



## STUDY OF REGULATION OF ANTHOCYANIN PRODUCTION FROM CONFEDERATE ROSE BY SPECTROSCOPIC METHOD AND THEIR NON LINEAR OPTICAL CHARACTERIZATION

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### ABSTRACT

Flavanoids are major constituents of flower colour and anthocyanins are the coloured class of Flavanoids. We report on the study of the regulation of anthocyanin compound from confederate rose, a *Hibiscus mutabilis*. The third order non linear optical (NLO) properties dye extracted from confederate rose in methanol has also been studied using a Q-switched Nd:YAG laser at 532 nm. The third order non linearity of these samples show multiple saturable absorption (SA) within the reverse saturable absorption (RSA), suggesting that these dyes are promising candidate for the development of photonic non linear optic devices.

**KEYWORDS:** flavanoids, anthocyanin, saturable absorption, photonic non linear optic devices *etc.*

### INTRODUCTION

Flower colour in many red, purple, and blue flowers is a result of a complex interaction among anthocyanin, co-pigments, and pH of the vacuole, all of which is under genetic control<sup>[1]</sup> and exists as a chemical complex in the vacuole which is held together by hydrophobic interactions. While the anthocyanidins (anthocyanin without sugars attached) and anthocyanin provide the basis for floral pigmentation, flowers with the same anthocyanidins can have diverse colours, conditional on the uniqueness and concentration of co-pigments<sup>[2,3]</sup> and pH of the vacuole<sup>[4-6]</sup>. Current research is focussed on the study of regulation of anthocyanin production from confederate rose by spectroscopic method and their non-linear optical characterization. Confederate Rose, a *Hibiscus mutabilis*, is an old-fashioned southern shrub that often is seen flowering in the late summer, is not a rose at all, but a member of the Malvaceae family, that open

white, and then deepen to shades of pink as they age, (shown in Fig 1) the rapidity of this change suggests the possibility of direct reduction of flavonol glycoside (found in the white flower) to anthocyanin<sup>[7]</sup>, provides an excellent model system for our studies. The *Hibiscus mutabilis* has a little basal spot pigmented with cyanidin aglycone<sup>[8]</sup> and the compounds present have been reported to be quercetin 7-glucoside and quercetin 3-diglucoside in the white flower, and cyanidin 3,5diglucoside in the red flower<sup>[9]</sup>. Subsequently the presence of 3-glucoside, 3-rutinoside quercetin 3-galactoside and 4'-glucoside in flowers during the colour change has been reported<sup>[10]</sup>. Because of the wide diversity in glycosidic type, direct conversion of flavanol to anthocyanin appears improbable. Ishikura<sup>[11]</sup> also found that cyanidin 3-sambubioside compound to be the predominant anthocyanin of *Hibiscus mutabilis*.



FIGURE 1: different stages of confederate rose on a single plant\*

Under laboratory conditions, colour change of petals was slower than that of flowers under open-air conditions<sup>[12]</sup>. Temperature may be an important factor affecting the rate of colour change as white flowers kept in the refrigerator remain white until they are taken out to warm, whereupon they gradually turn pink<sup>[13]</sup>. There is little research done on the optical properties of flowers during colour change. The need for nonlinear optical materials for applications such as phase conjugation, image processing, optical switching and optical limiting are increasingly become important. Recently, a large number of organic  $\pi$ -conjugated molecules have been investigated due to their large nonlinear optical susceptibility and the possibility of tailoring their properties which allow these materials to be used to protect optical detection elements such as human eyes and optical sensors, by controlling the fluency on the image plane below the desired level. The outcome of such studies has helped to establish certain guidelines for molecular design of the third-order nonlinear optical materials with desired properties. However, most of the synthesized materials require elaborated preparation procedures and safety measures, use or generation of hazardous materials, costly materials, as well as fragile or chemically unstable structures beyond certain threshold irradiance. Therefore, we suggest natural dye extracts as environment friendly, safe, and inexpensive materials, as well as having high chemical stability during optical excitations with coherent light sources.

By using the Z-scan technique the optical nonlinearity of many organic materials has been explored. It is well known that the Z-scan technique, which was present by Sheik-Bahae *et al.*, 1990<sup>[14]</sup>, has been extensively used as an effective and convenient tool for exploring the nonlinear absorption properties of various materials. In this paper we report the techniques used to prepare the natural dye extracts, optical characterizations, and determination of absorption coefficients will be discussed. Also, we are presenting some of our findings and suggestions.

#### Experimental details

The extracts of the confederate roses were obtained from fresh biological materials. Plant samples were transported to the laboratory in a chilled ice-box. Pigments can be extracted (1.38 g) using methanol (10ml), after filtration the residues are removed. The pigments extracts must be protected from direct light exposure should be kept in cool

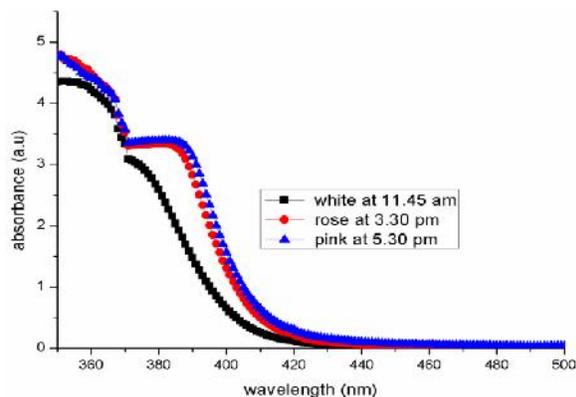


FIGURE 2: linear absorption spectra of confederate rose at three different times

place. The UV-VIS absorption spectrum of the dyes in methanol was recorded using a UV/Vis spectrophotometer (Jasco V-570 UV/VIS/IR.).

The dye solutions at 5.30 pm were carried out by the single beam Z scan technique. The transmission of a laser beam that changes near the focal point during the sample translation along the propagation path through an open-aperture (OA) was measured. A Q-switched Nd: YAG laser (Spectra Physics LAB-1760, 532 nm, 7 ns, 10Hz) was used in the z scan experiment to study the optical non linearity. A 20 cm converging lens was used to focus the laser beam. The radius of the beam  $\omega_0$  was calculated to be 35.4 $\mu$ m. The Rayleigh length,  $z_0 = \pi\omega_0^2/\lambda$ , was estimated to be 7.4 mm, which is much greater than the thickness of the sample, and is an essential prerequisite for z-scan experiments. The sample was fixed on a computer-controlled translation stage, so that it could be accurately moved through the focal region of the laser beam over a length of 6cm. The transmitted beam energy, reference beam energy and the ratios were measured simultaneously using an energy ratio meter (Rj7620, Laser Probe Corp.) having two identical pyroelectric detector heads (Rjp735). The detected signals were acquired, stored and processed by the computer. Optical density filters were used to vary the laser intensity at the lens focus.

#### RESULTS & DISCUSSION

Optical properties of organic molecules utilized in scientific and technological applications can strongly depends on properties of the surrounding media and the interaction of the dancing molecules. For liquid solutions, solvent plays fundamental role in optical processes, and various modes of energy transfer are involved in sample solution are collision between base fluid molecules, thermal diffusion in the molecules suspended in fluids, collision between the samples due to the Brownian motion, thermal interactions of dynamic or dancing samples with base fluid molecules and light induced aggregation of samples, leading to the modifications of ground state and excited states of the molecules. Such solvent effects mainly the interaction of dye molecules to the solvent surroundings which depends on the arising forces which can be determined by the charge distribution and polarizability of the solvent and solute molecules<sup>[15, 16]</sup>.

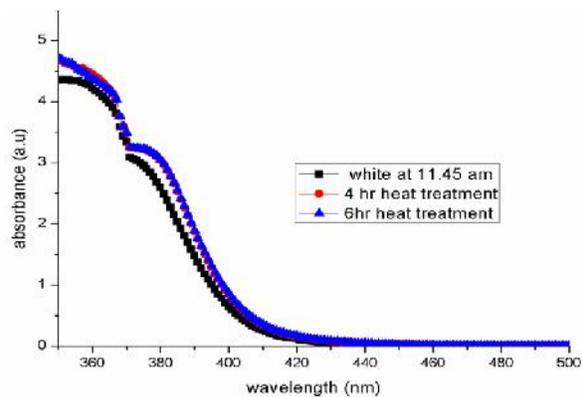


FIGURE 3: linear absorption spectra of confederate rose at different heat treatment

Methanol is the appropriate solvent in the extraction of phenolic compounds from plant tissues due to its facility to inhibit the action of polyphenol oxidases that cause the oxidation of phenolic compounds and its ease of evaporation compared with water<sup>[16]</sup>. The linear absorption spectrum of confederate rose at three different times is shown in fig 2. We further determined whether the dye from confederate rose at 11.45am is subjected to LED light illumination and external heat treatment (40°C) would show differential colour change. The linear

absorption spectra for the external heat treatment and the LED illumination are shown in fig 3 and 4. Light and heat treated dye from the flowers on the same plant revealed the shift in the spectrum may attributed to the production of the anthocyanin compound. But from fig 3 and 4 it was clear that the external factors not so much trigger the biosynthesis of anthocyanin compound. The response of these triggers is slow but regulates the anthocyanin biosynthetic pathway.

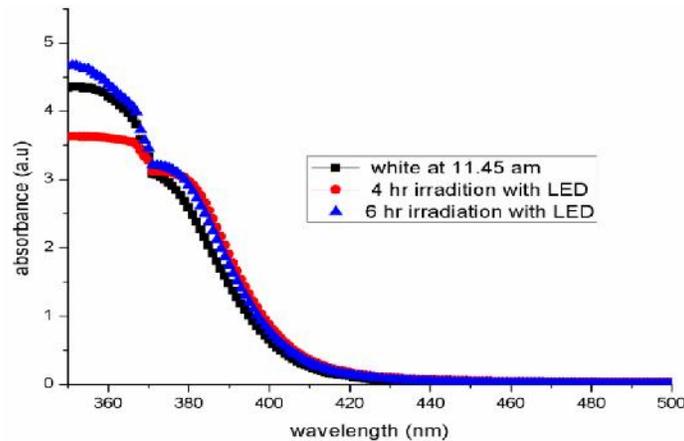


FIGURE 4: linear absorption spectra of confederate rose irradiated with super luminescent LED

The basic absorption processes in dyes can be divided into linear and nonlinear absorption. Nonlinear absorption is a phenomenon defined as a nonlinear change (increase or decrease) in absorption with intensity. This can be of either two types: saturable absorption (SA) and reverse saturable absorption (RSA). Depending on the pump intensity and on the absorption cross section at the excitation wavelength, most molecules show nonlinear absorption. If, however, the excited state has strong absorption compared with that of the ground state, the transmission will show RSA characteristics, or else SA characteristics. Therefore, it is necessary to identify their nonlinear absorption effects, and to determine their nonlinear absorption parameters, such as the saturable intensity for saturable absorber, the TPA coefficient for two-photon absorbing material. These properties are commonly defined in terms of intensity dependant non linear absorption coefficient  $\alpha(I)$ , which can be written in terms of linear absorption coefficient  $\alpha$  and two photon absorption coefficient  $\beta$  as

$$\alpha(I) = \alpha + \beta I \tag{1}$$

Fig 5 shows the open aperture Z scan curve of dye from confederate rose at 5.30 pm. The data is analyzed using the procedures described by Sheik-Bahae *et al.* for a two-photon absorption (TPA) process.

The transmitted OA Z scan signal is given by

$$T(z, S = 1) = \sum_{m=0}^{\infty} \frac{[-q_0(z, 0)]^m}{(m+1)^{\frac{3}{2}}} \tag{2}$$

Where

$$q_0(z, t) = \frac{\beta I_0(t) L_{eff} z_0^2}{z^2 + z_0^2} \tag{3}$$

$$L_{eff} = [1 - \exp(-\alpha l)] / \alpha \tag{4}$$

Where  $m$  is an integer, the parameter  $q_0$  can be obtained by fitting the experimental results to the equation (2). where  $Z_0$  and  $Z$  are the Rayleigh range and the translated length parallel to the beam propagation, respectively.  $I_0$  denotes the intensity of the incident beam at the focal point.

When SA is present eq (1) modifies to  $\alpha(I) = \alpha_0 / (1 + I/I_s)$  (5)

If excitation intensity  $I_0$  is less than  $I_s$ , we can consider SA as a third order process and in such cases  $-\alpha_0 / I_s$  is equivalent to nonlinear absorption coefficient  $\beta$ .

The mechanism of non linear absorption is a combination of SA and RSA. the RSA arises due to two photon absorption and photochemical reaction takes place which converts one chromophore to another. This photochemical change is transient and reversible below a threshold intensity. when irradiation is 290MW/cm<sup>2</sup> the intensity at -0.5 with respect to the focal point we have sufficient intensity to saturate  $S_0$  to  $S_1$  transition which appears as peak inside the RSA at intermediate level. At 508MW/cm<sup>2</sup> the power level is so high at the positive side which is symmetric with respect to the negative side, exhibiting a SA around 0.5 due to ground state bleaching. At 363 MW/cm<sup>2</sup>, the intensity is well above the threshold at the focal point causing the photochemical reaction as well as RSA. The simultaneous occurrence of the non linear behaviour of dye from confederate rose can be made use of in developing various photonic devices.

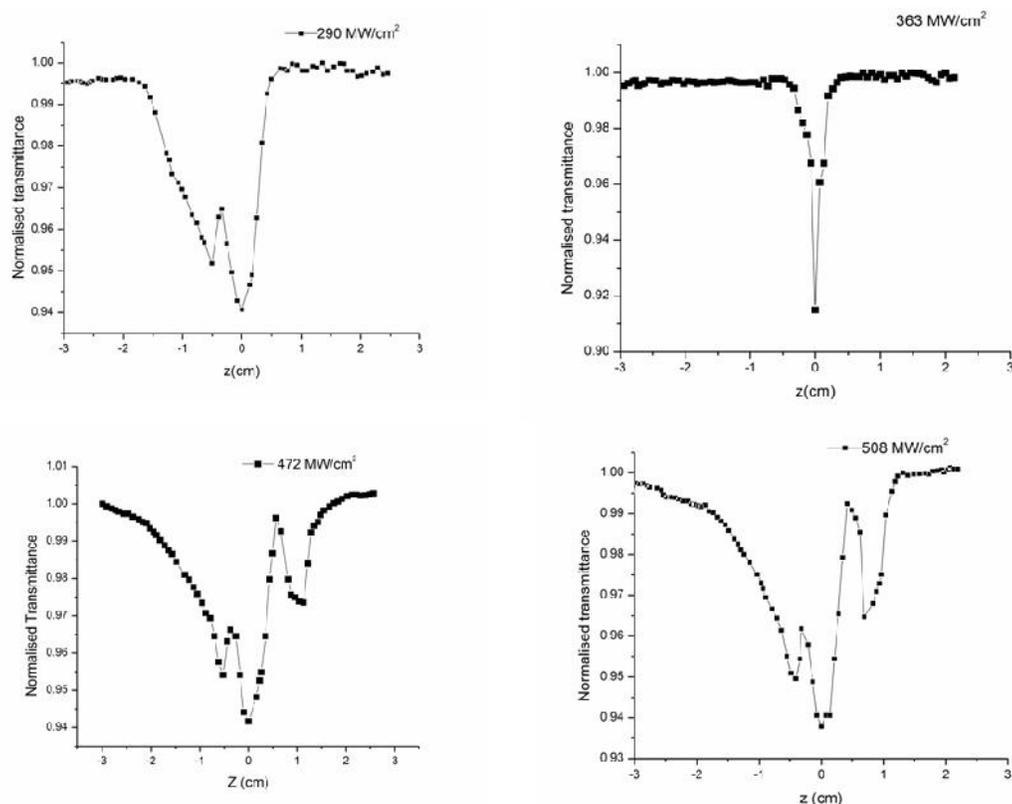


FIGURE 5: Open aperture Z scan curve of dye from confederate rose

## CONCLUSION

Flowers on plants subjected to LED illumination and the external heat treatment did not change colour, while flowers of equivalent age on plants maintained in the light did change colour, were characterized by UV-VIS spectroscopy. The effect of super luminescence LED and the external heat treatment are different from that corresponding to sunlight. Unlike sunlight LED has discrete spectral lines, some of which will be absorbed. The mechanism of non linear absorption is a combination of SA and RSA. Due to the simultaneous occurrence of the non linear behavior this can be made use of in developing various photonic devices.

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