Comparison of the Fluorescence Properties of Biological Solutions and Aerosols

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***Abstract*—The identification of biological aerosols is a subject of worldwide interest because of the potential for biological terrorism and the increase in public health emergencies. Laser-induced fluorescence is widely used to investigate the fluorescence properties of biomolecules and spores in solution; however, data on the fluorescence properties of the aerosolized forms are lacking. In this study, we report the fluorescence properties of tryptophan, serotonin, tyrosine, vitamin B6, and *Bacillus thuringiensis* and *Bacillus subtilis* spores in solution and aerosol form. Our results show that the shapes of the fluorescence spectra were generally consistent between the solution and aerosol states despite some wavelength shifts in the emission spectra. In contrast, the fluorescence intensity of the emissions varied significantly between the solutions and aerosols. Given the growing global interest in accurate measurement of airborne biological molecules and particles, our findings provide a useful baseline for further research into the development of laser-induced fluorescence-based bioaerosol detection instruments and can guide the ongoing design of effective measurement and engineering technologies.**

***Index Terms*—Emerging technologies, hardware, laser-induced fluorescence method, optical and photonic technologies**

I. Introduction

Owing to the emerging threat of international biological terrorism and the increase in public health emergencies, the identification of biological aerosols has become a subject of growing interest worldwide [1]. Aerosolized microorganisms (bacteria, fungi, and viruses) and particles (e.g., pollen) have been found in both outdoor and indoor air [2, 3]. As these aerosol-phase particles, particularly those ≤5μm or with pathogenic properties, have major effects on human health [4, 5], there is a need for real-time instrumentation to monitor biological particles in different environments. Bioaerosol detection has piqued the scientific community's interest.

Laser-Induced Fluorescence (LIF) is widely applied for the classification of biological and non-biological particles [6–8]. This technique is based on the excitation of fluorescence with an ultraviolet laser beam and the subsequent measurement of the emitted fluorescence light spectra [9, 10]. Several biochemicals emit fluorescence light. Fluorophores such as amino acids (tryptophan, tyrosin), coenzymes (NADH, NADPH), and flavins. (riboflavin, FAD, FMN) are found in association with airborne microorganisms, and they exhibit intrinsic fluorescence, enabling their detection using LIF [11]. However, while the fluorescence properties of fluorophores in solution are well documented [12], data on the fluorescence properties of aerosolized forms are lacking [13]. For example, Kunnil *et al.* [14] showed apparent differences in the fluorescence properties of dried and wet *Bacillus subtilis* spores, while the fluorescence properties of other biomolecules and spores in solutions and aerosols remain ambiguous.

To address this gap, we compared the fluorescence properties of various biomolecules found in association with airborne microorganisms as well as bacterial spores in both solution and aerosol forms using LIF. Therefore, this study is one of the first few comprehensive studies on the fluorescence properties of important biomolecules, and it has the potential to contribute to the development of new measurement and identification technologies.

II. Methods

A. Instrumentation

A schematic of the bioaerosol fluorescence detection system used is shown in Fig. 1. The system consisted of a solid-state Nd: YAG laser with a wavelength of 266nm, single pulse energy of 10μJ, and repetition frequency of 2.5kHz as the emission light source. After passing through the shaping optical path structure, the laser beam was shot toward the cloud generated by the bioaerosol generator above the middle section of the sample cell. Backward fluorescence signals were then received through a lens and converged into the optical fiber through an off-axis mirror. The fluorescence signals were filtered through the monochromator and were finally received by the Photomultiplier Tube (PMT). All control signals were controlled uniformly by a computer, and the signals collected by the PMT were transmitted back to it for data processing.

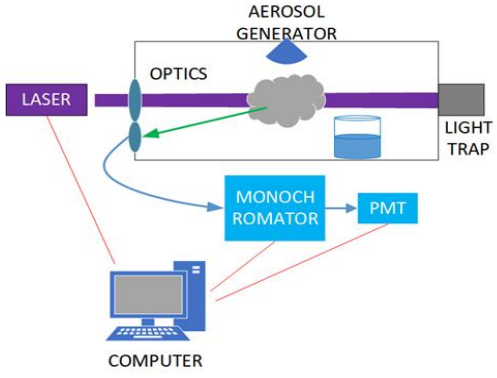

Fig. 1. Bioaerosol fluorescence detection system.

Manuscript received April 24, 2023; revised June 4, 2023; accepted July 10, 2023.

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B. Biological and Chemical Agents

All experimental chemicals, biological agents, and water were of analytical grade. The fluorophores used were L-tryptophan, tyrosine, vitamin B6, serotonin (Solarbio® Life Science, Beijing, China), *B. thuringiensis* (BT; ATCC33679), and *B. subtilis* (BS; ATCC6633) spores. All materials were stored in the dark at 2–8 °C.

C. Solutions and Aerosol Preparation

The fluorophores were prepared in deionized water solution 30 min before the measurements. The concentration of each compound was 0.1 mg/mL. The BT and BS solutions contained 1×10^7 colony forming units (CFU)/mL, and their pH was approximately 7; they were stored in the dark at 22–25 °C. Fluorescence spectra were obtained for the solutions in quartz cuvettes and the bioaerosol generator. A nebulizer (NSF-6A; Tawang, Shanghai, China) was used to generate aerosol particles from the fluorophore/water solutions (0.1 mg/mL). The NSF-6A system generates aerosol particles with an average mass median aerodynamic diameter of 2.818 μm. The nebulizer flow rate was set to 15 L/min, and the rate of solution consumption was measured in real-time.

Tryptophan is the dominant source of intrinsic protein fluorescence and has a high fluorescence quantum yield. Thus, it is widely used as a convenient standard for the measurement of fluorescence quantum yields of organic and bioorganic molecules. Therefore, we aimed to provide insight into tryptophan fluorescence and demonstrate the influence of solution concentrations on the emission spectra. In this study, we selected tryptophan among the six biomolecules and compared different concentrations of tryptophan in solution and aerosol (100–500 ppm).

III. Results

The biological fluorophores showed different absorption and emission properties. A comparison of the fluorescence spectra of the solutions and aerosols of the six studied biological agents is shown in Fig. 2. To demonstrate the influence of solution concentrations on the emission spectra, the fluorescence properties of different concentrations of the tryptophan solution and aerosol are shown in Fig. 3.

As shown in Fig. 2, tryptophan, serotonin, and tyrosine showed consistent fluorescence curves and peaks in both solutions and aerosols, with emission maxima at 355, 350, and 310 nm, respectively. Wavelength shifts in the fluorescence emission maxima between the solutions and aerosols were observed for vitamin B6, and BT and BS spores. The emission maximum of the BT aerosol was red-shifted by 12 nm compared with that of the solution. However, the emission maxima of vitamin B6 and BS aerosols were blue-shifted by approximately 15 and 12 nm compared with that of their solutions, respectively. The fluorescence intensities of the Trp, 5-HT, Tyr, vitamin B6, and BT and BS solutions were approximately 6.14>100. 65, and >10 times higher than those of their aerosol equivalents, respectively.

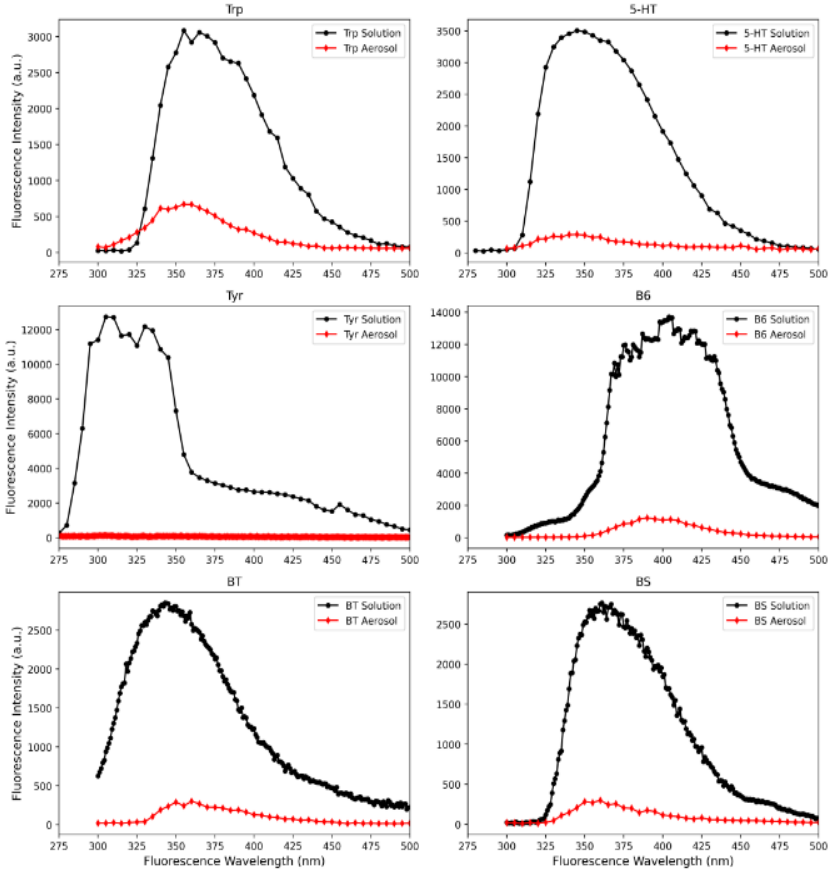

Fig. 2. Comparison of the fluorescence properties of different biological agents in solution versus aerosol excited at 266 nm laser wavelength. Trp = tryptophan; 5-HT = serotonin; Tyr = tyrosine; B6 = vitamin B6; BT = *B. thuringiensis*; BS = *Bacillus subtilis*.

The results for tryptophan showed that at relatively low concentrations (100–400 ppm), the fluorescence intensity increased with the increasing solution and aerosol concentrations with a maximum of 400 ppm. However, the fluorescence intensity of the 500-ppm solution was lower than that of the 400-ppm solution, as shown in Fig. 3.

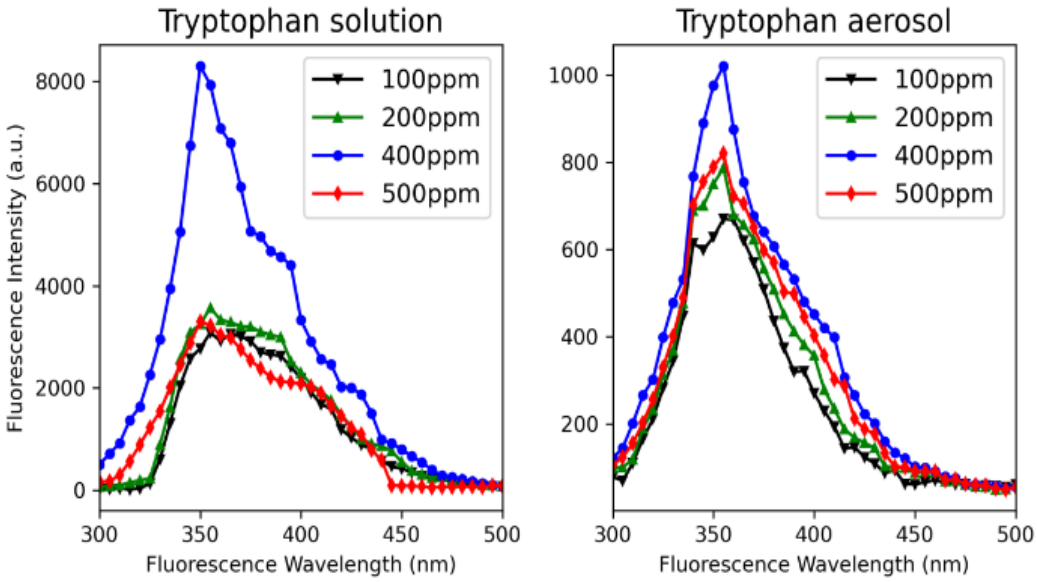

Fig. 3. Fluorescence properties of tryptophan solution and aerosol at different concentrations (100, 200, 400, and 500ppm).

IV. Discussion

Our measurements showed that the fluorescence intensity of the emissions significantly varied between the solutions and aerosols. However, the fluorescence curves remained broadly similar, despite some wavelength shifts in the emission spectra.

To avoid the uncertainty of the measurement, we took at least three times of measurements to make sure the repeatability for each substance is good. For all studied biological fluorophores, the fluorescence intensities of the solutions were considerably greater than those of their aerosol equivalents. Concentration quenching is more probable for aerosol particles, whereby fluorophore molecules are relatively close to each other and, thereby, lower the fluorescence quantum yield. This likely had a considerable effect on the fluorescence intensity and could explain the lower fluorescence intensity of the aerosols than that of the solutions.

Tryptophan, serotonin, and tyrosine showed similar fluorescence curves and peaks in both forms, which is consistent with the findings of previous studies [9, 11]. Wavelength shifts in the fluorescence emission maxima between the solutions and aerosols were observed for vitamin B6 and the bacterial spores. In the case of bacterial spores, the dominant features of the corresponding spectra were amino acid peaks [15], which were similar for both solutions and aerosols. The emission maximum of the BT aerosol was red-shifted by 12nm compared with that of the BT solution, whereas the converse was true for the BS solution. The fluorescence properties of fluorophores depend on their temperature, humidity, and pH [16, 17], which may further lead to minor changes in the emission fluorescence properties of vitamin B6 and bacterial spores in the present study.

Notably, the microenvironment around the fluorophore molecules in the aerosols would have differed from that of the solutions [18]. Depending on the differences in solution concentrations and laser parameters adopted, emissions can shift by a few wavelength units. Therefore, to demonstrate the influence of solution concentrations on the emission spectra, we compared different concentrations of tryptophan in solution and aerosol (100–500 ppm). We found that at 100–400 ppm, the fluorescence intensity increased with concentration, reaching a maximum at 400ppm. In contrast, the 500-ppm tryptophan preparations had lower maximum fluorescence intensity than the 400-ppm solution. This was probably because fluorescence was partially quenched by collisions in the solution at the higher concentration [19, 20]. Future studies involving additional types of fluorophore molecules are required to confirm the influence of solution concentrations on the emission spectra [21, 22].

There are some limitations that have to be considered. First of all, we estimated the concentrations of the aerosols from the solution concentrations rather than using direct measurement, which can result in minor deviations [23, 24]. Furthermore, we could not evaluate the suspension duration of the nebulized fluorescent bioaerosol particles and the corresponding fluorescence attenuation over time [25, 26]. Future research could explore these variables more comprehensively.

V. Conclusions

We examined the fluorescence spectra of various biologically associated fluorophores in both solution (deionized water) and aerosol forms. Our results showed that the curves of the fluorescence emission spectra were consistent between the solutions and aerosols, whereas the corresponding fluorescence intensities varied significantly. These results provide a useful reference for further research on the development of LIF-based bioaerosol detection

instruments.

VI. Future Research

Future studies involving additional types of fluorophore molecules in association with bioaerosols in different environments are required to confirm the influence of solution concentrations on the emission spectra. Moreover, in order to further explore the variation of bioaerosol fluorescence attenuation, future work could explore the suspension duration of the nebulized fluorescent bioaerosol particles and observe the corresponding fluorescence attenuation over time.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

CX, YL, and YC designed the study and wrote and revised the manuscript; ZY, ZZ, DY, and WF designed the optical system and offered technical support; HC and ZZ, and YW performed data analysis and offered software support; YL, YC, and ZY performed the experiments; and CX interpreted the spectral data and revised the manuscript. All authors have read and approved the final manuscript.

Funding

This study was supported by the Anhui Provincial Natural Science Foundation (grant no. 1908085MH265) and the Anhui Provincial Key Research and Development Project (grant no. 2022b13020002).

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