Identification Studies of *Bacillus* Spores Using Fluorescence Spectroscopy

A thesis submitted in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy in Medical Physics

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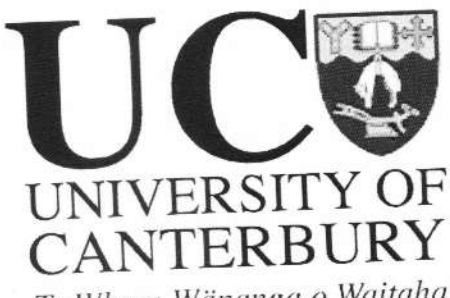

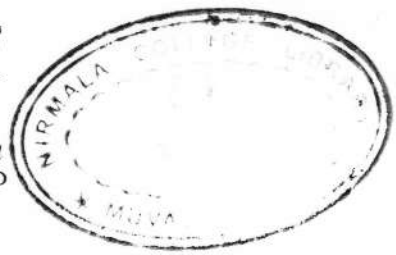

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Abstract

Fluorescence spectroscopy was examined as a potential technique for identifying aerosol particles like bacterial spores. This technique was used for laboratory measurements on some common biological agent simulants. We have measured the intrinsic steady-state fluorescence emission spectra as a function of the excitation wavelength for several bacterial spores (washed and unwashed) in dry and aqueous suspensions at room temperature using excitation wavelengths from 200 to 600 nm. These measurements were compared to those of common, naturally occurring biological components like fungal spores and pollen and non spore samples like ovalbumin. The spectra of samples were combined into fluorescence profiles or fluorescence fingerprints. Different substrates were used for collection and detection of spores. Each bacterium produces a unique *in vitro* fluorescence profile when measured in dried and aqueous suspension and exhibits a strong maximum in its fluorescence emission spectrum near 330-340 nm. The fluorescence profiles were reproducible. The complexity of microorganisms made the interpretation of their spectral signature a difficult task. Principal components analysis (PCA) and cluster analysis were done as a data reduction technique for detection and identification from different backgrounds. PCA illustrates that linear combination of detected fluorescence intensities, which are present in different ratios in each of samples studied, can be used to discriminate biological agent simulants from other biological samples. The hydration effects, washing effects and the role of tryptophan on spore fluorescence were also investigated. The emission spectra of the dried spores showed a maximum near 330 nm, suggesting a hydrophobic environment for its tryptophan residues. The aqueous solution of tryptophan showed fluorescence shifted to 360 nm and in ethanol solution the maximum was shifted to 340 nm, suggesting a rather more polar average location of the tryptophan. To find the limit of detection we measured the quantum efficiency (QE) of a few samples. We concluded that spectroscopy techniques coupled with effective interpretation models are applicable to biological simulants agents.

Index Heading: Bacteria; Spores; Identification; Fluorescence; Fluorescence Quantum Efficiency; Principal Components Analysis; Cluster Analysis.

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