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Measurement of Beet Root Extract Fluorescence Using TR-LIF Technique

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Laser induced fluorescence is a powerful spectroscopic technique commonly used to study the structure and internal state distributions in molecules of biological interest. Betanin ($C_{24}H_{26}N_2O_{13}$) is a specific violet betacyanin and the most prominent pigment in the red beet root where it contributes to 75–95% of the total visible color. Our method of excitation of the beet root extract is based on the tunable (320 nm to 475 nm) Nd:YAG laser system. Fluorescence images of beet root extract excited at 320, 340, 360 and 400 nm were obtained. The fluorescence is observed in range from 580 nm to 660 nm. The influence of the solution concentrations on the fluorescence intensity is also analyzed.

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1. Introduction

A variety of different pigments are produced by nature. The main food pigments found in the common red beet *(Beta vulgaris)* are the betalains, water soluble pigments. Two main groups of betalains are red-violet betacyanins and the yellow betaxanthins. Betaxanthins are relatively stable and do not have antioxidant properties. The most important betacyanin in red beet is betanin, which is a betanidin 5-O-β-glucoside.

Betanin ($C_{24}H_{27}N_2O_{13}$) makes up 75–95% of the total colouring matter found in the beet root, therefore it is used as a natural food coloring agent [1]. This pigment, like other betacyanins, is highly susceptible to changes induced by both pH and temperature [2]. As a powerful antioxidant pigment, betanin may provide protection and reduce risk of cardiovascular disease and cancer [3]. Bioassays in the mouse skin and lung clearly revealed betanin to be a potent cancer chemopreventive agent [4, 5]. High antioxidant activity of betanin is associated with phenolic and cyclic amine groups. Both of which are very good electron donors, acting as antioxidants [6]. Some compounds in beet root juice emit a strong fluorescence suitable for direct measurements [7, 8]. The growing interest in betalains is demonstrated by the floral fluorescence effect that is crucial for pollination. In flower of *Mirabilis jalapa* there is observed that visible fluorescence is emitted by yellow pigment betaxanthin and is absorbed by another, violet betacyanin [9, 10]. Investigation of natural corrosion inhibitors is attracted because they are not toxic, biodegradable and not expensive. An aqueous extract of beet root in presence of Zn^{2+} is used to control and inhibit the corrosion of carbon steel [11].

In the present study, beet root juice was investigated in relation to its fluorescent properties. Also, we study the changes of the fluorescence wavelength range and intensity, with the different laser excitation wavelengths and different solution concentrations.

2. Experimental setup

The experimental time resolved laser-induced fluorescence (TR-LIF) setup is shown in Fig. 1. Sample in quartz cuvette was illuminated using a tunable Nd:YAG laser system (Vibrant models 266-I made by Opotek, Inc.). This system incorporates the optical parametric oscillator (OPO) that is pumped by the fourth harmonics of the Nd:YAG Brilliant laser at 266 nm and control electronics. The output of the OPO can be continuously tuned over a spectral range from 320 nm to 475 nm. The samples were illuminated by 5.4 ns pulses with energy up to ≈ 50 mJ at 266 nm at repetition rate of 10 Hz.

The laser induced fluorescence in the samples was recorded using streakscope (Hamamatsu model C4334-01) with integrated video streak camera. The streak camera allows detection sensitivities in the photon counting region and enables a wide range of fluorescence lifetime measurements from ps to ms with high accuracy. For all spectra measurements, the emission was collected at 90° from the excitation and dispersed by a 0.3 m focal length triple grating imaging spectrograph (SpectraPro-2300i). The spectrograph is added to

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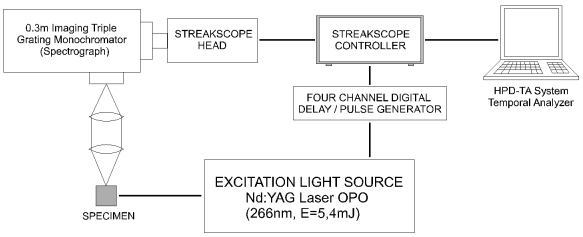

Fig. 1. Schematic illustration of experimental setup for TR-LIF spectroscopy.

the system for simultaneous wavelength and time measurements ($I_f(\lambda, t)$). The fluorescence data have been acquired in photon counting mode using HPD-TA software. All the measurements were made at room temperature.

The juices of fresh beet-root were prepared by separation of the juice in a juice maker. After that, juice was filtered to remove impurities. The 0.25 ml native beet root juice extract was diluted with 2 ml distilled water (solution volume proportion 1:8). Such obtained solution was diluted 2 times, 4 times and 8 times and we measured the fluorescence of these solutions.

As shown in literature [8, 9], betanin, the main pigment of the beet root extract, shows no fluorescence, it absorbs light and weakens measured fluorescence. Fluorescence is due to the presence of other beet-roots pigments.

3. Results and discussion

Streak images of the fluorescence spectrum at 360 nm tunable laser excitation of beet root water solution are shown in Fig. 2. We measured $I_f(\lambda, t)$ using a high MCP gain and a 500 s accumulation time in photon counting mode. We varied the solution concentration, seeking the maximum intensity of fluorescence. Maximum intensity of fluorescence is observed in Fig. 2b, so this concentration is regarded as optimal. We did not determine the concentration of pigments in solution, but comparing our results with results presented in [10], concentration of about 6 μ M should be a good guess.

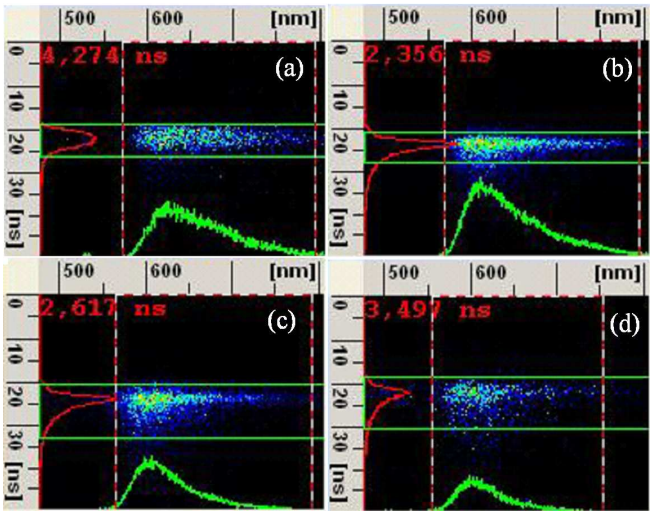

Fig. 2. Streak images of the fluorescence spectrum at 360 nm tunable laser excitation of beet root water solution: (a) 0.25 ml beet-root juice + 2 ml distilled water, (b) 2 times diluted, (c) 4 times diluted, (d) 8 times diluted.

We also compare characteristics of fluorescence as a function of excitation wavelength. Instead of showing all streak images, fluorescence intensity of beet-root juice (optimal concentration) as a function of OPO tuned excitation wavelength is shown in Fig. 3a, and as a function of time in Fig. 3b. As can be seen from Fig. 3a, b, the intensity of fluorescence is the most prominent at the 340 nm laser excitation. The intensity decreases gradually as the excitation wavelength increases up to 400 nm excitation. However, the result obtained at the 320 nm excitation showed the least intensity of the fluorescence spectra.

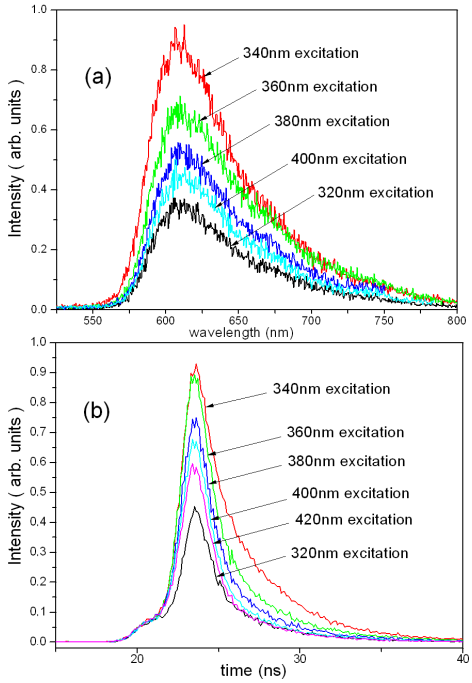

Fig. 3. (a) Fluorescence spectra of beet-root juice (optimal concentration) as a function of OPO tuned excitation wavelength. (b) Fluorescence spectra as a function of time.

4. Conclusion

We presented the beet root extract fluorescence images obtained by the TR-LIF detection system. The dependence of fluorescence intensity on the solution concentrations was analyzed. The fluorescence spectra of beet root water solution were acquired at different excitation wavelengths.

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