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Final Report

Biological Particle Analysis by Mass Spectrometry

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Summary

This report is a formal summary of the collaborative research performed by Dr. Mahadeva Sinha's Mass Spectrometry Laboratory at JPL and Dr. Vincent L. Vilker's Biochemical Engineering Laboratory at UCLA. The effort was directed toward adapting the PAMS system to the analysis of biological particles including amino acids, peptides, and whole bacteria cells. The report covers the period of 21 October 1981 through 20 October 1992 during which time Dr. Vilker held a subcontract with JPL (JPL Contract No. 956144). Although the formal subcontract ended effective 20 October 1982, a collaborative effort has continued with financial support being furnished by an NSF Grant to Drs. Friedlander and Vilker at UCLA and Dr. Sinha at JPL.
The objective of this project was to develop an instrument that analyzes the chemical composition of biological particles in aerosol or hydrosol form. During the past year, efforts have been directed toward the acquisition of mass spectra from aerosols of biomolecules and bacteria. For this initial study the filament ion source was installed on the particle analysis by mass spectrometry (PAMS) system. Modifications of the vacuum system improved the sensitivity of the mass spectrometer. After the modifications were incorporated, detailed mass spectra of simple compounds from the three major classes of biomolecules, proteins, nucleic acids, and carbohydrates, were obtained. A method of generating bacterial aerosols was developed for this laboratory. The aerosols generated were collected and examined in the scanning electron microscope to insure that the bacteria delivered to the mass spectrometer were intact and free from debris.

Description of PAMS System

The sample aerosol is introduced to the mass spectrometer by means of a particle beam which is formed by the expansion of the aerosol through a capillary nozzle. The carrier gas is stripped from the particles as the aerosol expands through the nozzle. The particles, by virtue of their higher momentum, form a well focused beam which is directed at the ion source of the mass spectrometer. In the recent studies, a hot rhenium filament was used to volatilize the particles. The impaction of the particles on the filament produces a burst of molecules which are ionized by electron bombardment. The ions are analyzed in a quadrupole mass spectrometer (UTI 100C), which is manually scanned over the mass range and the data acquired wherever a significant signal can be recorded. The signal pulses are accumulated on a pulse height analyzer and the average signal intensity is obtained. Therefore, the spectrum generated represents the mass spectrum of an average particle.
Improvements to the PAMS System

To proceed with the mass spectrometric analysis using the hot filament, two major modifications of the vacuum system were made in the last year to reduce the background signal and thereby improve the sensitivity of the mass spectrometer. A second skimmer was added to the aerosol beam generator. This provided a second vacuum chamber prior to the introduction of the beam to the source region. As a result, the operating vacuum at the mass spectrometer was improved from $10^{-4}$ torr to $10^{-6}$ torr. The second modification was the introduction of liquid nitrogen cold traps on the three vacuum lines. This significantly reduced contamination originating from residual pump oils.

The improvement in sensitivity was demonstrated using 1.8 μ particles of potassium biphthalate. These particles could not be detected before the modifications were made. With the improved vacuum system a detailed mass spectrum of potassium biphthalate was obtainable. The smallest peaks recorded were 5% of the most intense, which corresponds to well over an order of magnitude improvement in the sensitivity of the instrument.

With the introduction of the second skimmer to the particle beam, the transmission of the particles through the beam had to be redetermined. The system at JPL was no longer identical to the to the beam system at UCLA where the beam characteristics had originally been determined. In the transmission efficiency experiments performed at JPL, the number of particles impacted on the rhenium filament was determined by monitoring the 105 amu/e peak for potassium biphthalate particles. This result was compared with the number of particles introduced to the particle beam as determined by optical particle counter measurements. The percent of filament collection was obtained for particles ranging from 1.0 to 3.0 μ in diameter. The results show that 35-45% of the particles introduced to the beam were collected at the rhenium
filament and the percent collection was independent of particle diameter over the size range studied. The spot size of the particle beam at the position of the filament, obtained by the collection of particles on a grease coated slide, was determined to be 2.0-2.5 mm in diameter. Given that the filament ribbon has a 1 mm width, the total transmission through the beam is estimated to be 60-80%.

Mass Spectrometry of Biomolecules by PAMS

Mass spectrometric studies on aerosols generated from solutions of biomolecules was undertaken to demonstrate that the spectra recorded are characteristic of the samples and are reproducible. Sample aerosols were produced from a Berglund-Liu vibrating orifice generator. The aerosol was monitored in an optical particle counter to determine particle size and quantity delivered to the beam. Detailed mass spectra were obtained for a variety of simple biocompounds, with representatives from each of the three major classes of biomolecules, proteins, nucleic acids and carbohydrates.

Mass spectra of adenine and thymine, common nucleic acid bases, were characterized by predominant peaks from the parent molecule. Mass spectra of a polymer of the carbohydrate, glucose, and polymers of the amino acid, glycine, exhibited peaks characteristic of their respective monomers as well as a few additional peaks. Wherever comparisons with the literature were available, the mass spectra recorded from the PAMS system were remarkably similar to mass spectra obtained from electron impact ionization instruments.

Bacterial Aerosol Generation and Mass Spectrometry

Spores of the common bacteria, Bacillus subtilis were chosen to demonstrate the application of particle mass spectrometry to the detection of
bacteria. The bacteria were grown for 48 hours then heated to 70°C for one half hour to complete sporulation. The spore suspension was washed three times in distilled water to remove the culture medium and stored under refrigeration until needed.

Aerosols of the B. subtilis spores were prepared in a Bird Products micronebulizer (Model 4144). The nebulizer effluent is passed through a Kr\textsuperscript{85} charge neutralizer and a silica gel diffusion drier before introduction to the particle beam. Sample aerosols that typically contain 50-100 particles/cc were generated from a starting suspension which contained 10\textsuperscript{9} spores/cc. The particle density and size distribution were obtained from an optical particle counter. The size distribution recorded from the bacterial aerosol was similar to the size distribution obtained from an aerosol of polystyrene latex spheres. This indicates that the aerosol produced from the nebulization of the bacterial spore suspension was monodisperse.

The ability to monitor only one mass peak at a time, a limitation of the quadrupole mass spectrometer, requires that the sample aerosol be free of any foreign particulate matter. Bacterial aerosols which had been collected on Nuclepore filters after the diffusion drier were examined in a scanning electron microscope. This verified that the aerosol particles were predominately single spores, 20% being doublets, and were free of particulate debris. The electron micrographs show that the spores remain intact and that their shape remains unchanged by the aerosolization process.

When the aerosol of B. subtilis spores were introduced into the particle beam for analysis by mass spectrometry, no signal could be detected. Further investigation will be conducted to determine whether the difficulty lies in the inadequate volatilization/ionization of the particle in the
mass spectrometer or in the inability to generate a particle beam from bacterial spores.