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ORIGINAL PAPER

Laser Induced Fluorescence of Biochemical for UV LIDAR Application

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Abstract Laser induced fluorescence spectroscopy in the ultraviolet regime has been used for the detection of biochemical through a fiber coupled CCD detector from a distance of 2 m. The effect of concentration and laser excitation energy on the fluorescence spectra of nicotinamide adenine dinucleotide (NADH) has been investigated. The signature fluorescence peak of NADH was centred about 460 nm. At lower concentration Raman peak centred at 405 nm was also observed. The origin of this peak has been discussed. Detection limit with the proposed set up is found to be 1 ppm.

Keywords Laser induced fluorescence · Biochemical · Detection

Introduction

Biological agents have emerged as a real and potentially immediate threat. They are relatively cheap to manufacture and deploy, and they have tremendous potential impact as terror tools against society [1, 2]. Biological agents (BA) may be spread in form of aerosol cloud. The infectious and lethal doses of these BA for causing the damage are very small. Thus there is a need to develop a system to provide warning of a remote biological impact before getting exposed. UV-LIDAR system based on laser induced fluorescence (LIF) technique has emerged as a potential technology for standoff-detection of BA. Fluorescence methods may be used not only to detect but for quantitative estimates of fluorophores as well. BWA tends to fluoresce at its maximum when excited in the absorption wavelength band for that target [3–5]. The key constituent molecules evident from fluorescence spectra of bi- aerosols are the amino acids tryptophan, tyrosine, and nicotinamide adenine dinucleotide (NADH) contributing to the molecule's total fluorescence in amounts relative to the wavelength of the incident beam as well as their concentration and location within the molecule. Tryptophan, for example, is highly fluorescent when excited with a 266 nm wavelength and [6, 7]. The absorption maximum for NADH is around 340 nm. NADH is the reduced form of nicotinamide adenine dinucleotide (NAD^+) and is important in cell respiration and hence metabolism and this reduced form shows fluorescence when excited. NADH is a protein and an important part as the universal energy carrier for cells and is therefore present in spores or viruses, bacteria and other living cells. It is excited around 340–355 nm, and has an emission band in 400–600 nm. Thus, biologically active bio-agents are best detected with a ~355 nm wavelength. Therefore, a UV-LIF LIDAR based on Nd: YAG laser emitting at 355 nm is an excellent candidate to perform standoff UV-LIF LIDAR bioaerosol monitoring [8].

In this paper, we present the experiments of measured fluorescence spectra of NADH at varying concentrations (ppm level) and laser energy and their preliminary data analysis. The origin of Raman peaks will also be discussed. The paper will also discuss the relevance of above studies for determining the limit of detection, a parameter, to be used in development of UV LIDAR for detection of biological agents. The present studies have been performed in the lab environment from a distance of 2 m with fiber coupled detection. The results and analysis of present studies will help in the future experiments to be performed in field scenario.

Experimental Set-Up

The experimental set-up used in the experiment is shown in Fig. 1. Fourth harmonic Nd:YAG (Make: Quanta) laser emitting at the fundamental wavelength of 1,064 nm with maximum

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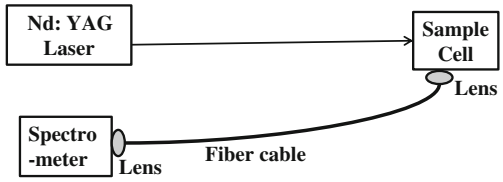

Fig. 1 Schematic of experimental set up

energy 0.7 J @1,064 nm has been employed. The laser wavelengths of 355 nm (third harmonics of 1,064 nm) were used as excitation source in the experiment. Laser pulse energy and pulse repetition frequency are variable and maximum energy is 30 mJ @ 266 nm at 10 Hz repetition rate. The pulse width of the laser is ~5 ns. The laser beam diameter is 8 mm (at $1/e^2$) and it has a divergence of 0.5 mrad. A fiber coupled CCD spectrometer (Make: Ocean Optics) is used as a detector with a spectral width of 200 to 900 nm. The fluorescence signal was coupled to the detector via a fiber cable having 600 μm slit from a distance of 2 m. The fluorescence spectra were recorded in 90º geometry in UV range through visible range (200 nm–700 nm). The samples of different concentration (up to 1 ppm) were prepared and kept in a quartz cuvette placed in cuvette holder. The cuvette holder was kept at distance of 2 m from the laser exit to form a spot size of ~8 mm diameter at the cuvette surface. For each spectrum the sample was exposed only for the single laser pulse. The cuvette holder is designed such that no light is exposed on the sample except the laser energy. Also the experiments were performed in dark lab environment.

Results and Discussion

The UV-LIF spectra of NADH at various concentration levels in the spectral range 200 nm to 700 nm have been recorded. The NADH was excited at laser wavelength of 355 nm closely matching absorbance peak at ~346 nm. Each spectrum was recorded thrice and an average is taken in order to minimize the error. The raw and spectrally processed (background subtracted and fitted) spectra of NADH at one ppm concentration is also shown in Fig. 2. At the higher concentration (50 ppm and above) only the fluorescence signature peak of NADH centred around 460 nm. But as the concentration is lowered signature of a new peak peaking at 403 nm observed which is clear in Fig. 2.

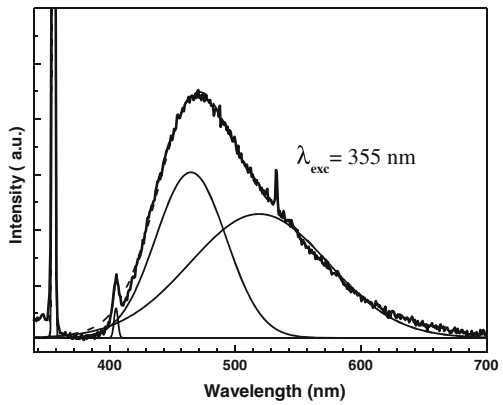

Fig. 2 LIF spectra of NADH in aqueous solution with concentration of 1 ppm. Resolved peaks after peak fitting are also shown peaking about (i) 405 nm (ii) 460 nm and (iii) 525 nm

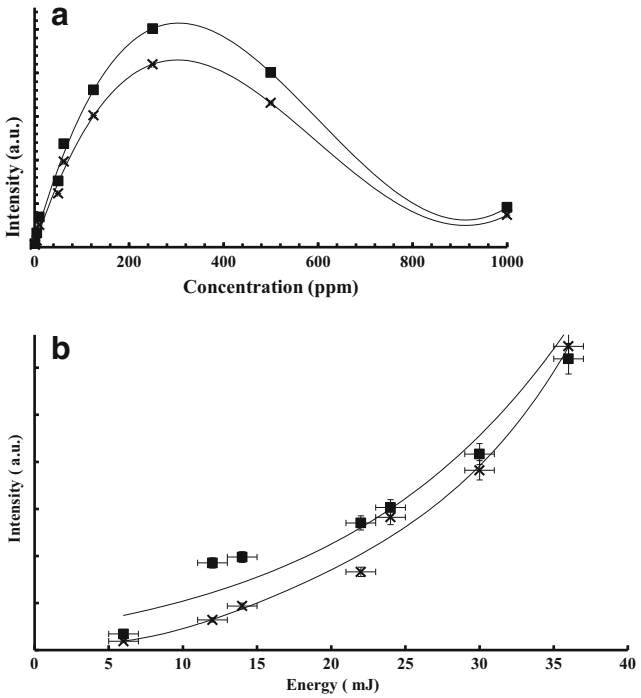

Fig. 3 **a** Intensity of NADH fluorescence peaks with peak centre about 460 nm (*black square*) and 525 nm (**x**) vs. concentration. **b** Intensity of NADH fluorescence peaks with peak centre about 460 nm (*black square*) and 525 nm (**x**) vs. laser energies at 1 ppm concentration

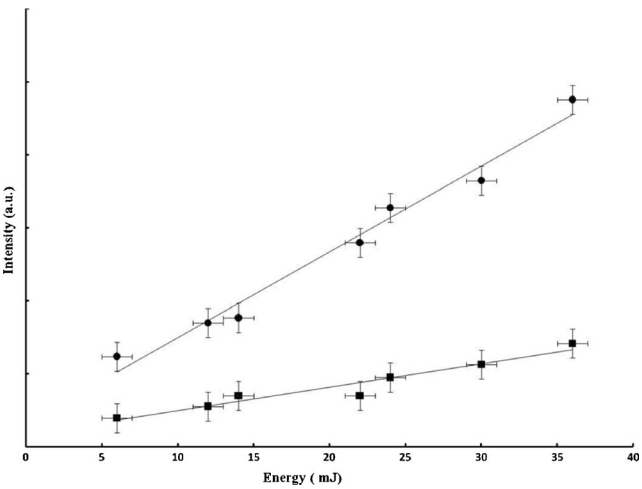

Fig. 4 Intensity of Raman peak (*black square circle*) and ratio of intensties of fluorescence peak (~460 nm) with Raman peak Intensity (*black square*) vs. laser energies

From the shape of the peak and peak position it is revealed that this is due to Raman scattered excited light on the water molecules. The region from 2,700 to 3800 cm^{-1} relates to the O-H stretching modes of liquid water. There are five components in this regime [9]. The component with Raman shift of 3484 cm^{-1} (corresponding to 403 nm) with the highest intensity is observed in the spectra in Fig. 2. At higher concentrations the Raman peak gets masked by the fluorescence spectra of the NADH. The asymmetry at higher side is attributed to another weak fluorescence peak centred about 525 nm which was obtained by peak fit. This fluorescence peak may be due to presence of flavin [10]. Figure 3(a) and (b) shows the concentration and laser energy dependence of the LIF intensities of both these fluorescence peak. The fluorescence peak follows the same concentration and laser energy trends as the signature fluorescence peak of NADH with lower intensities. Thus the signature fluorescence peak remains prominent in the observed concentration and energy regime. The fluorescence intensity increases sharply as the concentration increases till 250 ppm. High concentrations of NADH tend to absorb a significant amount of the excitatory light. As a result of incomplete light saturation of the NADH at the excitation wavelength of 355 nm, at higher NADH concentrations quenching of NADH fluorescence is observed. It was observed during experiment that at concentration below 1 ppm fluorescence signal could not be observed. This is due to increased absorption to fluorescence ratio of the laser photon density at very low concentration. It was also observed that the fluorescence intensity decreases for the samples which were first exposed to UV for longer time and then LIF was recorded. This fact may act as interferent in detecting the standoff signal in actual field scenario, whereby fluorescence intensity may quench due to UV radiation present in the sunlight. A very good correlation between concentration and fluorescence is observed using a 4-parameter logistic fit of the data. Unknown concentrations can safely be determined in this range with a high degree of confidence as the coefficient of determination (r^2) value was calculated to be 0.999. With the present set-up after optimizing the experimental parameters, we could detect the spectral signature of this peak till 1 ppm concentration below which its intensity falls off rapidly. Figure 3(b) shows the LIF spectra of NADH for various excitation laser energies at a concentration of 1 ppm. The intensity however keeps increasing with increasing excitation energy within the observed energy regime. This increase also suggest that though the NADH is photo chemically active but within the present laser energies and exposure limit fluorescence properties of this remains unaltered as is clear from Fig. 3(b). No shift was observed in the peak position with energy. Unaltered peak position feature with energy will be fruitful to detect NADH from longer distances by increasing energy. The intensity of the Raman peak of water also increases with the increased laser energies. The ratio of intensities of fluorescence and Raman peak are plotted in Fig. 4. The ratio increases linearly which may become a parameter in the design of UV-LIDAR system for distinguishing the fluorescence spectra at higher concentration (above which quenching effects comes into play) from that observed at lower concentration. Detection limits and in turn standoff detection distance can be further enhanced using more sensitive detector viz. multichannel PMT and collection optics.

Conclusion

The LIF spectra of NADH were recorded from a distance of 2 m and analyzed after peak fit in order to ascertain the true intensities and centre positions of all the peaks. Fluorescence peaks of were observed around 460 nm for NADH and around 525 nm for the FAD. New peaks centred at 405 nm were also observed in the spectrum of NADH at lower concentration which is assigned to O-H stretching of water molecules. The concentration and energy effects on the LIF of NADH have been investigated. The detection limit with the existing fiber coupled CCD detector is found to be 1 ppm.

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