

Detection of bio-chemicals for UV-LIF Lidar application

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Laser induced fluorescence spectroscopy in the ultraviolet (UV-LIF) regime has been used for the detection of bio-chemicals like tyrosine, tryptophan, NADH through a fiber coupled CCD detector. It is observed that the spectrum of the mixture of these bio-chemicals resembles more with that of the tryptophan. The effect of concentration and laser excitation energy on the fluorescence spectra of tryptophan has been investigated. Detection limit with the proposed set-up is found to be one ppm. The study is a part of development of UV-LIF LIDAR system for the standoff detection of biological warfare agents.

Keywords: Laser induced fluorescence, Biochemical, Detection

Introduction

In today's world there is increasing awareness to vulnerabilities to biological attack not only to military assets but civilian targets as well. Thus there is a need to develop a system to provide early warning of a remote biological warfare attack so that protective measures can be taken before getting exposed to infectious and lethal doses of the aerosol cloud.¹⁻² UV-LIDAR system based on laser induced fluorescence (LIF) technique has emerged as a potential technology for standoff-detection of biological warfare agents (BWA)³⁻⁵. The fluorescence spectra of biological agents have been attributed to the proteins and aromatic amino acids specially NADH, riboflavin, tryptophan, tyrosine and phenylalanine etc. which are present in cell material of all biological organisms and the cell wall of bacterial endospores⁶⁻⁷. Thus in the context of detection of biological aerosols; tryptophan, NADH, DPA and tyrosine molecules are of particular interest and it is important to study the fluorescence spectra of these bio-chemicals. Tryptophan ((S)-2-Amino-3-(1H-indol-3-yl)-propionic acid) is an amino acid, with chemical formula $C_{11}H_{12}N_2O_2$ and one of the building blocks in the protein synthesis that occur in cells and present in all biological material, including viruses⁷. Tryptophan has absorption peaks at 230 nm and 280 nm, the latter being of particular interest in standoff detection applications. When excited at 280 nm, tryptophan emits fluorescence with a peak around 350

nm. Tryptophan can also be excited at other UV wavelengths, but with reduced fluorescence intensity as a possible result. Also other biological molecules emit fluorescence, but to a lesser degree and thus less relevant for standoff detection. NADH is the reduced form of nicotinamide adenine dinucleotide and is important in cell respiration and hence metabolism. NADH is a protein and an important part as the universal energy carrier for cells. NADH is therefore present in bacteria and other living cells, but to a lesser degree in spores or viruses. NADH is excited around 340-350 nm, and has an emission peak around 450 nm⁸. Dipiclonic acid (DPA)⁹ is formed during sporification and its absorption and emission spectra resemble that of tryptophan, but it is weak and is expected to be completely masked by emission from tryptophan.

In the present work, LIF studies were performed for three bio-chemicals namely NADH, tryptophan and tyrosine in aqueous solution. The LIF signatures of tryptophan were recorded by varying concentrations (ppm level). The excitation was done using a laser source from a distance of two meter and LIF was recorded through a fiber coupled CCD detector. The fluorescence of tryptophan in aqueous solutions has a maximum at ~350 nm. 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 warfare agents.

Experimental set-up

The experimental set-up used in the experiment is shown in Fig.1. Fourth harmonic Nd:YAG (Make:

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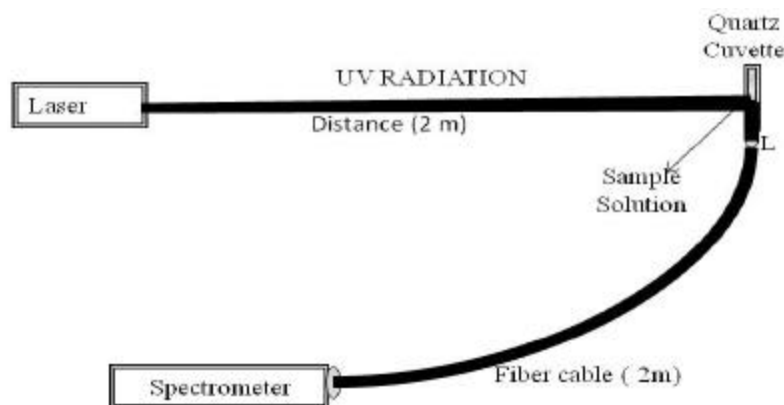


Fig.1—Experimental Set-Up

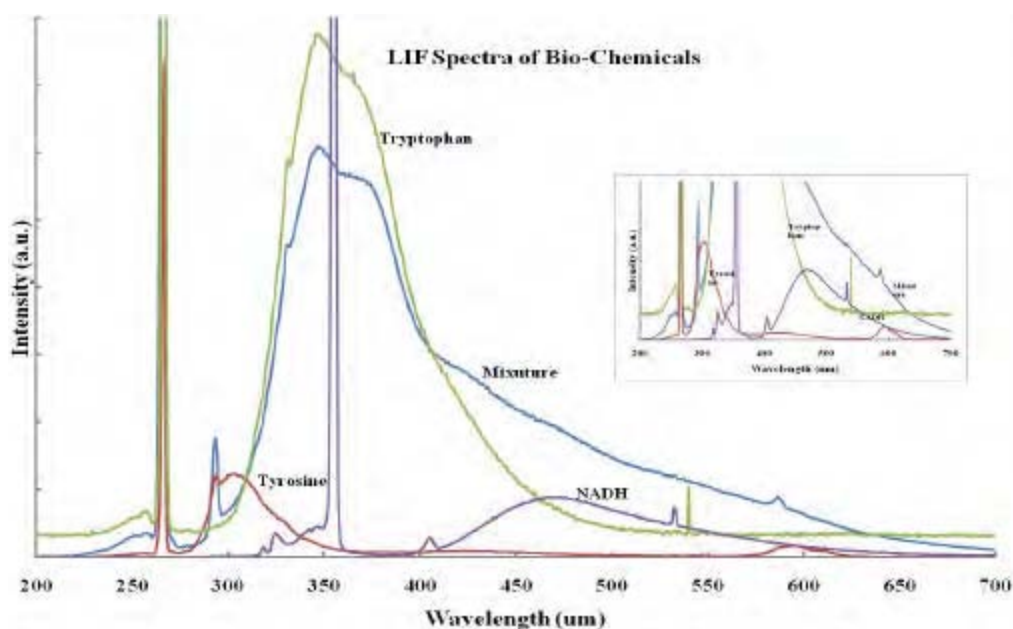


Fig.2—LIF Spectra of Bio-Chemicals Tryptophan, Tyrosine, NADH and mixture at one ppm concentration. Inset shows the enlarged view of same spectra

Quanta) laser emitting at the fundamental wavelength of 1064 nm with maximum energy 0.7 J @1064nm was used. The laser wavelengths of 266 nm and 355 nm (fourth and third harmonics of 1064 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 ~5ns. The laser beam diameter is eight mm (at $1/e^2$) and it has a divergence of 0.5 mrad. A fiber coupled CCD spectrometer (Make: Ocean Optics, Model # QE 65000) is used as a detector with a spectral width of 200 to 900 nm. The fluorescence signal was coupled to the detector via a two meter long fiber having 600 μ m slit. The fluorescence spectra were recorded in 90° geometry in UV range through visible

range (200nm - 700 nm). The samples of different concentration (up to one ppm) were prepared and kept in a quartz cuvette placed in cuvette holder. The cuvette holder was kept at distance of two meter from the laser exit to form a spot size of ~8 mm diameter at the cuvette surface in order to maximize the fluorescence cross-section area. The fluorescence spectra were also recorded by varying the laser energy keeping the concentration constant (one ppm).

Results and Discussion

The UV-LIF spectra of tryptophan, NADH and tyrosine at one ppm concentration in the spectral range 200nm to 700nm are shown in Fig. 2. Also shown are the spectra of three chemicals mixed together and excited

with 266 nm laser wavelength. The laser excitation was done in UV range at 266nm for tyrosine and tryptophan as they have absorbance peak at ~270nm and 280 nm respectively which are near to available excitation laser wavelength. The NADH was excited at laser wavelength of 355nm closely matching absorbance peak is at ~346nm. Each spectrum was recorded thrice and an average is taken in order to minimize the error. The incident laser energy used in the present experiment was ~8.5 mJ. The correction for background was done by subtracting the background from each spectrum. The signature peak of NADH at 457 nm and a new peak at 405 nm at lower concentration observed from the figure. The signature peak of tyrosine is observed at 303 nm in addition to many other peaks (at ~428 nm and ~598 nm) which may be due to different conformers.

The detailed analysis of these spectral features will be published elsewhere. It is clear that spectral features of the mixture resemble that of tryptophan. The signature peak of tryptophan peaking at ~348 nm is observed. The absorbance, fluorescence peaks^{2,10} of these bio-chemicals and available nearest laser excitation wavelength are listed

Table 1

Bio-Chemical	Absorption peak (nm)	Laser Excitation (nm)	Fluorescence (nm)
Tyrosine	273	266	303
Tryptophan	280	266	348
NADH	346	355	457

in Table 1. Due to tryptophan's greater absorptivity, higher quantum yield, and resonance energy transfer, the fluorescence spectrum of a protein containing three bio-chemicals usually resembles that of tryptophan. However the presence of NADH is indicated at the asymmetric tail of the peak. Figure 3(a) shows the concentration profile of tryptophan LIF spectrum. The concentration was varied up-to one ppm. The fluorescence intensity increases sharply below ~200 ppm till one ppm. At concentration higher than 200 ppm fluorescence quenching effects comes into play and intensity shows saturation effect. This is due to high biochemical concentration. The intensity is increased when the concentration is decreased below 200 ppm level due to more fluorescence signal and no quenching. It was observed during experiment that at concentration below one ppm fluorescence intensity decreases. This is due to increased absorption to emission ratio of the laser photon density at very low concentration. It was also observed that with more UV exposure also the fluorescence intensity decreases. 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. With the present set-up after optimizing the experimental parameters, we could detect the spectral signature of this peak till one ppm concentration below which its intensity falls off rapidly. Figure 3(b) shows the LIF spectra of tryptophan for various excitation laser energies at a concentration of one ppm. The intensity however

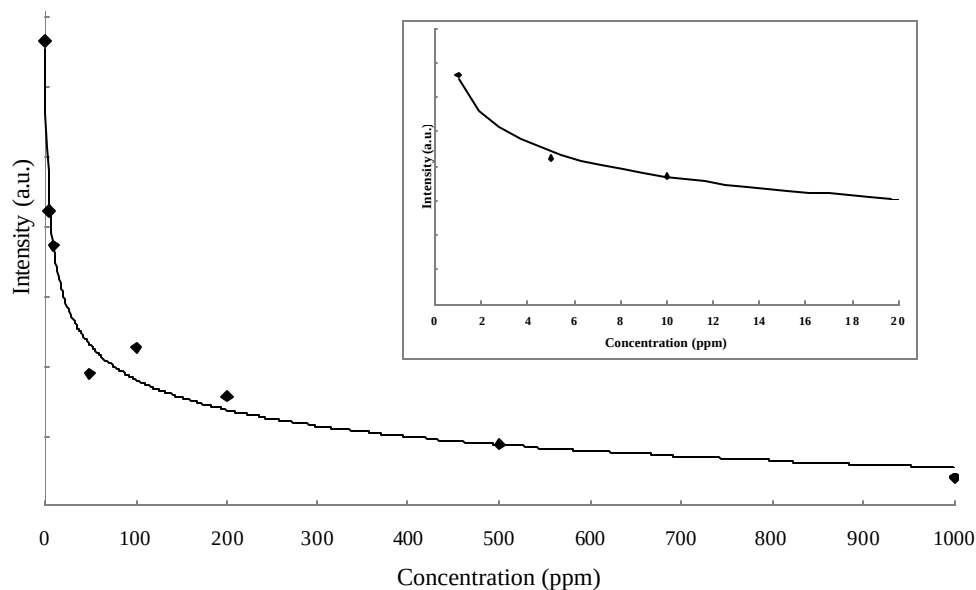


Fig.3a—Concentration vs. LIF intensity at laser energy ~ 8.5 mJ. Solid line is guide to eye

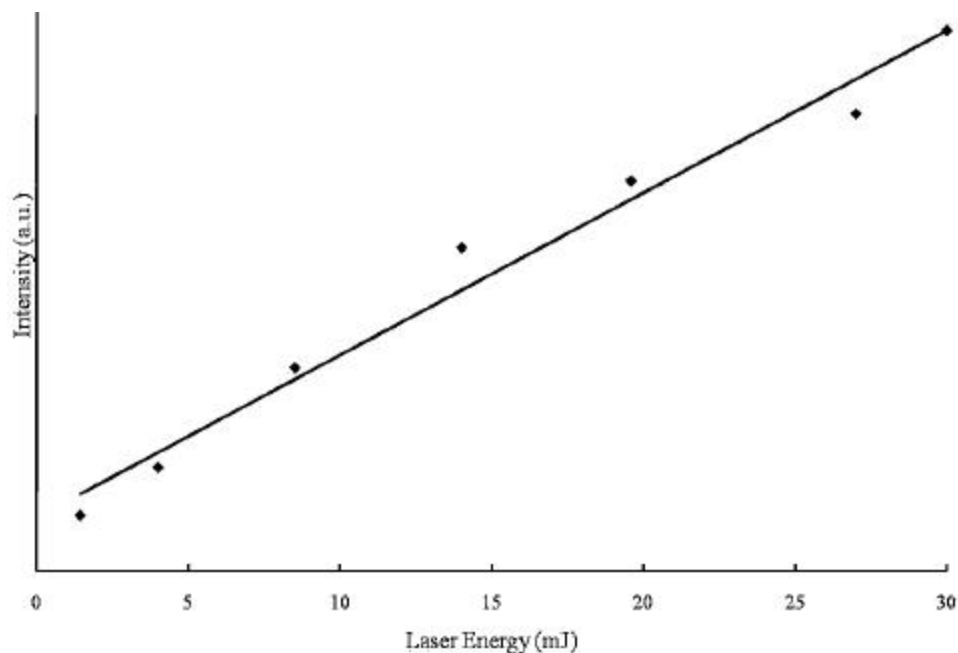


Fig.3b—Excitation energy vs. LIF-Intensity at concentration of one ppm. Solid line is guide to eye

keeps increasing with increasing excitation energy within the observed energy regime. No shift was observed in the peak position with energy. Unaltered peak position feature with energy will be fruitful to detect tryptophan from longer distances by increasing energy. 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 bio-chemicals individually and as a mixture were recorded. Signature peaks of tyrosine at 303 nm, of tryptophan at 348 nm and NADH at 457 nm were observed. New peaks were also observed in the spectrum of NADH and tyrosine. It was observed that spectral features of the tryptophan mask the signature peaks of other bio-chemicals in the mixture. The concentration and energy effects on the LIF of tryptophan have been investigated. The detection limit with the existing fiber coupled CCD detector is found to be one ppm.

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