Monitoring of pathogen carrying air-borne *Camellia sinensis* dust particles by light scattering

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In order to investigate if *Camellia sinensis* dust particles are a possible carrier of the species *Mycobacterium* a biotechnical procedure was used and to verify the possibility of monitoring this dust, a laser-based setup was designed and fabricated and experiments were carried out. *Mycobacterium smegmatis* mc^2 155 was used as a model organism to study the effect on *Camellia sinensis* dust particles.

INTRODUCTION

Light scattering [1] is an important tool for the optical characterization of small particles suspended in air or when dispersed in a medium. The study of the angular scattering dependence of such particulate matter helps in the investigation of the nature of the scattering particle and to understand the radiative transfer through a medium containing the scatterer [2,3]. A number of different experimental setups have been made in the past to investigate the scattering behavior of small particles [4].

Tea is an indispensable beverage used all over the world and is mainly brewed from the leaves of the plant species *Camellia sinensis*. *Camellia sinensis* is grown in many tropical regions of the world. During the processing stage from raw leaves to commercially packaged tea, tea dust is released into the atmosphere as an effluent. Such organic dust has the possibility of acting as a carrier for asthmatic triggers and also a carrier for pathogens [5]. It has been reported that tuberculosis, which is caused by the pathogen *Mycobacterium tuberculosis* is also prevalent in such tea gardens, the reasons being given as low socio-economic status, overcrowding of residential area and low literacy. *Mycobacterium tuberculosis* is non-motile and can live up to a few weeks in a dry state. The bacteria range from 0.2 to 0.4 microns in size.

This paper reports the work done in Assam in India, where tea is grown in large gardens and processed on a very large scale, to investigate if air-borne *Camellia-sinensis* dust particles are a possible carrier of *Mycobacterium* pathogens and if monitoring of such non-spherical particles can be done by light scattering techniques. An original laboratory light-scattering instrument, which could be used to measure the volume scattering of *Camellia sinensis* air-borne nonspherical dust was designed and fabricated.

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EXPERIMENTAL DETAILS

Light scattering setup

The setup was designed to study the light scattering characteristics of small particles. The essential components of the setup (Fig. 1) are a laser source, controlled sample holders, a photomultiplier tube (H 5784-20, Hamamatsu, Japan), data acquisition system (Vinytics, PCI-9812) and associated instrumentation. The scattered light intensity is sensed by the photomultiplier tube. The system uses three He-Ne laser sources used alternatively for three different wavelengths of 632 nm, 594 nm and 543 nm respectively, for studying the scattering properties as a function of scattering angle. The system can measure scattered light signals from an angle of 10º to 170º in steps of 5º for θ, and from 0º to 50º in steps of 10º for φ to account for recording the volume scattering. The setup is covered by a black metallic enclosure to cutoff electromagnetic noise and the beam stops are used at strategic points to minimize the intensity of stray reflections.

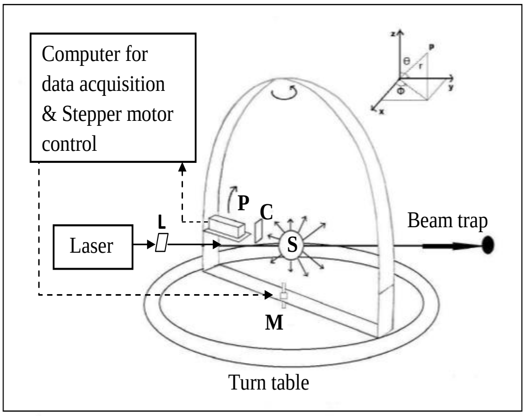

Figure 1. Schematic diagram of the scattering setup. P – Photomultiplier tube; L-Polarizer; C – Analyzer; S – Sample; M – Stepper motor

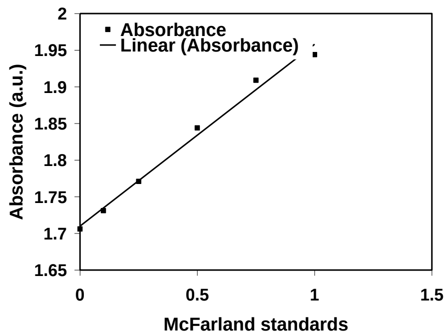

Figure 2. Absorbance of *Camellia sinensis* dust particles at 600 nm

Sample preparation and Antimycobacterial Assay of Camellia sinensis dust

The samples of *Camellia sinensis* dust particles were collected from areas around tea factories (Nonoi Tea estate and Hatikhuli Tea estate situated in Assam, India). Using scanning electron micrograph images the size distribution of these particles was calculated and the results were extrapolated for the population of dust particles. These dust particles are non-spherical and have a large size distribution that is nearly Gaussian. The interaction of *Camellia sinensis* dust, when exposed to *Mycobacterium* species, was studied by using the Agar Well Diffusion Method [6]. In order to maintain safe laboratory procedures, the non-pathogenic species *Mycobacterium smegmatis* which has all the characteristic properties of the pathogenic species *Mycobacterium tuberculosis* was used for the investigations. The strain *Mycobacterium smegmatis* mc^2 155 was cultured in Mueller Hinton Broth 2 media at 37° C for 18 hours. The bacterial cells were suspended in a saline solution (0.85 % NaCl) and the McFarland standard of the cells were adjusted to a turbidity of 0.5 (approximately10^8 CFU/ml). The suspension was inoculated in Mueller Hinton Agar media and four wells (6 mm) were punched. 50 µl of the sterile organic tea dust of two concentrations i.e., 20 mg/ml and 50 mg/ml, dissolved in 1 % (v/v) DMSO were added to the wells. Growth of the bacterial cells was not affected by 1 % DMSO as shown by our control experiments. 1 % DMSO was used as negative control and Streptomycin was used as an antibiotic control. The plates were incubated at 37° C for 18 h. The experiments were conducted in triplicate. An attempt was made to differentiate sterile tea (*Camellia sinensis* (L) Kuntze.) dust from *Mycobacterium smegmatis* contaminated tea dust by investigating the absorbance at 600nm by the sterile tea dust and tea dust inoculated with *Mycobacterium smegmatis* culture at different concentrations. A 16 hour *Mycobacterium smegmatis* culture was harvested by centrifugation at 3000 rpm, washed twice with sterile Phosphate buffer Saline(PBS) pH 7.4, and resuspended in PBS (pH 7.4). The suspension was further diluted and mixed properly in PBS (pH 7.4) containing 20 mg/ml of the tea dust to achieve McFarland standards of 0.1, 0.25, 0.5, 0.75 and 1.0 corresponding to approximately 0.3×10^8 CFU/mL, 0.75×10^8 CFU/mL, 1.5×10^8 CFU/mL, 2.25×10^8 CFU/mL respectively. The absorbance of the samples was measured at 600nm (Cecil Aquarius Spectrophotometer, Sl. No.146 -276). The plot between the McFarland Standards and Absorbance at 600 nm presented in Fig. 2. indicates that the absorbance of the tea dust increases with the increase in bacterial load on the tea dust.

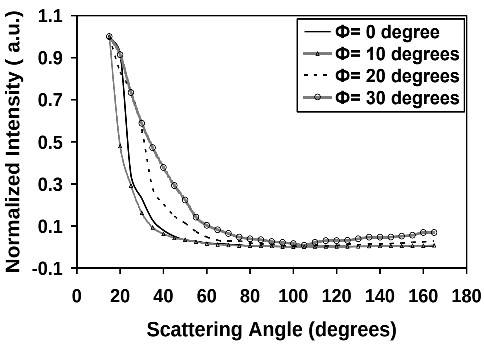

Figure 3. Scattering Intensity profile of uncontaminated *Camellia sinensis* dust particles at 594 nm.

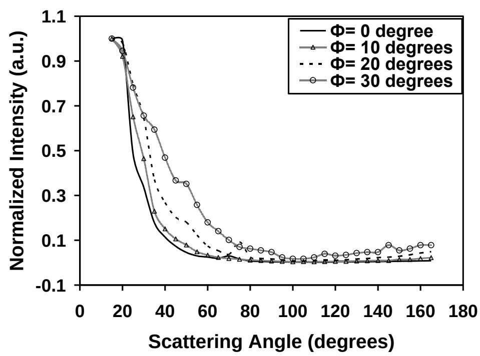

Figure 4. Scattering Intensity profile of *Mycobacterium* contaminated *Camellia sinensis* dust particles at 594 nm.

RESULTS

We have studied the light scattering from *Camellia-sinensis* dust particles at 543 nm, 594 nm and 632 nm laser wavelengths respectively. Scanning electron micrograph images were taken to observe the morphology of the particles. UV-vis absorption spectroscopy was performed to obtain the absorption spectra. The choice of 543 nm, 594 nm and 632 nm as probe wavelengths was employed because UV-vis absorption spectra had shown negligible non-resonant absorption at these particular wavelengths. It was observed that the tea dust particles did not exhibit any antimycobacterial activity and might act as a potent carrier. It was also observed that the light scattering behavior of *Mycobacterium* contaminated *Camellia sinensis* dust particles significantly vary for the three different laser wavelengths. The scattering results for *Mycobacterium* contaminated and uncontaminated dust sample shows marked difference for θ=0º, θ= 10º, θ=20º and θ=30º (Fig. 3 and 4) at wavelength of 594 nm. Preliminary results are shown for the light-scattering behavior of tea dust particles. These will be described in more detail in the presentation.

CONCLUSIONS

In this paper we report the design and fabrication of a low-cost, light-weight and miniaturized light-scattering instrument. The light-scattering behavior of both *Mycobacterium* contaminated and uncontaminated *Camellia sinensis* dust particles has been studied as a function of scattering angle. The instrument has proven itself to be efficient for performing light-scattering experiments on small particulate matter, so extensive investigations will be further carried out on other small particles, aerosols and hydrosols.

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