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A High Sensitivity and Fast Responsive Fluorescence-Based Device for Detection of Biomolecules to Enhance Security against Biowarfare and Pandemics

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Abstract: ***A highly sensitive fluorescence-based device has been developed for the detection of biomolecules to enhance security against biowarfare. In response to the escalating threat of biowarfare due to rapid development and heightened international competition, quick detection of hazardous viruses or bacteria becomes crucial for evacuating contamination zones promptly. The device presented in this work is a prototype utilizing photoluminescence spectral analysis principles. Notably, the device exhibits exceptional detection selectivity through the incorporation of different LEDs of wavelengths along with 266 nm laser for excitation and a photomultiplier-based high sensitive detection system. The author highlights the device's responsiveness using standard optically active amino acids, forming the basis for establishing a standard data set for the detection mechanism. The instrument is specifically designed to detect biomolecules in aerosol form, with the added capability of detecting liquid samples. Moreover, the instrument is cost-effective, enabling commercial use in critical locations such as trains, parliaments, and other areas prone to bio attacks. Its deployment promises significant advancements in the security systems of nations.***

1. Introduction

In the face of many global health challenges, the rapid and reliable detection of pathogens is of paramount importance for public health and safety. Traditional methods that have been used to detect pathogens have not been real-time and involve costly equipment, hindering the ability to detect these pathogens efficiently and swiftly at a reasonable price. This research paper introduces a novel approach to real-time pathogen detection, leveraging fluorescence-based technology to achieve high sensitivity and selectivity at a cost-effective scale. The primary objective of this research is to develop a device capable of detecting various pathogens in real time, emphasizing affordability without compromising sensitivity or selectivity. The potential applications of such a device are far-reaching, with one notable example being the detection of airborne pathogens, potentially allowing for rapid wide-scale detection of the SARS-CoV-2-virus in the air. This capability could revolutionize public health protocols, providing an early warning system for the presence of contagious agents in shared spaces.

The prevalent technique used for SARS-CoV-2-virus detection is the reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR experiments are typically separated into three steps: the first step is the extraction of viral RNA, the second step is a reverse transcription into complementary DNA (cDNA), and the third step is the amplification of cDNA for detection using the polymerase chain reaction (PCR) [3]. While highly sensitive and accurate, it requires extensive specialized equipment and complex sequence comparison primer construction, which takes time and should be performed in a well-trained laboratory [2]. Our research paper focuses on a more efficient and economical method of pathogen detection, which is why the technique used in this paper revolves around the principles of photoluminescence. Photoluminescence is of paramount importance due to its flexible sensing mechanism, high specificity and sensitivity, quick real-time detection and plenty of other features that have sparked wide-spread interest in nanotechnology and biosensor research [2]. Photoluminescence involves the absorption of photons by the sample in its ground state, which transitions it to a higher energy state in an upper level conduction band [1]. By exposing our samples to a 266 nm laser excitation, we induce the emission radiation's characteristic of the target pathogens. Once the electrons are in their excited states following the exposure to the laser, they are reverted back to their ground state, releasing energy in the form of light emission or photons [2]. Additionally, during the transition there is a relatively small amount of energy lost in the form of heat or phonons. This is taken note of in order to avoid inaccuracies in the calculation of the wavelength of light emitted. For detection, Instrumentation such as photoluminescence spectrometers play a crucial role in conducting these experiments, enabling precise measurements of emission spectra by systematically scanning the wavelengths of both the excitation and emission radiation, generating comprehensive spectra unique to each biological pathogen present in the sample. This technique allows for the correlation of spectral patterns to specific pathogens, enabling a rapid and accurate identification process.

The SARS-CoV-2 pathogen, responsible for COVID-19, is primarily composed of genetic material in the form of single-stranded RNA enclosed within a lipid bilayer. This lipid envelope is embedded with spike (S), envelope (E), membrane (M), and a nucleocapsid phosphoprotein [4]. These proteins are comprised of the amino acids being optically active due to their chiral nature. Optical activity arises from the presence of asymmetric carbon atoms in amino acids and the 6 amino acids known to be optically provable are tryptophan (Trp), tyrosine (Tyr), phenylalanine (Phe), Histidine (His), Cysteine (Cys), and Proline (Pro). The detection of SARS-CoV-2 often involves identifying specific amino acid sequences within these proteins. Utilizing our photoluminescence technique, we can target and analyse optically active amino acids to detect the presence of the virus. This molecular understanding of the pathogen's composition and the optical properties of its amino acids

forms the basis for developing effective diagnostic tools in the ongoing efforts to combat the spread of COVID-19.

Others have attempted to carry out the same optical bio-sensing detection using the Raman spectroscopy technique. In this method, a monochromatic light source is directed onto a sample. When photons interact with molecular vibrations, they undergo a shift in energy, known as the surface-enhanced Raman spectroscopy (SERS) effect. This shift is unique for different molecular bonds, enabling the identification and quantification of specific biomolecules. This method has held great value as an optical bio-sensing technique, which under optimal conditions, enables deep and high-resolution volumetric imaging [5]. However, SERS-based bio-imaging is not feasible in our experiment due to the requirement of a very expensive laser. Additionally, SERS-based bio-imaging is quite underdeveloped [6] due to the lack of specialized Raman instruments tailor-made for volumetric bio-imaging, [7] toxicity challenges, and poor stability of SERS probes due to enzymatic degradation or desorption [8].

This study introduces a practical, cost-effective solution for detecting pathogens in real-time, thereby aiding the ongoing endeavors to enhance worldwide health protection. The device, developed and constructed, operates on photoluminescence principles and utilizes standard data derived from NADH and Tryptophane. The findings demonstrate the device's impressive sensitivity, suggesting it could make a valuable contribution to the progress of similar safety technologies.

2. Experimental Methods

This study includes the development of a highly sensitive and cost-effective fluorescence-based device for rapid in-situ detection, specifically designed to raise alerts during potential biological warfare or pandemic events in extremely short duration of time. To enhance cost-effectiveness and prevent false alarms, various light-emitting diodes (LEDs) with different wavelengths are utilized to excite biomolecules, and optical filters are employed to capture the desired spectrum through a photomultiplier tube [9].

The schematic diagram illustrates each component of the device. Fabrication involves using a rectangular aluminium cavity coated with black silicon to minimize reflections. The cross-section dimensions are 15cm x 20cm, with a length of 40cm. A 266nm laser mount are fabricated on one of the cross-section faces, and three LEDs mounts are machined on the side faces of the aluminium cavity. A Teflon-made black cuvette holder is placed just below the photomultiplier tube (PMT) detector for liquid sample analysis. On the top face, a 50mm diameter optical filter holder is fabricated and mounted, supporting a Hamamatsu head-on type PMT R375 fitted with a voltage divider circuit and input-output BNC connectors. Two 5mm diameter holes for aerosol inlet and outlet are fabricated, connecting a Phillips nebulizer and a vacuum pump. The PMT output is monitored using LabVIEW 7 computer program on a window-based computer [10]. Standard variable voltage LED supply serves as the driver for LEDs, and a 1500V DC supply from SES Instruments, India, is used as input to drive the PMT voltage divider circuit [11].

In the second part of the experimental work, standard optically active amino acids, representing the main constituents of biomolecules, are utilized to calibrate and test the instrument. Due to safety considerations, only the primary constituents of biomolecules are used, avoiding actual bacteria or viruses that would require high-safety labs. For analysis, reagent-grade Tryptophan and NADH (≥98% HPLC) procured from Sigma-Aldrich, USA, are mixed in 10 ml double-distilled water to generate aerosols using a nebulizer kit [12,13]. For the data acquisition entire system is activated, and while continuously monitoring PMT current, the nebulizer is initiated to introduce NADH and tryptophan aerosols into the duct and the variation in PMT current has been monitored. For the liquid sample testing same solution as discussed above has been used after dilution with distilled water to get desired concentration and the emission florescence has been monitored using PMT and the results has been represented in following sections.

The Device and Schematic Diagram

This device is engineered for the detection and identification of biological pathogens using a method that combines laser/LEDS-induced fluorescence and spectral analysis. First a sample is nebulized into an aerosol and injected in the device cavity where it gets exposed to a 266 nm laser and LEDs of various wavelengths (280 nm, 365 nm, and 430 nm) using a controlled voltage power supply, which excites the sample and causes the sample to emit photoluminescence radiation. This emitted radiation is filtered using an optical filter of desired spectral band based on literature survey, where the filtered light is then detected by photomultiplier tubes (PMTs) from Hamamatsu Photonics, which are highly sensitive to the emission radiation. The photons captured are converted to an electrical signal and amplified through a Keithley Pico-ammeter, which measures the current associated with the intensity of emitted radiation from biomolecule. By measuring intensity of emission of desired wavelengths using various excitations sources, the device correlates specific spectral signatures to known pathogens, thus identifying them within the sample. This integration of optical and electronic components allows for precise and rapid pathogen detection, crucial for applications in healthcare, biosecurity, and research.

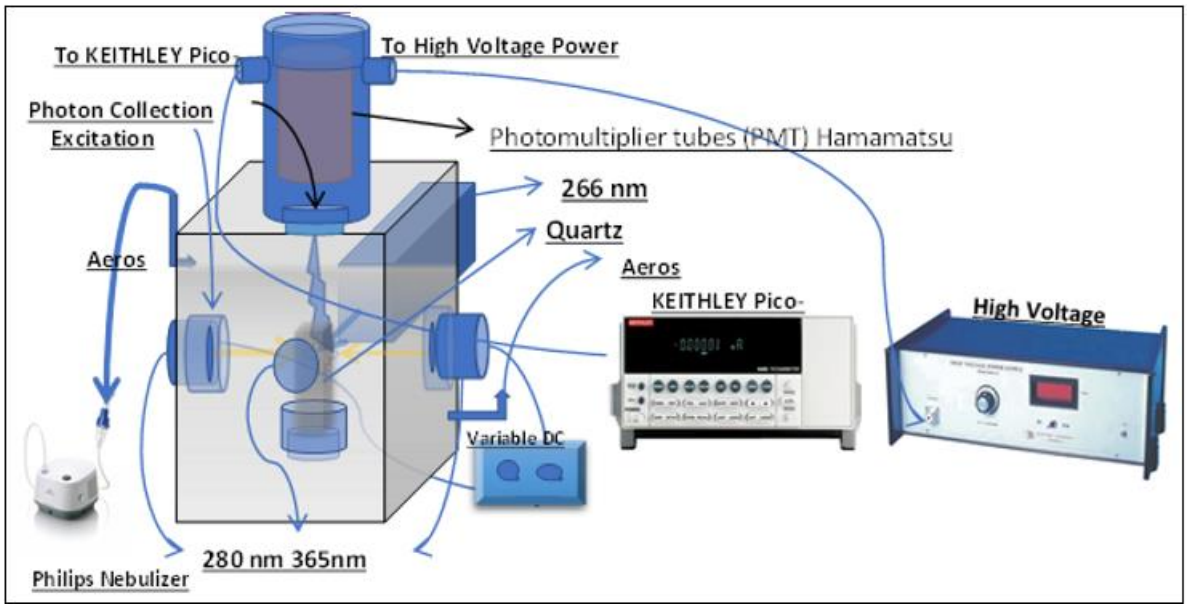

Figure 1: Schematic diagram of florescence-based device fabricated for biomolecule detection

Figure 1 represents the schematic diagram of the device, it includes a aluminium cavity in light transparent sky blue colour with various mount stands fabricated on it to mount all excitation sources detector and sample inlet outlet. On all the faces of this cavity the mounted three LEDs and a rectangular laser shown in slightly daker blue colour as compared to cavity colour has been shown. On the top of cavity, a filter holder along with PMT is shown sing blue colour and pin/blue colour. A Keithley Pico ammeter image has been shown in between the main device and high voltage supply driving the PMT voltage divider circuit. The pico-ammeter is connected to output of PMT. There are two more things a variable voltage supply shown in blue derives the LEDs and a Phillips nebulizer.

3. Results and Discussion

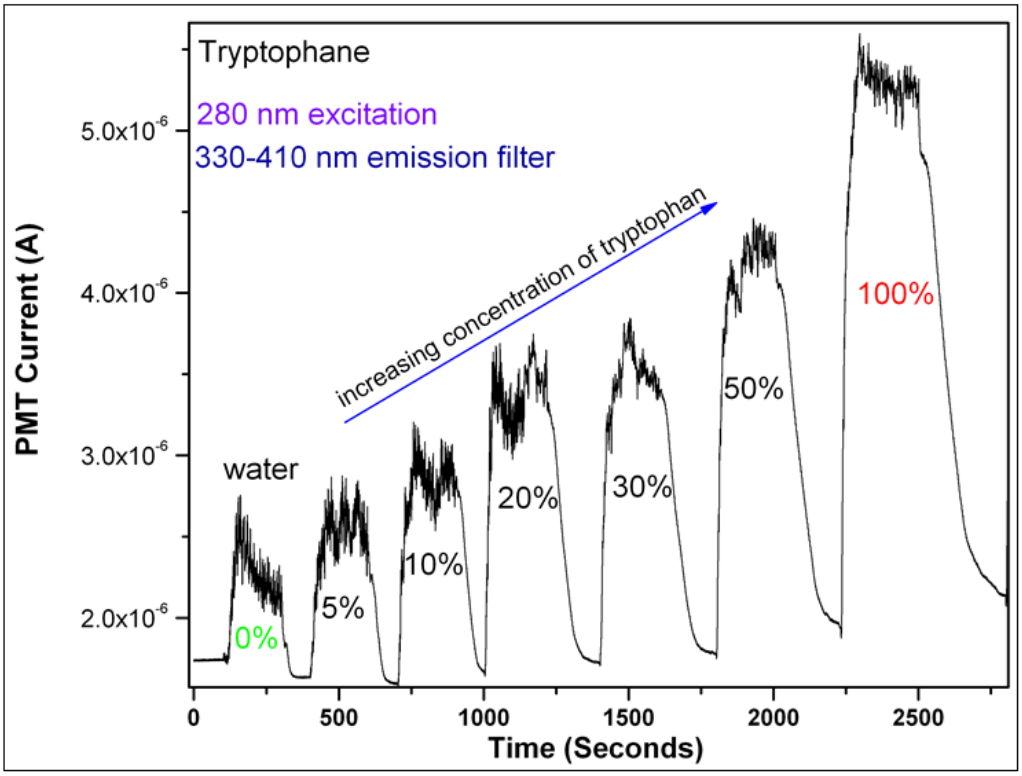

Figure 2: Florescence from various concentrations of tryptophan in aerosol form. 280 nm LED used as excitation source and 330 to 410 nm filter used for collection of emitted photons (repeatability test)

Figure 2 represents the variation in PMT current corresponds to fluorescence emission from tryptophane aerosol monitored for 3000 seconds by increasing the concentration of tryptophane in solution used for creation of aerosol in nebulizer kit. Results from different concentrations of tryptophan in aerosol form, with a 280 nm LED used for

excitation [14]. This figure shows a linear variation in fluorescence intensity with tryptophan concentration in aerosol. The dark current of PMT was below 1.5 µA and as the aerosol generated switched on at 250 seconds in graph the emission from tryptophane in wavelength range 330-410 nm increases and a careful observation shows there is some time lag to reach maximum current it occurs because aerosol generator purge aerosol with a limited speed and it takes some time to stablish a uniform aerosol in cavity. The observed PMT current variation at 0% i.e. only water droplets purged occurs because of scattering of source light and no-optical filter this range is available which can 100% cutoff the tail ends. The down fall in the PMT current occurs results from evacuating the chamber using a vacuum pump attached at other end of cavity after saturation of monitored current to increase the concentration of tryptophane in aerosol kit. A linear increase in PMT current was observed as a function of Tryptophane concentration and the 100% represents the saturation concentration of tryptophane in 10ml water i.e.1.2 gm tryptophane.

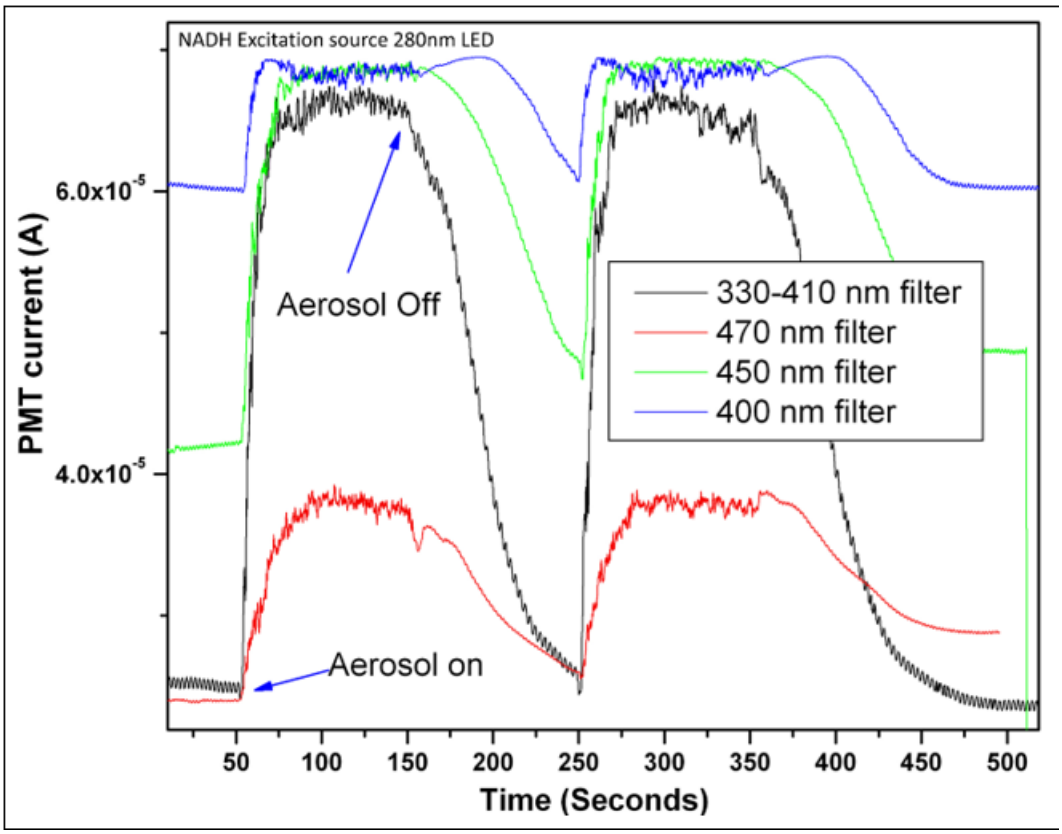

Figure 3: Florescence from NADH in aerosol form. 280 nm LED used as excitation source and various filters are used to study spectral properties of emitted photons.

Figure 3 illustrates the fluorescence emitted by NADH in aerosol form, employing a 280 nm LED for excitation and employing various bandpass filters [15]. The graph displays the comparative emission values across different wavelength ranges. The most notable variation is observed with the emission filter spanning 330-410 nm, while the least variation is noted with the 10 nm band filter cantered at 400 nm.

The terms "aerosol on" and "aerosol off" indicate the activation of the nebulizer to introduce aerosol into the cavity and the activation of the vacuum pump to remove aerosol from the cavity, respectively. The evacuation time for aerosol is comparatively longer than the aerosol introduction, which is expected as completely removing aerosol particles from the cavity poses inherent challenges. The findings parallel those of the tryptophan case, although in this section, the focus has been on optimizing the instrument with respect to filter selection rather than concentration effects.

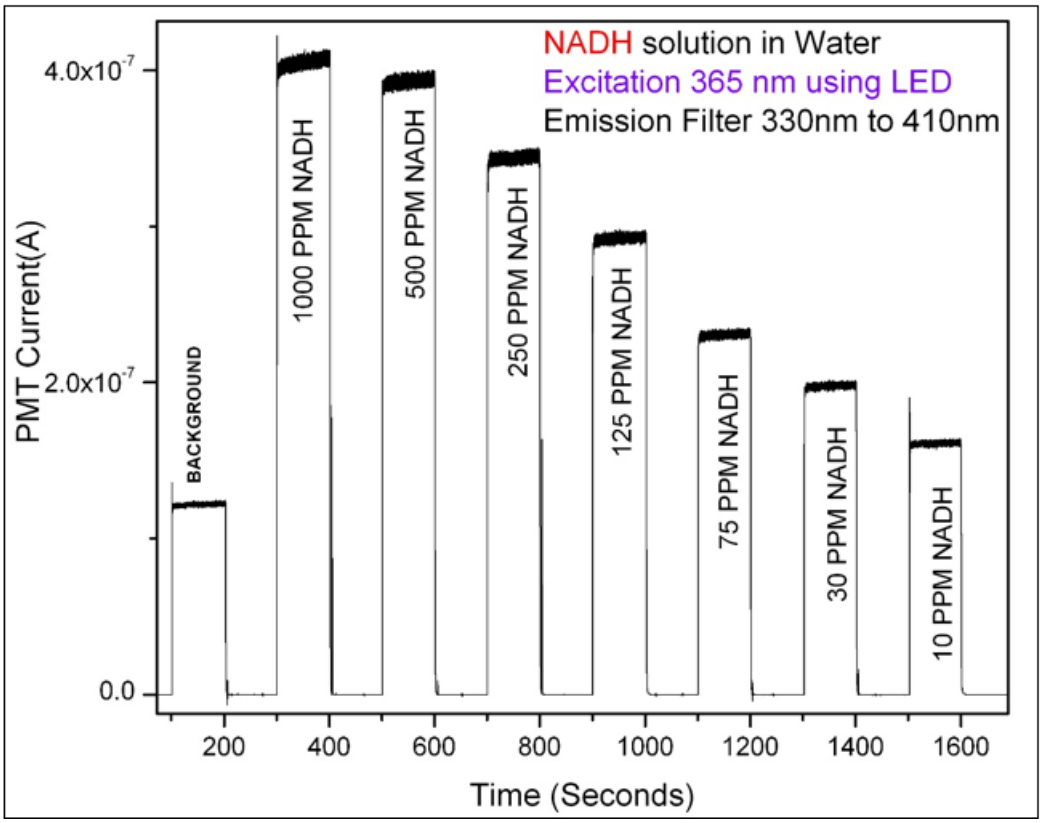

Figure 4: Florescence from various concentrations of NADH dissolved in water. 365 nm LED used as excitation source and 330 to 410 nm filter used for collection of emitted photons

Figure 4 depicts the intensity of fluorescence emission from varying concentrations of NADH dissolved in water, utilizing a 365 nm LED for excitation. The initial peak in the graph is attributed to scattering from water molecules and is identified as the background signal. The experiment commenced with a 1000 PPM NADH solution in double-distilled water, registering a PMT current of 40 µA. Subsequently, the NADH concentration was systematically reduced by halving the solution and replenishing it with pure water, resulting in a 50% less concentrated solution. This process iterated until reaching a concentration of 10 ppm. Notably, a linear correlation in the fluorescence radiation intensity was observed in the case of NADH, affirming the successful detection of such molecules in a water medium

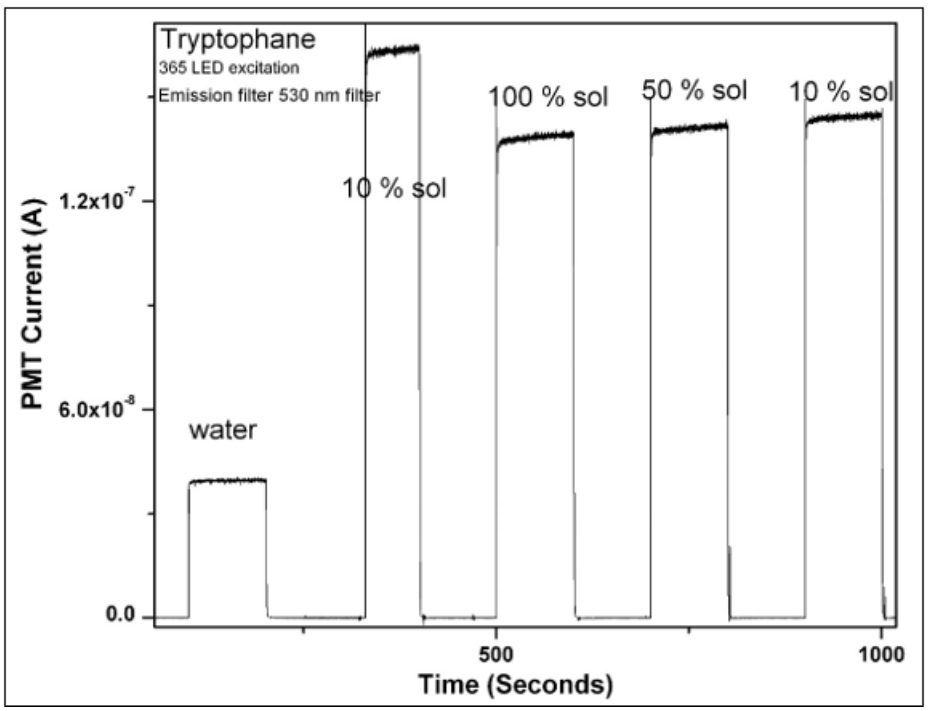

Figure 5: Florescence from various concentrations (100 % means 1000 PPM) of tryptophan dissolved in water. 365 nm LED used as excitation source and 530 nm filter used for collection of emitted photons

Figure 5 depicts the intensity of fluorescence emission from varying concentrations of tryptophane dissolved in water, utilizing a 365 nm LED for excitation. Similar to the NADH/water graph the initial peak in the graph is attributed to scattering from water molecules and is identified as the background signal. The experiment commenced with 100% saturated solution of tryptophane in double-distilled water, registering a PMT current of 15 µA. Subsequently, the tryptophane concentration was systematically reduced by halving the solution up to 10 times less concentrated solution. Notably, Almont no variation in the fluorescence radiation intensity was observed in the case of tryptophane, and it may be because tryptophane reaction with water [16].

4. Conclusion

In conclusion, the development of a sensitive and fast responsive device for biomolecule detection is essential as a protection towards biowarfare threats and pandemic situations. This prototype device designed and fabricated, incorporating photoluminescence and spectral analysis principles, holds immense significance for prompt evacuation of contamination zones, addressing the urgent need for quick detection of hazardous viruses or bacteria. The device's unique features, including exceptional selectivity achieved through a combination of different LEDs and an optional 266 nm laser for excitation, coupled with a extremely high sensitive photomultiplier-based detection system fitted with desired optical filter. The responsiveness of the device has been checked for tryptophane and NADH as standard optically active amino acids, It validates its detection capabilities and establishes a foundation for a standardized data set. Significantly, the device is designed to detect biomolecules in both aerosol and liquid forms, making it versatile in various scenarios. Its cost-effectiveness further positions it for widespread commercial use in critical locations susceptible to bio attacks. The deployment of this instrument holds the promise of significantly advancing national security systems, providing an effective deterrent against potential biowarfare threats.

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