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A correlation between fluorescence of chlorophyll solution in some medicinal plant leaves and absorption in liquid phase

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ABSTRACT

Red chlorophyll fluorescence of a number of medicinal plants Azardicta indica, Clerodendron colebrookonium, Leucas linifolia, Mesua ferua, Leucas cephalotes/lini folia have been photographed with the help of a two prism glass spectrograph and a 500W hellogen lamp as continuum. The corresponding absorption spectra of this sample have also been photographed. The salient points of the observation are that the fluorescence intensity depends on the concentration of the sample but at the same time it also depends on the absorption of the sample. When the red fluorescence intensity is maxima the absorption band also exhibits maximum extinction. The fluorescence intensity has been estimated with the help of a photodiode connected to a optical fiber. The result so obtained in the work can be used to estimate the fluorescence content of the solution of the medicinal plants. We have also used Ar^+ laser to excite fluorescence spectra and worked out a correlation between absorption and fluorescence.

Key words: absorption, fluorescence, chlorophyll.

INTRODUCTION

Fluorescence is a process in which an atom or molecule emits radiation in the course of a transition from a higher to a lower electronic state. A more restricted definition, applicable particularly to atomic processes, excludes the special case, known as resonance radiation, in which the wavelength of the emitted radiation is the same as that of the exciting radiation. The term fluorescence is further restricted to phenomena in which the time interval between the acts of excitation and emission is small, of excitation and emission is small, of the order of 10^{-8} –10^{-3} second. This distinguishes fluorescence from phosphorescence, where the time interval between absorption and emission may extend from 10^{-3} second to several hours. The phenomenon of fluorescence was known by the middle of the century. It was the British scientist Stokes who first made the observation that the fluorescing light has longer wavelengths than the excitation light, a phenomenon that has become to be known as Stokes-shift. Fluorescence microscopy is an excellent method of studying material that can be made to fluoresce either in its natural form or when treated with chemicals capable of fluorescing. One of the most prominent processes is the chlorophyll fluorescence, which is readily

observed when a solution of chlorophyll (green leaves) is illuminated with a strong source of continuum radiation. The red colour characteristics of fluorescence readily appear in a sample cuvette. Light energy is absorbed by chlorophyll within plant tissues and used to drive photochemistry of photosynthesis and thus become chemical energy available to the plant for growth. Light in the waveband 400-700 nm is absorbed by chlorophyll and used for photochemistry. This light is termed photosynthetic ally Active Radiation (PAR). Although fluorescence emission from whole leaf system is too weak to be viewed with the naked eye, it can be observed from the illuminated extracts of a chlorophyll solution. Peak fluorescence occurs in the red region of the spectrum (685 nm) and extends into the infrared region to around 800nm. The fluorescence from chlorophyll has been used extensively in the past to characterize and investigate agricultural plants [1-5]. In the present work we report an experimental investigation, which correlates between absorption and fluorescence for the same chlorophyll extracts of medicinal plants. This correlation is not exactly known in the investigations of the early workers. The approach presented in this work is simple, as we have used the linear dimension of the absorption and the intensity of the fluorescence for our correlation.

MATERIALS AND METHODS

We have used five specimens of medicinal plants namely (a) *Azardicta Indica*, (b) *Clerodendron colebrookorium*, (c) *Leuca linifolia*, (d) *Mesua ferua L* and (e) *Leucas cephalotes-linifolia* for our studies of fluorescence and the corresponding absorption .In all the cases immersion of the crushed leaves in a glass vessel containing a suitable solvent like acetone enables the pigments responsible for their green colour to be conveniently and quickly extracted . After filtering, the extract may be transferred to a cuvette of suitable length .The cuvette with the solution is held in front of the slit of a two prism glass spectrograph. A bright source of halogen lamp (500W) is used as the source of continuum radiation for observing fluorescence and as well as absorption. The absorption spectrum of a sample solution may be visually observed on the position of the plate holder with the help of a ground glass.

Figure 1: Well known red fluorescence from a chlorophyll extract

Fig. 1 shows the characteristic picture of the red coloured fluorescence, which appears when the radiation is incident on it. This is the well-known chlorophyll fluorescence. It has already been described in chapter3. It is worthwhile to observe that the red colour becomes very intense when the concentration of the extract is more and at the same time the corresponding

absorption is maximum. Fig. 2 shows the absorption and fluorescence spectra of five specimens when the concentration of the extract is maximum in each case. For our correlation between absorption and fluorescence we have used a graphical method, which relates the linear dimension of the absorption band and fluorescence intensity. It is observed that (and it is usually the case) absorption length is minimum when the concentration is very low. The corresponding fluorescence intensity is also very low. The corresponding fluorescence intensity is also very low. The intensity of the fluorescence is measured with the help of a photodiode connected through an optical fiber. Fig 3 shows the correlation between the fluorescence intensity and absorption lengths. The specimen used in this case is *clerodendron colebrookorium*. Similar graphs may also be obtained for other samples.

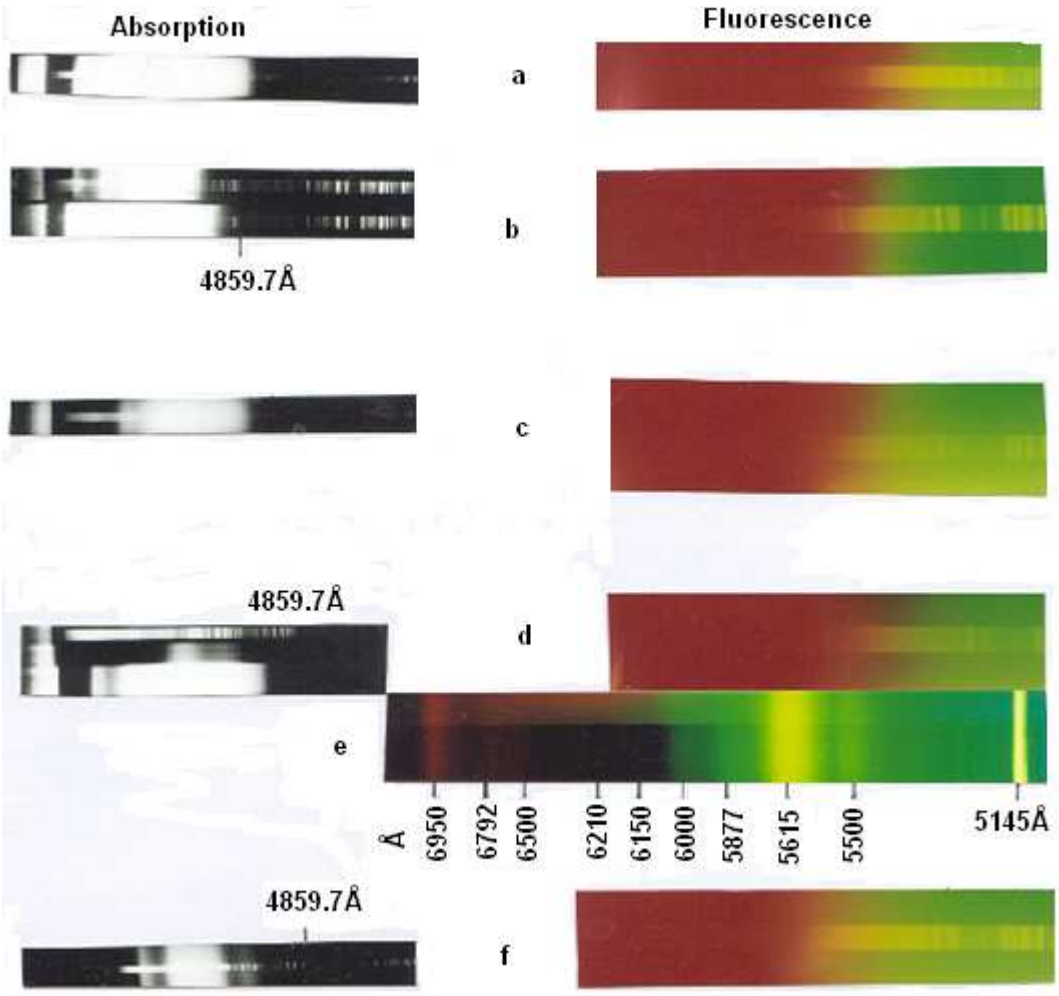

Figure2: Absorption and the corresponding fluorescence from five chlorophyll extracts of medicinal plants

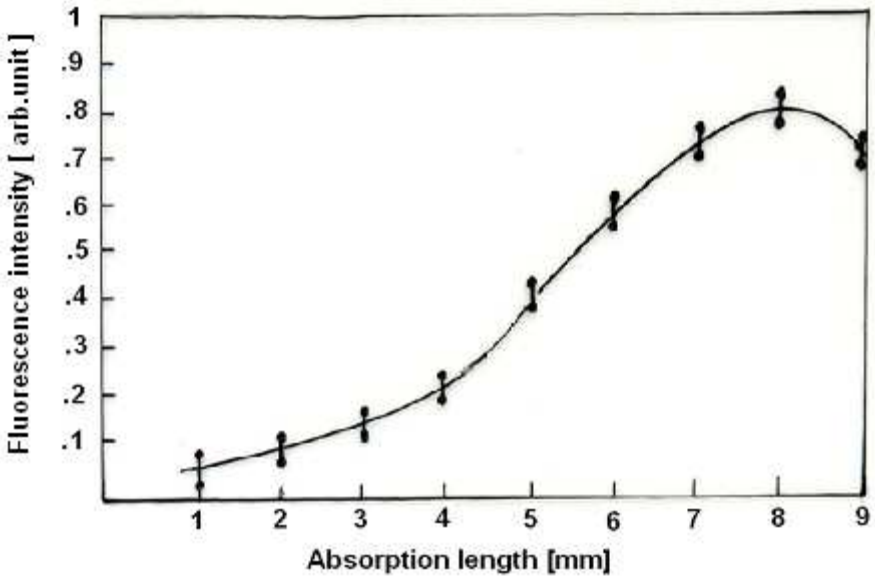

Figure 2 The relation between absorption bands width and fluorescence intensity

RESULTS AND DISCUSSION

The extent to which the red fluorescence colour penetrates in the cell before the green colour appears depends on the particular chlorophyll extract chosen. As for example for a cell of 15mm breath the red and green colour combination in terms of their penetration depths are shown in the following Table 1 for five chlorophyll extracts considered in the present work. It is scarcely surprising that the red green combinations for different chlorophyll extracts are different. This simple fact may be used to characterize different chlorophyll extracts by a method entirely different from absorption. As shown in Fig2, all the fluorescence show nearly continuum structure while in the case of Fig. 2 (e) fluorescence spectrum of *Mesua ferra L* leaf shows more than ten discrete bands when the leaf is excited with the help of Ar^+ laser (500w).

S. N.	Sample	colour	Penetration depth
1.	*Azardicata indica*	Red	5mm
		Green	10mm
2.	*Cherodendron colebrookorium*	Red	5mm
		Green	10mm
3.	*Leucas linifolia*	Red	2mm
		Green	13mm
4.	*Mesua ferua*	Red	8mm
		Green	7mm
5.	*Leucas cephalotes/ linifolia*	Red	11mm
		Green	4mm

Table 1: The penetration length versus colour of fluorescence observed in a *Sample cell of dimension 1 cm x 1 cm x 6 cm*

This clearly differentiates between the fluorescence excited with the help of a broadband source and narrow band or single line source. As may be inferred from Fig 3 when the concentration is at maximum the fluorescence intensity as measured from the digital meter is also minimum. The Fluorescence intensity **I** gradually increase as the absorption length **L** (that is the horizontal spread of the absorption region in the spectrogram) also increases. It may be noted from the **I-L** curve that the fluorescence intensity **I,** at a particular stage, does

not increase even after the increase of absorption length **L** but in reality the fluorescence intensity decreases. This occurs primarily due to self-absorption, which is always present whenever a beam of light penetrates a certain distance of the medium. As concentration of the medium is maximum the process of self-absorption is complete and under this circumstance no transmitted light is observed. We must also consider the process of scattering and fluorescence because in all the cases both may be operating. Fluorescence and absorption are familiar phenomena in nature, but surprisingly enough, attention does not appear to have been drawn to particular type of relation, which we have worked out using simple parameters.

CONCLUSION

The present experimental investigation shows that the fluorescence intensity from a chlorophyll extract may be correlated with the absorption bandwidth by a simple empirical relation, which is not linear and closer to Beer's law.

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