamu Shimomura	1962
9	Using of green fluorescent protein as a marker for gene expression	Martin Chalfie	1994 [10]

2 Absorption of Light and Subsequent Phenomena

If a photon is absorbed by a molecule, the molecule will be raised to an excited state. Then, the excited molecule will try to return to the ground state, and the same can be achieved by many ways. One possibility is the occurrence of fluorescence (Fig. 1).

The various possibilities are summarized below:

It can return to the ground state without emission of photons, and the extra energy of the excited state is released via vibrational relaxation and collision with the surrounding medium. As a result, the energy of the photon is converted ultimately to the thermal energy. If during this whole process, the spin state of the excited and the ground state remains same, the process is called the internal conversion. Since no photon is emitted in this process, it becomes a radiation-less decay.

There may occur a transition from singlet to triplet state in the excited state. It is known as intersystem crossing, and it is generally followed by phosphorescence or delayed fluorescence.

The excited molecule may participate in chemical reaction which is the basis of photochemistry.

The excited molecule may transfer the extra energy to another system and reverts back to ground state. De-excitation may also be accompanied by proton transfer, electron transfer or conformation change of the molecule. Other fates of the excited molecule may be the formation of excited state complex, i.e., exciplex or the excited state dimer known as excimer. Lastly, one of the probabilities is the emission of the photons from the excited molecule leading to the phenomenon of fluorescence.

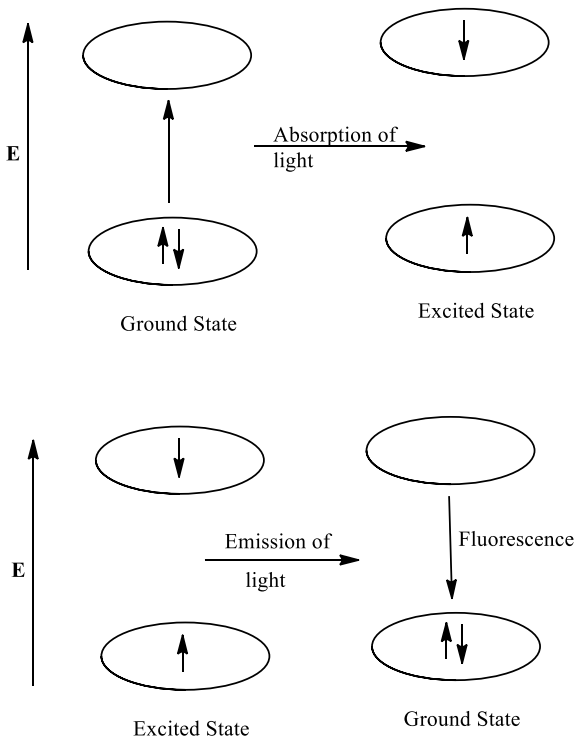


Fig. 1 Light absorption and emission of fluorescence

3 An Overview of Singlet and Triplet State

In a molecular singlet state, all electrons remain paired. Let us explain the concept with Fig. 2.

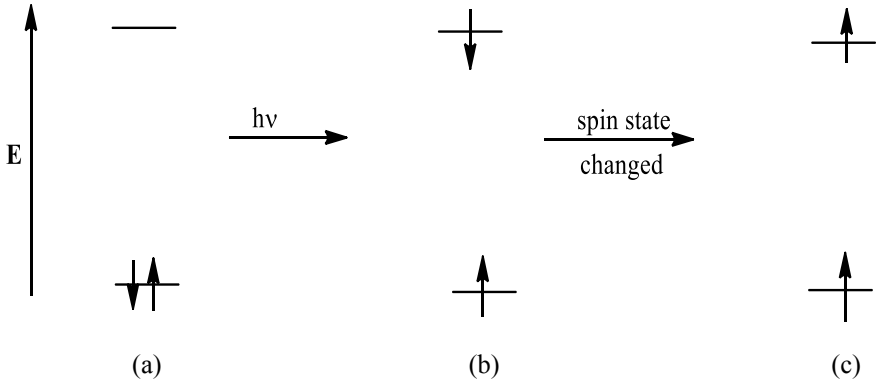
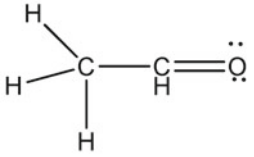


Fig. 2 Singlet and triplet state

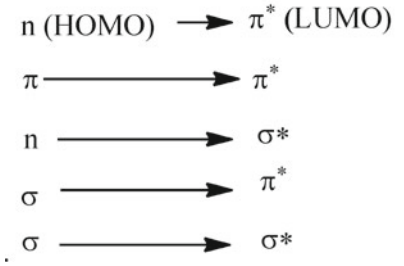
In '*A*', two electrons are remaining in the same orbital, and they are in spin-paired state. So, the total quantum number is zero $[(+1/2) + (-1/2) = 0]$. Therefore, the spin multiplicity will be $2S + 1 = 2 \times 0 + 1 = 1$. Thus, the state '*A*' represents a singlet ground state which is symbolized as So.

In '*B*', one electron has been promoted to a higher energy level by absorption of photon, but spin state of the electron in the higher orbital remains same. Hence, it is also a singlet state but in excited condition. It is symbolized as *S*1 (first excited singlet state). But in '*C*', the case is different; here, both the electrons have the spin in the same direction. Hence, the spin multiplicity is 3, and this state is called the triplet state. Let us elaborate this idea in some more details by considering an example of a molecule, say, acetaldehyde.



Here, there are three types of molecular orbitals: (i) σ—molecular orbital, (ii) Π—molecular orbital and (iii) *n*—(non-bonding) molecular orbital. We can draw a simple energy diagram as shown in Fig. 3.

Now, as shown in Fig. 3, many types of electronic transitions are possible upon absorption of light such as



During the transition if the spin state of the promoted electron remains same, then the total spin quantum number ($S = \sum si$) will also remain unchanged, for all paired up ground state, its value will be equal to zero, in this case, its spin multiplicity will be one, and accordingly, as discussed earlier, it should be termed as a singlet state. Since upon excitation, the spin state is also remaining same, and it is also said to be an excited state singlet. The ground state is designated as So, and the excited state involving lowest energy (*n* to π^*) is designated as *S*1. Similarly, (π to π^*) is *S*2 and so on. These types of transition are called singlet–singlet transition. It may happen that promoted electron has undergone a conversion process and the spin state has changed in such a way the two electrons now have parallel spin (Fig. 4).

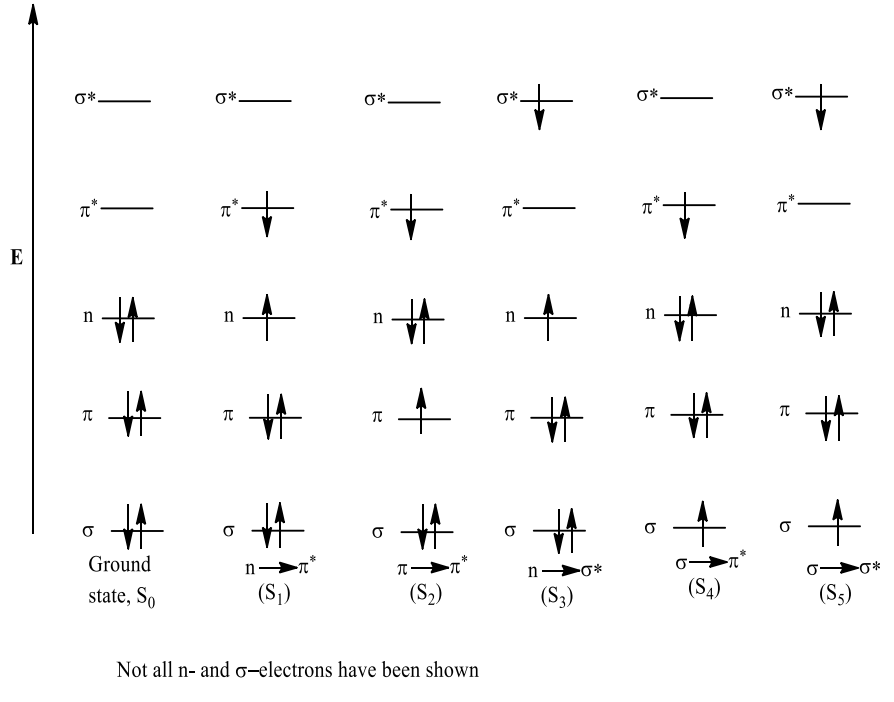


Fig. 3 Various singlet states

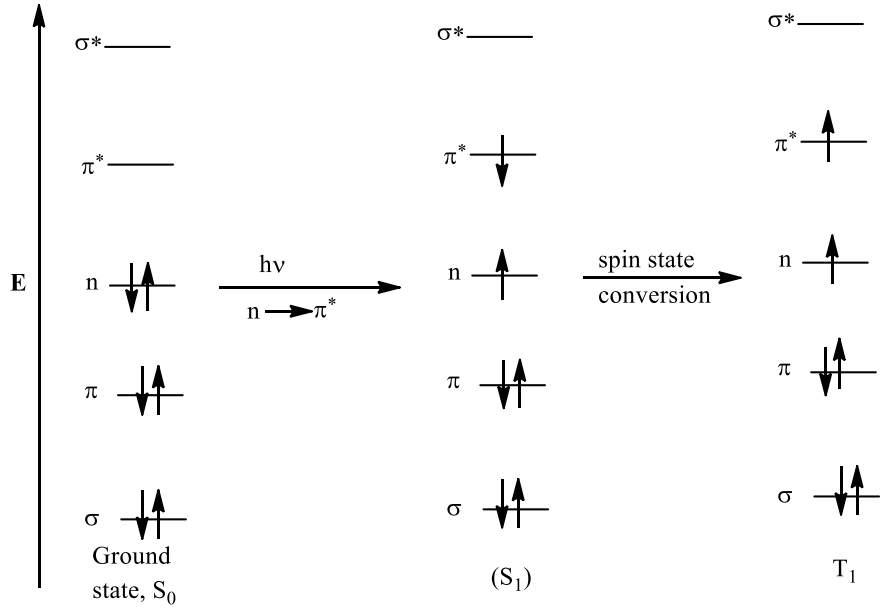


Fig. 4 Singlet–singlet transition and generation of triplet state

Since the spin multiplicity after the change of spin state is three, it is called a triplet state. Now, the triplet state associated with the $n \rightarrow \pi^*$ transition is designated as T1, $\pi \rightarrow \pi^*$ Is T_2 and so on. According to the Hund's rule, energy associated with the triplet state is lower than the singlet state having same electronic configuration, i.e., $T_1 < S_1$, $T_2 < S_2$, $T_3 < S_3$ and so on.

4 Jablonski Diagram: An Interpretation of Fluorescence

In a molecule, each electronic state is associated with a number of vibrational states. According to Boltzmann distribution, the majority of the molecules remains in the lowest vibrational state (zero vibrational level) of the ground state. After absorption of the light, many interesting phenomena may occur which can be beautifully shown by Jablonski diagram. Here, the lowest vibrational energy level of any electronic state is shown by a thick line, and other vibrational energy levels are shown by thin lines. Fluorescence phenomenon can easily be interpreted with the help of Jablonski diagram (Fig. 5) [11].

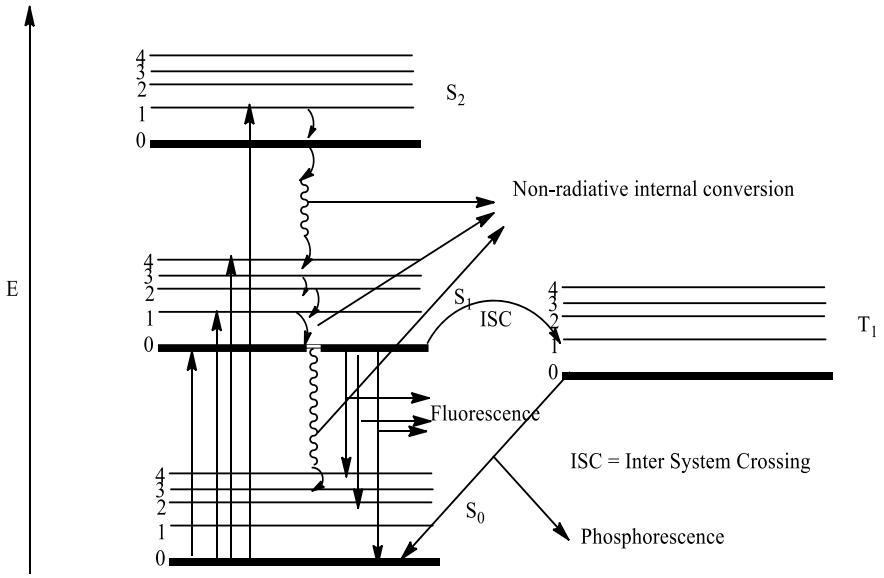


Fig. 5 Jablonski diagram

5 Conclusion

This mini-review on fluorescence spectroscopy may be helpful to (i) those who are the beginners in this field, (ii) those who belong to different discipline other than physics, chemistry but curious about the term 'fluorescence', (iii) those who are graduating and post-graduating in engineering and interested in biomedical imaging processes.

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References

1. O'Haver, T.C.: Development of luminescence spectrometry as an analytical tool. J. Chem. Edu. **55**(7), 423–428 (1978)
2. Brewster, D.: Trans. Roy. Soc. Edinburgh. **12**, 538–545 (1834)
3. Herschel, J.F.W.: Formula No. I. On a case of superficial colour presented by a homogeneous liquid internally colourless. Phil. Trans. **135**, 143–145 (1845)
4. Stokes, G.G.: On the change of refrangibility of light. Phil. Trans. **142**, 463–562 (1852)
5. Valeur, B.: Molecular Fluorescence: Principles and Applications. First Edition. Wiley-VCH, New York (2001)
6. Renz, M.: Fluorescence Microscopy—A Historical and Technical Perspective. Cytrometry Part A. **83A**, 767–779 (2013) and the references cited therein
7. Davenport, D., Nicol, J.A.C.: Luminescence in hydromedusae. Proc. R. Soc., London B. **144**, 399–411 (1955)
8. Shimomura, O., Johnson, F.H., Saiga, Y.: Extraction, purification and properties of Aequorin, a bioluminescent protein from the luminous hydromedusan. Aequorea. J. Cell. Comp. Physiol. **59**, 223–239 (1962)
9. Perrin, F.: Doctoral Thesis, Paris, Annales de Physique.**12**, 2252–2254 (1929)
10. Chalfie, M., Euskirchen, Y., Tu, G., Ward, W.W., Prasher, D.C.: Green fluorescent protein as a marker for gene expression. Science **263**, 802–805 (1994)
11. Jablonski, A.: Efficiency of anti-stokes fluorescence in dyes. Nature **131**, 839–840 (1933)