Sub-Nanosecond Fluorescence Lifetimes Using Light Pulses Of Several Nanoseconds Width

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Abstract: This work demonstrates the possibility of obtaining fluorescence lifetimes in the sub-nanosecond timescale using excitation light pulses with few nanoseconds width. Measured and calculated fluorescence lifetime values where confirmed using more sophisticated systems with pico-second and-femto second pulse width excitation sources. For demonstration purposes the variation in fluorescence lifetimes for α-tocopherol in polar and nonpolar solvents is presented.

Keywords: Fluorescence lifetime, benzimidazole, αα-tocopherole, fluorescence decaytime, pesticides, TCSPC.

I. Introduction

Fluorescence lifetime is usually defined as the time needed for the light intensity to decrease by the fraction **I/e,** where ***e*** is the base of the Napierian logarithm system [1]. Fluorescence lifetime measurements are extremely and exceptionally useful tool for investigating the dynamics of excited states in biology, chemistry, and physics [2-6]. It is also used to probe the environment around molecules, study mechanisms of complexation between guest and host molecules or to understand molecular charge transfer, (inter or intra molecular) [7,8]. Two main techniques are used for obtaining time-resolved fluorescence. The most commonly used is Time-Correlated Single-Photon Counting (TCSPC) while the other is Multi-Frequency Phase-Modulation Fluorometry (MFPMF) [9]. Both techniques yield essentially the same information but differ mainly in how time-resolved fluorescence data are obtained. While MFPMF measures the phase difference the excitation light wave and emitted light wave, TCSPC is built around the use of extremely narrow width light pulses, delta function for ideal cases, to excite the sample molecules and measure the time difference between excitation and emission. Until recently, the sources of light excitation were limited to either thyratron gated gas discharge flash lamps or Q-switched lasers. The minimum pulse width that can be achieved by gas discharge based flash lamps is about 2-3 nanoseconds, while Q-switched lasers, combined with special techniques, pulse width down to few hundred femto-seconds could be produced [10]. Practically, flash lamps are more commonly used for their low cost, easy operation, easy maintainance, simple construction and small size. Q-switched lasers, however, are very costly, difficult to operate and maintain, in addition to special technical experience. As a conclusion, sub-nanosecond lifetime measurements are difficult and costly as the use of sub-nanosecond light pulses is a must for such measurements.

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The main purpose of the present work is to show the possibility of obtaining sub-nanosecond fluorescence lifetimes using excitation light pulses, generated by gas discharge flash lamps, with time duration much longer than the expected sample lifetime. Mathematical deconvolution software is used to extract the fluorescence decay from the combined decay curve containing light source & sample. The measured lifetime values were confirmed by pico-second and femtosecond excited TCSPC systems. The reported results encourage the use of less sophisticated excitation light sources such as thyratron controlled flash lamps to measure fluorescence lifetimes that was only possible by sophisticated pico and femto second pulsed laser sources.

II. Experimental

- **Chemicals and glassware,**
  Spectroscopic pure hexane, acetonitrile and methanol solvents were purchased from Aldrich Company. There purity was confirmed by spectrofluorometry where no emission was detected when solvents were excited between (270 –500 nm). Solid benzimidazole standard was given as a gift by a local pesticides production company MOBEDCO. Pure α-tocopherol sample was purchased from Sigma Aldrich and used as received. UV and mass spectra confirmed the purity of all samples. Fused silica sample cuvettes of 3.0ml volume and 1cm optical path length were used in all measurements.
- **Instrumentation,**
  Mass spectra measurements were performed on VG- England E/B sector field system model 7070E. EI mode of ionization set at 70eV energy was used. Labomed UV-Vis spectrophotometer Model Dual-PC was used to record the absorption spectral data. All fluorescence measurements were recorded on a home-assembled spectrofluoremeter described in a previous work [11]**.** Fluorescence lifetime measurements were performed using Edinburgh time correlated single photon counting system (TCSPC) model 199. The excitation light source employed is a thyratron controlled low pressure flash lamp usually filled with high purity hydrogen or nitrogen gas. The lamp generates intense UV pulses of 2-3 ns width, fast rise time and 25 kHz repetition rate. The time difference between excitation and emission is determined by

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a device known as Time to Amplitude Converter (TAC), its simplified block diagram is depicted in Figure (1) and full details about the instrument are described elsewhere, [12]. Decays were processed against lamp decay using mathematical deconvolution software provided by Edinburgh Instruments. The software generates functions composed of one or more exponential that best fits with the experimentally obtained decay. Chi-square values as close as possible to unity are the measure for the accepted life time values. The software is capable of processing up to 3 exponential decays in the same fit. For confirmation purposes, LED excited TCSPC (Jobin-Yvon model IBH-5000U) and laser excited TCSPS, (Tsunami, mode-locked Ti:Sapphire laser) were used for fluorescence lifetime measurements, [13,14].

III Results and discussion

In an effort to characterize a number of benzimidazole based pesticides using UV spectral analysis the fluorescence lifetime for the parent molecule namely 1H-benzimidazole could not be found in the literature. Patricia C. Tway' et. al. reported the fluorescence lifetime for several molecules except 1H-benzimidazole which was attributed to instrumental limitations [15]. For this purpose the absorption and emission spectra for a pure sample of 1H-benzimidazole dissolved in acetonitrile were measured and shown in Figure-2. The structural features and values of absorption and emission maxima agree with those found previously [16]. For further confirmation of sample identity the mass spectrum for a solid sample of the compound was found to agree with that reported in NIST-MS, [17]. The fluorescence lifetime for 1H-benzomidazole, was measured using flash lamp excited TCSPC with a minimum pulse width of 3-nsec. The resulting sample spectral decay appeared to have a very fast decay that is very close to that of the lamp profile. In processing the spectral decay data using the mathematical deconvolution, the calculated fluorescence life time came to be 0.7 nsec. As this value is much shorter than the excitation pulse width it was difficult to trust, Figure-3. The value was considered as a meaningless mathematical coefficient generated for fitting purposes. When much shorter excitation light pulse (0.7 nsec) was employed using the new nano-LED sources the lifetime value of benzimidazole came to be similar to that produced by the hydrogen flash lamp, Figure-4. Such result encouraged us to trust the value obtained by the hydrogen flash lamp and to think about further confirmation using a femto-second TSPC instrument. The fluorescence decay for the same molecule is shown in Figure-5 and similar lifetime value was obtained. Fluorescence lifetimes for other standard molecules were made for molecules were made using flash lamp and compared with those obtained by the LED excited TCSPC system. The measured life time values were in good agreement with those reported by Boens et. al [9]. For demonstration purposes, the effect of solvent on fluorescence lifetime of α-tocopherol (Vitamin E) using hydrogen flash lamp is shown in Figure-6 where a lifetime values of 0.8, 0.9 and 1.0 nsec for the molecule in hexane acetonitrile and methanol respectively. These results clearly indicated that flash lamps are still powerful enough to show the effect of solvent polarity on fluorescence lifetime by resolving the small differences in the fluorescence decay without the need of sophisticated instrumentation.

IV. Acknowledgement:

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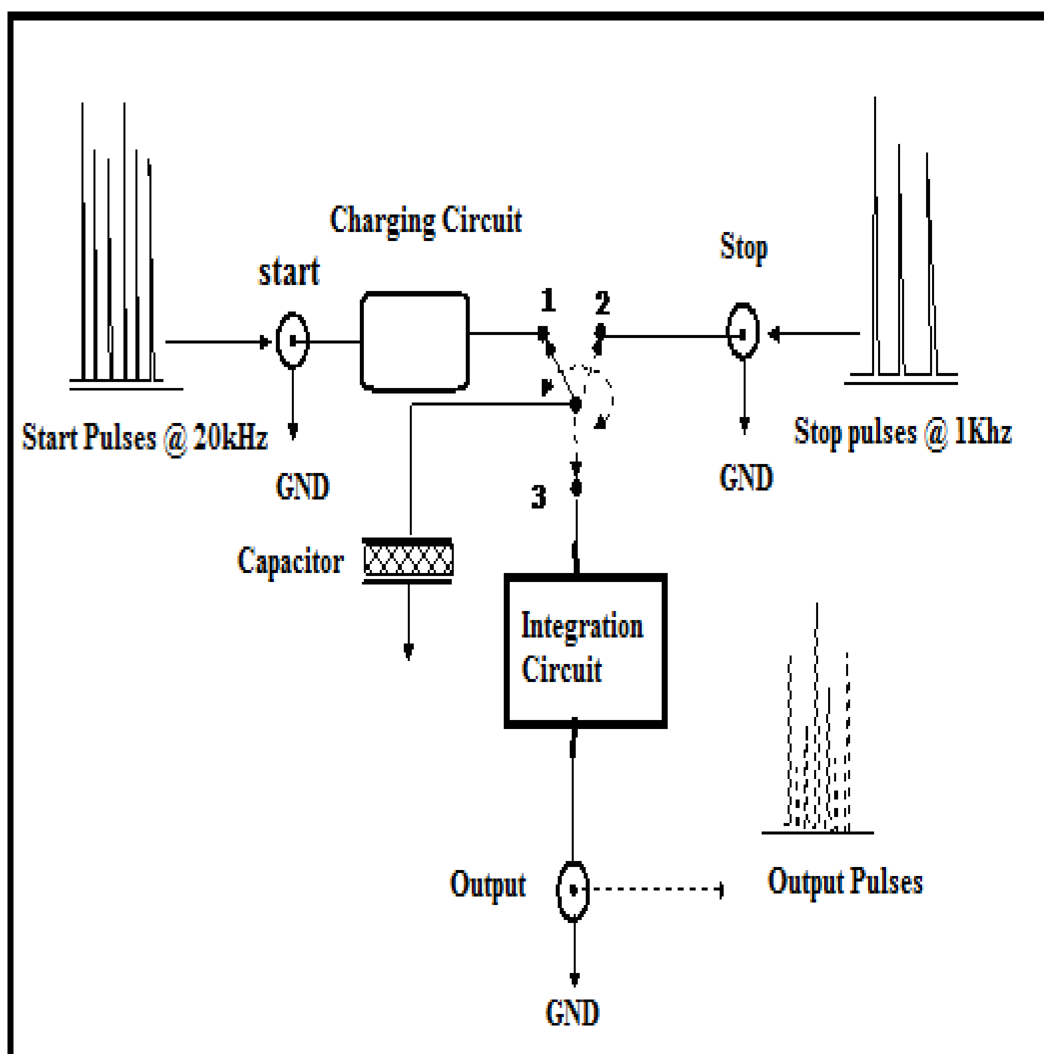

Fig. 1 Components of a typical TAC unit

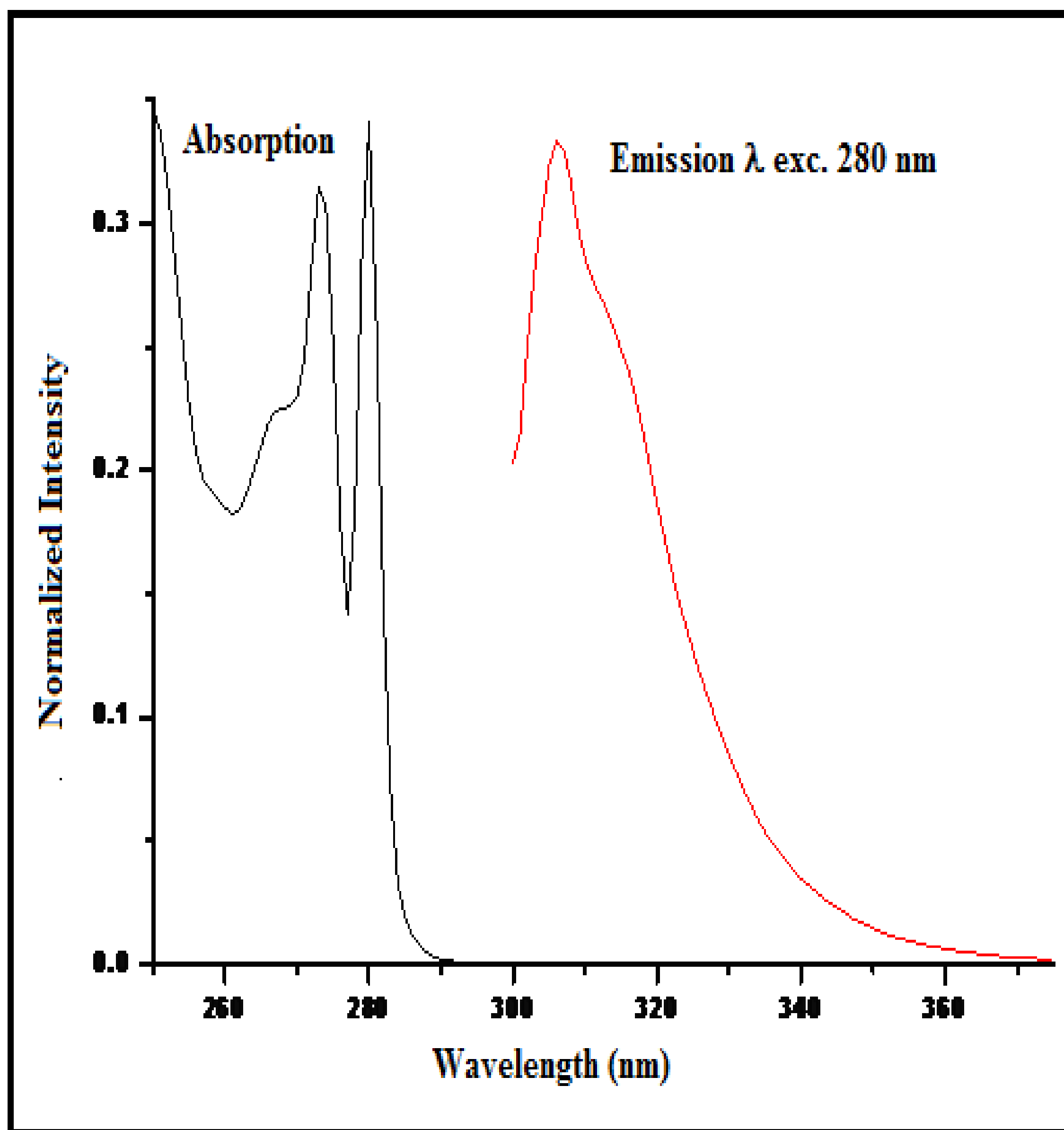

Figure-2: UV absorption and fluorescence spectra for 1H-benzimidazole in acetonitrile solution,

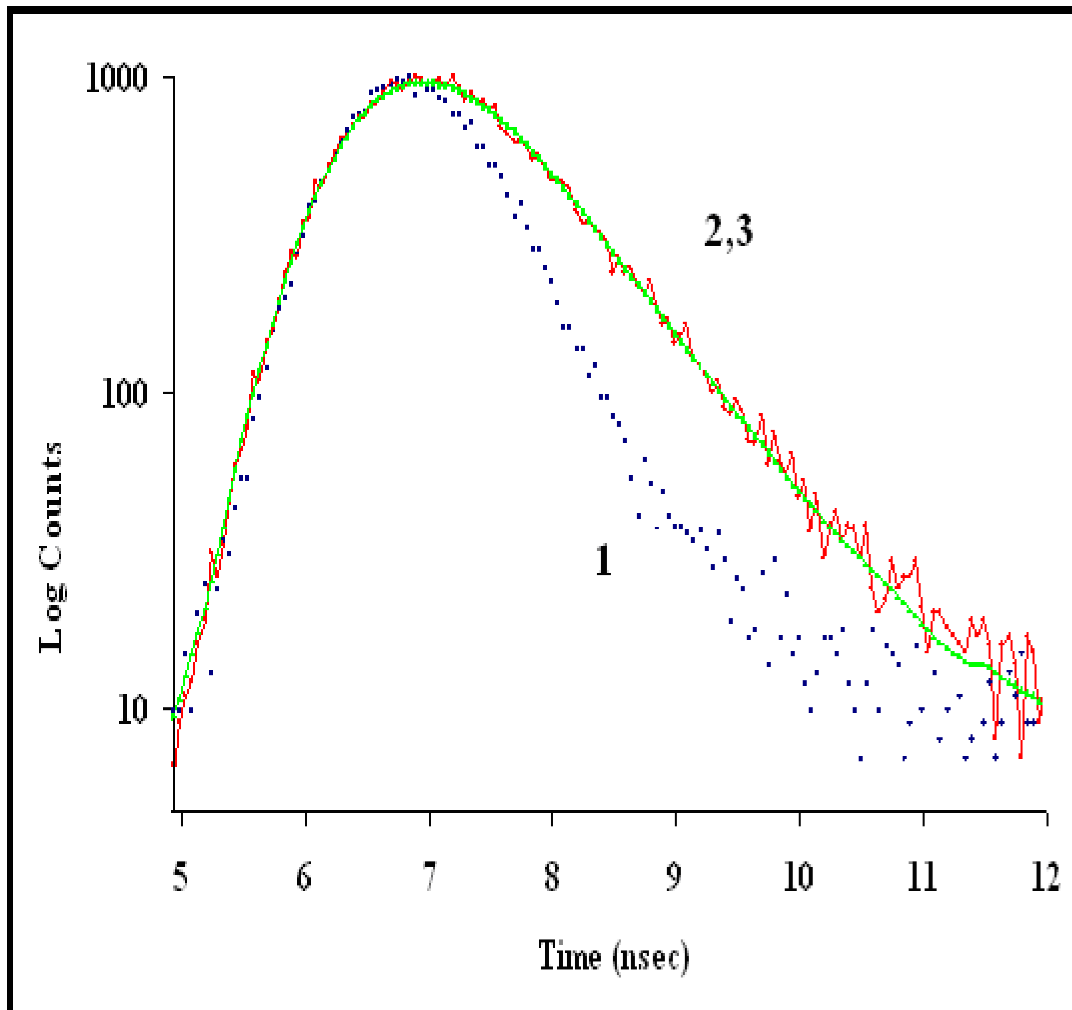

Figure-3: Fluorescence decay curves: H_2 flash lamp (1), 1H-Benzimidazole (2) and theoretical curve fit (3), in acetonitrile solution.

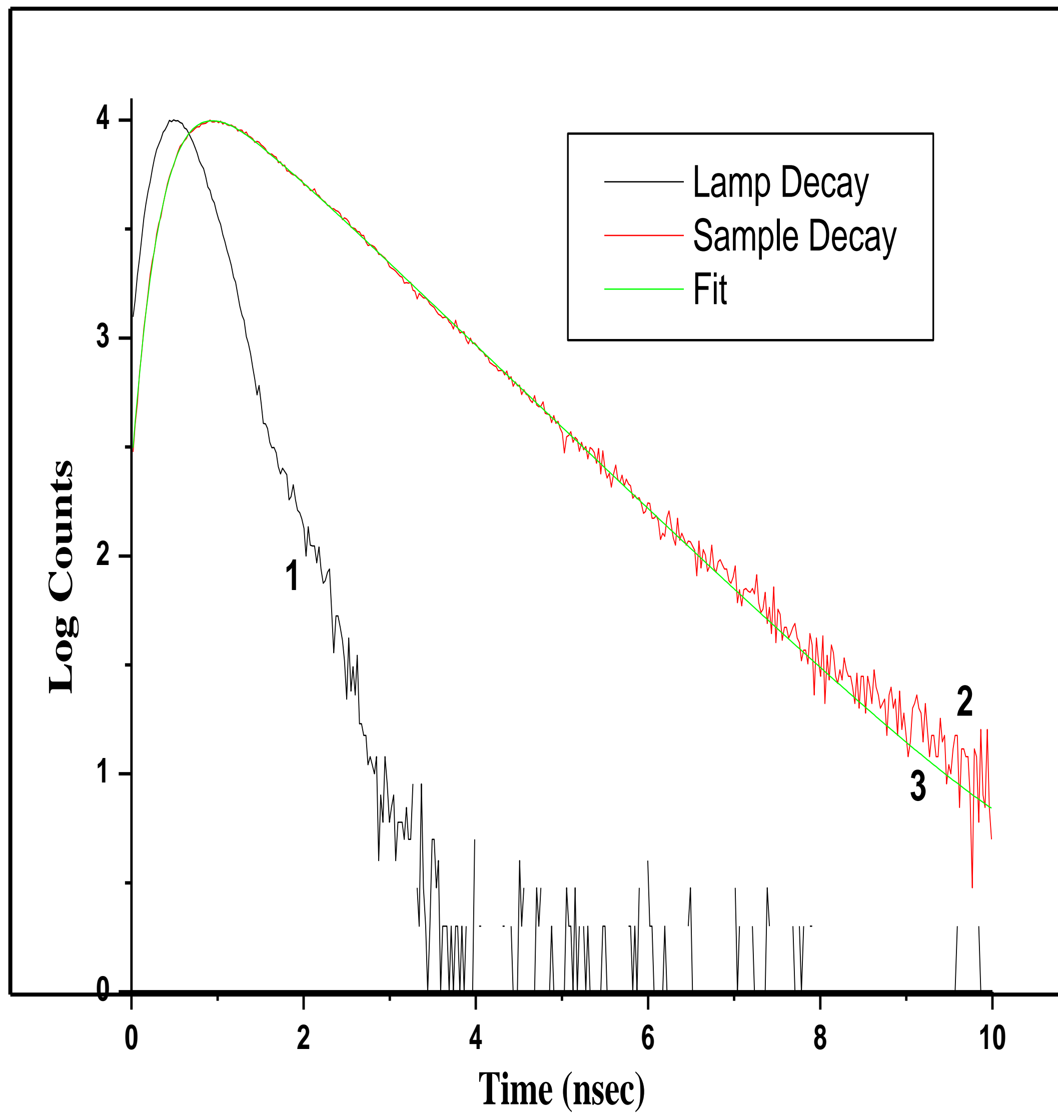

Figure-4: Fluorescence decay curves: nano-LED (1), 1H-Benzimidazole (2) and theoretical curve fit (3), in acetonitrile solution.

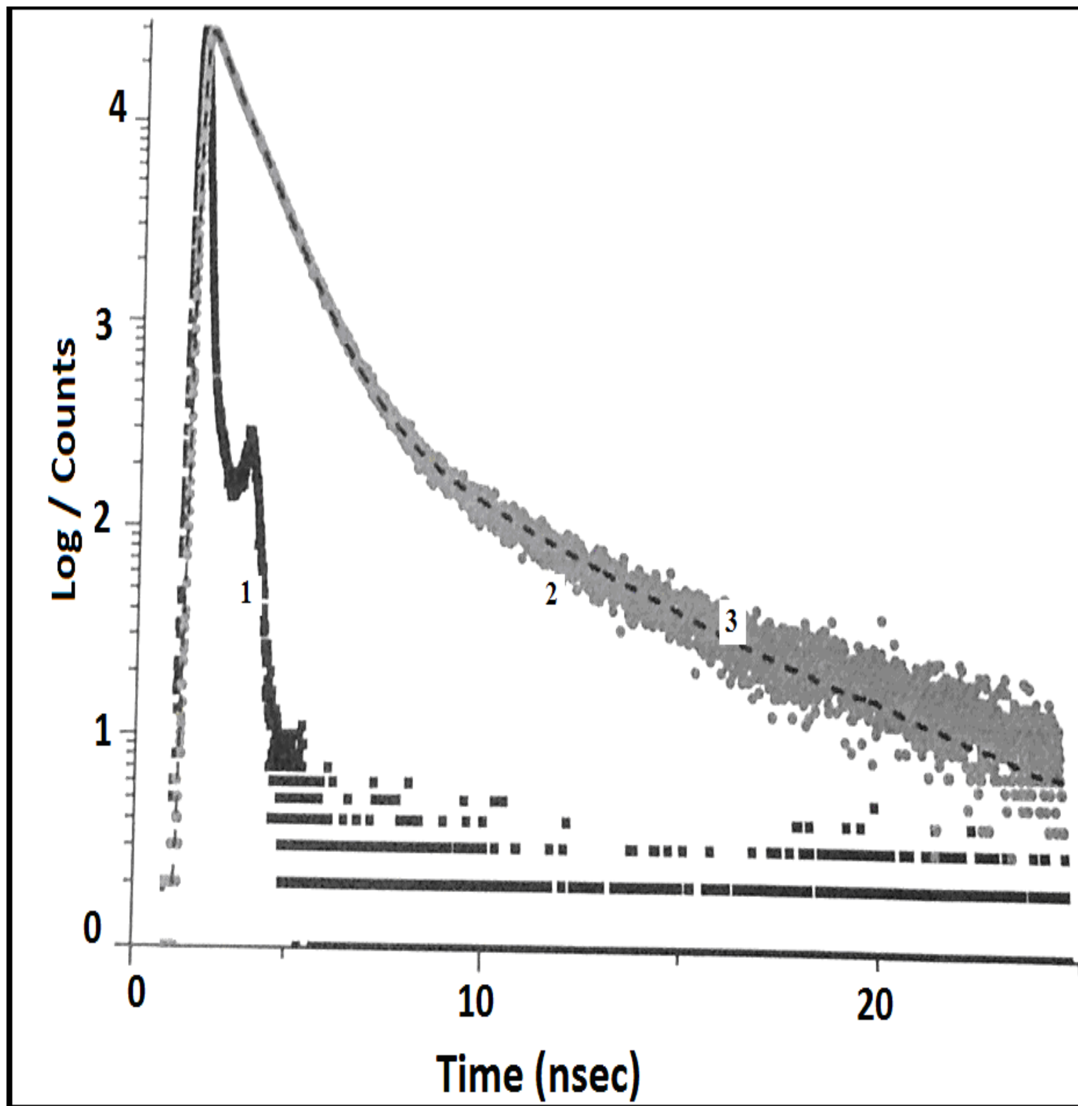

Figure-5: Fluorescence decay curves: femtosecond laser (1), 1H-Benzimidazole (2) and theoretical curve fit (3), in acetonitrile solution.

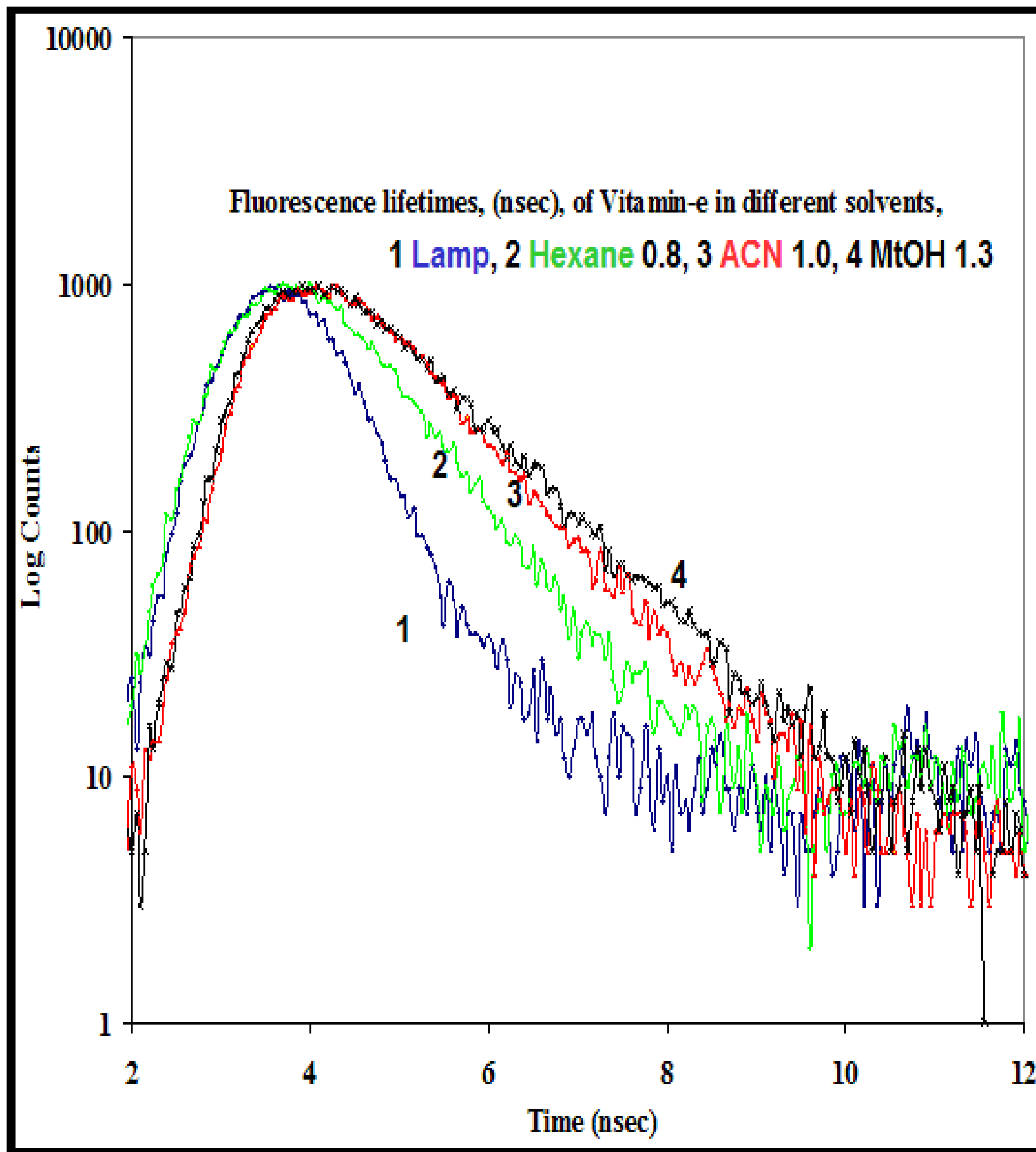

Figure-6: Fluorescence decay curves H_2 flash lamp (1), α-tocopherol in Hexane (2), α-tocopherol in acetonitrile (3), α-tocopherol in methanol solution (4).