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ANALYSIS OF BIOLOGICAL PARTICLES BY MASS SPECTROMETRY

FINAL TECHICAL REPORT

M. P. Sinha

October 23, 1984

U. S. ARMY RESEARCH OFFICE

MIPR NO. 50-81

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A new method has been developed for the rapid characterization of aerosol particles of biological nature using the technique of Particle Analysis by Mass Spectrometry (PAMS). PAMS is a combination of particle beam generation with mass spectrometry. Mass spectral measurements were made on individual biological aerosol particles on a continuous, real-time basis using this method. The generation of characteristic mass spectra from single particles introduced into the ion source directly from aerosols makes it a unique method for the detection and identification of biological particles in air on an instantaneous basis.

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EXECUTIVE SUMMARY

A new method has been developed for the rapid characterization of aerosol particles of biological nature using the technique of Particle Analysis by Mass Spectrometry (PAMS). PAMS is a combination of particle beam generation with mass spectrometry. Mass spectral measurements were made on individual biological aerosol particles using this method. The generation of mass spectra directly from aerosols on a single particle basis makes PAMS a unique method for the detection and identification of biological particles in air on an instantaneous basis.

Aerosols of various organic compounds, and microorganisms were produced by nebulizing their suspensions, and were introduced into the ion source of a quadrupole mass spectrometer in the form of a beam. It is found that the particle beam method provides a well-controlled means for introducing one particle at a time into the ion source. Two methods for the volatilization and ionization of particles were studied: thermal volatilization followed by electron impact ionization, and laser-induced volatilization and ionization.

Originator Furnished Keywords Include:

Mass spectra of aerosol particles of different microorganisms were measured. Spectra were generated by successively measuring the average intensities of different mass peaks on individual particle basis. Bacillus cereus, Bacillus subtilis and Pseudomonas putida were analyzed. The results for Pseudomonas putida and Bacillus subtilus are comparable with data found in the literature. However, peaks at higher masses (>120 amu) were found to be more intense in the present studies. These fragments may contain much information about the identity of the parent species. Significant differences between the relative intensities of the various mass peaks in the spectra were also observed. The results obtained on the above bacteria particles demonstrate the potential of the PAMS technique for detecting and identifying biological particles.

The preliminary nature of the work should be emphasized. Additional work on different biological samples present in various environments needs to be done. Mass spectroscopic measurements on biological macromolecules should also be made. These studies will help in assigning their characteristic mass peaks in the biological particle spectra and providing a basis for their detection and identification. The limitation of measuring only one mass peak at a time from each particle by the quadrupole-PAMS system can be overcome by using a focal plane mass spectrograph equipped with an electro-optical ion detector. All the mass ions (28-500 amu) from a single particle can be simultaneously measured by this instrument. Such studies have been proposed separately to the Army Research Office.

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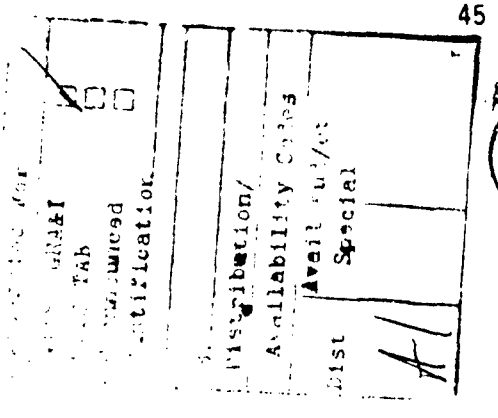
TABLE OF CONTENTS



I. INTRODUCTION

The goals of this project were to make basic studies toward the development of a method for the rapid characterization of aerosol particles of biological nature using the technique of Particle Analysis by Mass Spectrometry (PAMS). The PAMS method involves a combination of the techniques of particle beam generation and mass spectrometry. It provides a method for the generation of mass spectrum of micron-size individual aerosol particles on a continuous, real-time basis. This generation of characteristic mass spectrum from single particles makes PAMS a unique method for the detection/identification of biological particles in air on an instantaneous basis, a method which here-to-fore was unavailable.

The major objectives have been accomplished. Beams of particles composed of biological compounds and different microorganisms were prepared for their introduction into the ion-source of the mass spectrometer. The physical characteristics of the particle beam were studied in detail. Use of a particle beam offers the advantage of introducing one particle at a time into the ion source in a well controlled fashion. Two methods for the volitalization and ionization (V/I) of particles in the source have been studied. The first one involves the thermal volatilization of the particle followed by an electron impact ionization of the resulting vapor. The other uses a high-energy laser for both V/I of particles. Aerosols of several biologically important compounds, and different microorganisms were generated in the laboratory and mass spectrometric measurements were made on them.

Most of the results of the above investigations have been either published or submitted for publication.* The present report summarizes the

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*Publications are listed on page 43.

important results from these studies. A brief description of the PAMS methodology is included. References are cited for the details. Spectra from different microorganisms obtained in this research work have been found to possess several distinguishing features making it possible to differentiate microbial particles from nonbiological ones and also enabling differentiation among themselves. Some suggestions for further work are also described in this report.

II. BACKGROUND

Due to the complex nature of particulate material present in the atmosphere, it is important that the analytical methods used for detecting and identifying microbial particles in air should not only be sensitive to biological compounds but should also be specific enough to eliminate interferences. Methods which use chromatography, mass spectrometry (MS), and various combinations of these two techniques meet such requirements and have attracted much attention for the rapid analysis of microorganisms.[1,2,3,4] In particular, direct application of mass spectrometry to the pyrolysis (Py) product of biological samples has been used by various workers[5,6] to characterize a wide-range of biological materials.

The earlier studies by others for bacterial identification by mass spectrometry involved separate steps of pyrolysis of bulk quantities (milligram) of bacteria samples, collection or separation of the volatile pyrolysis products, followed by their introduction into a mass spectrometer.[8] Mass fragments were recorded for nearly all integer masses between 14 and 140 amu. The complex mass spectra permitted determination of up to 100 components, but did not give structural information useful for bacterial differentiation.

A more recent design of a pyrolysis mass spectrometer for characterization of complex biological material is that of Meuzelaar and coworkers.[6,9] In this instrument, bulk samples of (>10 μg) are pyrolyzed in a glass reaction tube using the technique of Curie-point pyrolysis. The volatilized products leak through an orifice into the mass spectrometer (ms) where low-voltage electron-impact ionization and mass analysis is performed. The mass spectrum obtained with this instrument is limited to the m/e range of 30 to 140. The instrument has been demonstrated to successfully differentiate among several distantly related bacteria on the basis of relative intensities of various masses. It has also been used to study the effects of growth media on the composition of the cell wall of B. subtilis[10]. Wieten et al.[11] incorporated multivariant factor analysis into the comparison of the spectra from this instrument. This permitted differentiation of a variety of closely related species from the genus Mycobacterium.

However, the application of the above methods to aerosol particles require the collection of a sample from air and its preparation in a suitable form before analysis. These entail some preanalysis time, and the collection of all particles without any discrimination may introduce background noise in the analytical procedure. Since particles other than the microorganisms of interest, are in most environments numerous in air, a desirable way to analyze the airborne microbial particles in the midst of a host of other particles would be to work on individual particles. This would provide a major improvement in the signal-to-noise for the bioparticle analysis. In some circumstances, the speed of analysis is critically important and a real-time monitoring of the environment for the presence of microorganisms may be desired. Such a method for the analysis of individual bioparticles on a continuous real-time basis has been developed

under the present Army Research Office (ARO) sponsored research. Known as Particle Analysis by Mass Spectrometry (PAMS), the technique seems to hold promise for detecting, quantifying, and identifying airborne microorganisms.

III. PARTICLE ANALYSIS BY MASS SPECTROMETRY

PAMS may be classifed as a Pyrolysis-mass spectrometry (Py-MS) technique with unique features; the direct introduction of aerosols particles in the form of a beam into the ion source of the masss spectrometer; the volatilization and ionization of one particle at a time under optimum conditions of pyrolysis; and the mass spectral measurement of individual particles. These features enable the analysis of single particles on a continuous, real-time basis and are described below.

A. Particle Beam Generation

The aerosol particles are delivered to the ion source of the mass spectrometer in the form of a beam. It is formed by an aerosol expanding into a vacuum through the generator that consists of a capillary nozzle (100 μm in diameter and 5 mm in length) and a set of two skimmers with orifice diameters of 350 μm and 500 μm. The particles, being much heavier than the carrier gas molecules, remain concentranted along the central axis. The skimmers allow an efficient transmission of the particles into the ms chamber and provide for differential pumping of most of the carrier gas upstream. The details of the beam generator have been described elsewhere[12,13]. However, in the original system, the beam tube was arranged in the horizontal direction, and only one skimmer was used. Since the particles are massive compared to the gas molecules, they tend to be lost along the sample delivery line and physical losses occur on the surface because of settling, convective flow, or diffusion.[14] The predominance of any one of these mechanisms depends on the particle size and flow velo-

city. For particles of diameter greater than 0.5 μm, the gravitational settling has the highest contribution to the particle loss. Several modifications to the PAMS system, noted below, were made for the present work in order to minimize the particle loss in the delivery system and also to increase the sensitivity of the analytical method.

1. The beam generator was reconfigured so as to make the beam tube lie along the vertical direction. This was done to minimize the sedimentation loss of larger particles, in particular.

2. Arrangements were made for the isokinetic sampling of the aerosol particles in the beam tube.

3. The second skimmer was added between the first skimmer and the MS to reduce the gas load to the MS chamber. The region between the two skimmers was differentially pumped. The size and location of the second skimmer was selected such that it did not reduce the particle throughput to the MS to any significant extent.

4. Liquid-nitrogen-cooled traps were installed above the vacuum pumps in order to cut down the backstreaming of oil into the system.

Figure 1 shows the schematic of the PAMS system.

B. Mass Spectrometer Subsystem

Mass spectrometric measurements on particle involves volatilization and ionization of the particles, mass separation of the ions, and quantitative measurement of ion currents for different masses. The sensitivity of the method depends on each of the above steps. Different methods of V/I of particles have been applied. In particular, the method of thermal volatilization and electron-impact ionization have produced highly encouraging results for the analysis of aerosol particles. Some preliminary work on laser V/I of particles have also been done[15,16] and is described

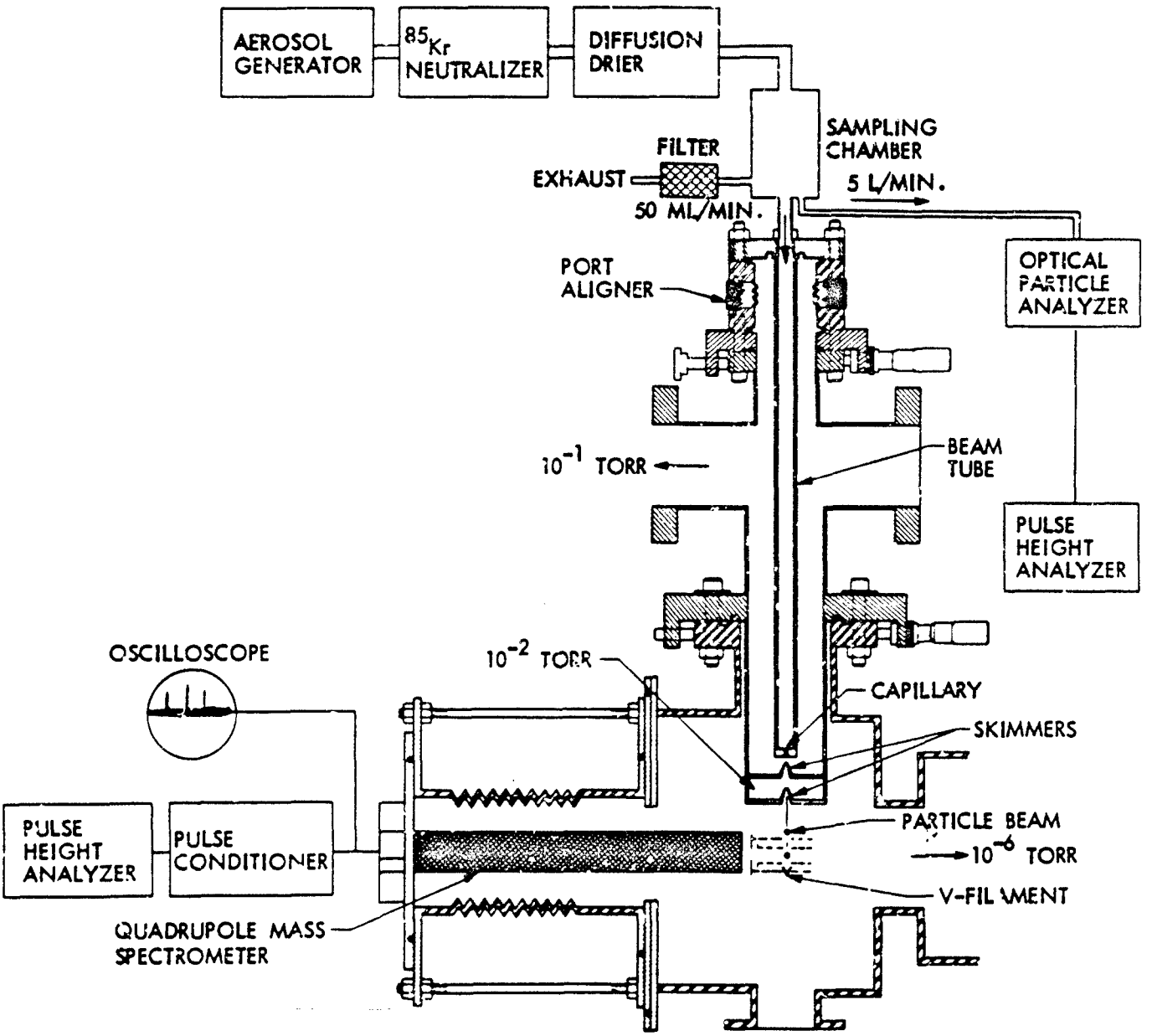


Fig. 1: Schematic of PAMS Systems

in Section V.

1. Thermal Volatilization and Electron-Impact Ionization

The particle beam enters the main spectrometer chamber through the skimmers and impinges on a V-shaped zone refined rhenium filament (Fig. 1) located between the grid and the repeller of the ion source of the quadrupole mass spectrometer (Uthe Technology, Inc., Model 100C). The filament is resistively heated and maintained at a constant temperature in the range of 200-1400° C. A particle striking the filament produces a plume of vapor molecules; these are ionized by electron impaction in an in situ fashion. Volatilization and ionization of each particle is a discrete event in time and results in a burst of ions for each particle, as shown in Fig. 2. The ion-pulse width has been found to be ~100 to 200 μs. The brevity of the pulse does not allow for the scanning of the mass spectrometer for different mass ion measurements.

2. Data Acquisition

A quadupole mass spectrometer measures the intensities of different masses by scanning them in time. Since the available scan speed is too slow for a complete mass analysis of a single ion pulse, only one mass peak is monitored from each particle. The mass range is scanned manually, and the intensity measurement is made wherever the signal is observed. The scheme for average intensity measurement is shown in Fig. 3. Measuring the average intensity of a mass peak is particularly important when the input aerosol is polydispersed. The ion pulse at a particular mass is fed into a charge integrator that integrates the signal pulse for a preset time interval. Immediately after this, the background noise is integrated for the same length of time and subtracted from the stored signal. The distribution of the peak voltages of the integrated signal pulses is then measured with a a pulse-height analyzer. The distribution

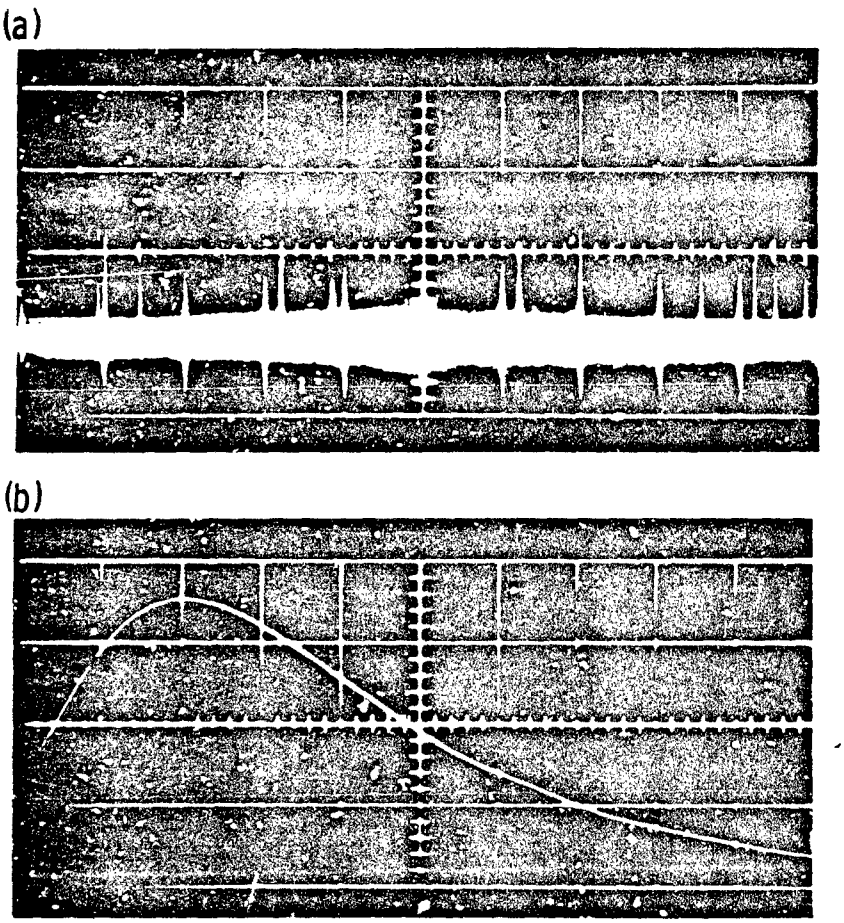


Fig. 2: Oscilloscope traces of: (a) the pulses showing the burst of ions produced from the volatilizing and ionizing of individual dioctyl phthalate particales of 1 μm diameter; (b) the fast scan of a single pulse. The horizontal scale is 100 ms per division for (a) and 20 μs for (b). Mass peak at 149 u is monitored. Reproduced with permission.

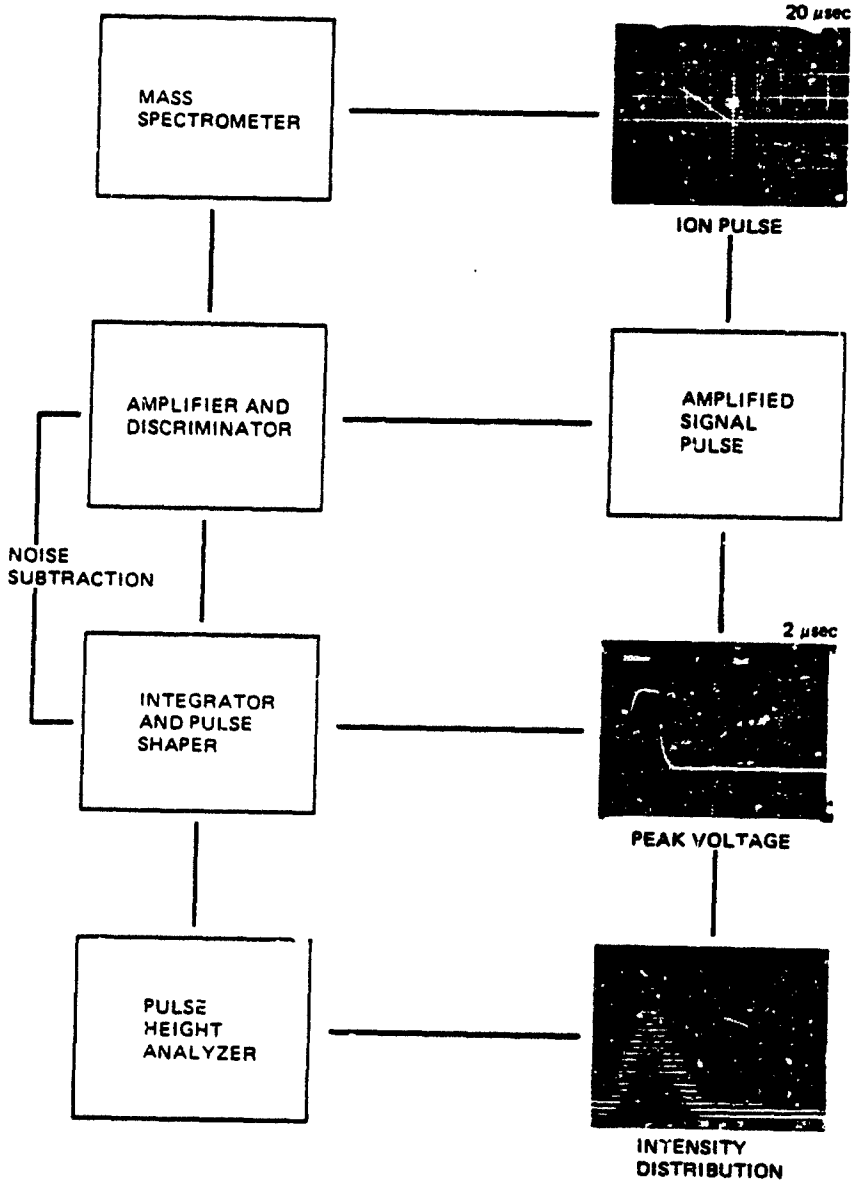


Fig. 3. Average intensity measurement scheme for a mass peak.

is sufficiently narrow for the monodisperse aerosol and shows the reproducibility of the volatization process. The peak of the distribution measures the average intensity of the particular mass peak. For a polydisperse aerosol, the intensities in various channels of a distribution are weighted by their respective number of particles. The sum of the number weighted intensities is then divided by the total number of particles to obtain their weighted average intensity. At a selected mass, ~1000 pulses are processed to obtain a distribution of single intensities. The mass spectrum is a collection of the average intensities of individually measured mass peaks and represents the spectrum of an average individual particle. One such spectrum of 1.7μm diameter potassium biphthalate aerosol is shown in Fig. 4.

C. Generation of Aerosols

The aerosols were generated by various techniques in the laboratory. The choice of the method depends on the nature of the material to be aerosolized. For compounds which are soluble in water or alcohol, a vibrating orifice aerosol generator (Thermo System, Inc. Model 3050) was used. This consists of forcing a dilute solution (~0.01% v/v) of the compound through an orifice (10 to 20 μm diameter) in a plate; the resulting liquid jet is broken by vibrating the plate in contact with a piezo-electric crystal at a fixed frequency. This generated droplets of uniform size, that after drying in a stream of air or nitrogen, produce a highly monodispere aerosol. The particle size can be changed by properly adjusting the concentration of the solution, its feed rate, and the frequency of vibration. A micronebulizer (Bird Model 9993) was used for the generation of aerosols of bacteria particles.

The droplets acquire electrical charges during their generation and

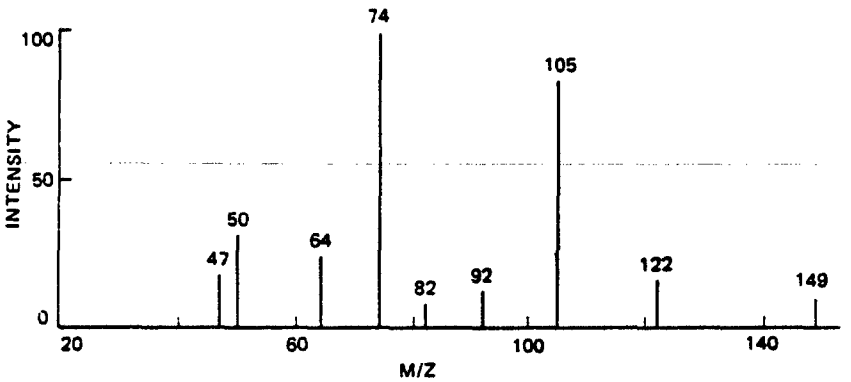


Fig. 4: Mass spectrum obtained from potassium biphthalate particles of 1.7 μm diameter.

therefore were passed through a cylinder containing Kr^{85} where the charge is neutralized by capturing ions of gas molecules. The aerosol was finally dried by passing it through a silica gel diffusion drier (Fig. 1). After drying, the aerosol stream passed into the sampling chamber where a portion of it was isokinetically sampled into the beam tube.

IV. RESULTS

A. Particle Beam Characterization

The objective of this study was to characterize the performance of the beam generator. This involved the determination of the transmission efficiency of the generator and the divergence of the beam for different size particles. Various monodisperse potassium biphthalate (KBP) aerosols were generated and the transmission efficiencies --- ratio of number of particles in a given volume of the aerosol to the number of particles in the beam generated from that volume --- were determined. This was accomplished using light scattering techniques[14]. A He-Ne laser was used as the light source. When a particle in the mass spectrometer chamber passes through the laser beam, it scatters light predominantly in the forward direction of the laser beam. Scattered light, between the angles of 20 and 65°, was collected and focused on a photomultiplier tube. The output pulses were counted on an electronic counter. The particle number density and the volume flow rate of the aerosol at the input of the beam tube were measured by an optical particle counter and a flow meter, respectively. High transmissions of particles were observed for different size particles. For example, the transmission efficiency for 3μm particles was found to be ~70%.

Transmission efficiencies of monodispere polystyrene latex and KBP aerosol particles have also been measured by Estes et al.[17] using a beam

generator nearly identical to that in the PAMS system. The difference being that only one skimmer was used in their studies. The particles in the beam were collected on a Vaseline-coated-glass-slide and their number was counted under a microscope. The average transmission efficiency of ~80% is found for particles in the size range of 0.3 to 5 μm. The divergence of the beam was determined by measuring the size of the beam deposit on the slide and the distance of the slide from the capillary. The divergence of the beam does not change drastically between particle diameter in the range of 1 to 5μm.

Figure 5 shows a deposit of the beam generated from Bacillus subtilis aerosol on an Apiezon-grease-coated-glass slide in the mass spectrometer chamber of the PAMS system. The beam has a divergence of 1.6×10^{-3} sr.

The measurement of transmission efficiencies of the beam generator and the beam divergence is particularly important when the beam is generated from a polydisperse aerosol (natural aerosol) and if only a part of the cross section of the beam is analyzed. The results show that the input aerosol (of particle size range 1-5 μm) is not significantly modified in the process of its beam generation. The weak dependence of the divergence of the particles in the beam on their size was further confirmed by measuring the fraction of particles in the beam that impacted on the V-filament. This was found to be 35 to 45% and independent of the particle diameter over 1.5 μm to 3.9 μm size range studied. Fig. 6 shows the filament collection efficiency as a function of particle diameter.

B. Mass Spectral Measurements

The PAMS technique was applied to the mass spectral measurements of a variety of aerosols generated from organic compounds (which constitute the biological macromolecules), and aerosols of microorganisms. The long

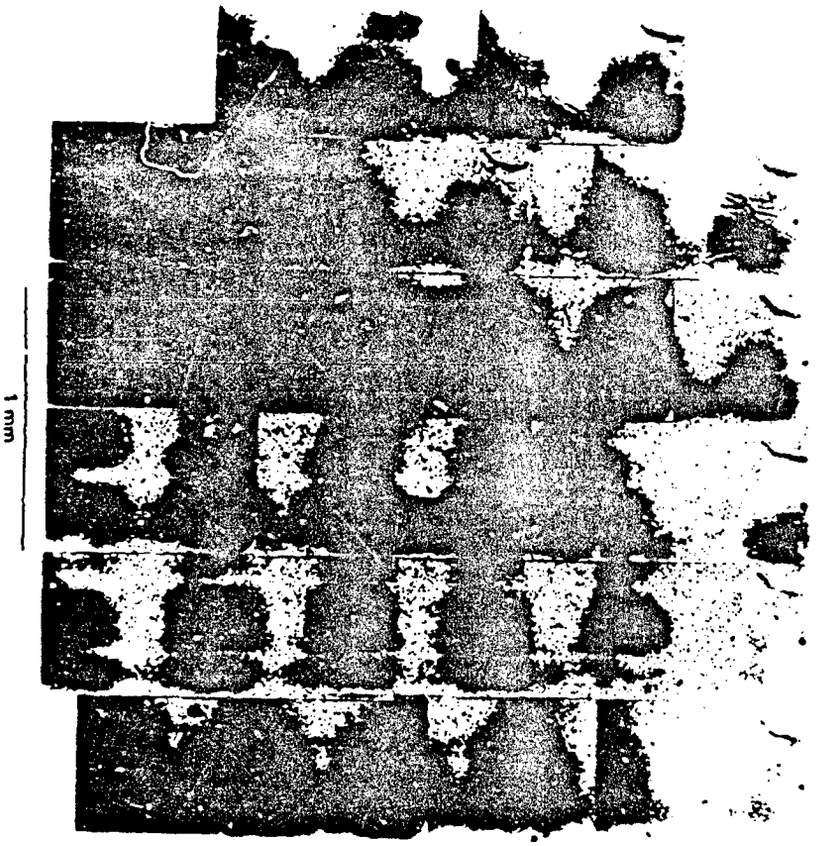


Fig. 5: Deposits of Bacillus subtilis particles from a beam on a glass slide coated with Apezion grease in the mass spectrometer chamber.

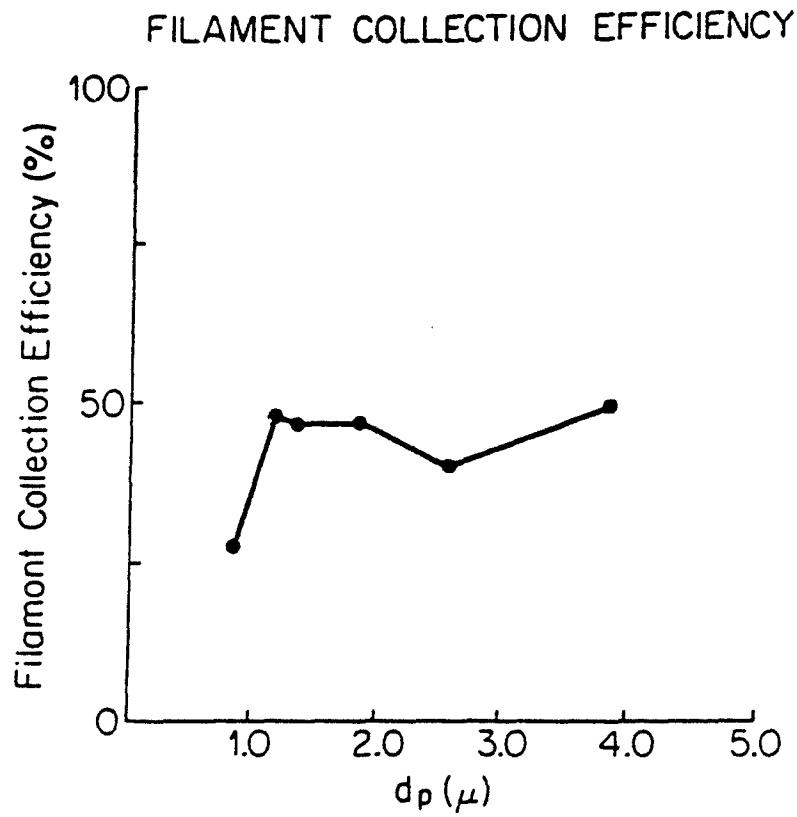


Fig. 6: Filament Collection Efficiency

term objective of studying aerosols composed of simple organic molecules is to identify the presence of these molecules in their macromolecules. These can provide as markers in the identification of different microorganisms.[18]

1. Organic Aerosols

Aerosols of biologically important organic compounds were prepared with the vibrating orifice aerosol generator. Particle beams from these aerosols were prepared and their mass spectra obtained. Figures 7 through 9 show some of the results. The results on these particles are very encouraging. Where comparisons are available, results match with the existing spectra in the literature (EPA/NIH Mass Spectral Data Base, 1978) except that the relative intensities of the peaks are different in some cases. It may be due to the difference in the operating conditions. In the PAMS technique, the volatilization is a very fast process (~30 μsec) whereas the library spectra were run with a conventional thermal probe taking several seconds for the vaporization of these compounds. The ionizing electron energies are also different.

We observe the retention of the characteristic mass peaks obtained from aerosol particles of simple molecules in the mass spectra of their polymer particles (Fig. 9). This mass spectral feature may prove to be useful for the identification of different components present in the aerosol particles of complex biological nature.

2. Biological Particle Analysis

The PAMS technique has been applied to the real-time mass spectral analysis of single biological particles[19]. The aerosols of Pseudomonas putida, Bacillus subtilis and Bacillus cereus were generated by nebulizing their ethanol suspensions.

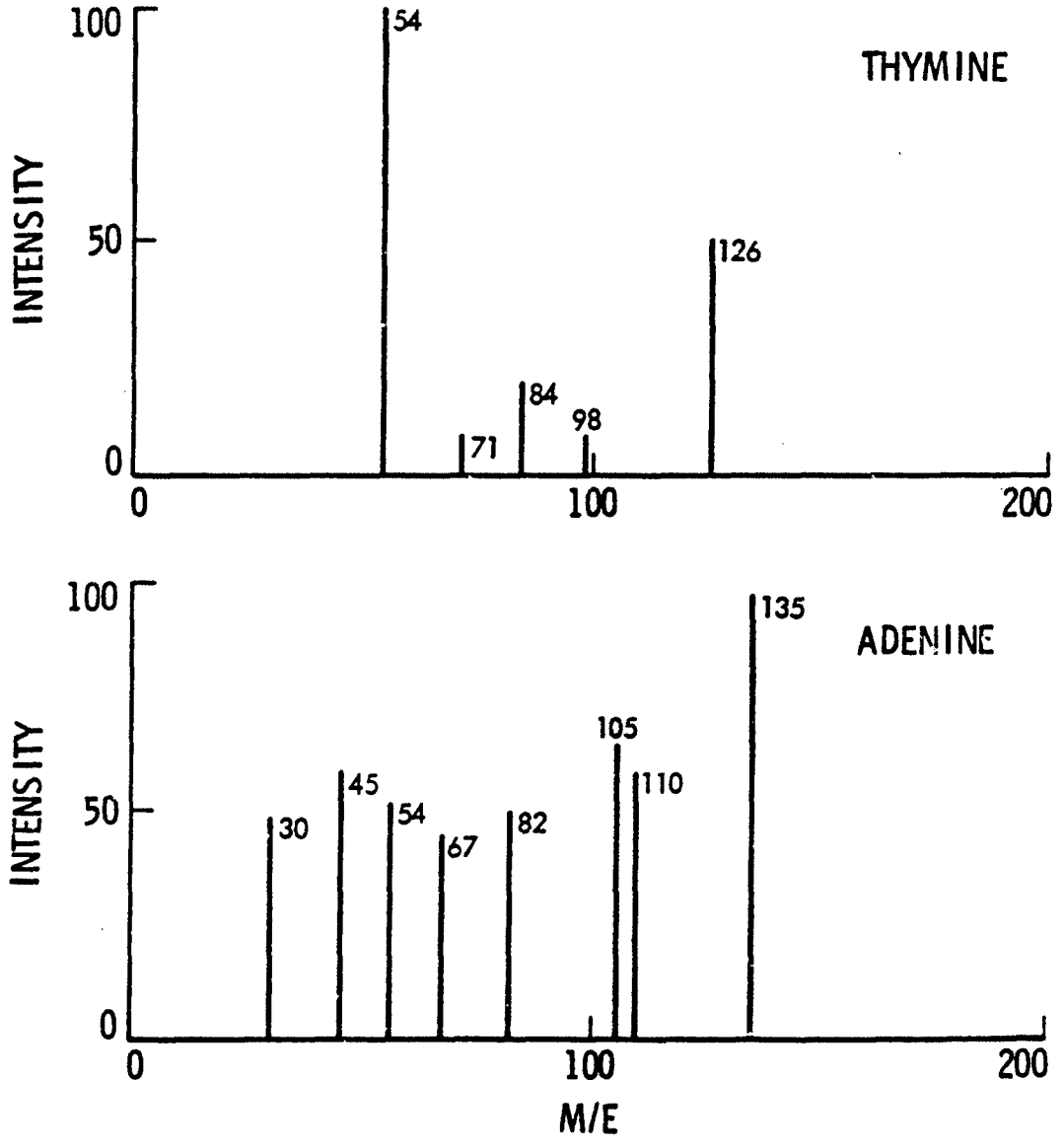


Fig. 7 Spectra from 1.2 μm diameter particles.

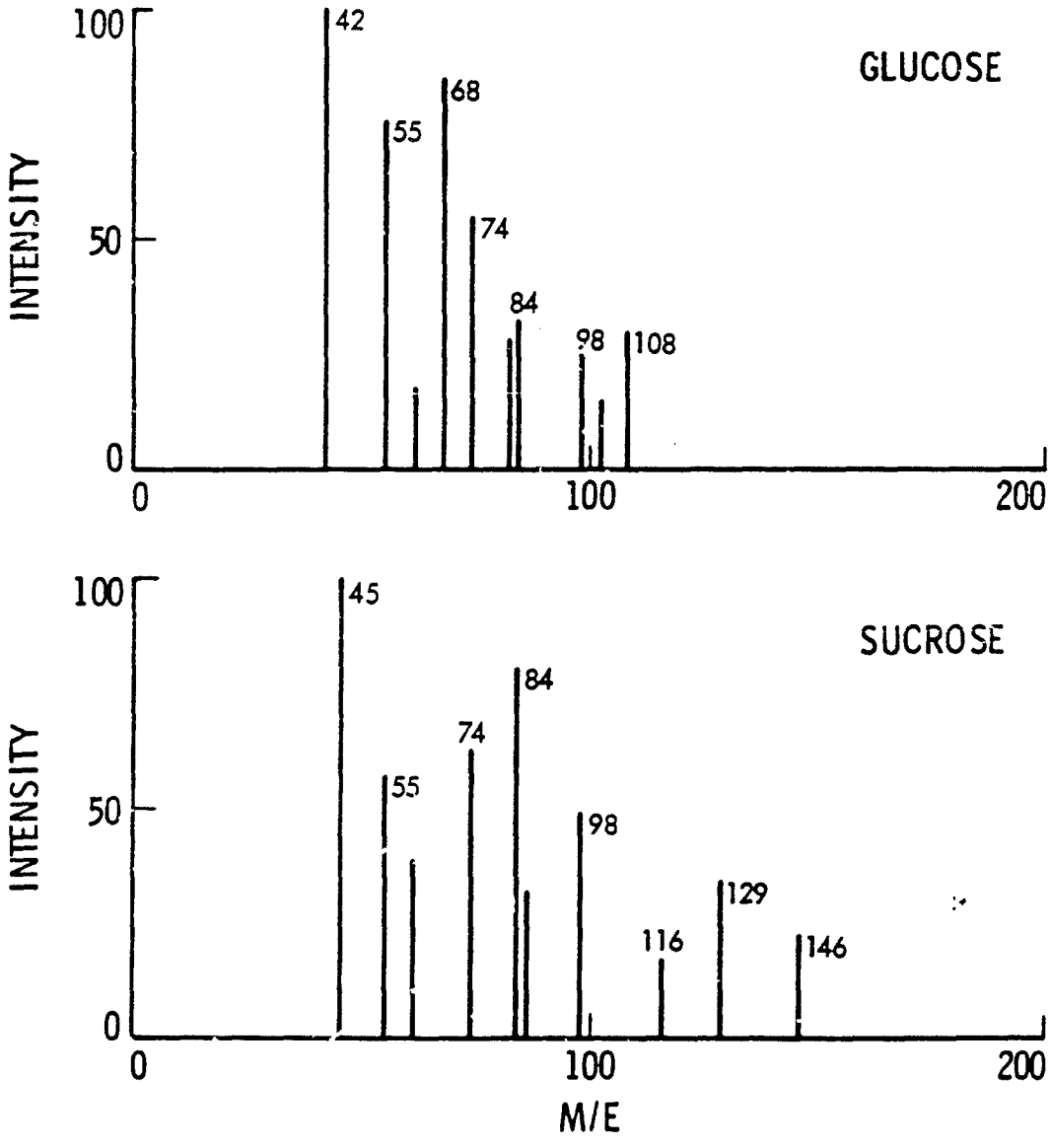


Fig. 8: Mass spectra obtained from glucose and sucrose particles (1.7 μm diameter).

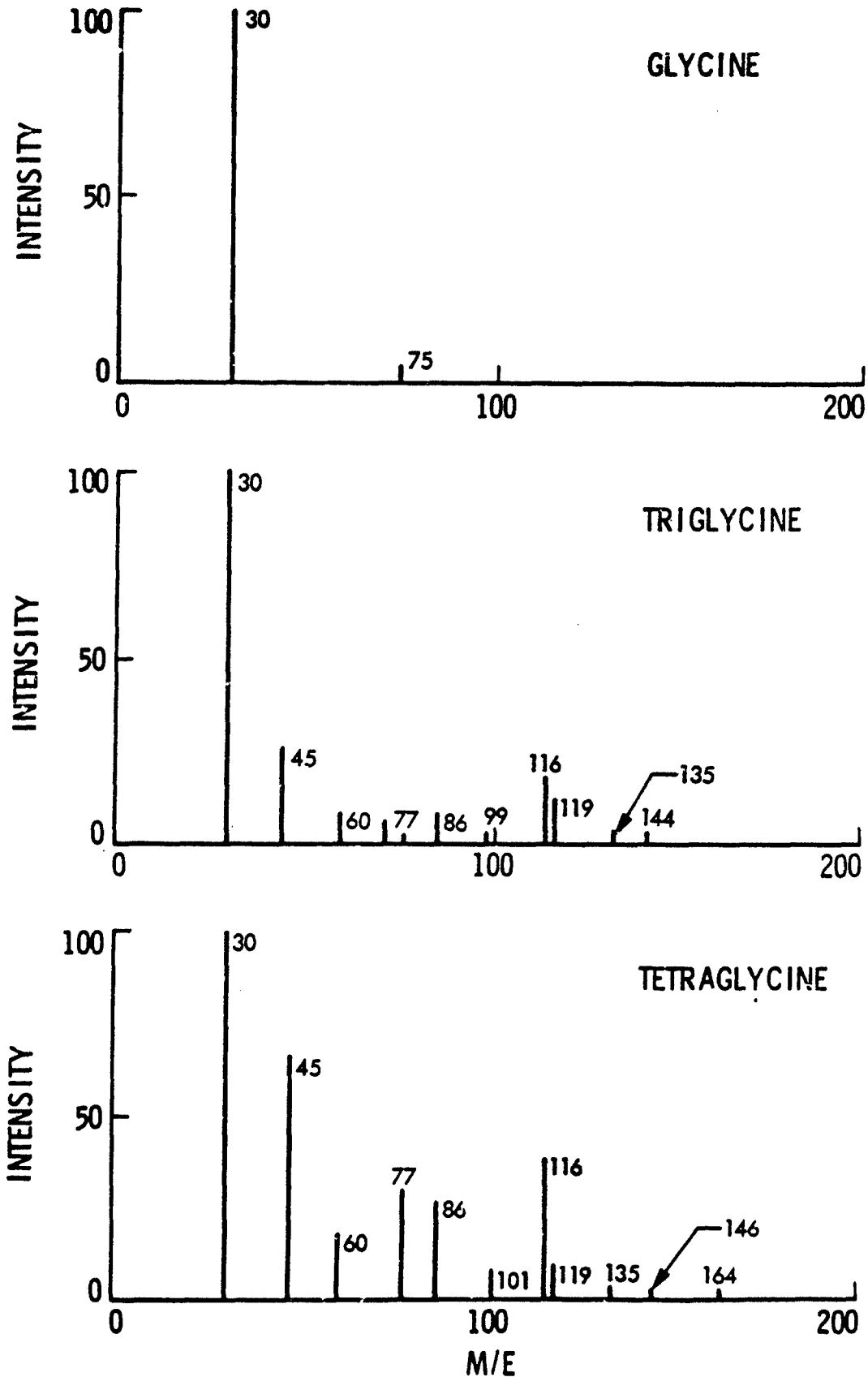


Fig. 9: Mass spectra of different peptide particles of 1.7 μm diameter.

The aerosol was introduced into the beam tube which resulted in an array of particles in a form of a beam in the MS chamber. The particles were collected on an Apiezon-coated-glass-slide. Figure 10b shows an electron micrograph of the deposit which was made with Bacillus subtilis spores. Figure 10a is a deposition of the spore particles on a nucleopore filter after aerosolization. It can be seen from Fig. 10 that the cells retain their integrity during the process of aerosolization and beam generation in the vacuum. We believe this is the first time that a beam of cells has been made for direct introduction into the ion source of a mass spectrometer. The generaation of a beam is not limited to spores possessing hardy characteristics. Beam particles of Pseudonomas putida (a non-spore-forming bacteria) were prepared and used for the mass spectral analysis.

The particles are injected in the form of a beam into the mass spectrometer where they are individually volatilized by impaction on a rhenium filament and ionized by electron bombardment. The average intensity of a mass peak is obtained from the pulse height distribution of about a thousand ion pulses from different particles. Figure 11 shows the mass spectra thus obtained. Several high mass peaks (>125 amu) are conspicuous in the spectra which are different from the previous pyrolysis-mass spectral work [8,10] The larger mass fragments have higher information contents and can be effectively used as a signature for their parents. These fragments may arises due to the difference in the pyrolysis conditions. In the PAMS method, the duration of pyrolysis of a particle is extremely small (~30 usec). Furthermore, the vapor molecules are produced in a collision-free condition in the ion source and the small amount of sample (~10^{-13} g/particle) assures a uniform temperature for the vaporization.

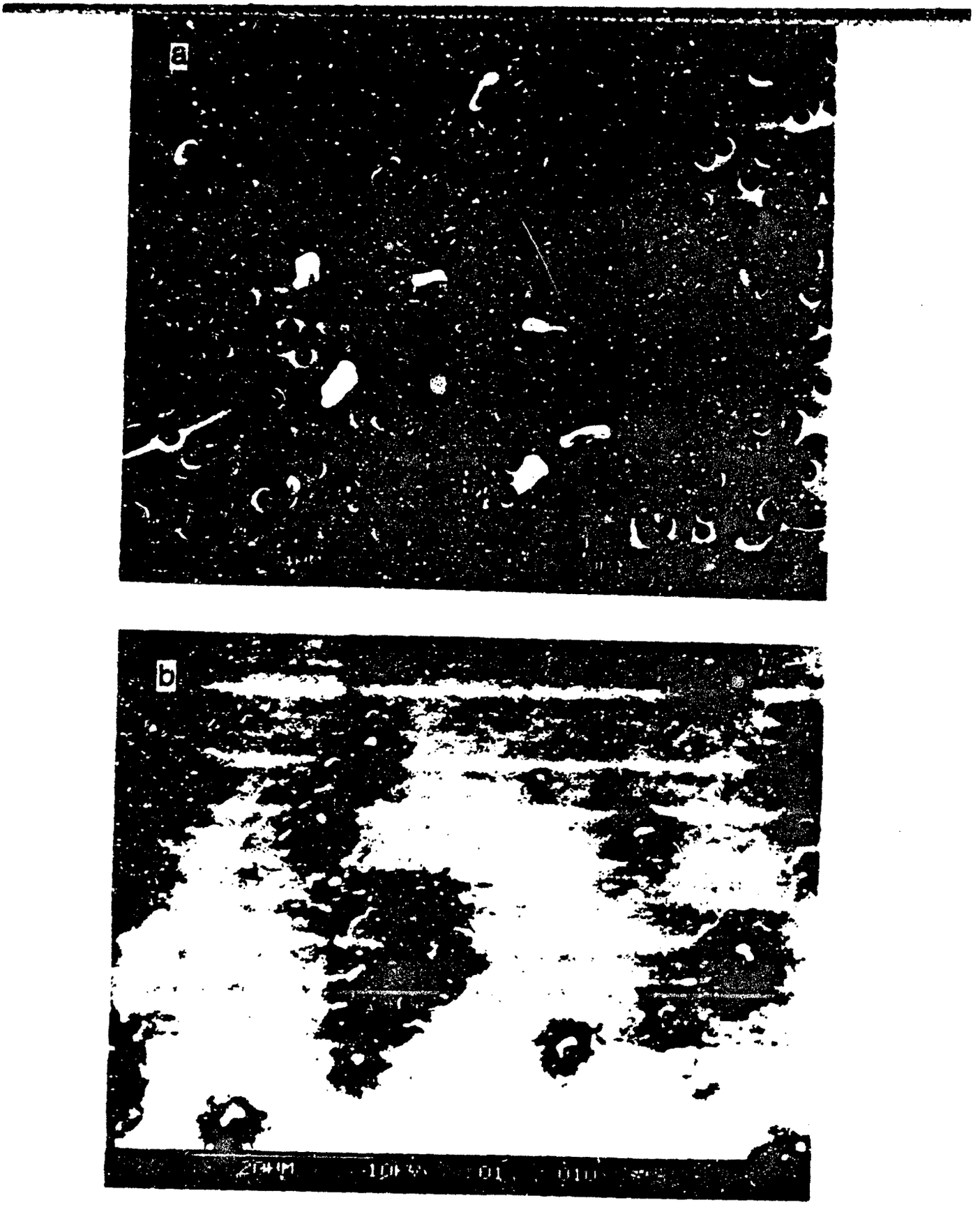


Fig. 10: Electron micrographs of Bacillus subtilis spores collected on (a) Nucleopore filter from aerosol and (b) Apiezon coated glass slide from particle beam in MS chamber.

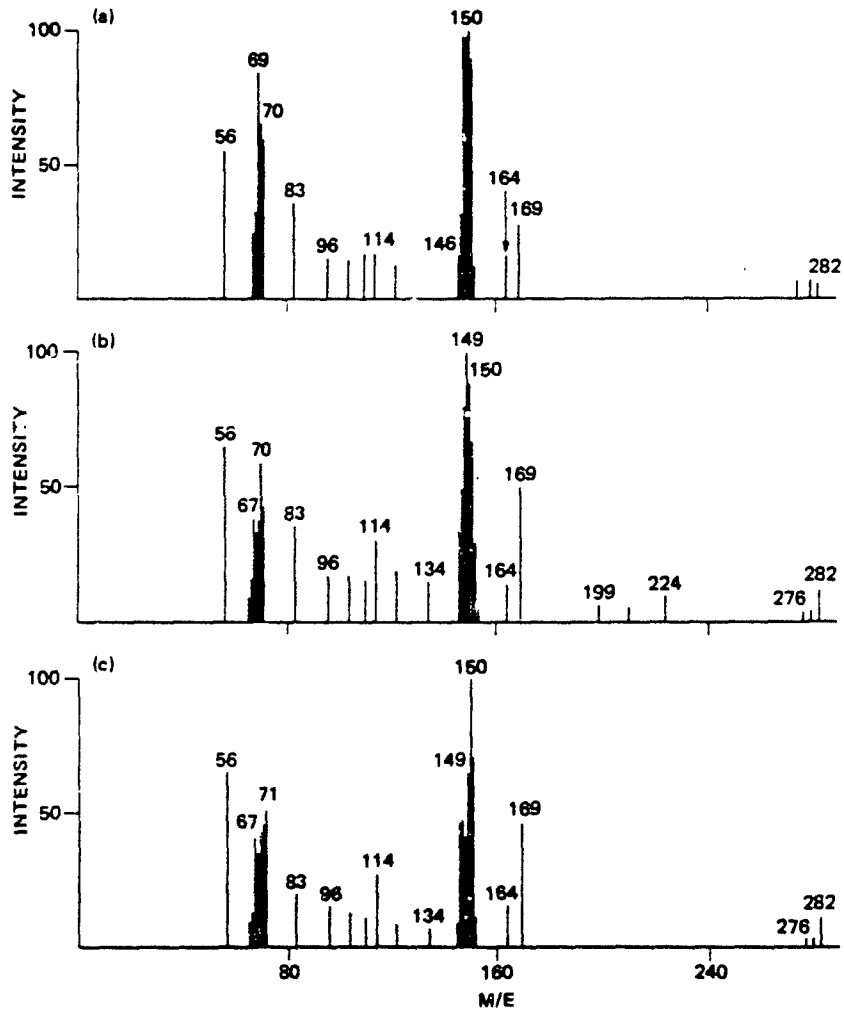


Fig. 11: Mass spectra of bacteria particles: (a) Pseudomonas putida; (b) Bacillus cereus; (c) Bacillus subtilis

A striking similarity between the mass spectra of P. putida, B. subtilis and B. cereus bacteria particles can be seen, which is not surprising since the microorganisms have essentially the same major chemical building blocks; however, some visual differences can be seen in them. The mass peaks between 192 and 275 u were weak for Pseudonmonas putida and Bacillus subtilis, and a good intensity measurement of these peaks could not be made. The spectra may be distinguished by considering the relative intensities of different peaks. For example, the relative intensities of mass peaks at 67, 114, 169, and 282 compared to the intensity at 149 are higher in the spectrum from Bacillus cereus than those in the spectrum from Pseudomonas putida. Another difference between the P. putida and the Bacillus samples lies in the presence of m/z 134 peak in the Bacillus samples. This could be related to differences in the composition of the bacterial cell walls; bacilli are gram-positive and P. putida is Gram-negative.[20]

Such difference in intensities may be used for the differentiation of biological particles. An objective comparison of mass spectra and their reproducibility can be made by applying statistical procedures.[21] One such preliminary analysis of the mass spectra based on the criterion of the degree of correspondence[9] has been made.

3. Statistical Analysis of the Mass Spectra

In order to examine system reproducibility for samples of like species of bacteria and system capability to differentiate between samples of unlike species, we used a statistical method for comparing the visually similar mass spectra. This method, based on the degree of correspondence, was adapted from Kistemaker et al.[9] who used it to differentiate among spectra of distantly related bacteria. More advanced statistical

programs could not be used for the analysis of mass spectral data due to limited amount of data from our experiments. In the degree of correspondence method, one spectrum is arbitrarily designated as the "reference" spectrum with peak intensities $\{R_i\}$ at the several i values of m/e. The peak intensities comprising each "sample" spectrum to be compared with the reference are $\{S_i\}$. The peak intensities of the sample are first normalized to those of the reference spectrum by multiplying the sample by a scaling factor α which minimizes the error E in the squares of the difference of comparable peaks in the two spectra, each consisting of n total peaks:

$$E = \sum_{i=1}^{n} (R_i - \alpha S_i)^2$$

Setting $dE/d\alpha = 0$, gives the value of the scaling factor to be:

$$\alpha = \sum_{i=1}^{n} R_i S_i / \sum_{i=1}^{n} S_i^2$$

In the case of comparing two spectra from <u>like</u> species, perfect reproducibility means that the intensities for each pair of peaks are equal and α is unity. In practice, differences in culture growth, particle beam characteristics, volatilization/ionization conditions and spectrometer signal processing lead to non-unity values of α. In the case of comparing two spectra from <u>unlike</u> species, differences in the masses of individual bacteria particles are also taken into account in the α calculation.

The degree of correspondence between the normalized sample spectrum and the reference spectum is the average of the ratios of the minor peak intensities to the corresponding major peak intensities:

$$C = \left(\sum_{i=1} A_i/B_i \right) / n$$

where A_i is the minor peak intensity in spectrum $\{R_i\}$ or $\{\alpha S_i\}$ and B_i is the respective major peak intensity in spectrum $\{\alpha S_i\}$ or $\{\alpha S_1\}$. In case of perfect matching, the degree of correspondence is one. A correspondence of zero indicates the spectra have no peaks in common. The value of C from comparing spectra of like species is a measure of reproducibility and is a characteristic of the system as a whole. The system is capable of differentiating between species if the comparison of spectra from unlike species results in a value of C which is significantly different from the characteristic value.

Two more cultures of B. cereus in addition to the spectrum shown in Figures 11 were analyzed in our system. The spectrum of one of these three was chosen as the "reference" and the degree of correspondence between this reference and each of the other sample spectra was determined. All the mass peaks were used in the calculation for C without discriminating for their sensitivity to the intensity variations in different runs. The results of the comparison are shown as the histogram in Figure 12. The results for the B. cereus samples indicate that the characteristic degree of correspondence for our system is in the range of 0.70-0.77. Since C = 0.70 for B. subtilis, it cannot be differentiated from B. cereus in our system. However, for P. putida, C = 0.64 indicating that this species can be differentiated from the bacilli samples.

V. LASER-INDUCED VOLATILIZATION AND IONIZATION OF PARTICLES

The use of lasers in mass spectrometric analysis has attracted much attention because of the advantages it offers over the other methods;[22] namely:

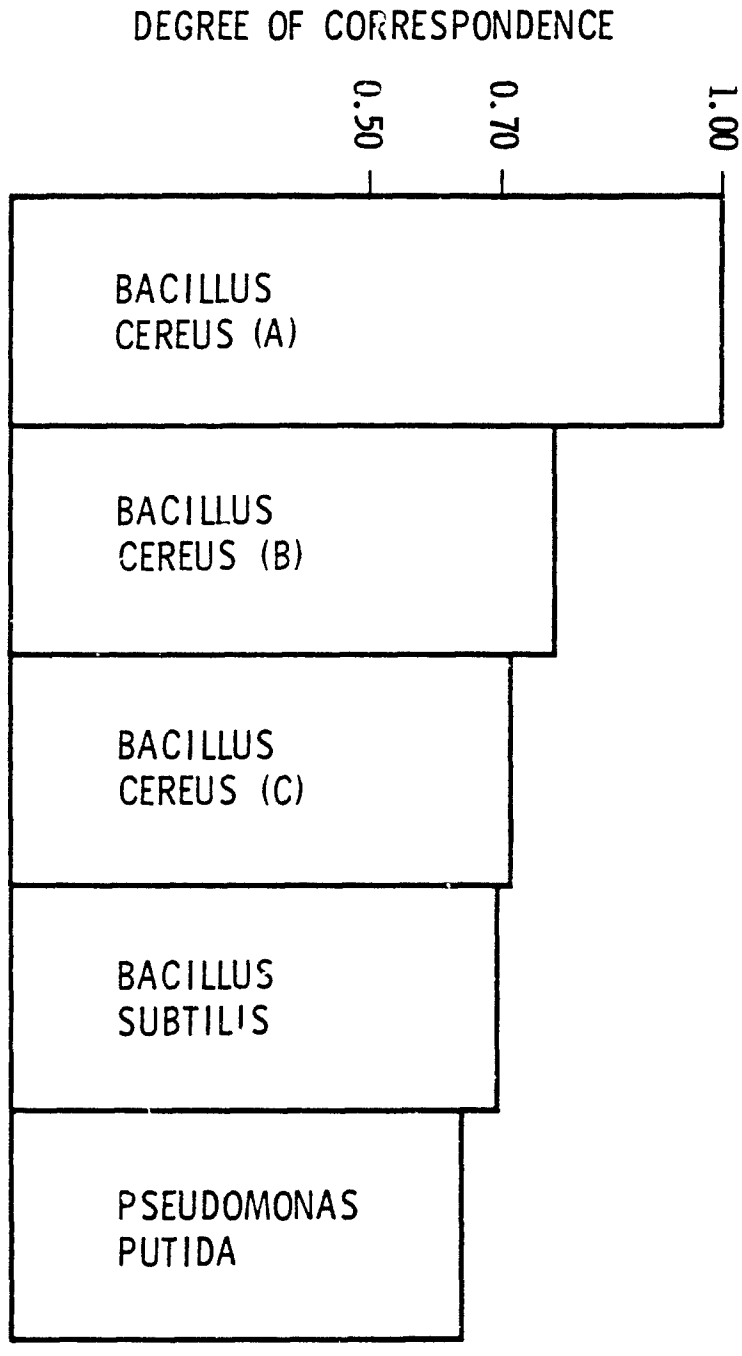


Fig. 12: Histogram of the degree of correspondence for the five bacteria samples. Each sample is a separate culture and B.cereus (A) was selected as the reference sample.

(a) the high efficiency for vaporization and ionization of solids.

(b) Its general applicability.

(c) The fragmentation of molecules during vaporization and ionization can be controlled making it suitable for analysis of relatively involatile, thermally labile compounds.

(d) The rapidity of analysis.

The above features of the ionization process may prove to be very helpful in generating characteristic fingerprint spectra for complex analytes, e.g., biological particles. With this in mind, preliminary studies were made on the feasibility of the use of high energy laser pulse for the volatilization and ionization of particles in the beam. The unique feature of this work being that the particles are vaporized and ionized while in flight in the beam instead of being collected on a substrate before hitting them with laser pulse. The real-time capability of the PAMS method is thus retained and the method eliminates any possible substrate-sample matrix interferences in the analysis.

The optical arrangement for the experimental set up for the laser-induced V/I is shown in Figure 13. For the synchronization of the particle with the YAG laser pulse, the velocity of the particles in the beam was determined. This was accomplished by measuring the time-of-flight of monodisperse aerosol particles between two He-Ne laser beam I & II. As a particle traverses through the laser beam, it scatters light. The scattered light pulses are monitored by the two photomultiplier tubes (PMT I,II). The

time interval between the pulses from laser beams I and II provide the velocity of the particle.

The synchronization of a particle with a high energy Nd-YAG laser (J. K. Lasers, system 2000 FQY2) pulse was accomplished by detecting the particle up stream of the ion-source. The scattered light pulse monitored by PMT I provided the reference time for the arrival of the particle moving with a known velocity in the ion source. A suitable timing circuit was developed such that the YAG laser is fired at the appropriate time for hitting the particles with the YAG pulse in the ion source. The details of the method are described in reference 16. The resulting ions were then mass analyzed with the quadrupole mass spectrometer.

A. Results

1. Velocity Measurements

The particle velocities in the beam corresponding to various particle sizes are listed in Table I. The standard deviation in the velocity is also given in Table I . The laser beams I and II are of 3 mm diameter and introduce some uncertainty in the flight distance for the particle between the two lasers. The measured center-to-center distance of 66.04 mm may vary between 66.04 ±1.5 mm depending on where in the rising portion of the PMT pulses the comparator threshold in set. In addition to this, the digital circuit used for measuring time also involves an uncertainty of two clock periods (±1 μs) in the TOF. The maximum spread in the velocity due to the above effects is also listed in Table I. This may account for most of the uncertainties in the velocity measurements.

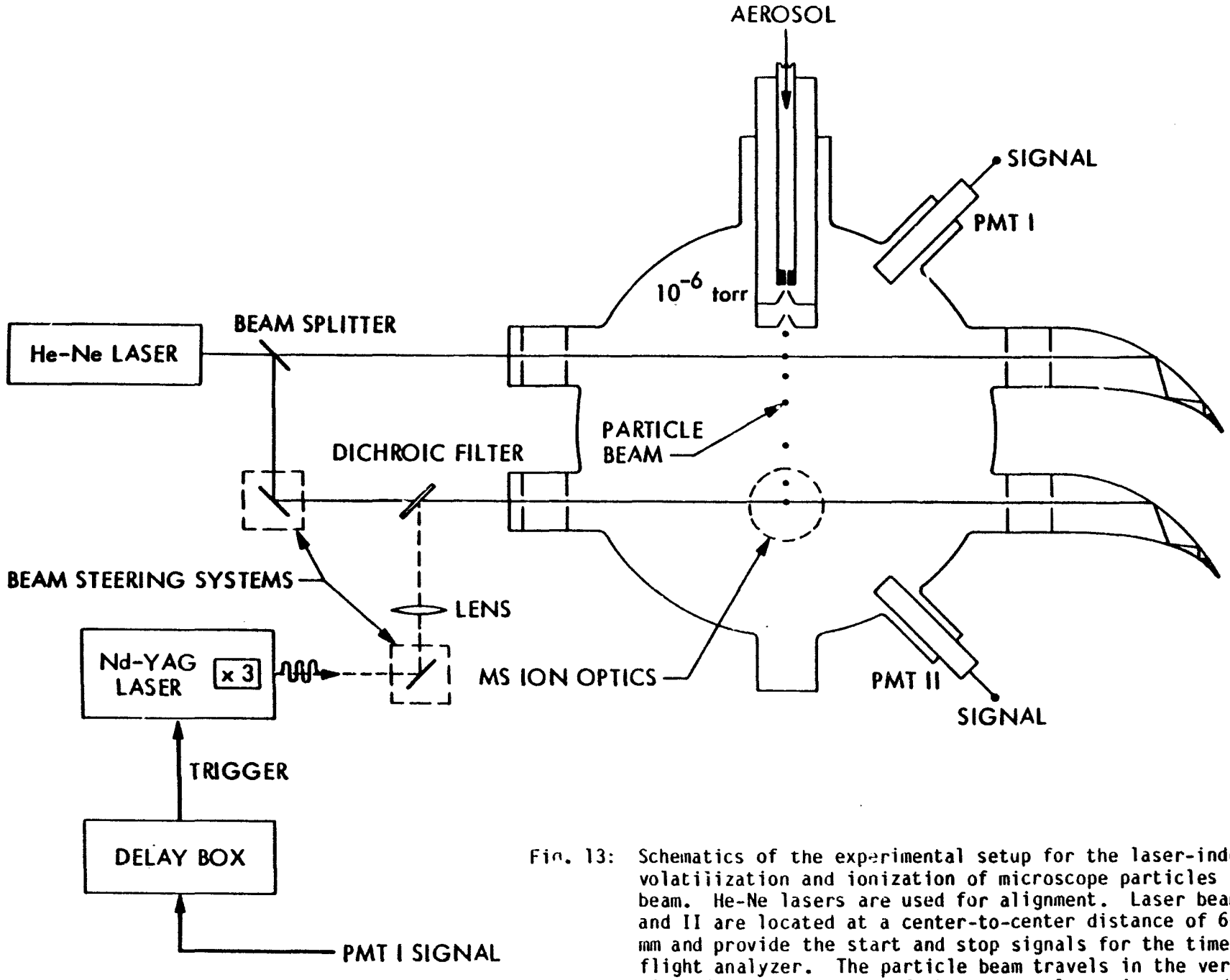


Fig. 13: Schematics of the experimental setup for the laser-induced volatiiization and ionization of microscope particles in a beam. He-Ne lasers are used for alignment. Laser beams I and II are located at a center-to-center distance of 66.04 mm and provide the start and stop signals for the time-of-flight analyzer. The particle beam travels in the vertical direction and the axis of the mass analyzer is perpendicular to the particle beam and the laser beam axes.

TABLE I: Velocity Measurement Results

Aerodynamic Particle Diameter (μm)	Particle Velocity (m/s)	Maximum Error Due to Flight Path, and Clock Pulse Counts (m/s)
1.27	355 ±9	±10
1.64	332 ±10	±10
2.06	299 ±11	±10
2.30	287 ±18	±9
2.87	267 ±11	±8
3.00	262 ±5	±8

Figure 14 shows the plot of particle velocity versus their aerodynamic diameter, d_a. d_a was calculated by the equation $d_a = \sqrt{\rho} \times d_g$ where ρ is the density of KBP salt and d_g is the geometric diameter which was obtained from the droplet diameter and the salt concentration used in aerosol generation. It can be seen that the velocity decreases with the increasing size of the particle and possesses a narrow distribution about their most probable velocity (a standard deviation of less than 6%). This feature may be used to measure the aerodynamic size of aerosol particles and may provide a method of relating the optical diameter with the aerodynamic diameter. The former can be obtained after suitable calibration from any one of the scattered light pulses from He-Ne laser beams and the latter from the velocity measurement. A sharp velocity distribution also helps in the synchronization of the particle and the Nd-YAG laser pulse for volatilization and ionization studies.

2. Volatilization and Ionization of Particles

The Nd-YAG laser used for the volatilization and ionization of the aerosol particles delivers 1 Joule of energy per pulse at a repetition rate of less than 20 per second, the pulse width being ~100 μsec. The time interval between the external triggering of the laser and the appearance of the pulse was measured. The YAG beam was aligned colinear with the He-Ne laser beam II (Fig. 13) and focused to a spot size of about 2.5 mm onto the particle beam axis. For the vaporization and ionization studies, the He-Ne laser II and the alignment lasers were turned off.

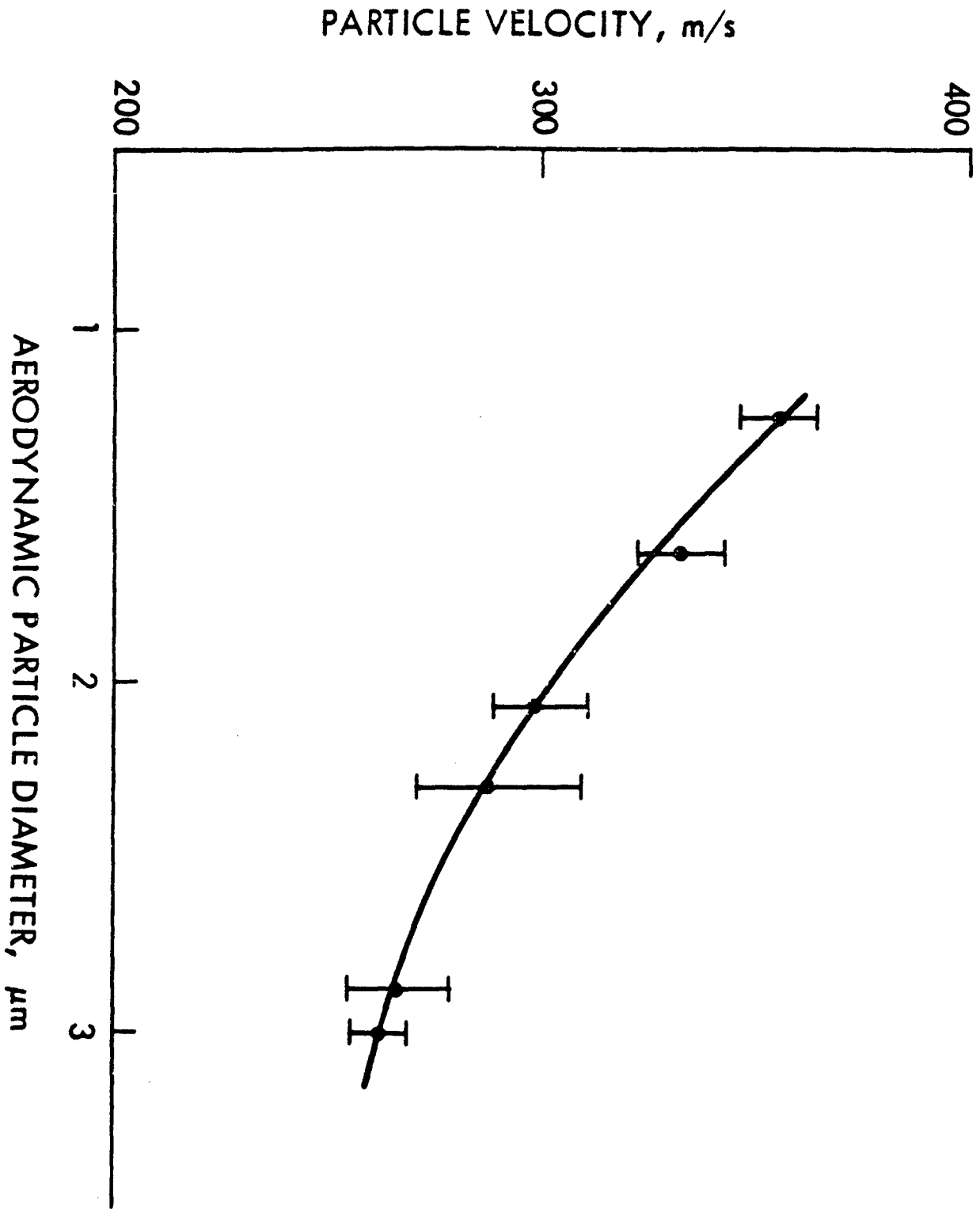
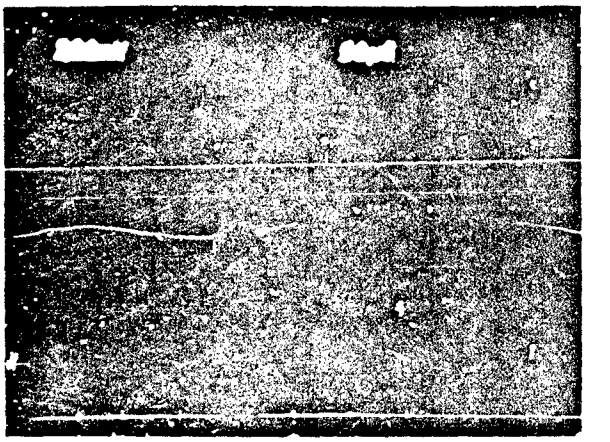


Fig. 14: Plot of velocity vs aerodynamic diameter of KBP particles. The error bars indicate one stardard deviation.

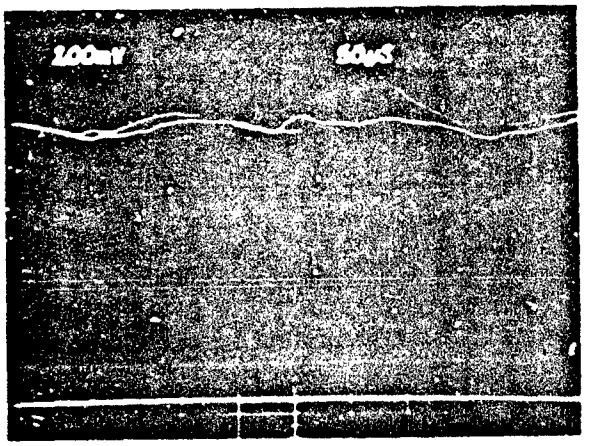
A beam of KBP particles of 1.96 μm in diameter was made. KBP was selected for the study because of the fact that the presence of alkali metals in organic salts have been found to facilitate the ionization of the salt.[23]

Figure 15 shows the oscillograms of the mass spectrometer signal for K^+ ion (upper trace) resulting from the laser volatilizatin and ionization of a single 1.96 μm diameter KBP particle. Oscillogram 15B shows that not all particles detected by the sensor laser I are hit by the YAG laser pulse. This results due to the divergence of the particle beam. The mass spectrometer was set manually at 39 amu. Manual operation is necessary because of the fast generation of ions from the laser pulse. The combined efficiency of vaporatization, ionization, and detection is about 10^{-6} i.e., 1 in 10^6 molecules present in the particle could be detected. Weak signals at masses 23 and 28 were also observed. We believe the signal at 23 amu arises from sodium present as impurity in KBP, and at mass 28 from CO fragments of the carboxylic groups. No other ion signal from the organic counterpart of the KBP molecules could be detected. The reason may be a combination of factors. The average power density in the focused beam spot is about 2×10^5 W cm^{-2} which is likely to be low for the ionization of organic molecules.[13] Also, the mass spectral features of the molecules in the laser-induced volatilization and ionization may be different from the conventional electron impact ionization and could be missed in the manual scanning of the MS.

The results obtained show that the method described above can be used for the volatilization/ionization of individual micron size particles in flight in a vacuum. The method may also provide selection of a narrow size range of particles in a beam generated from a polydisperse aerosol (natural aerosol). Such a size discrimination is possible because of the character-



(a)



(b)

Fig. 15: The upper trace in each photograph shows the mass spectrometer signal for K+ ions produced from potassium biphthalate particles (1.96 μm in diameter). The input signal to the delay generator (Fig. 14) was used to trigger the oscilloscope and is shown as the lower trace. The current was measured at $x10^{-7}$. A scale of the MS. The absence of MS signal in one of the upper traces in (b) shows that the particle is missed by the YAG laser pulse due to the divergence of the particle beam. The vertical scales in (a) and (b) are 200 mV per division and 100 mV per division, respectively, and the horizontal scale for both (a) and (b) is 50 μs per division.

istic velocities of particles in a beam. The method of volatilization and ionization of particles without any substrate may also find applications in other spectroscopic and chemical kinetic studies.

VI. CONCLUSIONS

PAMS has the following advantages:

1. Aerosol particles can be directly introduced into the ion source of a mass spectrometer in the form of a beam, thus eliminating the need of sample collection and preparation.
2. The bacteria particles remain intact during the process of beam generation.
3. Individual particles can be volatilized within a V-type filament.
4. The pyrolysis provides fast heating, uniform pyrolysis temperature, and proximity of the pyrolyzer to the ion source, and it assures that one extremely small sample at a time is pyrolyzed in a collision-free environment.
5. Mass spectra of individual biological particles can be obtained on a continuous, real-time basis.
6. The mass spectra contain peaks at high masses which can provide more information about their parents.
7. Initial results on the laser ionization of particles in the beam show the feasibility of the technique. However, more work remains to be done in order to demonstrate its usefulness for the volatilization and ionization of biological particles. The power density in the focused laser beam needs to be increased before it can be used as a general method for the vaporization and ionization of particles.

The results obtained on P. putida, B. subtilis, and B. cereus demonstrate the potential of the PAMS technique for the detecting and possibly identifying biological particles. The preliminary nature of the work should be emphasized, however; more work on different biological samples needs to be done. The advanced data analysis technique of chemical pattern recognition developed by various workers[4] should be incorporated in the technique.

It is encouraging to note that other pyrolysis-mass spectrometric methods for the analysis of biclogical material have found considerable success. In view of this and the advantages of the PAMS technique, it is safe to conclude that the PAMS method holds promise for biological particle analysis. The real-time analytical capability of the method makes it uniquely suitable for monitoring a protected environment (e.g., a clean room) or a general field environment.

VII. FUTURE DEVELOPMENT AND RECOMMENDATIONS

One of the limitations of the PAMS method arises from the mode of its data acquisition. One mass peak is measured per particle. In order to obtain the average intensity of a mass peak from a polydisperse aerosol, a large number of particles must be measured, and the process has to be repeated for all the mass peaks. This entails a long analysis time, particularly for the ambient air sample where the number density of biological particles is expected to be low. This limitation is not inherent in the PAMS technique but lies in the use of a quadrupole mass spectrometer. A quadrupole mass spectrometer measures the intensities of different masses by scanning them in time and is too slow for a complete scan of an ion

pulse from a particle. Moreover, a scanning instrument is, in general, less sensitive than a nonscanning one because in the scanning mode, a particular signal is measured for only a small part of its total time of generation. However, in a restricted environment where not much interference exists, one characteristic mass peak, possibly in high m/z range, could be selected for the particle, and a quadrupole instrument could still be used for the detection. The problem associated with the use of a quadrupole mass spectrometer could be overcome by replacing it with a focal-plane mass spectrograph (nonscanning); then all the ions of different masses arising from a single particle could be measured simultaneously.

A miniaturized mass spectrograph (Mattauch-Herzog type) with an electronic detector known as electro-optical ion detector[25] covering the entire focal plane has been developed and built at the Jet Propulsion Laboratory. Figure 16 shows a photograph of this instrument. It as a 5-in. long focal plane and covers a mass range of 28 to 500 u. This instrument has demonstrated sensitivity at the femtogram (10^{-15} g) level, which, for a unit-density material, is equivalent to a spherical particle of 0.1 μm in diameter. The mass spectograph with the particle beam generator is being assembled. This system will provide a complete mass spectrum from individual particles. A computerized pattern-recognition technique of analysis will help differentiate the mass spectra of different microorganisms.

Since lasers have been found to provide a controllable and efficient method of ionization, they may help generate characteristic mass spectra (fingerprints) of different particles. Preliminary results on the application of laser-induced volatilization and ionization of individual particles in the beam on the PAMS system have been obtained. The lower ionization efficiency and the absence of other mass signals from the organic part of

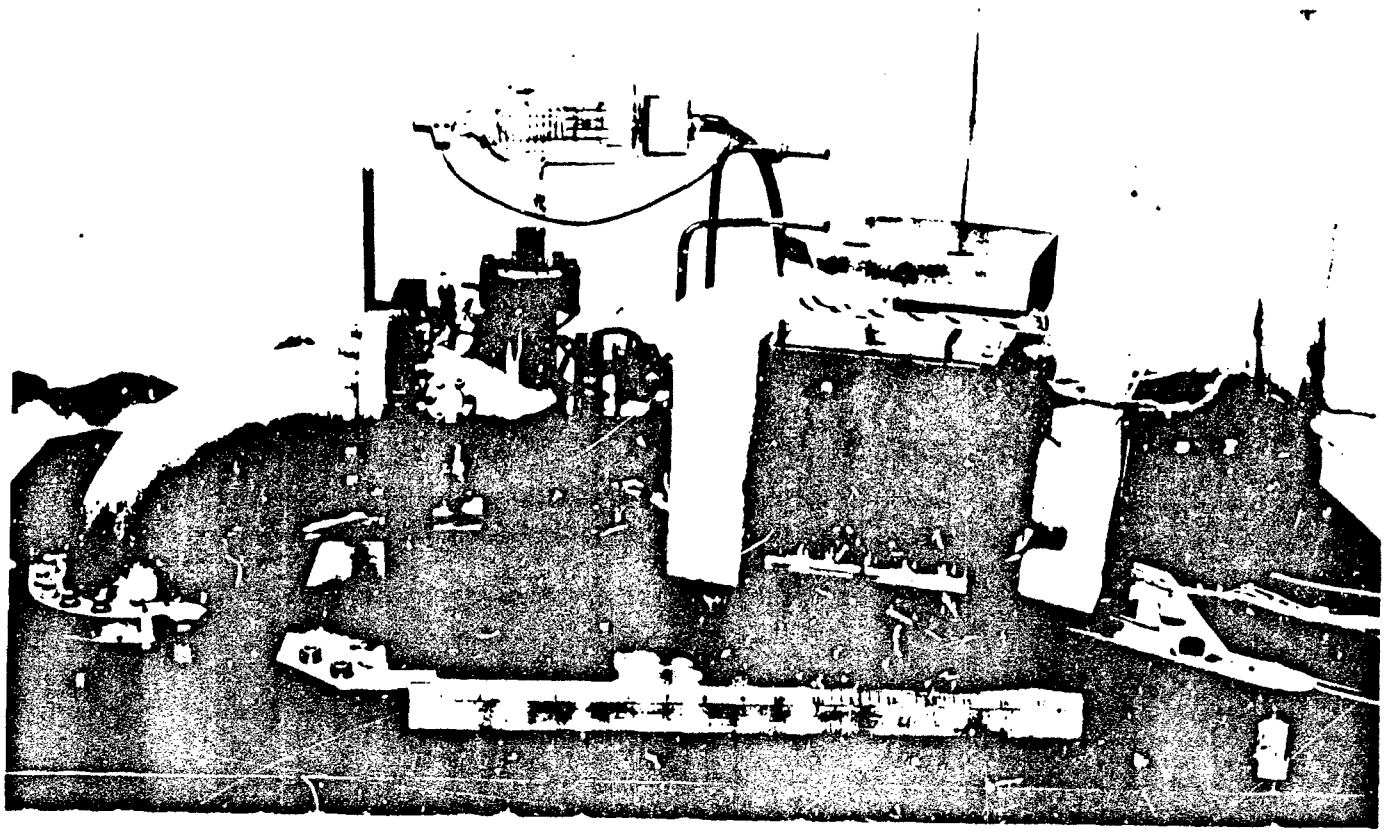


Fig. 16: Photograph of a miniaturized mass spectrograph with electro-optical ion detector.

KBP molecules are attributed to the low laser power density (~2 x 10^5 W/cm^2) as well as to the incomplete vaporization of the particle. Work should be performed on the study of laser-induced ionization with increased power density on the particles while in the beam. This can be accomplished by Q-switching the high energy laser.

Microorganisms generally consist of four broad classes of organic compounds: lipids, carbohydrates, proteins, and nucleic acids. Proteins and nucleic acids make up the inner portion of the cell while the phospholipids, polysaccharides and proteins constitute the plasma membrane and the wall layers of the cell. Differences in the structure of these components in the cell have been utilized as criteria for the broad classification of bacteria and viruses. For example, organisms always contain both DNA and RNA whereas viruses contain either DNA or RNA. Cell wall composition have been suggested as a basis of chemical taxomony [20] of bacteria. Amino acids present in the cell wall seem to be characteristic of the genus and the sugars and aminosugars seem to be characteristic of the species within the genus. It is, therefore, important that a catalog of mass spectra of the fundamental building units of the above biopolymers and as well as of their model polymers be made as the first step. The mass spectral measurements on the biopolymers should be made as the second step prior to the final measurement made on the microbial particles. The examination of the mass spectra of microbial particles and relating the mass peaks and their intensities to the characteristic peaks of the spectra from the catalog will help in their assignment and provide a basis for the detection and identification of microorganisms.

A library of mass spectral data of the different microorganisms should be compiled for identification by comparison. It is expected that biologi-

cal particles in air may be mixed with nutrients, attached to dust particles, or mixed with other substances deliberately to obscure their identity; sometimes more than one kind of particles might be mixed together. Such interferences should also be studied.

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IX. SCIENTIFIC PERSONNEL SUPPORTED AND DEGREE AWARDED*

Principal Investigator:	M. P. Sinha
Co-Investigator:	C. E. Giffin
Engineer:	J. Hill
Engineer Associate	V. Taylor
Technician:	W. Uhl

Graduate Research Assistant: Robert M. Platz *M.S. 1983

X. PUBLICATIONS AND AWARDS

A. Papers

1. M. P. Sinha, R. M. Platz, S. K. Friedlander and V. L. Vilker "Characterization of Single Bacteria by Particle Beam Mass Spectrometry," (Accepted for publication in J. Appl. Env. Microbiol. 1984).
2. M. P. Sinha, "Laser-Induced Volatilization and Ionization of Microparticles," Rev. Sci. Instrum. 55, 886 (1984).
3. M. P. Sinha, R. M. Platz, V. L. Vilker, & S. K. Friedlander, "Analysis of Individual Biological Particles by Mass Spectromery," Int. J. Mass Spectrom. Ion Processes 57, 125 (1984).
4. M. P. Sinha and R. M. Platz, "Analysis of Biological Particles by Mass Spectrometry," J. Aerosol Sci. Technol. 2, 256 (1983); Annual Conference Issue of American Association of Aerosol Research (AAR).
5. M. P. Sinha, D. A. Chatfield, R. M. Platz, V. L. Vilker and S. K. Friedlander, "Analysis of Aerosol Particles by Mass Spectrometry," in Proc. of 30th Annual ASMS Conference of Mass Spectrometry and Allied Topics, Honolulu, 1982.

B. Thesis

1. Robert M. Platz - MS Thesis, Department of Chemical Engineering University of California at Los Angeles, 1983. Title: "Particle Analysis by Mass Spectrometry for Detection of Single Bacteria in Air Suspension"

C. Book

1. M. P. Sinha, "Analysis of Individual Biological Particles in Air," in Rapid Detection and Determination of Microorganisms by Physical Methods, W. H. Nelson ed.; Verlag Chemie International Inc.: Florida, 1984 (in press).

D. NASA Innovation Awards

1. NASA Tech. Brief (1984, NPO-163549)	"Method for the Detection and Identification of Bacteria and Other Biological Particles in Air"
2. NASA Tech. Brief (1984, NPO-15808)	"Laser-Assisted Particle Analysis by Mass Spectrometry"

APPENDIX

PUBLICATIONS AND MANUSCRIPTS

International Journal of Mass Spectrometry and Ion Processes, 57 (1984) 125–135
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ANALYSIS OF INDIVIDUAL BIOLOGICAL PARTICLES BY MASS SPECTROMETRY

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ABSTRACT

A method has been developed for the measurement of the mass spectrum of single biological particles introduced in aerosol form into a quadrupole mass spectrometer. The aerosol is generated by nebulizing an ethanol suspension of bacteria. The particles are injected in the form of a beam into the mass spectrometer where they are individually volatilized by impaction on a rhenium filament and ionized by electron bombardment. The average intensity of a mass peak is obtained from the pulse height distribution of about a thousand ion pulses from different particles. Results are reported for *Pseudomonas putida*, *Bacillus cereus* and *Bacillus subtilis*. The results for *Pseudomonas putida* and *Bacillus subtilis* are comparable with the data reported in the literature [8,10]. However, peaks at higher masses in the present work are found to be more intense, probably because of the difference in the pyrolysis conditions. Significant differences between the relative intensities of mass peaks in the spectra from the two species are found. This may provide a method for differentiation between different micro-organisms.

INTRODUCTION

There are various methods, biological, physical, and chemical, for the detection and identification of micro-organisms in air. The physical methods which depend on size and shape determination or use of a lidar technique, are relatively rapid but suffer from non-specificity. The methods based on biological activity are in general slow and need collection of viable bacterial particles for a long time in order to acquire a sufficient quantity of sample. The other class of methods, which does not require the particles in the viable state, depends on the detection of key biological substances in them. Of these methods, use of pyrolysis–gas chromatography (Py–GC) and pyroly-

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sis–mass spectrometry (Py–MS) have attracted much attention for rapid analysis of micro-organisms [1–3].

The success of Py–MS in the detection, identification, and structure elucidation of complex samples depends largely on the characteristics of the pyrolysis process that the sample undergoes before mass analysis. Some of the desirable criteria of Py–MS have been discussed by Schulten [4]. It is preferable to use a method of pyrolysis which would provide a controllable uniform temperature, a maximum heating rate, a minimum heating time, and a minimal amount of sample. Reproducibility is enhanced if the entire sample experiences the same temperature and the pyrolysis products are exposed to a collision-free environment. Recently, Curie-point pyrolysis [5], laser heating [6] and loading the sample on the activated emitter of the field-desorption ion source [4] of a mass spectrometer have been employed. Very encouraging results have been obtained with Curie-point pyrolysis–MS and in some cases even differentiation between micro-organisms has been achieved [7,8].

We report here a novel method developed in our laboratory for the detection and identification of biological particles in air on a continuous, real-time basis using the technique of particle analysis by mass spectrometry (PAMS). It involves a combination of methods of particle beam generation and mass spectrometry [9]. The particles are introduced into the ion source of the mass spectrometer in the form of a beam, where they are individually volatilized under some of the desirable pyrolysis conditions described earlier, for their mass spectral analysis.

EXPERIMENTAL

Figure 1 shows the experimental set-up for the PAMS system. Its main components include a particle beam generator and a quadrupole mass spectrometer housed in differentially pumped chambers. The ionizer of the mass spectrometer (MS) was modified to accommodate a V-type rhenium filament and holes were cut into the repeller and grid cages for the passage of the particles. The beam generator consists of a capillary nozzle and a skimmer. The details of this system and its characteristics have been described elsewhere [9]. However, some modifications were made for the present studies. As shown in this figure, a second skimmer having a 0.5 mm diameter orifice was added to reduce the gas load into the MS chamber and its distance from the first skimmer was adjusted such that it did not provide any additional collimation for the beam of particles. The region between the two skimmers was pumped separately. The beam generator was reoriented such that it lies along the vertical direction in order to minimize the loss of particles due to gravitational settling. Arrangements were made for the

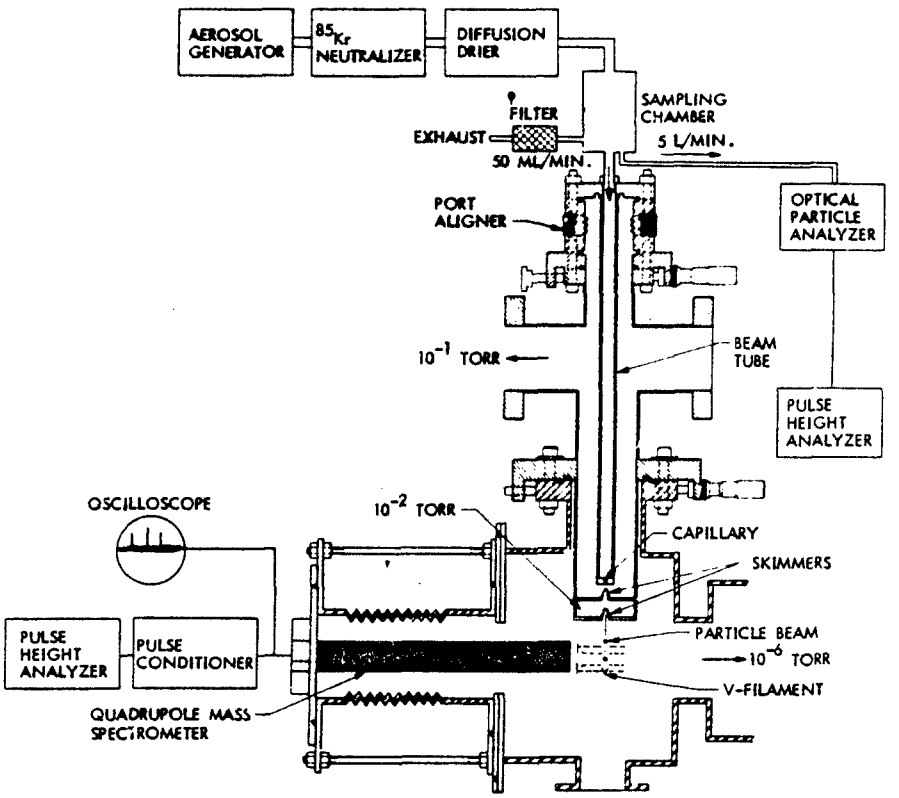

Fig. 1. Schematic of PAMS Systems.

isokinetic sampling of the aerosol in the beam tube and liquid-nitrogen-cooled traps installed above the vacuum pumps. These modifications resulted in a marked increase in the transmission of particles and the sensitivity of the system. The transmission efficiency (fraction of the number of aerosol particles in air reaching the mass spectrometer) of the beam generator was found to be as high as 70% for even larger particles of about 3 μm in diameter.

A beam of particles is produced by the expansion of an aerosol through a capillary nozzle into the vacuum. The volumetric flow through the capillary is about 50 atm-cm^3 min^{-1}. The particles which are heavier than the carrier gas (N_2) molecules remain confined to the beam axis and are efficiently led into the ionizer. The V-type rhenium filament installed inside the ionizer is heated resistively. The particles impinging on the hot Re filament are volatilized, and subsequently ionized by electron impaction in an in situ fashion.

Pseudomonas putida, *Bacillus subtilis*, and *Bacillus cereus* bacteria were used in the studies reported here. *Pseudomonas putida* (ATCC 29607) bacteria were grown in a liquid suspension at room temperature for 40 h under continuous agitation. The culture medium contained tryptone, yeast extract, glucose, and sodium chloride, and the pH of the medium was initially adjusted to 7.8. The culture was harvested by repeated washing with distilled water. After the final wash, the sample was resuspended in distilled water and refrigerated until used. Before aerosolization of the bacterial suspension, ethanol was substituted for water. This was accomplished by successive washing of the sample with a series of water–ethanol solutions of increasing ethanol concentration. The final suspension in 100% ethanol was used for the experiment. The bacterial aerosol was generated by nebulizing the liquid suspension. The aerosol was carried in a stream of dry N_2, first through a charge neutralizer containing Kr^{85} and then through a silica gel diffusion drier. The ethanol suspension must be used for complete drying of the aerosolized droplets containing the cells before being introduced into the particle beam generator. An efficient transmission of cell particles through the beam tube was found, whereas aerosolization from aqueous suspension resulted in a poor transmission. This may result from the incomplete drying of water droplets which are subsequently lost in the beam tube. Aerosols, of *Bacillus cereus* (203a) and *Bacillus subtilis* (168), obtained from UCLA Biology Department, were also prepared by the above method. The *Bacillus* samples were grown in liquid suspension for 48 h at 34°C. Sporulation was completed by placing the culture in a 70°C water bath for 30 min.

RESULTS AND DISCUSSION

The aerosol was introduced into the beam tube which produced, after expansion through the capillary nozzle, a beam of particles in the MS chamber. In order to characterize the physical state of the bacteria in the beam, the particles were collected on an Apiezon-coated glass slide in this chamber. Figure 2(b) shows an electron micrograph of a deposit. This deposit was made with *Bacillus subtilis* spores. Figure 2(a) is a deposit of the spores on a nucleopore filter collected from the aerosol upstream of the beam tube. It can be seen from Fig. 2 that the cells retain their integrity during the process of aerosolization and beam generation in the vacuum. We believe this is the first time that a beam of cells has been made for direct introduction into the ion source of a mass spectrometer. The generation of a beam is not limited to spores possessing hardy characteristics; *Pseudomonas putida* is a non-spore-forming bacteria.

A burst of ions is produced from *individual cell particles* after being volatilized on the hot rhenium filament (740°C) and ionized by electron

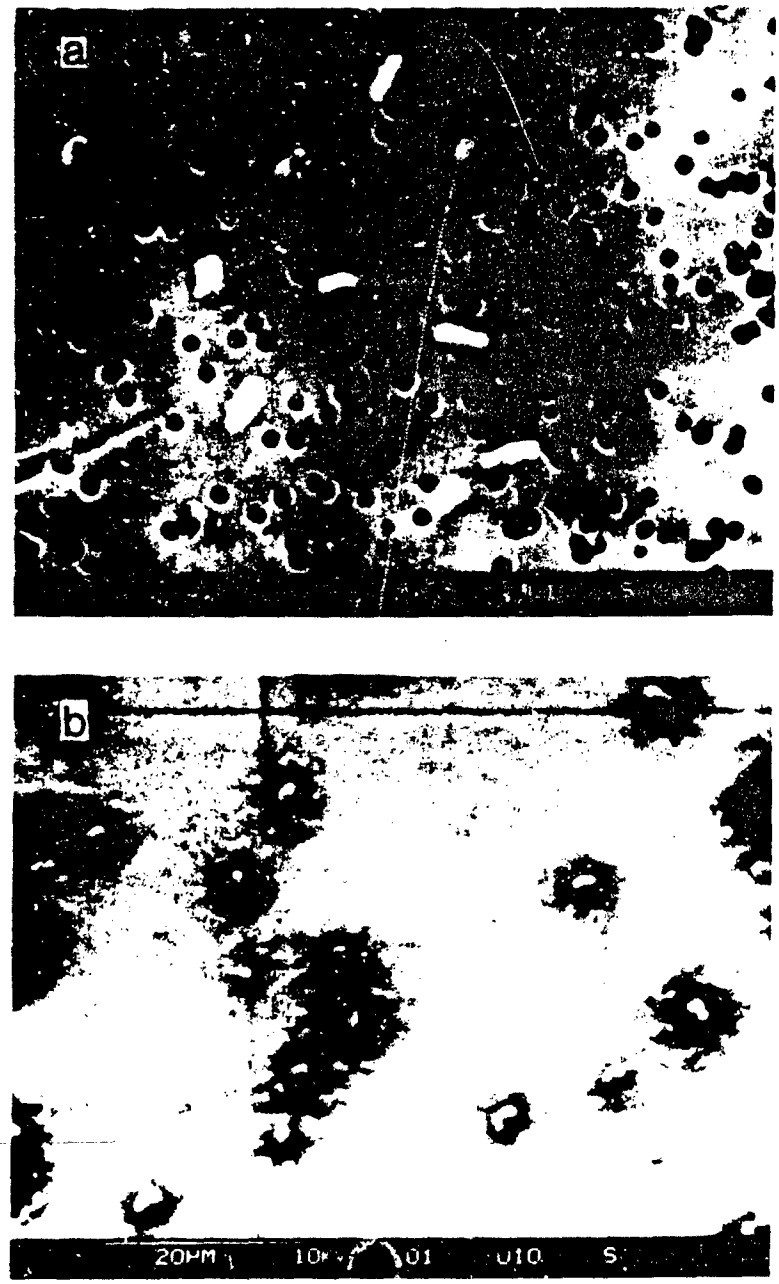

Fig. 2. Electron micrographs of *Bacillus subtilis* spores collected on (a) a nucleopore filter from the aerosol and (b) an Apiezon-coated glass slide from the particle beam in the MS chamber.

impaction (~ 40 eV in energy) in the ion source of the quadrupole mass spectrometer. The ion pulse width is about 100 μs which necessitated monitoring a single mass peak from each particle. The total charge of the particular mass under each ion pulse is then measured by integration, and an output voltage proportional to this is used for determining the pulse height distribution. The average intensity of a mass peak is obtained from the pulse height distribution of about a thousand ion pulses from different particles. The intensities of different mass peaks were measured successively.

Signals were observed at several masses throughout the spectrometer mass range of 30–300 u. A common set of more than 30 mass peaks was selected for intensity measurements. The mass spectra for the three bacteria so obtained are shown in Fig. 3. A striking similarity between them can be seen. This is not surprising in view of the fact that the micro-organisms have essentially the same major chemical building blocks. The peaks around m/z 150 are found to be the most intense. The mass peaks between 192 and 275 are too weak for faithful intensity measurements in the case of *Pseudomonas putida* and *Bacillus subtilis*. A mass peak of 5% intensity of the base peak could be measured with good signal-to-noise ratio in our experiments. These peaks are much more pronounced for *Bacillus cereus*. The important points of differentiation between the spectra, however, lie in the relative intensities of various peaks. For example, the intensities of mass peaks at m/z 114, 134, 169, and 282 relative to the intensity of 149 u is greater in the spectrum of *Bacillus cereus* than those in that of *Pseudomonas putida*. The differences are real and greater than the experimental uncertainties. Such analysis of intensities can provide for the identification and differentiation of micro-organisms and have been successfully used in the literature [8].

The spectrum obtained from *Pseudomonas putida* agrees reasonably well with that observed by Schulten et al. [10]. However, there exist some marked differences between the two spectra particularly in the high mass range. These possibly result from the difference in pyrolysis conditions. In their work, a sample of 5 mg of freeze-dried *Pseudomonas putida* bacteria was pyrolyzed in a flask for 2 min at 500°C and its mass spectrum was obtained by field-ionization mass spectrometry, a method which produces prominent molecular ions [11] during ionization. The low intensity of higher mass peaks arises from fragmentation due to the prolonged and severe primary pyrolysis conditions. The spectrum of *Bacillus subtilis* has been obtained by Boon et al. [12] using the method of Curie-point pyrolysis–mass spectrometry with low electron energy (14 eV) ionization. The Curie-point pyrolysis [5] provides a method for faster heating (50–100 ms) of the sample. Here also the most intense peaks lie in the low mass range (40–60) and the intensities at higher masses (> 125) are weak. The pyrolysis conditions in our studies differ in that each cell is vaporized separately in the source and, consequently, many

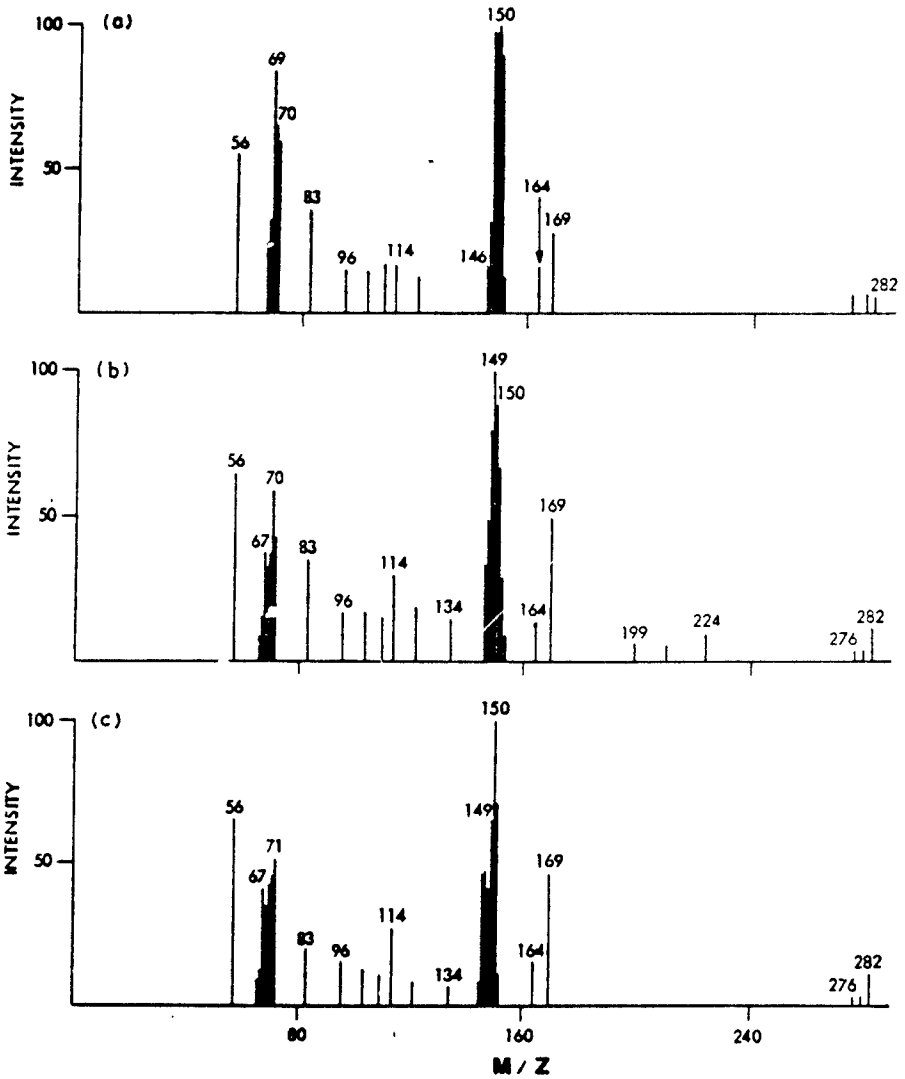

Fig. 3. Mass spectra of bacteria particles. (a) *Pseudomonas putida*, (b) *Bacillus cereus*, (c) *Bacillus subtilis*.

of the desirable conditions for pyrolysis are met naturally. The duration of pyrolysis is extremely small. It is estimated from the ion pulse width of 100 μs to be about 30 μs. Furthermore, the vapor molecules produced are essentially in a collision-free condition in the ion source. A cell particle of 0.5 μm in diameter and 1.5 μm in length will produce a pressure of less than 10^{-6} torr in an ionization volume of 1 cm^3. There is a minimum transit time

that a quadrupole mass spectrometer measures the intensities of different masses by scanning them in time and the available scan speed is too slow for mass analysis of a single ion pulse. The problem can be overcome by using a focal plane mass spectrograph (non-scanning) in place of the quadrupole MS. All the ions of different masses from a particle can be monitored simultaneously. One such miniaturized mass spectrograph (Mattauch–Herzog-type) with an ultrasensitive electro-optical ion detector [14] covering the entire focal plane, has been developed and built at the Jet Propulsion Laboratory. This instrument has the capability of analyzing extremely small amounts of samples (8×10^{-15} g). The particle beam generator when interfaced with this system should provide a complete mass spectrum from individual particles. Such an experimental system is presently being constructed for particle analysis.

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Characterization of Bacteria by Particle Beam Mass Spectrometry

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ABSTRACT

A technique is described for detecting and characterizating bacteria on a single particle basis by mass spectrometry. The method involves generation of a particle beam of single whole cells which are rapidly volatilized and ionized in vacuum in the ion source of a quadrupole mass spectrometer. The particle beam can be generated, with minimal sample handling, from a naturally-occurring aerosol or from a solution of bacteria that can be dispersed as an aerosol. The mass spectrum is generated by successively measuring the average intensities of different mass peaks. The average intensity is obtained by measuring the ion intensity distribution at the particular mass (m/e) for ion pulses from more than one thousand bacteria particles. Bacillus cereus, Bacillus subtilis and Pseudomonas putida samples were analyzed to test the instrument's capability for differentiating among species of bacteria. Significant ion intensity information was produced over the m/e range of 50 to 300 which is an improvement over previous pyrolysis/mass spectrometry results. The complex mass spectra contained few unique peaks which could be used for the differentiation of the bacteria. A statistical analysis of the variations in peak intensities among the three bacteria provided a quantitative measure of instrument reproducibility and its applicability to differentiate among bacteria. The technique could lead to a new rapid method for the analysis of microorganisms and could be used for the detection of airborne pathogens on a continuous, real-time basis.

INTRODUCTION

This application of particle beam mass spectrometry to bacteria characterization is an extension of a technique we are developing for real-time analysis of the composition of aerosol particles (9,19). The method has been used successfully for the analysis of micron size particles made up of inorganic salts or small organic molecules including amino acids (19).

The earliest studies by others for bacteria identification by mass spectrometry involved separate steps of pyrolysis of bulk quantities (milligram) of bacteria samples, collection or separation of the volatile pyrolysis products, followed by their introduction into a mass spectrometer (17,18). Mass fragments were recorded for nearly all integer masses between 14 and 140 amu. The complex mass spectra permitted determination of up to 100 components, but did not give structural information useful for bacteria differentiation.

A more recent design of a pyrolysis mass spectrometer for characterization of complex biological material is that of Meuzelaar and coworkers (14,16,21). In this instrument, bulk samples of (>10 µg) are pyrolyzed in a glass reaction tube using the technique of Curie-point pyrolysis. The volatilized products leak through an orifice into the mass spectrometer (ms) where low-voltage-electron-impact ionization and mass analysis is performed. The mass spectrum obtained with this instrument is limited to the m/e range of 30 to 140. The instrument has been demonstrated to successfully differentiate among several distantly related bacteria on the basis of relative intensities of various masses (14). It has also been used to study the effects of growth media on the composition of the cell wall of b. subtilis (2). Wieten et al. (21) incorporated multivariant factor analysis into the comparison of the

spectra from this instrument. This permitted differentiation of a variety of closely related species from the genus Mycobacterium.

In our system, the sample is introduced to the site of volatilization (pyrolysis) and ionization as a beam of particles consisting of mostly single bacteria. The rapid volatilization of the particles by a heated rhenium filament and the proximity of the ionizing electrons extend the range of the mass spectrum above m/e 140. These larger fragments contain important structural information that greatly enhance the capability for particle differentiation.

The particle beam can be generated, with minimal sample handling, from a naturally-occurring aerosol or from a solution containing a non-volatile solute that can be dispersed as an aerosol. This provides a method for volatilization/ionization of single bacteria on a continuous real-time basis. This leads to a more characteristic mass spectra for microorganism differentiation. When applied to aerosol sampling, the technique would be directly useful for detecting airborne pathogens such as those associated with transmission of disease in hospitals (3), cotton mills (4), mushroom harvesting (15), wastewater treatment (5,6,12,13), and Legionnaires' Disease (10).

MATERIALS AND METHODS

Bacteria and media. Bacillus subtilis (168) and Bacillus cereus (203A), obtained from the UCLA Biology Department, and Pseudomonas putida (ATCC 29607) were grown in non specific cell-culture media containing 10 g/ℓ Bacto tryptone and 1 g/ℓ yeast extract (Difco), 4 g/ℓ glucose (Baker) and 5 g/ℓ sodium chloride. The media pH was initially adjusted to 7.8. The Bacillus samples were grown for 48 hours at 34°C followed by placing the culture in a 70°C

water bath for 30 min to complete sporulation. P. putida was grown at room temperature for 40 h. The bacteria were washed three times with distilled water and refrigerated in distilled water suspension. Immediately prior to use, water was replaced by ethanol as the suspending liquid in order to improve the performance of the particle beam generator. This was done by washing the bacteria in a series of ethanol/water solutions of successively increasing ethanol concentration. Aggregates of cells formed during the washing/centrifugation steps were removed by filtration through a 20 μm mesh screen. The turbidity of the final suspensions in 100% ethanol indicated approximately 10^9 bacteria/mℓ.

Particle beam generation. The generation of the bacteria particle beam involved the sequence of operations shown on the upper part of the apparatus schematic, Figure 1. A similar experimental setup for generating non-biological particle beams has been previously reported (9,19). The first step was aerosolization of the bacteria from the ethanol solutions using a microne-bulizer (Bird Space Technology, Model 9993) with nitrogen gas flow of 20 liters min^{-1}. The static charge on the aerosol droplets was neutralized by passing them through a ^{85}Kr charge neutralizer (TSI Model 3470). Solvent was evaporated by passing the aerosol through the annular region of a diffusion drier. This drier is a 0.75 m long cylindrical tube containing silica gel held in an outer coaxial shell by a fine mesh screen. The dried aerosol was introduced to a sampling chamber from which a small portion was drawn off through a sampling probe for introduction to the particle beam tube; another portion was drawn off for measurement of particle concentration using an optical particle counter (Climet Instruments Model 208). The remaining aerosol from the sample chamber was filtered and exhausted. The particle

concentration in the sample chamber was maintained in the range of 75-100 particles/ml by regulation of the N_2 dilution-gas flow to the nebulizer.

The particle beam was formed by passing the aerosol through a capillary nozzle and two skimmers into the vacuum chamber. The aerosol travels down the beam tube (350 mm long x 10.7 mm i.d.) and the gas expands through the capillary nozzle (5 mm long x 0.1 mm dia.) into the first vacuum region. As the gas expands through the capillary, most of it is pumped off upstream of the skimmers while the particles, having much higher inertia, tend to continue in a focused beam through the two skimmer apertures. In our earlier work with non-biological particles (9), we found that the efficiency of particle transmission through the capillary nozzle-skimmer system depended upon nozzle-skimmer separation distance, as well as particle size (0.22-4.28 μm) and density. Based in part on those results, this apparatus for use with bacteria particles was designed with the first skimmer (0.350 mm aperture) positioned 3 mm below the capillary and the second skimmer (0.500 mm) positioned 12.5 mm below the first. The particle beam cross section at the filament position was determined to be 2.0-2.5 mm for potassium biphthalate beams of controlled particle size (1-4 μm). Alignment of beam tube components with the volatilization filament target was accomplished with a He-Ne laser beam. Mechanical vacuum pumps were used to reduce the pressure to about 10^{-1} torr and 10^{-2} torr in the beam exhaust chambers and the region between the two skimmers, respectively. In the ms chamber after the second skimmer, an oil diffusion pump was used to maintain $\sim 10^{-6}$ torr vacuum. Liquid-nitrogen-cooled traps were installed on all pumps to reduce contamination of the system by the pump oil.

Mass spectrometry. Bacteria particles from the beam generator were volatilized to molecular fragments upon impact on the V-shaped heated rhenium filament located between the two grids of the ion source of the quadrupole mass spectrometer (Uthe Technology, Inc., Model 100C). The filament cross section perpendicular to the particle beam was 1 mm x 7 mm. The neutral fragments were ionized by electron bombardment in an in-situ fashion. Optimum performance for analysis of bacteria particles was found for filament temperature 730-740°C and electron ionization energy of 52 eV. The ions were then focused into the quadrupole mass analyzer for their mass separation and detection. The mass range of the quadrupole is 10-300 m/e, although background interference in the low mass region reduced the effective operating range to 50-300 m/e.

Impaction of each bacteria particle on the hot filament and the ionization of the resulting vapor plume produced a discrete burst of ions from each particle. For each burst event, which lasted from 100 μs to 300 μs, the quadrupole mass spectrometer recorded a single m/e intensity. Typically the signals from 1000-5000 events, each of which corresponded to volatilization/ionization of a single bacteria particle, were averaged to obtain the intensity at that m/e setting. The electronic components which performed this signal processing are shown at the bottom of Figure 1. The various steps in the measurements of the average intensity at a given mass are shown schematically in Figure 2. These involve the integration of the charge under the ion-pulse and the measurement of the resulting peak-voltage on the integrator. The distribution of peak voltages from ion-pulses was then measured on a pulse height analyzer (Tracor-Northern, Model TN 1710). The mass spectrometer output pulse (step 2A) is amplified and discriminated from noise by comparing it with a

suitably adjusted threshold voltage level (step 2B). The rising edge of the comparator pulse was used to close the switch on the input of the integrator in order to integrate the amplified signal pulse. The integration was performed for a preset time interval. The background was also integrated for the same length of time immediately following this operation and was subtracted from the stored signal. The peak voltage pulses of the integrator (proportional to the accumulated charge) was shaped (step 2C) in order to meet the input signal requirements of the pulse height analyzer. Each event output was recorded by the pulse height analyzer (step 2D). We found that 1000-5000 burst events were sufficient to define an intensity distribution at a particular m/e setting. A typical intensity distribution is shown in Figure 2D. The average intensity $\bar{I}$ was calculated from the weighted average of the distribution:

$$\bar{I} = \left[\sum_{j=1}^{m} f_j v_j \right] / n$$

where v_j is the voltage of pulses in interval j, f_j is the corresponding number of pulses in j, m is the number of intervals and n the total number of pulses (1000-5000).

A common set of 30 mass peaks, covering the mass range of 50-300 m/e, was selected and their intensities measured using the above procedure for the three bacteria samples. The mass spectrometer was manually tuned to these peaks. The compilation of the intensities of these peaks represents the mass spectrum of a single "average" bacterium in the particle beam. It should be mentioned that this does not represent the complete mass spectrum of the bacteria particle since it does not include all the mass peaks. However, the set

of 30 masses provides a good data base for comparing the mass spectra from different bacteria samples.

Sample preparation for microscopy. Bacillus subtilis spores were collected at three positions in the process for observation in the scanning electron microscope (SEM). Samples of the cell suspensions were prepared by the conventional SEM techniques of alcohol substitution and critical point drying. Aerosolized bacteria were collected after the diffusion drier on 0.2 μm Nuclepore filters. Bacteria that had passed through the particle beam were collected on Apeizon grease-coated glass slides in the ms chamber. All samples were coated in a sputter coater with approximately 20 nm thick layers of gold/palladium.

RESULTS

Particle beam characterization. The objectives of this part of the study were to determine the efficiency of particle transmission through the particle beam generation system, and to characterize the morphology of the bacteria particles at several points in the system.

Particle transmission efficiency is the ratio of number of particles which reach the filament target position to the number of particles that enter the beam tube during the same time. This efficiency was determined to be greater than 80% for nonbiological bacteria-sized particles in our earlier studies using an identical apparatus to that described here except for the absence of the second skimmer 12.5 mm below the first (9). In the present work, the second skimmer was added to improve the vacuum at the mass spectrometer, thereby decreasing the background interference during bacteria particle analysis (20). The transmission efficiency for bacteria particle beams was unaffected by the second skimmer. However, due to the smaller rhenium filament cross-section (7 sq. mm) relative to the particle beam cross section (16 sq. mm), the mass spectrometer analyzed only about one-half of the particles arriving at the filament target position for an overall filament collection efficiency of 35-40%.

Scanning electron micrographs of B. subtilis spores were taken from samples collected on Nuclepore filters from the suspension before aerosolization (Figure 3A), from the aerosol stream after the diffusion drier (Figure 3B), and in the particle beam on Apeizon grease-coated glass slides at the filament target position (Figure 3C). These micrographs verify that the bacteria samples were predominantly single spores, 20% occurring as doublets, and were

free from particulate debris. Grease-coated slides had to be used for collection of particles at the filament target position in the ms chamber because the particles are moving at nearly sonic velocity in the beam and bounce off nonsticky surfaces. The details are not well resolved in these micrographs from the grease-coated slides; however, the shape and size of the particles were still discernible and they were estimated to average 0.5 μm x 1.5 μm. These features suggest that the spores remain intact during beam generation and the collection process.

Mass spectrometry of bacteria. The mass spectrometer signal from the bacteria in the beam was observed throughout the mass range of 10-300 amu. Several clusters of mass peaks were present below 170 amu, similar to results previously found in the pyrolysis mass spectra of bacteria samples (2,14,16,17,18,21). Intensities of all the mass peaks were not measured in our experiments due to their large number and also due to their low intensities at some of these masses. A set of ~30 intense mass peaks was selected and their intensities measured for all the bacteria samples. This included two clusters of mass peaks around 70 amu and 150 amu. The intensities of signals below m/e of 50 could not be measured quantitatively due to the interference from ethanol and other background contaminants. Strong signals from ethanol, which was used as the dispersion medium of bacteria, were present. The mass spectra of P. putida, B. cereus, and B. subtilis are shown in Figure 4. Peak intensities are given as a fraction of the most intense peak, which occurred in the cluster of peaks around m/e 150 in each of the samples. The intensities of peaks around m/e 280 are about 5% of the most intense peak and were the weakest signals recorded. No correction for the decrease of transmission of higher masses through the quadrupole analyzer was made.

In general, there is significant similarity in the spectra of the three bacteria. Also, a considerable amount of new information has been gained over previous pyrolysis mass spectrometry by the presence of peaks at m/e greater than 140. Only four peaks differ among the samples. The m/e 134 peak observed in both bacilli spectra is not present for P. putida. B. cereus is the only sample with peaks at m/e 199, 210, and 224.

There are also a few readily observed differences in the peak intensity patterns among the samples. The bacilli spectra, relative to the P. putida spectrum, have enhanced signals at m/e 67, 114, 169, and 282, as well as m/e 56 peak intensity greater than the intensity of peaks clustered around m/e 70.

Statistical analysis of the mass spectra. In order to examine system reproducibility for samples of like species of bacteria and system capability to differentiate between samples of unlike species, we used a statistical method for comparing the visually similar mass spectra. This method, the degree of correspondence, was adapted from Kistemaker et al. (14) who used it to differentiate among spectra of distantly related bacteria. More advanced statistical programs could not be used for the analysis of mass spectral data due to the limited amount of data from our experiments. In the degree of correspondence method, one spectrum is arbitrarily designated as the "reference" spectrum with peak intensities $\{R_i\}$ at the several i values of m/e. The peak intensities comprising each "sample" spectrum to be compared with the reference are $\{S_i\}$. The peak intensities of the sample are first normalized to those of the reference spectrum by multiplying the sample by a scaling factor α which minimizes the error E in the squares of the difference of comparable peaks in the two spectra, each consisting of n total peaks:

$$E = \sum_{i=1}^{n} (R_i - \alpha S_i)^2$$

Setting $dE/d\alpha = 0$, gives the value of the scaling factor to be:

$$\alpha = \sum_{i=1}^{n} R_i S_i \Big/ \sum_{i=1}^{n} S_i^2$$

In the case of comparing two spectra from <u>like</u> species, perfect reproducibility means that the intensities for each pair of peaks are equal and α is unity. In practice, differences in culture growth, particle beam characteristics, volatilization/ionization conditions and spectrometer signal processing lead to non-unity values of α. In the case of comparing two spectra from <u>unlike</u> species, differences in the masses of individual bacteria particles are also taken into account in the α calculation.

The degree of correspondence between the normalized sample spectrum and the reference spectrum is the average of the ratios of the minor peak intensities to the corresponding major peak intensities:

$$C = \left[\sum_{i=1}^{n} A_i / B_i \right] \Big/ n$$

where A_i is the minor peak intensity in spectrum $\{R_i\}$ or $\{\alpha S_i\}$ and B_i is the respective major peak intensity in spectrum $\{\alpha S_i\}$ or $\{R_i\}$. In case of perfect matching, the degree of correspondence is one. A correspondence of zero indicates the spectra have no peaks in common. The value of C from comparing spectra of <u>like</u> species is a measure of reproducibility and is a characteristic of the system as a whole. The system is capable of differentiating between species if the comparison of spectra from <u>unlike</u> species results in a value of C which is significantly different from the characteristic value.

Two more cultures of B. cereus in addition to the spectrum shown in Figure 4B were analyzed in our system. The spectrum of one of these three was chosen as the "reference" and the degree of correspondence between this reference and each of the other sample spectra was determined. All the mass peaks were used in the calculation for C without discriminating for their sensitivity to the intensity variations in different runs. The results of the comparison are shown as the histogram in Figure 5. The results for the B. cereus samples indicate that the characteristic degree of correspondence for our system is in the range of 0.70-0.77. Since C = 0.70 for B. subtilis, it cannot be differentiated from B. cereus in our system. However, for P. putida, C = 0.64 indicating that this species can be differentiated from the bacilli samples.

DISCUSSION

The use of particle beams and the method of pyrolysis (volatilization/ionization) are the principal features which make this system different from previous applications of mass spectrometry to microorganism characterization. Of the several techniques available for generating particle beams (8), we selected the capillary-skimmer design for its capability to form directed, tightly-focused streams of single non-interacting particles. We are not aware of previous uses of this design to generate beams of biological materials. Our previous development work (9) used beams of nonbiological materials which formed particles whose size and density were comparable to bacteria, but whose spherical shapes differed from the rod-like bacteria. In the previous studies, about 80% or more of the spherical polystyrene latex (PSL) particles (less than 3 μm diameter) were focused to a beam diameter of 5 mm or less at the filament target position. The transmission efficiency for these particles was 80% or greater. Beams of the non-spherical bacteria particles used in the present work appear to have comparable characteristics. An important difference between polystyrene latex and bacteria arises in aerosol generation and drying prior to particle beam formation. Whereas monodisperse aerosols of single PSL particles could be easily generated from aqueous suspension, similar generation techniques resulted in a marked decrease of particle beam transmission efficiency for bacteria from aqueous solutions. We suspected that the cause for this problem was due to inadequate drying of the bacteria during aerosolization. Substitution of ethanol for water as the suspending liquid corrected this difficulty, but ethanol is likely not to be a suitable choice in application to other microorganisms. A modified diffusion drier will be used in future work to eliminate the need for ethanol.

All previous applications of mass spectrometry to microorganisms identification have used some form or another of bulk sample pyrolysis. The most developed of these is one which uses Curie-point pyrolysis of ~10 μg bulk bacterial samples (14,16,21). In this technique, the sample is placed on a ferromagnetic wire positioned in a low vacuum chamber. The wire is heated by high frequency induction to the Curie temperature within 100 ms. Volatile pyrolysis products diffuse to the high vacuum region of the quadrupole mass spectrometer where they are ionized by low energy (15 eV) electron impaction. A spectrum is accumulated over approximately 10 sec, the time the sample pyrolyzates remain available. Boon et al. (2) used this technique in a study of the effects of growth media on the composition of the cell wall of B. subtilis. Figure 6 is a reconstruction of the mass spectra for comparing the two techniques applied to the same bacterium (different strains). Although the two spectra have a number of intensity peaks or clusters of peaks in common (e.g. m/e 56, 71, 83, 96), they differ significantly in the observed mass range and relative intensities. Mass fragments above 140 m/e observed in particle analysis were not detected in Curie-point pyrolysis. The ability of the particle beam technique to obtain information in the higher mass range is likely to be due to more rapid heating of the sample (11)--- 30 μs vs. 100 ms. Also, the placement of the filament within the ionization source eliminates the losses of pyrolysis products incurred by molecular diffusion to the ionization region (14). The vaporization of single particles in the ion source itself, maintained at a background pressure of $\sim 10^{-6}$ torr, minimizes the secondary fragmentation by collision of the pyrolyzates in the vapor phase. A final distinction between the two techniques is that in Curie-point pyrolysis, ion intensities at each m/e are accumulated over the whole sample pyrolysis

event; in the particle beam technique, these ion intensities represent averages for a distribution over more than one thousand separate events.

Usually compounds are differentiated in mass spectrometry by examining the spectra for the presence or absence of unique ionized fragments. For example, in our results of Figure 4, the 134 m/e peak may be useful for distinguishing P. putida from bacilli. This distinction between the two spectra could be related to differences in the composition of the bacteria cell walls; bacilli are Gram-positive bacteria and P. putida is Gram-negative (7).

It is more likely however, that differentiation of bacteria from complex mass spectra requires analysis of peak intensity variations in addition to the usual criteria of presence or absence of unique ionized fragments. Both the particle beam volatilization method and the Curie-point pyrolysis method yield insufficient molecular fragments that are unique to a specific bacterium. Kistemaker et al. (14), using the Curie-point pyrolysis method, were able to differentiate among 12 Streptococcus strains by analyzing the degree of correspondence of peak intensities. The characteristic degree of correspondence was 0.80 or more for most replicate cultures and strain differentiation was readily achieved when the degree of correspondence between different strains was in the range 0.55-0.70. In our work, the comparable analysis is made for a much smaller data set--triplicate cultures of B. cereus and one culture each of B. subtilis and B. putida. The degree of correspondence between the spectra of P. putida and one of the B. cereus reference spectra (0.64) was different enough from the characteristic value (0.70-0.77 from pair comparisons of B. cereus spectra) to suggest that P. putida would be differentiated when compared with a larger file of reference spectra.

We conclude that particle beam mass spectrometry presents a new opportunity for developing a rapid automated method of microorganism identification. Its most significant advantage is the ability to generate spectra in a mass range that encompasses the molecular size of basic biological monomers (e.g. amino acid residues). The replacement of the quadrupole mass spectrometer by a highly sensitive focal plane mass spectrograph (1) in this system will further increase the accessible mass range to 28-500 amu. Such a system is being developed in our laboratory. This addition, plus more advanced statistical techniques for comparing spectra intensity differences (21) should lead to a powerful new method of microorganism identification.

ACKNOWLEDGMENT

This work was supported by the National Science Foundation, grant no. CPE 80-08686, and by the U.S. Army Research Office, grant no. 18137-C.

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Figure Legends

Fig. 1 Schematic of particle beam mass spectrometer apparatus.

Fig. 2 Mass spectrometer signal processing sequence. (A) Oscilloscope trace of mass spectrometer signal at specific m/e setting during volatilization/ionization of a single particle, 25 mV to 2 V amplitude, 100-300 μs duration; (B) comparator circuit signal superimposed on amplified trace of mass spectrometer signal, 200 mV to 4 V amplitude, 100-200 μs duration; (C) pulse shaping for reducing signal duration and for smoothing signal amplitude, 200 mV to 4 V amplitude, 5 μs duration; (D) distribution of 1000-5000 integrated pulse signals from the pulse shaper corresponding to volatilization/ionization of an equal number of particles at a specific m/e setting of the mass spectrometer.

Fig. 3 Scanning electron micrographs of B. subtilis spores collected on (A) a Nuclepore filter from suspension before aerosolization (B) a Nuclepore filter from the aerosol after the diffusion drier and (C) an Apeizon grease-coated glass slide from the particle beam at the filament target position.

Fig. 4 Mass spectra of bacteria particles: (a) Pseudomonas putida, (b) Bacillus cereus, (c) Bacillus subtilis

Fig. 5 Histogram of the degree of correspondence for the five bacteria samples. Each sample is a separate culture and B. cereus (a) was selected as the reference sample.

Fig. 6 Bacillus subtilis mass spectra from (A) Curie-point pyrolysis of

bulk bacteria sample, spectrum reconstructed, with permission, from Figure 1a of reference (2), and (B) pyrolysis (volatilization) of single bacteria from particle beam, spectrum reconstructed from Figure 4c of this work.

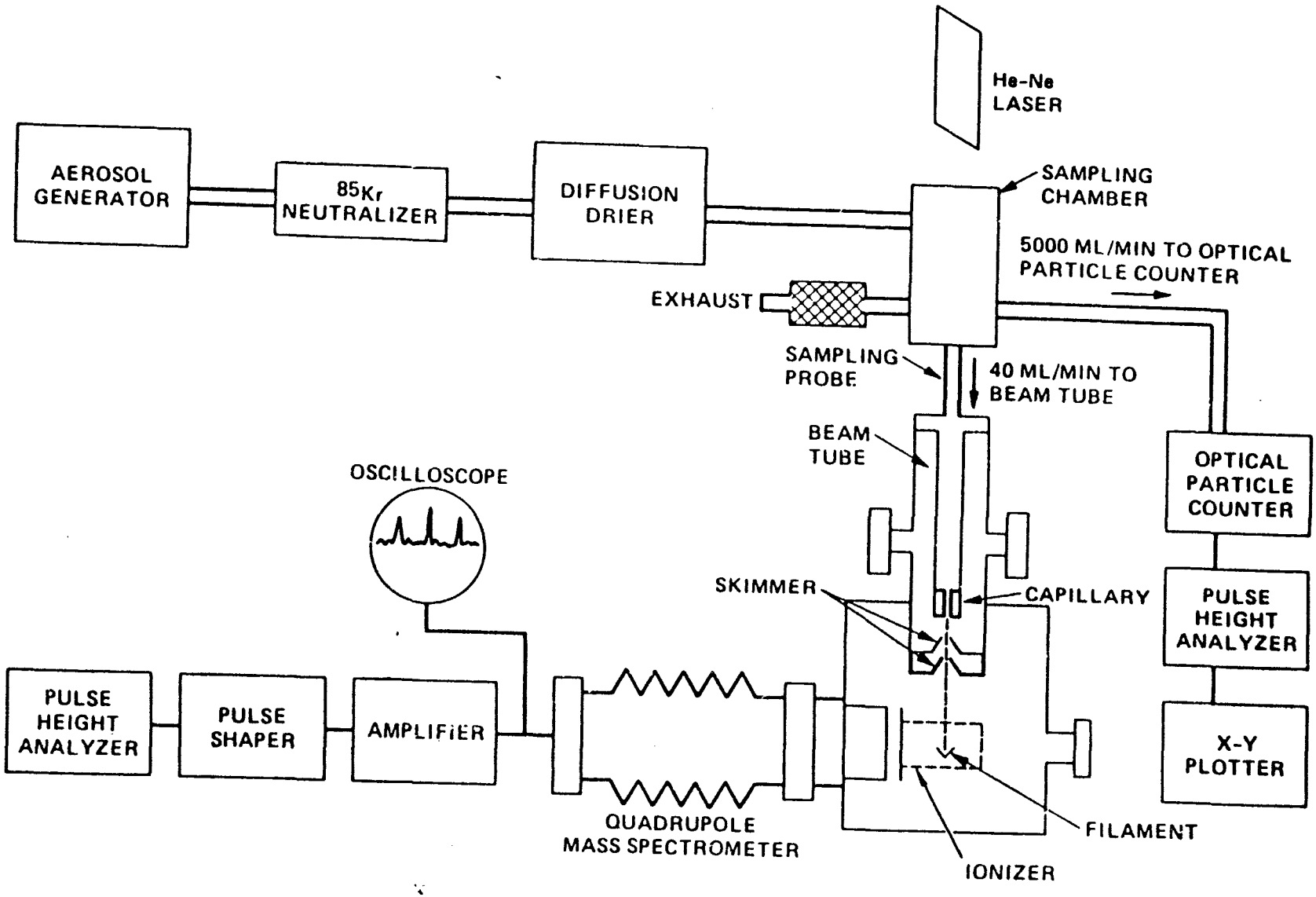

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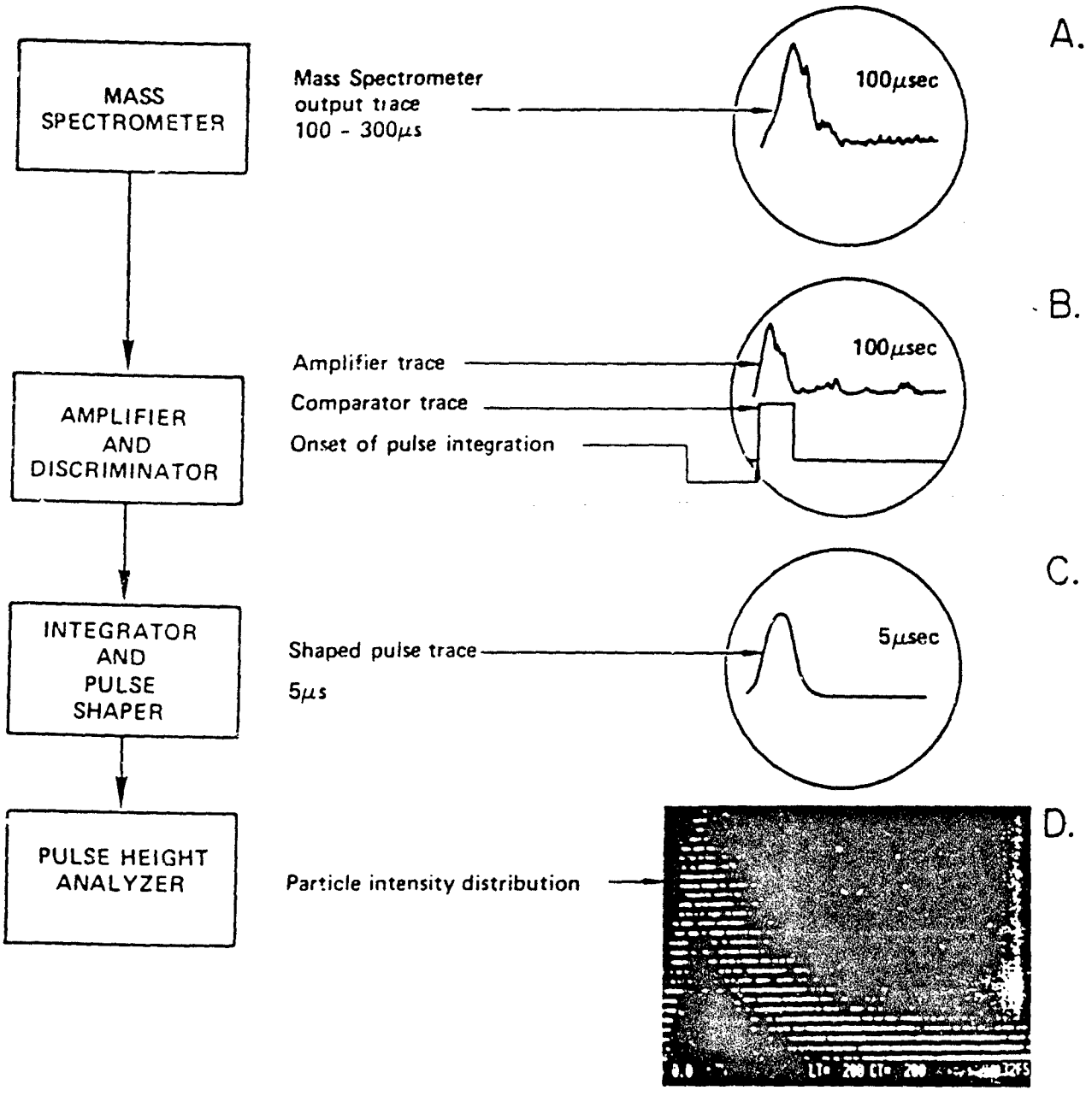
Fig. 1


MASS SPECTROMETER
AMPLIFIER AND DISCRIMINATOR
INTEGRATOR AND PULSE SHAPER
PULSE HEIGHT ANALYZER
Mass Spectrometer output trace 100 - 300μs
Amplifier trace
Comparator trace
Onset of pulse integration
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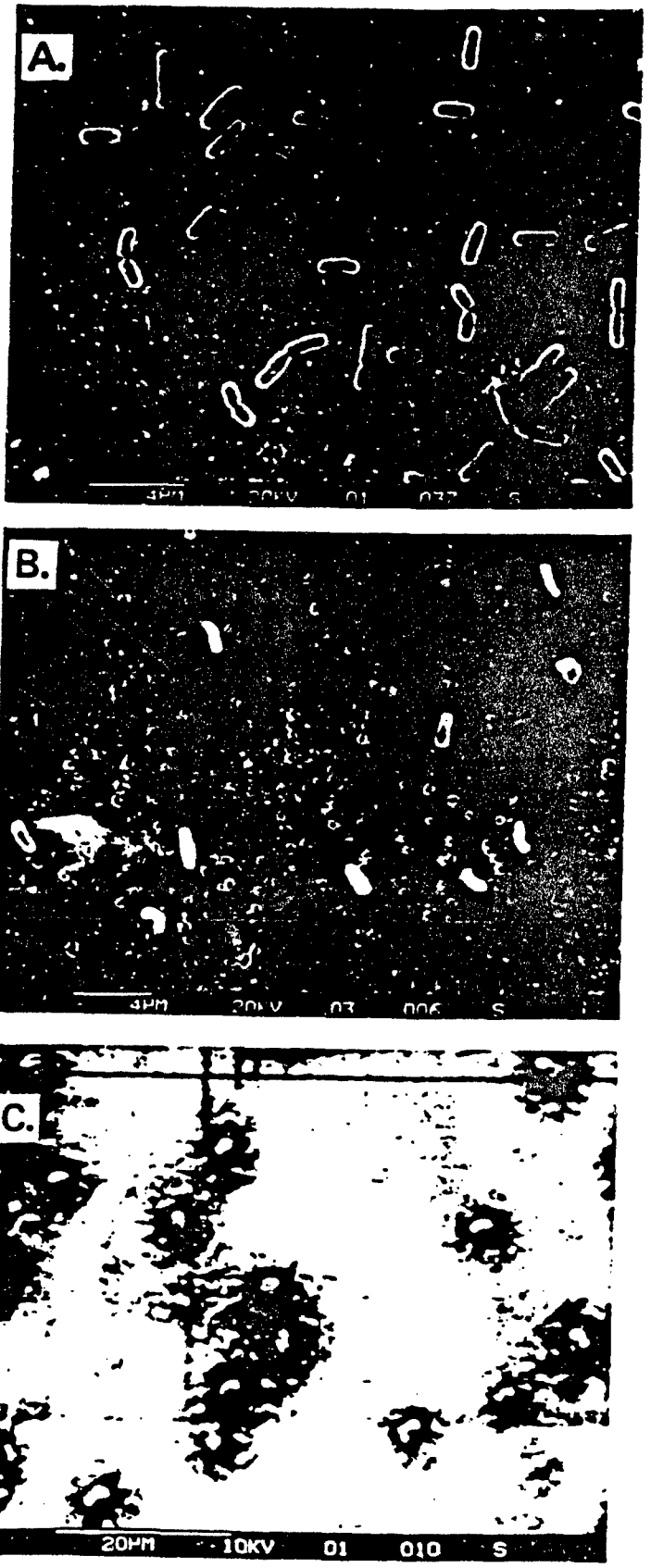

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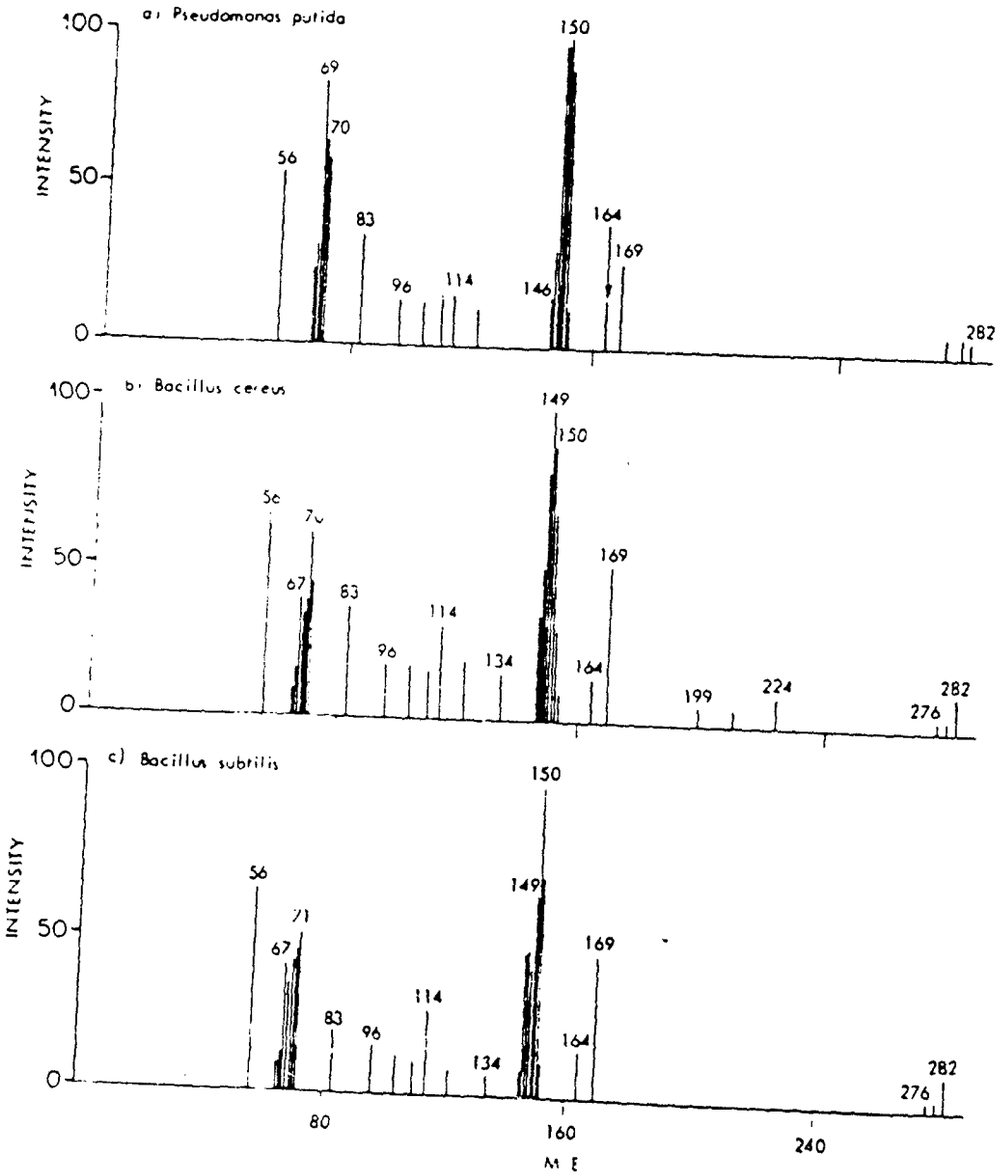
Fig. 3



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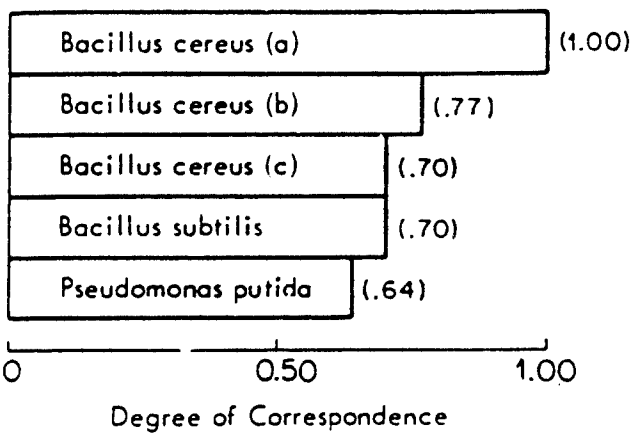

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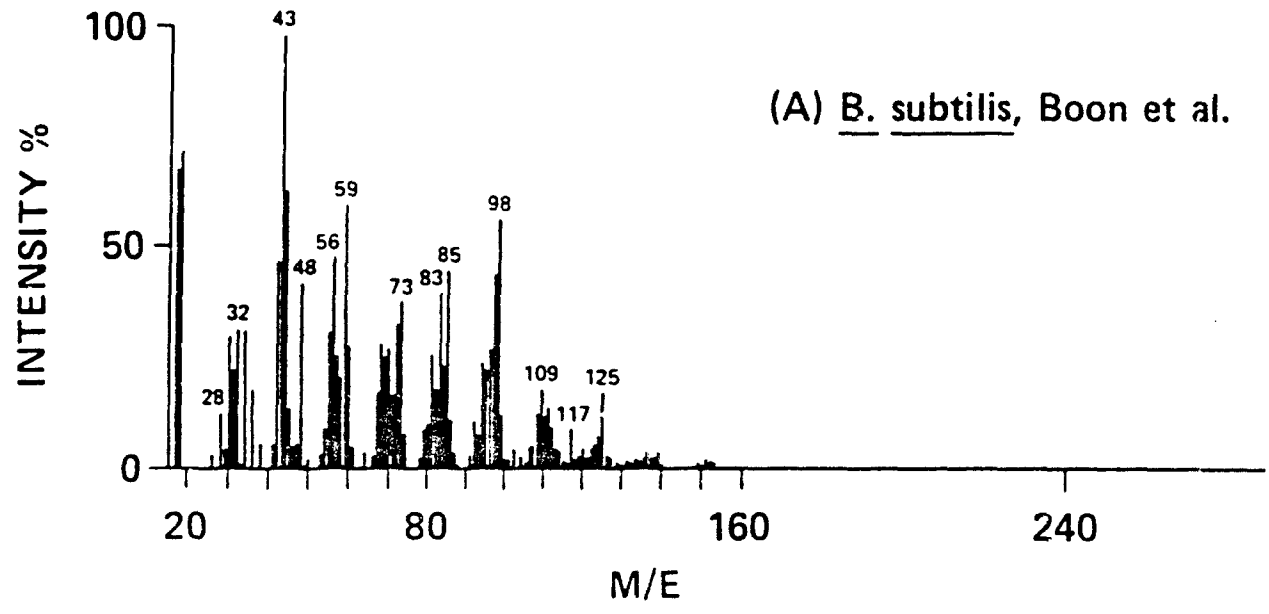
Fig. 5



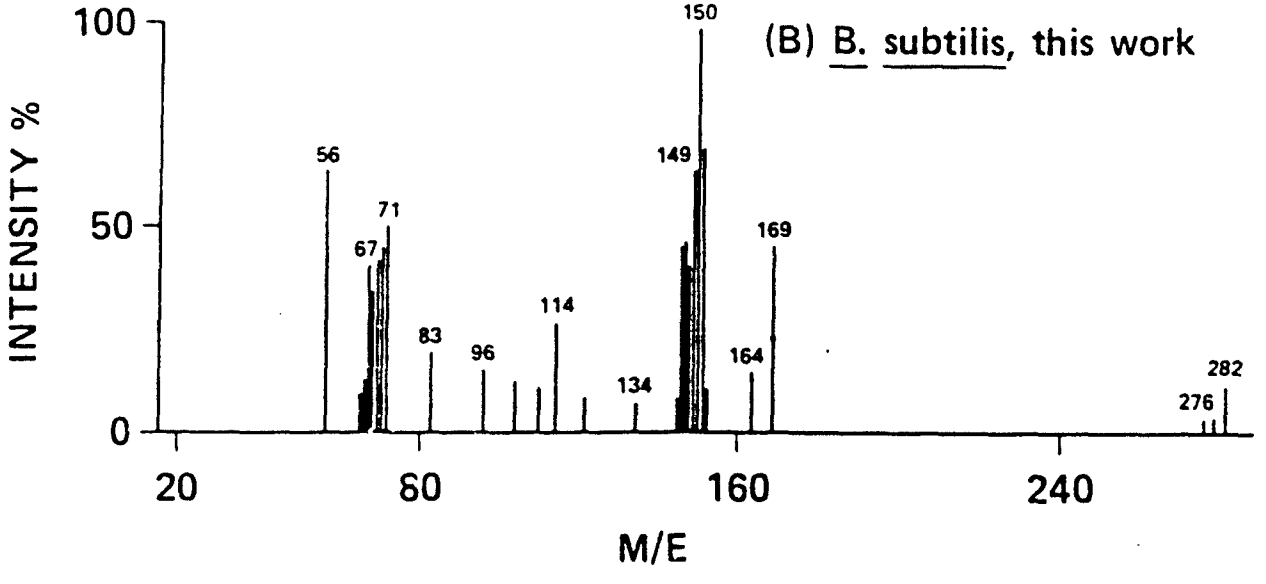


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Fig. 6

Laser-induced volatilization and ionization of microparticles

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We have developed a method for the laser-induced volatilization and ionization of individual microscopic particles on a continuous, real-time basis. A beam of particles is produced by the expansion of an aerosol into a vacuum through a capillary nozzle and skimmer system. The particle in the beam is then hit by a high-energy Nd-YAG laser pulse for its volatilization and ionization. Mass spectral measurements using a quadrupole mass spectrometer on the ions thus generated from potassium biphthalate particles (1.96 μm in diameter) have been made. The combined ionization efficiency for K^+ ion is found to be $\sim 10^{-6}$. The advantages of laser-induced volatilization and ionization along with the real-time capability of the present method make it very useful for the chemical analysis of aerosol particles by mass spectrometry. For the synchronization of the particle with the YAG laser pulse, the velocity of the particles in the beam has also been determined. This was accomplished by measuring the time-of-flight of monodisperse aerosol particles between two He–Ne laser beams. The velocities of different size particles are found to decrease with their increasing diameters and the velocity distribution for a given size is sharply peaked. The characteristic velocity may be used for the aerodynamic size measurement of aerosol particles and for relating the aerodynamic size to the optical size. Volatilization and ionization of microparticles in the beam eliminates any possible substrate-sample matrix interferences in the analysis. The method may also find applications in other spectroscopic and chemical kinetic studies.

PACS numbers: 82.80.Ms, 79.20.Ds

INTRODUCTION

Recently there has been a growing interest in the use of high-energy lasers for the vaporization and ionization of solids. Lasers provide a controlled means, both temporally and spatially, of depositing a predetermined amount of energy into the target. Several applications of laser–solid interaction have been made. These include vaporization and/or ionization of small and precise areas of samples for analysis by different spectroscopic methods,[1] laser fusion and plasma research[2], welding and cutting of metals, simulation of cometary particle impact on spacecraft[3], and thin film deposition.[4] In particular, the use of lasers in mass spectrometric analysis[5] has attracted much attention because of the advantages it offers over the other methods; namely: (i) the high efficiency for evaporation and ionization, (ii) its generally applicability, (iii) the fragmentation of molecules during vaporization and ionization can be controlled making it suitable for the anaysis of relatively involatile, thermally labile compounds, and (iv) the rapidity of analysis.

Our own interest in the laser ionization arises from the chemical analysis of aerosol particles. The methods generally used for analysis of these particles provide their average composition over various sizes and over the period of collection. Much more information is contained in the chemical composition of individual particles which may vary significantly even within a given size range.[6] The health effects of aerosols depend on the chemical composition of individual particles rather than on their average composition. The individual particle composition also provide a signature of their emitter source[7] and help understand the mechanism of their formation and chemical transformation.

A continuous, real-time analysis of individual particles will provide the desired detailed information on an aerosol sample. Such a technique for determining the chemical composition of single particles is being developed in our laboratory.[8] It utilizes the methods of mass spectrometry and particle beam generation. Different methods for the volatilization and ionization of particles for their mass spectral analysis have been studied. In earlier work,[8] the particles were introduced into the ion source in the form of a beam where they impinged on a hot rhenium filament and converted into vapor. The ensuing vapor from individual particles was ionized by electron impaction. The method has been found to work well for aerosols made up of both organic and inorganic compounds. However, it suffers from extensive fragmentation of molecules of the particles especially if the particles are composed of large and fragile molecules. This makes the identification of the parent molecules difficult. In order to overcome the limitation, we have investigated the use of a high-energy pulsed laser for the volatilization and ionization of particles. A Nd-YAG laser is used for this purpose. The advantages of the laser ionization mentioned above is expected to simplify the mass spectra and will be particularly useful in the analysis of multicomponent particles and for particles composed of complex organic molecules.

In this paper, we describe the experimental arrangements for the laser vaporization and ionization of individual micron size particles and some of the initial results obtained on this system. The novelty of the method lies in the fact that the particle is ionized by the laser pulse while in flight in the beam, unlike the usual mode of laser ionization where the sample is loaded on a substrate and then hit by a laser pulse.[9,10] Ionization of particles in the beam offers the real-

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time analytical capability of the technique and eliminates any possible substrate-sample matrix interferences in the analysis. The synchronization of the laser pulse with the particle requires a knowledge of the particle velocity. The velocity measurements of various size particles based on the laser light scattering technique have also been made and are described in Sec. II.

I. EXPERIMENTAL

A beam of particles is produced by the expansion of an aerosol into the vacuum (~0.3-Torr pressure) through a beam generator. The generator comprises a capillary (100 μm in diameter, 5 mm in length) and a skimmer with an orifice diameter of 350 μm. The particles being much heavier than the gas molecules remain confined along the beam axis. The skimmer allows an efficient transmission of the particles along the axis of the beam and provides for pumping most of the carrier gas. The beam is then led into the mass spectrometer (MS) chamber which is differentially pumped and maintained at $\sim 10^{-5}$ Torr of pressure during operation. The details of the particle beam generator and the MS system have been described previously.[8] However, some modifications in the system were made for the present studies. The axis of the beam generator in the earlier work was horizontal and the axis of the quadrupole mass analyzer was arranged vertical, orthogonal to the beam axis. This resulted in partial loss of larger particles due to gravitational settling. In order to eliminate this loss, the entire system was rotated through an angle of 90° making the particle beam axis vertical and the mass analyzer axis along the horizontal direction. Liquid-nitrogen-cooled traps were installed above the vacuum pumps to suppress the back streaming of pump oil into the beam exhaust and the MS chambers.

The velocity of a particle in the beam is measured by monitoring the scattered light pulses as it traverses through two cw He–Ne laser beams located at a known distance of separation. The particles are then individually vaporized and ionized by hitting them with high-energy Nd-YAG laser pulses (J. K. lasers, System 2000 FQY2) in the ion optics region of a quadrupole mass spectrometer (Uthe Technology, Inc. 100C) and the resulting ions are mass analyzed. The ion source of the MS was replaced with a different ion optics, designed to accommodate the laser beams and to focus the ions onto the entrance aperature of the mass analyzer. The axis of the quadrupole analyzer lies in the direction normal to the plane of the laser beams.

The optical arrangement of the experimental setup is shown schematically in Fig. 1. Two He–Ne laser beams designated as I and II of 4- and 7-mw, power, respectively, pass through $x3$ beam expanders to produce 3-mm diam (measured at $1/e^2$ intensity points) beams. They enter into the vacuum chamber through quartz windows. A set of knife edges are located inside the chamber to suppress the scattered and reflected lights. The laser beams exit into the Rayleigh horn light traps and are aligned such that they intersect the particle beam axis at right angles. This was accomplished by determining the particle beam axis (Z direction) with the help of a 2-mw He–Ne laser beam passing through the beam tube, capillary nozzle, and the skimmer system. The beam generator is provided with two pairs of micrometers which enable its movement in X, Y directions. Lasers I and II are

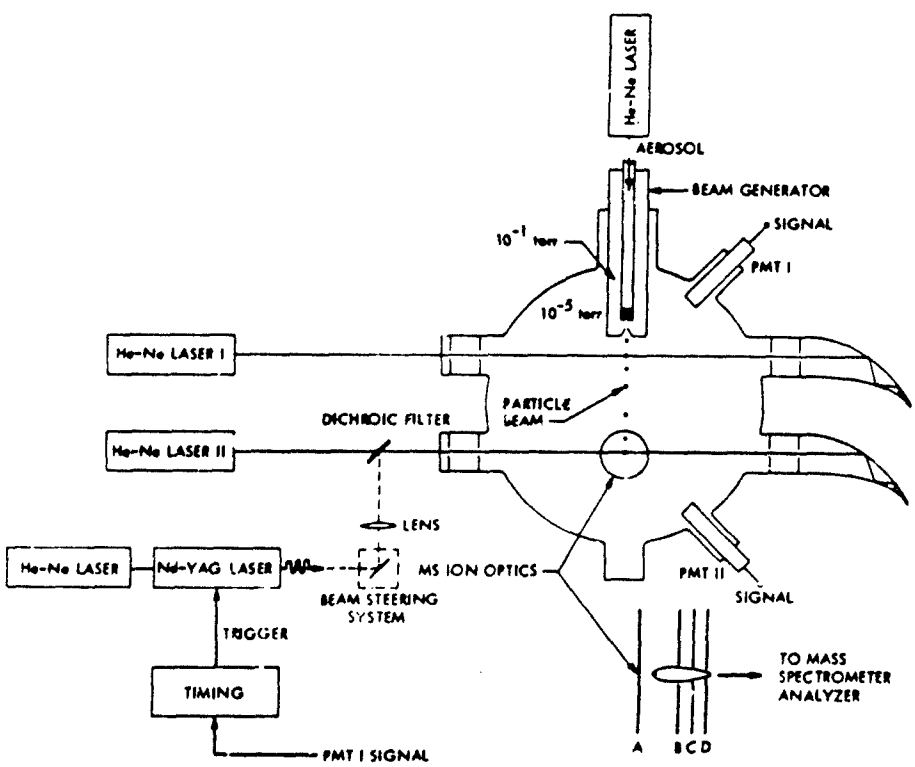

FIG. 1. Schematics of the experimental setup for the laser-induced volatilization and ionization of microscopic particles in a beam. He–Ne lasers are used for alignment. Laser beams I and II are located at a center-to-center distance of 66.04 mm and provide the start and stop signals for the time-of-flight analyzer. The particle beam travels in the vertical direction and the axis of the mass analyzer is perpendicular to the particle beam and the laser beam axes.

mounted on precision adjustable laser mounts with micrometers for Y, Z motion as well as for θ,ϕ rotations in order to facilitate their alignment. The center-to-center distance between beams I and II along the particle beam axis was carefully measured. Photomultipliers (PMT) I and II each with 19-mm-diam photocathode (Hamamastu Type No. R663) monitor the light pulses scattered by a particle while traversing through beam I and II, respectively. An optical baffle was put between the two laser beams in the MS chamber such that the light scattered by the particle in beam I is not seen by PMT II and also PMT I does not see the scattered light from beam II. The solid angle of light collection was 0.09 sr at an angle of 26° from the forward direction of the laser beam.

The Nd-YAG laser pulse is steered with a beam steering instrument (Newport Research Corp. Model 670), that uses a pair of elliptical first surface Pyrex mirrors with multilayer coating (NRC 13E20RM.4) for the YAG 1060-nm radiation. The top mirror holder assembly with micrometers was mounted on the post through a translation stage (NRC Model 430-1) to adjust the height of the laser pulse precisely. The YAG laser is then reflected from a dichroic filter (CVI Laser Corp). The filter possesses the characteristics of the maximum reflectivity of > 99% for the YAG radiation incident at 45°, and a maximum transmission (~82%) for 633-nm radiation. The optical system was aligned such that the YAG laser pulse was collinear with the beam II axis. It intersected with the particle beam axis at the same point as beam II did. A converging lens, f = 80 cm, was placed between the steering mirror and the dichroic filter which focused the YAG pulse to a spot size of about 2.5 mm at the particle beam axis. A Kodak burn paper was used during the alignment process to determine the YAG pulse location.

The YAG laser pulse and the beam II pass through the central region of the ion optics plates of the quadrupole MS. The ion-optical arrangement shown in Fig. 1 consisted of three lens plates, the bottom plate A being the ion repeller and was maintained at a positive potential with respect to the middle extractor plate B. Ions are generated by the laser interaction with the particle in between these two plates and are focused by the lens C, maintained at a negative potential, onto the entrance aperture D of the MS analyzer. D was kept at ground potential. The YAG pulse was synchronized with the particle in the ion source by sensing the latter upstream and from the knowledge of its velocity (see Sec. III). The particle velocity measurements are described below.

II. VELOCITY MEASURMENTS

Monodisperse aerosols containing potassium biphthalate (KBP) particles of known size were produced using a vibrating orifice aerosol generator (Thermo-Systems, Inc. Model 3050). It involved passing a dilute solution of KPB in 10% water and 90% isopropanol through an orifice into a disk to produce a liquid jet. The jet was then broken into uniform size droplets by vibrating the disk at a fixed frequency. The droplets were dispersed in a stream of nitrogen and the solvent then evaporated producing the particles. These particles acquire electric charges during their generation.

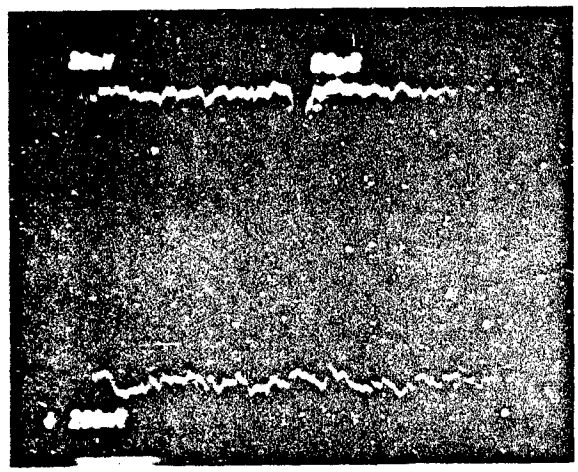

FIG. 2. Photograph of scattered light pulses. The bottom trace shows the output signal (inverted) from PMT I which monitors the light scattered from laser beam I by a particle while traveling through it. The top trace is the pulse scattered from laser beam II by the same particle and monitored by PMT II. The horizontal scale is 50 μs per division.

The aerosol stream was neutralized by passing through a neutralizer containing ^{85}Kr and further dried in a diffusion drier containing silica gel. The size distribution and number density of particles in the aerosol were determined using an optical particle counter (Climet Instruments, Model CI-208). Orifices of 10- and 20-μm diameters were used along with various concentrations of KBP in the solutions to produce particles of different sizes. A small amount of aerosol (50 cm^3 min^{-1}) passes through the beam tube and the remainder of it is discharged to the fume hood. The aerosol in the beam tube produced a beam of particles in the MS

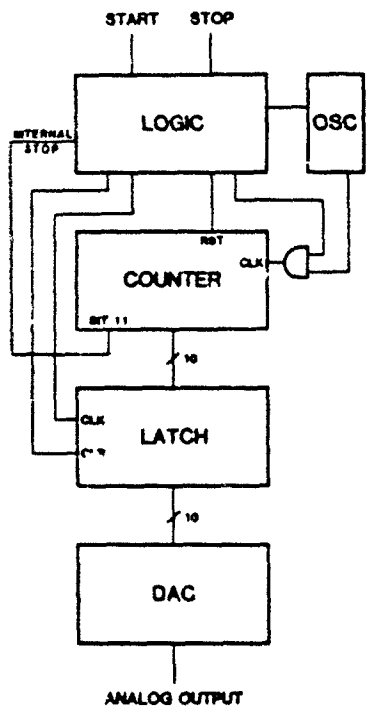

FIG. 3. Block diagram of the time-of-flight analyzer.

chamber after expansion into vacuum through the capillary and the skimmer system.

A particle in the beam while passing through laser beams I and II scatters light pulses which are monitored by PMT I and II, respectively, as shown in Fig. 2. The time interval between the two pulses provides the time-of-flight (TOF) for the particle. The distribution of the TOF for particles in the beam was measured using the TOF analyzer shown in Fig. 3. The signal from PMT I is amplified and shaped and is used to start the count of the clock pulses. PMT II signal was used to stop the count. The count was loaded into the data latch and then to digital-to-analog convertor (DAC, Burr–Brown, DAC 87). The analog voltage is fed into the pulse-height analyzer. (Tracor Northern, Model 1710) This was repeated for a large number of particles and their TOF distribution measured. A typical distribution for a 1.28-μm-size particle is shown in Fig. 4. Since the particle beam has a finite divergence, some particles which cross laser beam I miss beam II. Such particles do not provide the stop pulse for the counter. The counter in such cases counts to its full 10-bits capacity. The bit 11 of the counter is used to generate the stop-count pulse internally for such events. When this bit goes high, the zeros stored in the lower bits of the counter are loaded into the latch and the DAC, and the data are thus ignored. This also resets the TOF analyzer. The number density of particles in the input aerosol is less than 100 cm^{-3} and, therefore, the coincidence error due to the simultaneous presence of particles in the laser beams is not important.

The particle velocities in the beam corresponding to various particle sizes are listed in Table I. The TOF curve closely fits a Gaussian distribution (Fig. 4) and is sharply peaked. The standard deviation in the velocity is also given in Table I. Beams I and II are of 3-mm diameter and introduce some uncertainty in the flight distance for the particle between them. The measured center-to-center distance of 66.04 mm may vary between 66.04 $\pm$ 1.5 mm depending on where in the rising portion of the PMT pulses the comparator threshold is set. In addition to this, the digital circuit also involves an uncertainty of two clock periods ($\pm$ 1 μ) in the TOF. The maximum spread in the velocity due to the above effects is also listed in Table I. This may account for most of the uncertainties in the velocity measurements.

TABLE I. Velocity measurement results.

Aerodynamic particle diameter	Particle velocity (m/s)	Maximum error due to flight path, and clock pulse counts (m/s)
1.27	355 ± 9	± 10
1.64	332 ± 10	± 10
2.06	299 ± 11	± 10
2.30	287 ± 18	± 9
2.87	267 ± 11	± 8
3.00	262 ± 5	± 8

Figure 5 shows the plot of particle velocity versus their aerodynamic diameter d_a. d_a was calculated by the equation $d_a = \sqrt{\rho}\ d_g$ where ρ is the density of KBP salt and d_g is the geometric diameter which was obtained from the droplet diameter and the salt concentration used in aerosol generation. It can be seen that the velocity decreases with the increasing size of the particle and possesses a narrow distribution about their most probable velocity (a standard deviation of less than 6%) This feature may be used to measure the aerodynamic size of aerosol particles[11] and may provide a method of relating the optical diameter with the aerodynamic diameter. The former can be obtained after suitable calibration from any one of the scattered light pulses from He–Ne laser beams and the latter from the velocity measure-

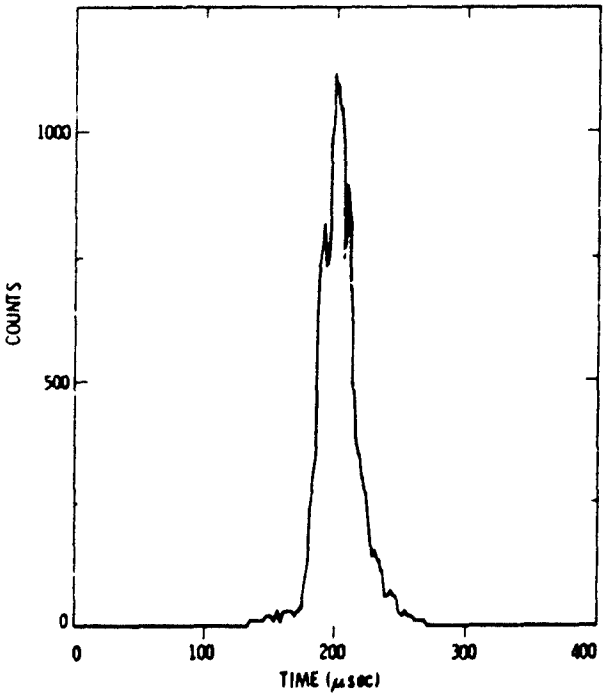

FIG. 4. Time-of-flight distribution of potassium biphthalate particles (1.28 μm in diameter) in a beam.

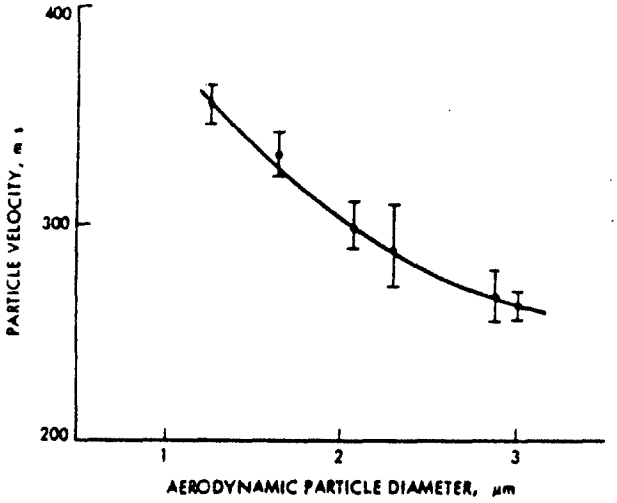

FIG. 5. Plot of velocity vs aerodynamic diameter of KBP particles. The error bars indicate one standard deviation.

ment. A sharp velocity distribution also helps in the snychronization of the particle and the Nd-YAG laser pulse for volatilization and ionization studies. The results on velocity measurements agree well with those obtained by Dahneke *et al.*[11]

III. VOLATILIZATION AND IONIZATION OF PARTICLES

The Nd-YAG laser used for the volatilization and ionization of the aerosol particles delivers 1 J of energy per pulse at a repetition rate of less than 20 per second, the pulse width being $\sim 100\,\mu$s. The time interval between the external triggering of the laser and the appearance of the pulse was measured. The YAG beam was aligned collinear with the He–Ne laser beam II and focused to a spot size of about 2.5 mm onto the particle beam axis. A He–Ne laser beam passing along the optical axis of the Nd-YAG laser cavity provided a visual path of the YAG laser beam. For the vaporization and ionization studies, the He–Ne laser II and the alignment lasers were turned off.

A beam of KBP particles of 1.96 μm in diameter was made. KBP was selected for the study because of the fact that the presence of alkali metals in organic salts have been found to facilitate the ionization of the salt.[12] The arrival of the particle was detected by PMT I upstream of the YAG location. PMT I looks at the pulse scattered by a particle from beam I. The signal was amplified and discriminated from noise by properly adjusting the threshold of a comparator. The comparator output was used as the external trigger for the YAG laser as shown in Fig. 6. An optimum delay using the pulse generator G (Intercontinental Instruments, Inc. Model PG-2) was introduced in the trigger line to account for the time the particle would take to reach the YAG location, and the time lag between the triggering and the appearance of the pulse. The former was calculated from the particle velocity and the distance of separation between the laser locations. The delay was adjusted such that the particle would arrive at the ion source in the middle of the YAG laser pulse. It may be noted that the particle takes about 9 μs to travel across the 2.5-mm waist diameter YAG laser beam and the YAG laser pulse width is $\sim 100\,\mu$s. This feature makes the synchronization of the particle and the YAG pulse essentially independent of the small spread in the particle velocity. The trigger circuit for the PMT I pulse was designed such that the trigger line was inhibited for 128 ms after allowing a pulse to pass through. This was done to avoid triggering the YAG laser at $>$ 20-Hz frequency and was accomplished by using the output pulse from G to set the flip-flop F. This stops any pulse from PMT I to pass past gate E. After a predetermined time of 128 ms, F is reset by the counter C and the circuit is enabled for subsequent triggering of the YAG laser.

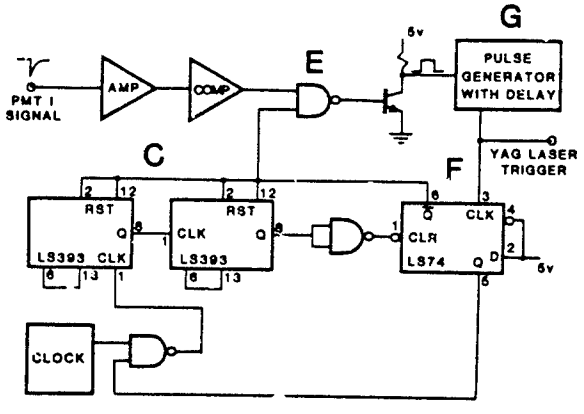

FIG. 6. Electronic circuit for the external trigger of the Nd-YAG laser.

Figure 7 shows the oscillograms of the mass spectrometer signal for K^+ ion (upper trace) resulting from the laser volatilization and ionization of a single 1.96-μm-diam KBP particle. The lower trace is the signal at the input of the delay generator G (Fig. 6) and was used to trigger the oscilloscope. Oscillogram 7(b) shows that not all particles detected by the sensor laser I are hit by the YAG laser pulse. This results due to the divergence of the particle beam. The mass spectrometer was set manually at 39 amu. Manual operation is necessary because of the fast generation of ions from the laser

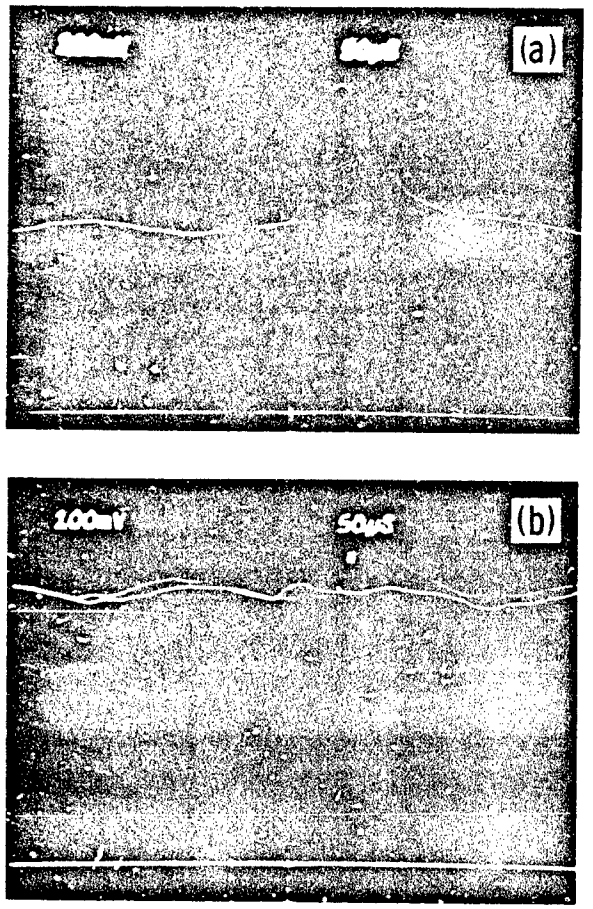

FIG. 7. The upper trace in each photograph shows the mass spectrometer signal for K^+ ions produced from potassium biphthalate particles (1.96 μm in diameter). The input signal to the delay generator G (Fig. 6) was used to trigger the oscilloscope and is shown as the lower trace. The current was measured at $\times 10^{-7}$ A scale of the MS. The absence of MS signal in one of the upper traces in (b) shows that the particle is missed by the YAG laser pulse due to the divergence of the particle beam. The vertical scales in (a) and (b) are 200 mV per division and 100 mV per division, respectively, and the horizontal scale for both (a) and (b) is 50 μs per division.

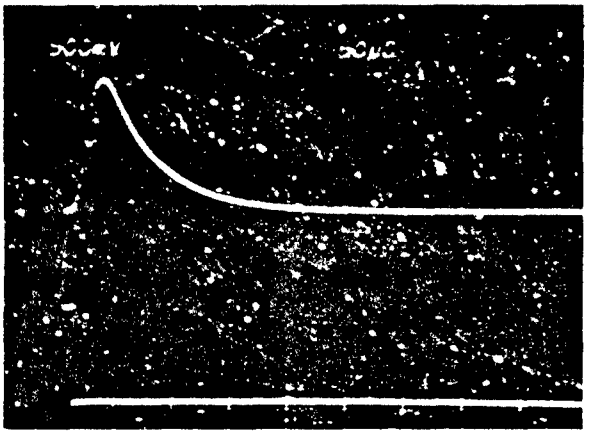

FIG. 8. Bottom trace: The voltage pulse on the light-emitting diode to generate a current pulse of 10 μs width and 10^{-7} A amplitude through a photomultiplier tube. Top trace: The current pulse measured by the mass spectrometer electronics.

pulse. The combined efficiency of vaporization, ionization, and detection is about 10^{-6}, i.e. 1 in 10^6 molecules present in the particle could be detected. Weak signals at masses 23 and 28 were also observed. We believe the signal at 23 amu arises from sodium present as impurity in KBP, and at mass 28 from CO fragments of the carboxylic groups. No other ion signal from the organic counterpart of the KBP molecules could be detected. The reason may be a combination of factors. The average power density in the focused beam spot is about 2×10^5 W cm^{-2}, which is likely to be low for the ionization of organic molecules.[13] Also, the mass spectral features of the molecules in the laser-induced volatilization and ionization may be different from the conventional electron impact ionization and could be missed in the manual scanning of the MS.

It can be inferred from the results on velocity measurements that a particle of 1.96-μm diameter remains in the YAG laser pulse for a period of about 9 μs and consequently, the ion production process should be of this duration. Figure 7, however, shows the ion-pulse width of $\sim 50\,\mu$s. The ion-pulse broadening arises from the time constant of the MS current measurement electronics. This was determined by feeding a current pulse of 10^{-7} A and 10 μs width into the MS in place of the output from the channeltron electron multiplier. The current pulse was generated by pulsing a light-emitting diode (LED, Xciton Inc., No. XL-556) with a 10-μs-wide voltage pulse and monitoring the light pulse with a PMT. The results are shown in Fig. 8. The lower trace is the voltage pulse which turns the LED on and the upper trace is the PMT current as measured by the MS system. The LED has a rise time of 500 ns and internal capacitance of 50 pf. The PMT output pulse was found to follow the voltage pulse on the LED. It can be seen from Figs. 7 and 8 that the pulse widths in both measurements are the same.

The time elapsed between the sensing of the particle by laser I and the peak of the MS ion pulse resulting from this particle by laser volatilization and ionization is about 277 μs (see Fig. 7). The time-of-flight of the particle between laser beam I and the YAG laser beam can be determined by subtracting the transit time of the ions through the analyzer and the time delay in the appearance of the peak of the current pulse due to the MS electronics (see Fig. 8) from the above time. The TOF is thus found to be about 235 μs and, therefore, the particles in the beam have a velocity of 280 m/s. This is in agreement with the velocity measurements described in Sec. II.

The results obtained show that the method described above can be used for the volatilization/ionization of individual micron-size particles in flight in a vacuum. The method may also provide selection of a narrow size range of particles in a beam generated from a polydisperse aerosol (natural aerosol). Such a size discrimination is possible because of the characteristic velocities of particles in a beam and can be accomplished by introducing a specific delay (depending on the velocity) in the pulse from PMT I that senses the arrival of the particle at a fixed distance upstream of the YAG laser beam and is also used to trigger the YAG laser.

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ANALYSIS OF INDIVIDUAL BIOLOGICAL PARTICLES IN AIR

Bioparticle Analysis (for running head)

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I. Introduction

Monitoring air for biological contaminants has attracted renewed interest due to the recent studies that have linked the spread of disease to the transfer of agents through an air medium.[1] Legionnaires' disease[2] typifies the air transfer of microbial agents and shows the difficulties in detecting and identifying unknown biological agents by conventional techniques.

Biological contaminants occur in air as aerosols, defined as solid or liquid particles suspended in air. These aerosols consist of bacteria, fungi, viruses, and pollen particles and cover a wide size range, the range of 1 to 10 μm being most important.[3] Particles larger than this, whether in the form of single cells or aggregates, do not remain suspended in air for a long time but settle on surfaces. They can multiply on congenial surfaces after settling and may again be made airborne by breaking as a result of external activities.[4] Microbial particles in air come from both artifically generated and naturally occurring sources: solid waste treatment,[5] industries (eg, cotton milling,[6] mushroom production[7]), wastewater treatment,[8] irrigation[9] projects, and bubbles bursting from microbially laden water layers,[10] to name some sources. Furthermore, these particles do not always remain confined to the proximity of their sources. A typical microbe of 1 μm can travel hundreds of kilometers downwind before settling and affecting the environment. Airborne contagions have been found to cause the spread of infections in hospital environments[11] where patients are likely to be more susceptible to disease: People disperse microbes while talking, coughing, and sneezing,[12]

and open wounds, skin diseases, and skin lesions[13] shed microbes that cause disease by air transfer. Analytical conditions under general field conditions would be more complex because of the interferences from substances normally present in air.

Obviously, it is desirable to monitor air for biological particles in a number of different environments, namely, hospitals, clean rooms, areas surrounding different industrial sites, and regions of military importance. The ideal method for such monitoring would be one that is fast, sensitive, and specific, but no single method possesses all of these features. Consequently, the choice of an analytical method depends on the circumstances. For example, speed may be the deciding criterion for an attack scenario, even at the expense of specificity (though a follow up by a more specific method would have to be made in order to take the appropriate countermeasures).

The objectives of this chapter are (1) to briefly review the principles of the various rapid methods of detection and identification of biological agents, along with their speed, sensitivity, and specificity; and (2) to describe in detail a new technique known as particle analysis by mass spectrometry (PAMS)[14] for the direct analysis of individual particles on a real-time basis.

II. Detection of Biological Particles

The methods of detecting microorganisms in air are the same as in any other medium. These can be classified into methods based on the following:

(1) Measuring physical characteristics of particles

(2) Measuring biological activities

(3) Detecting key biochemical substances

(4) Generating characteristic fingerprint specta

A. Physical Methods

The physical methods of detecting microorganisms depend on measuring the number, size, and shape of aerosol particles, and then the measurements indicate only the presence of aerosol particles in a suspected size range.[15,16] For example, a sudden increase in the ratio of the number of particles in the 1 to 5 μm range to the total number of particles could be construed as an indication of artifically generated biological aerosol. Such an interpretation would be based on the belief that the 1 to 5 μm size range will be preferred for dispersion of these agents because of their importance to enhanced pulmonary retention and their prolonged suspension in air. So, physical measurements are rapid but are not specific and make no distinction between a microbial particle and a particle of any other origin. The importance of physical methods, however, lies in their speed (seconds).

Instruments orginally developed for use in air pollution and industrial hygiene can be used for measuring microorganisms without any modification. Optical particle counters, aerosol photometers, nephelometers, and microscopes are widely used in bacteriology laboratory. The poor specificity as to particles of biological origin can be improved by combining a physical method with a method from other approaches listed above. A partichrome analyzer[15] is an example of such a combination. Here the particles are collected on a sticky tape and are stained with a dye. Biological particles retain the dye and can then be examined with an automated microscope.

B. Biological Methods

The methods based on biological activity measure life processes. The microorganisms are studied during their growth by monitoring the increase in their mass or number and by monitoring the evolution of their metabolic products. Several instruments that continuously measure such changes have been built. The Gulliver device[17,18] uses a growth medium containing C^{14} labeled sugar. During their growth, the microorganisms liberate $C^{14}O_2$, which is then measured by a Geiger-Muller counter. This particular method has the sensitivity to detect 10 to 100 cells. Measurements of the changes in turbidity and pH of the growth medium have also been used as indicators for biological activities. For example, Wolf Trap[19] is a miniature growth chamber whose turbidity is continuously monitored by a nephelometer and whose pH changes are monitored by a pH electrode. Other instruments (eg, multivator)[15,17] are based on the principle that certain media, upon decomposition by microbial enzymes during growth, form chromogenic or fluorescent substances that can be detected by fluorimetric or colorimetric techniques. Little information is available about the performance of these instruments, however.

Serological methods are highly specific and use antibody-antigen reactions[20] to detect and identify microorganisms. These methods apply to any microorganism for which antibody may be available, and a number of antibodies can be produced and extracted from laboratory animals. The antibody-antigen reactions are so specific that, even in a crude mixture, they can be used for the rapid detection of a single antigen. The reactions can be accelerated, bringing the antibody and antigen together using electric current (immunoelectrosmophoreris[20]). Also, a fluorescent antibody can be prepared by linking a fluoresecent dye, fluorescene, to the antibody gloublin. After reaction,

the fluorescent antibody-coated organism stands out as a brilliantly fluorescent cell under a microscope with an ultraviolet light source.[21,22,23] Cultivation and isolation, however, are usually performed to confirm the diagnosis because one microorganism can give rise to several antibodies, some of which may be similar to those produced by other species.

The methods for detecting and identifying microorganisms based on their biological activities have the advantages of high specificity and sensitivity. A single cell can be detected, given enough time, and viable particles can be differentiated from nonviable particles. These methods, however, suffer from a lack of speed (the growth of organisms takes time, at least a few hours), and their application is not general since microbes have specific requirements. Furthermore, these methods necessitate collecting particles from air in the viable state. Also, though changes in turbidity and the pH of the medium are normally caused by living things, sometimes nonliving substances imitate characteristic viable activity. For example, crystals can grow in the medium, and some nonviable material can absorb or desorb gases,[15] thus giving false indications of viability.

C. Biochemical Methods

Another class of methods depends on detecting biochemical components or unique chemical structures in microorganisms, chemical characteristics which distingish biological from nonbiological materials. Commonly used for this purpose are tests for the presence of nucleic acids, ATP, and proteins in the biological particles. For example, biological compounds (eg, proteins, nucleic acids, peptides, carbohydrates) react with certain dyes, such as thiocarbocyanins,[24,25] and produce a spectral shift in the light aborption characteristics of the dye. Measuring this shift by conventional spectroscopy has

been the basis for detecting biological compounds in an unknown sample. Acridine orange,[26] for instance, becomes bound to nucleic acids and produces fluorescence in the microbial particles under ultraviolet light. Another method uses ATP, present in all living cells as the carrier of energy, to induce the reaction of the firefly enzyme luciferase with its substrate luciferine. Luciferase does not react with luciferine in the absence of ATP and thus provides a test for ATP in a sample.[27,28,29,30] Another method of detection consists of heating the sample, which converts the protein in it to $NH4^+$. The ions are then measured in an ion-detection chamber.[15] This method is sensitive and can detect a sample as small as 0.1 μg of nebulized albumin but suffers from the noise generated by the presence of nonmicrobiological proteinaceous material in air.

The methods described above are fairly rapid and provide information about the biological nature of the sample, but they have several limitations. Notably, none of the methods require viability of the sample; consequently, these methods provide no information on the living state of the microorganisms and provide little information for differentiating among them. Furthermore, these methods cannot be used alone in field conditions for detecting biological particles because of possible interferences. For example, the method using acridine orange is compromised in the field by nonbiological particles that fluoresce either alone or in combination with the dye. Also, the complexities of the analysis of microorganisms in the field are increased because background material is present from natural sources, such as algae, pollen, and sawdust, and because a small number of nonpathogenic microorganisms usually exist in air. It is, therefore, important that the analytical methods used for detecting and identifying microbial particles in air

should not only be sensitive to biological compounds but should also be specific enough to eliminate interferences.

Methods which use chromatography, mass spectrometry (MS), and various combinations of these two techniques meet such requirements and have attracted much attention for the rapid analysis of microorganisms.[31,32,33,34,35] The composition of cell walls[36] and DNA content[37] differ markedly between bacterial species and therefore can be used for detection and identification. For the application of gas chromatographic and mass spectrometeric methods, the analyte has to be converted into the vapor phase. In addition, vaporization of the biological material must be preceded by fragmentation into smaller mass units. Two methods for the vaporization and fragmentation of large biological compounds have been used. In the first method, the macromolecules are converted into their small units by hydrolysis and are subsequently reacted with some suitable compounds to prepare their relatively volatile derivatives. The second method consists of heating a sample in nonoxidizing atmosphere (pyrolysis)[38] whereby the large molecules are fragmented, producing small units in the vapor phase. The fragmentation takes place at preferred junctions in the molecules, and the fragments contain information about their parent molecules.

Morgan and Fox[39] have recently used the derivatization gas chromatography (GC), high resolution capillary GC, GC-MS, and pyrolysis GC-MS for bacterial analysis. Specific compounds, for example, muramic acid, diamino pimelic acid, and D-amino acids, have been used as unique markers for the classification of bacteria. Anbar et al.[40] studied the feasibility of an identification method based on mass spectrometric analysis of the nucleic acids bases of microorganisms. The method consists of extracting nucleic acids from

microorganism, hydrolyzing the acids to quantitatively release purines and pyrimidines, and volatilizating and analyzing the purine-pyrimidine mixture using field-ionization mass spectrometry. The success of the analytical method following pyrolysis largely depends on the pyrolysis conditions. It is important that the fragments produced by the bond-scission do not undergo any reaction among themselves or with the background gases. These secondary reactions tend to produce noncharacteristic fragments and to decrease the reproducibility of the pyrolysis process. It is preferable to use a method of pyrolysis that provides a controllable uniform temperature, a maximum heating rate, and a minimum heating time and that uses a minimal amount of sample.[41] Reproducibility is enhanced if the entire sample experiences the same temperature and if the pyrolysis products are exposed to a collison-free environment. The transit time from the pyrolysis site to the analyzer should be minimized in order to decrease the chances of pyrozylates interacting among themselves or with the walls of the container.

Several designs of pyrolyzers have been reported in the literature and have recently been reviewed by Irwin,[38] and Wieten et al.[42] They are classified on their mode of operation, namely, the continuous mode (eg, furnace) and the pulse mode. The latter type of pyrolyzers are commonly used and include resistively heated pyrolyzers, Curie point pyrolyzers, and laser pyrolyzers. Very encouraging results have been obtained with Curie-point pyrolyzers. In this method a small amount of sample in the range of 5 to 20 μg is directly deposited on a wire made of ferromagnetic material. High-frequency inductive heating is employed to raise the temperature of the wire to its Curie temperature. It takes ~100 ms to reach its equilibrium

temperature, and typically the sample is pyrolyzed for a period of 1 to 10 s. Reliable and reproducible temperature-time profiles have been obtained. Meuzelaar and his co-workers have constructed fully automated systems for Curie-point pyrolysis-GC[43] and Curie-point pyrolysis-MS.[44]

Laser pyrolysis is still in its infancy; however, it possess many desirable features.[45] Some of these are high temporal and spatial resolution, a very fast heating rate, and the ability to pyrolyze extremely small amounts of sample ($\sim 10^{-12}$ gm) so that single-cell analysis is possible. A laser pyrolysis mass spectrometer is available commercially. The system, known as Laser Microprobe Mass Analyzer (LAMMA),[46,47] uses a frequency quadrupled Nd-YAG laser. The laser beam is focused to micron size and has a spatial resolution of this order. Applied to biological systems, the technique has been used for the analysis of intracellular electrolytes[48] and trace metals with high sensitivity. LAMMA has also been used for the analysis of single aerosol particles[49] deposited on glass substrates. The extent of the fragmentation of organic molecules is found to depend on the irradiance of the laser beam in focus. Also, laser desorption mass spectrometry[50,51] has been applied to the mass spectral measurements of nonvolatile bio-organic molecules. High mass fragments with good intensities are obtained, but the measurements seem to be qualitative in nature. Applicablity of lasers to the pyrolysis of biopolymers and cells is yet to be demonstrated.

D. Fingerprint Spectrum Methods

Pyrolysis gas-liquid chromatography (Py-GLC) and pyrolysis-mass spectrometry (Py-MS) have been used for fingerprinting complex biological material. Reiner[52] was able to visually distinguish the pyrograms of some of the closely related strains of microorganisms and pointed out the possibility of

Py-GLC forming a new basis of taxonomy in biology. Fingerprints of Salmonella bacteria were later matched by computer by Menger, Epstein, Goldberg, and Reiner.[53] The main drawbacks of fingerprinting microorganisms by Py-GLC are the lack of standardization and reproducibility of the results from different laboratories.[54] These deficiencies can be attributed to both the pyrolysis technique and the chromatographic column: the difficulty in reproducing the pyrolysis conditions, the insufficient column resolution, the degradation of the column, and the chemical selection by the column. It may be possible to minimize these problems by using a well-controlled pyrolysis and by applying MS directly to the pyrozylates. Direct application of MS to the pyrolysis product to obtain Py-MS fingerprints of albumin and pepsin was reported by Zemany in 1952.[55] Pyrolysis was performed by heating the proteins on a filament in a flask; no data on the reproducibility of the procedure was given. Recently, the combination of Curie-point pyrolysis with MS (using low-energy electron-impact ionization) has provided very promising results for the detection and identification of microorganisms.[34] High reproducibility in the pyrolysis pattern has been observed,[56] and the low-voltage electron-impaction ionization has helped to retain the chemical information on the building blocks of the biomacromolecules. Also Py-MS has been shown to possess high sensitivity, speed, and computer compatibility. The addition of pattern recognition programs for mass spectral analysis to Py-MS enhances specificity and speed. These advanced data analysis programs also help in identifying the chemical nature and the origin of mass peaks in a spectrum. The method has been successfully applied in the laboratory by Meuzelaar and others to characterize a wide range of biological materials, including bacteria, viruses, mammalian cells and tissues, body fluids, biopolymers, plant tissues, and others substances.[57] The detection and identi-

fication of microorganisms by comparing their fingerprint spectra require a compilation of standard fingerprints, so a library should be generated. An atlas of the reference spectra from complex biological compounds has been made by Meuzelaar et al.[57] A recent review of analytical pyrolysis in clinical and pharmaceutical microbiology has been made by Wieten, Meuzelaar and Haverkamp.[42] The use of the advanced statistical analysis programs[42] for mass spectral data analysis has the potential of making Py-MS a general method for biomaterial analysis.

However, all the above methods require the collection of a sample from air and its preparation in a suitable form before analysis. Methods of sampling airborne bacteria are in general the same as those used for any other particulate matter (eg, pollution aerosols). Commonly used methods of collection include impaction on solid surfaces (single- or multiple-stage impactors), impingement in liquids, and filteration.[58] The drawbacks of these sampling methods are that some preanalysis time is required and that the collection of all particles without any discrimination may introduce background noise in the analytical procedure. Since particles other than suspected pathogens are, in most environments, numerous in air, a desirable way to analyze the airborne microbial particles in the midst of a host of other particles would be to work on individual particles. This would provide a major improvement in the signal-to-noise ratio for the bioparticle analysis. In some circumstances, for example in biological warfare situations, the speed of analysis is critically important. Continuous, real-time monitoring of air samples for the presence of micoorganisms would, importantly, eliminate sample collection and preparation phases. Such a method has been reported by Sinha et al.[14] Known as particle analysis by mass spectrometry (PAMS), the technique seems to hold promise for detecting, quantifying, and

identifying airborne microorganisms.

III. Particle Analysis by Mass Spectrometry (PAMS)

PAMS may be classified as a Py-MS technique with unique features: the direct introduction of aerosol particles in the form of a beam into the ion source of the mass spectrometer; the volatilization and ionization of one particle at a time under the optimum conditions for pyrolysis; and the mass spectral measurement of individual particles. These features enable the analysis of single particles on a continuous, real-time basis.

The first direct introduction of airborne particles into a mass spectrometer was made by Meyers and Fite.[59] The work was extended by Davis[60] to analyze mainly the elemental composition of inorganic components of aerosol particles. Thermal ionization was used for the generation of ions, a method sensitive to elements with low ionization potential. Some organic amines could also be ionized by surface ionization.[61] However, no systematic study of the introduction of particles into the source was made, and the technique was limited to substances with low ionization potential. A more detailed study of the introduction of particles into the ion source of a mass spectrometer along with the different method of ionization has since been made by Sinha et al.[14] and Estes et al.[62] The method of electron-impact ionization is used, which makes the technique generally applicabile. Preliminary studies on laserinduced volatilization and ionization of the particles in the beam have also been made.[63,64]

A. Particle Beams

Particle beams, analogous to molecular beams, are produced when an aerosol expands through a nozzle into a vacuum. These beams are highly directed and forward peaked and provide a well-controlled means of sample

introduction into the mass spectrometer. Particle beams were first described by Murphy and Sears[62] and later studied in detail by Israel and Friedlander[63] and by Dahneke and Friedlander[64] for their physical characteristics. The properties and applications of particle beams have been reviewed recently by Dahneke.[65]

The main components of the PAMS system are the particle beam generator and a quadrupole mass spectrometer housed in differentially pumped chambers (Fig. 1). A beam of particles is produced by an aerosol expanding into a vacuum through the generator that consists of a capillary (100 μm in diameter and 5 mm in length) and a set of two skimmers with orifice diameters of 350 μm and 500 μm. The particles, being much heavier than the gas molecules, remain concentrated along the central axis. The skimmers allow an efficient transmission of the particles into the mass spectrometer chamber and provide for differential pumping of most of the carrier gas upstream. (The details of the PAMS system have been described elsewhere.[14,69]) Since the particles are massive compared to the gas molecules, they tend to be lost along the sample delivery line and physical losses occur on the surface because of settling, convective flow, or diffusion.[70] The predominance of any one of these mechanisms depends upon the particle size and the flow velocity.

The particle delivery system should be designed to minimize the line loss of the particles, and it is important to experimentally characterize its transmission efficiency for particles of different sizes. For particles of diameters greater than 0.5 μm, the gravitational settling has the largest contribution to the particle loss.[71] This loss was eliminated in the PAMS system by directing the beam vertically downward. The transmission efficiency -- ratio of the number of particles in a given volume of the aerosol to the number of particles present in the beam generated from

that volume -- was determined by making beams of various monodisperse aerosols. This determination was made by measuring the number density of particles and the volume flow rate of the aerosol at the input of the beam tube and by measuring the number of particles entering the mass spectrometer chamber in a given time interval. The physical properties of particle beams under experimental conditions similar to those for PAMS have also been made by Estes et al.[62]; the transmission efficiency measured is shown in Fig. 2.

The other important parameter of a particle beam is its divergence. The angle of divergence could be measured by collecting the particles from the beam on a glass slide coated with either Vaseline or Apiezon grease. Measuring the deposit size and the distance of the glass slide from the capillary provides the angle of divergence. Such measurements need to be made in order to ensure that the aerosol sample is not significantly modified in the course of its delivery to the mass spectrometer. Estes et al.[62] have made such measurements for various parameters of the beam generator and for various particle sizes. They found that the particle beam generator has high transmission efficiencies (80%) for particles in the size range of 0.3 to 5 μm and that the divergence of the beam does not change drastically between particle diameters in the range of 1 to 5 μm. However, particles of smaller sizes are better focused along the beam axis. The transmission efficiencies of the beam generator and the beam divergence are particularly important when the beam is generated from a polydisperse aerosol sample (natural aerosol) and only a part of the cross section of the beam is analyzed.

B. Volatilization and Ionization of the Particles

The particle beam enters the mass spectrometer chamber through the

skimmers and impinges on a V-shaped zone refined rhenium fialment (Fig. 3) located between the grid and the repeller of the ion source of the quadrupole mass spectrometer (Uthe Technology, Inc., Model 100C). The filament is resistively heated and maintained at a constant temperature in the range of 200-1400° C. A particle striking the filament produces a plume of vapor molecules; these are ionized by electron impaction in an in situ fashion. Volatilization and ionization of each particle is a discrete event in time and results in a burst of ions for each particle, as shown in Fig. 4. The ion-pulse width has been found to be ~100 to 200 μs. The brevity of the pulse does not allow for the scanning of the mass spectrometer for different mass ion measurements.

C. Data Acquisition

A quadrupole mass spectrometer measures the intensities of different masses by scanning them in time. Since the available scan speed is too slow for a complete mass analysis of a single ion pulse, only one mass peak is monitored from each particle. The mass range is scanned manually, and the intensity measurement is made wherever the signal is observed. The scheme for average intensity measurement is shown in Fig. 5. Measuring the average intensity of a mass peak is particularly important when the input aerosol is polydispersed. The ion pulse at a particular mass is fed into a charge integrator that integrates the signal pulse for a preset time interval. Immediately after this, the background noise is integrated for the same length of time and subtracted from the stored signal. The distribution of the peak voltages of the integrated signal pulses is then measured with a pulse-height analyzer. The distribution is sufficiently narrow for the monodisperse aerosol and shows the reproducibility of the volatization

process. The peak of the distribution measures the average intensity of tne particular mass peak. For a polydisperse aerosol, the intensities in various channels of a distribution are weighted by their respective number of particles. The sum of the number weighted intensities is then divided by the total number of particles to obtain their weighted average intensity. At a selected mass, ~1000 pulses are processed to obtain a distribution of single intensities. The mass spectrum is a collection of the average intensities of individually measured mass peaks and represents the spectrum of an average individual particle. One such spectrum of 1.7 μm diameter potassium biphthalate aerosol is shown in Fig. 6.

IV. Application of the PAMS Technique

The PAMS technique has been applied to the mass spectral measurements of a variety of aerosols. These consist of particles composed of chemical species present in the environmental pollution aerosols[14] (eg, ammonium salts, dibasic organic acids, lead salts, flyash[72]), aerosols generated from organic compounds[73] (which constitute the biological macromolecules), and aerosols of microorganisms.[69] The aerosols are generated by various techniques in the laboratory. For compounds which are soluble in water or alcohol, a vibrating orifice aerosol generator[74] is used. This consists of forcing a dilute solution of the compound through an orifice (10 to 20 μm diameter) in a plate; the resulting liquid jet is broken by vibrating the plate in contact with a piezoelectric crystal at a fixed frequency. This generates droplets of uniform size that, after drying in a stream of air or nitrogen, produce a highly monodispersed aerosol. The particle size can be changed by properly adjusting the concentration of the solution, its feed

rate, and the frequency of vibration. A nebulizer was used for generating aerosols of bacteria particles, and for the resuspension of flyash, a fluidized bed aerosol generator was used.

The quantitative nature of the PAMS technique has been investigated by using monodisperse aerosols. Beams of particles composed of different chemical species were made, and the intensities of their characteristic masses were measured for various size particles; Fig. 7 summarizes the results. The mass spectrometer signal increases linearly with the increase in the particle volume, which indicates a complete vaporization of the particle in the V-type filament. Since this observation was based on the measurements of particles in a rather narrow size range (0.9-2.1 μm), the investigation needs to be extended to other particle sizes and to aerosols composed of different chemical species. However, it is reasonable to expect from the nature of the compounds studied that bacteria particles of 0.9 to 2.1 μm will be completely volatilized within the filament.

V. Bacterial Particle Analysis

In applying the PAMS technique to the analysis of biological particles, mass spectrometric measurements of different bacterial particles have been made. The common bacteria Pseudomonas putida and spores of Bacillus subtilis and Bacillus cereus have been analyzed. These studies demonstrate the feasibility of analyzing individual bioparticles on a continuous, real-time basis. The preparation of aerosol samples, their introduction into the mass spectrometer, and the results obtained from them are summarized below.

A. Sample Preparation

A culture medium containing tryptone, yeast extract, glucose, and sodium

chloride was used for the growth of bacteria. The pH of the medium was initially adjusted to 7.8. Pseudomonas putida (ATCC 29607) bacteria were grown in liquid suspension at room temperature for 40 h under continuous agitation. Samples of Bacillus cereus 203A and Bacillus subtillis 168 were obtained from the UCLA Biology Department and were grown in liquid suspension for 48 h at 34° C. Sporulation was completed by placing the culture in a 70° C water bath for 30 min. The culture was harvested by repeated washing with water, and the water suspension of bacteria was refrigerated until needed.

It was observed that the aerosol generated from an aqueous medium generally results in a lower transmission of particles through the beam generator. The transmission efficiency is significantly increased if a volatile solvent (eg, alcohol) is used in place of water. The incomplete drying of water droplets containing particles seems responsible for the reduced transmission efficiency. In order to overcome this problem, bacterial suspensions in ethanol were prepared by successively washing the sample with a series of water-ethanol solutions of increasing ethanol concentrations. The final suspension in pure ethanol was used for aerosolization. Larger aggregates of cells, formed during the washing by centrifugation, were removed by filtering the suspension through a 20 μm mesh screen.

B. Aerosolization and Beam Generation

The alcohol suspension of bacteria was aerosolized in a micronebulizer in a stream of nitrogen gas. Since the droplets acquire charge during their generation, they were passed through a cylinder containing Kr^{85} where the charge was neutralized by capturing ions of gas molecules. The aerosol was finally dired by passing it though a silica gel diffusion drier (Fig. 1). The particle concentration in the aerosol was about 70 cm^{-3} as was determined with an optical particle counter. The electron micrographs in

Fig. 8 show that the bacterial aerosols are free from extraneous particles and debris and that the bacteria remain intact after aerosolization. The micrographs also indicate that the aerosol particles are predominantly single spores, 20% occurring as doublets.

After drying, the aerosol stream passes into the sampling chamber. A portion of the aerosol (50 atm cm^3 min^{-1}) is isokinetically sampled into the beam tube, which, after expansion through the capillary nozzle, produces a beam of bacteria particles. The particles having higher inertia than the gas molecules form a tightly focused beam and are led into the mass spectrometer chamber through the skimmers whereas most of the carrier gas is pumped off in this region. In order to characterize the physical state of the bacteria in the beam, the particles were collected on a glass slide in the mass spectrometer chamber. Particles in the beam attain high terminal velocity (~300 ms^{-1}),[63,64] which necessitates the coating of glass slide with Apiezon grease in order to avoid their bouncing off the glass slide. A photograph of the composite of the particle deposit under low magnification is shown in Fig. 9. The beam has a divergence of 1.6×10^{-3} sr. Light and electron micrographs of the particle beam deposit are also presented in Fig. 10. It can be seen that Apiezon grease is not a suitable substrate for electorn microscopy. However, the rod shape and size of the spores are clearly discernible, showing that the spores retain their integrity during the beam generation and particle collection processes. This is the first time that a beam of cells has been made in vacuum and directly introduced into the mass spectrometer. The generation of the beam is not limited to spores possessing hardy characteristics; Pseudomonas putida is a non-spore-forming bacteria.

C. Mass Spectral Measurments

The bacteria particles in the beam strike the hot V-type rhenium filament (740° C) and are pyrolyzed into small mass fragments. A burst of ions is produced from individual particles after volatilization and ionization by electron impaction (~40 eV in energy) in the ion source of the mass spectrometer. The pulse width of 100 to 200 μs necessitates the monitoring of one mass peak from each particle that is manually set at the mass spectrometer control unit. The average intensity of a mass peak is obtained from the pulse-height distribution of about a thousand pulses from different particles. Signals were observed throughout the mass range of 30-300 u. A set of 30 mass peaks was selected and their intensities measured for all three species (Fig. 11).

The intensities of the different mass peaks are normalized to the most intense peaks in their respective spectrum. A striking similarity between the spectra can be seen, which is not surprising since the microorganisms have essentially the same major chemical building blocks; however, some visual differences can be seen in them. The mass peaks between 192 and 275 u were weak for Pseudonmonas putida and Bacillus subtilis, and a good intensity measurement of these peaks could not be made. The spectra may be distinguished by considering the relative intensitities of different peaks. For example, the relative intensities of mass peaks at 67, 114, 169, and 282 compared to the intensity at 149 are higher in the spectrum from Bacillus cereus than those in the spectrum from Pseudomonas putida. Another difference between the P. putida and the Bacillus samples lies in the presence of m/z 134 peak in the Bacillus samples. A more objective comparison of mass spectra and their reproducibility can be made by applying statistical procedures[75].

One such preliminary analysis of the mass spectra based on the criterion of the degree of correspondence[34] has been made. The correspondence between P. putida and the Bacillus samples is significantly low. A value of .64 for the degree of correspondence between P. putida and a reference spectrum of B. cereus shows that the two spectra can be easily differentiated. However, a variability of ~25% is calculated for the replicate samples of B. cereus. It is believed that the polydisperse nature of the aerosol and variability introduced by the signal possessing, as well as some variability in the samples themselves, may be the contributing factors. The details of the analysis are given by Platz.[76]

D. Comparison with Other Pyrolysis Methods

The choice of Pseudomonas putida, Bacillus subtilis, and Bacillus cereus for study with the PAMS technique was made mainly because Py-MS data on them was available in the literature. Schulten et al.[77] made measurements on Pseudomonas putida. In their work, a continuous-mode pyrolyzer was used. A sample of 5 mg of freeze-dried bacteria was pyrolyzed in a flask for 2 min at 500° C and the pyrozylate was mass analyzed by field-ionization mass spectrometry. Field ionization, a softer method of ionization, produces predominantly molecular ions during ionization with minimal fragmentation. The spectrum so obtained, however, consists mainly of mass peaks of m/z less than 150. Boon et al.[78] have made mass spectral measurements of Bacillus subtilis. Their sample was volatilized in a Curie-point pyrolyzer. The pyrolyzate effused from the pyrolyzer through an orifice into the quadrupole mass spectrometer where it was ionized with electrons of low energy (14 eV) and the spectrum was accumulated by repeated scanning of masses for approximately 10 s. Here also the most intense peaks lay in the low mass

range (40-60) and were confined to m/z of less than 120. The fragmentation to low masses seems to result from the pyrolysis condition. Some fragmentation may also be possible during the collisions in the vapor phase. It must be added that the equilibrium temperature in the Curie-point pyrolyzer is attained very fast, which assures a reproducible uniform-temperature pyrolysis condition. Very encouraging results in the detection and identification of microorganisms have been obtained by this method.

In contrast to the above results, abundant molecular fragments are produced at high masses in the PAMS methods even though the ionization is made by the impaction of electrons of higher energy (40 eV) and the transmission of ions through the quadrupole analyzer decreases with increasing mass. The larger mass fragments carry more information about their parents and consequently should help in analysis. It is believed that the primary reason for high mass peaks is the pyrolysis process: Each cell is separatley volatilized, and many of the desirable conditions for pyrolysis stated in Section II.C are fulfilled. Individual particle pyrolysis is fast, with an estimated duration of less than 50 μs. A cell particle 0.5 μm in diameter and 1.5 μm in length is expected, after volatilization and fragmentation, to produce a pressure of less than 10^{-6} torr in an ionization volume of 1 cm^3, so the vapor molecules are essentially in a collision-free condition in the ion source, eliminating any secondary reactions in the vapor phase. The pyrolyzing filament is situated within the ionizer and, therefore, minimizes the transit time between the pyrolyzer and the ion source. Mass peaks of various monodisperse organic and inorganic aerosols have been found to have a relative standard deviation of 10% in their intensities, showing the reproduciblity of the pyrolysis method.[14]

VI. Conclusions

PAMS has the following advantages:

(1) Aerosol particles can be directly introduced into the ion source of a mass spectrometer in the form of a beam, thus eliminating the need of sample collection and preparation.

(2) The bacteria particles remain intact during the process of beam generation.

(3) Individual particles can be volatilized within a V-type filament.

(4) The pyrolysis provides fast heating, uniform pyrolysis temperature, and promixity of the pyrolyzer to the ion source, and it assures that one extremely small sample at a time is pyrolyzed in a collision-free environment.

(5) Mass spectra of individual biological particles can be obtained on a continuous, real-time basis.

(6) The mass spectra contain peaks at high masses which can provide more information about their parents.

The results obtained on P. putida, B. subtilis, and B. cereus demonstrate the potential of the PAMS technique for the detecting and possibly identifying biological particles. The preliminary nature of the work should be emphasized, however; more work on different biological samples needs to be done. The advanced data analysis technique of chemical pattern recognition developed by various workers[42] should be incorporated in the technique.

It is encouraging to note that other pyrolysis-mass spectrometric methods for the analysis of biological material have found considerable success. In view of this and the advantages of the PAMS technique, it is safe to conclude that the PAMS method holds promise for biological particle analysis.

The real-time analytical capability of the method makes it uniquely suitable for monitoring a protected environment (eg, a clean room) or a general field environment.

VII. Future Development

One of the limitations of the PAMS method arises from the mode of its data acquisition. One mass peak is measured per particle. In order to obtain the average intensity of a mass peak from a polydisperse aerosol, a large number of particles must be measured, and the process has to be repeated for all the mass peaks. This entails a long analysis time, particularly for the ambient air sample where the number density of biological particles is expected to be low. This limitation is not inherent in the PAMS technique but in the use of a quadrupole mass spectrometer. A quadrupole mass spectrometer measures the intensities of different masses by scanning them in time and is too slow for a complete scan of an ion pulse from a particle. Moreover, a scanning instrument is, in general, less sensitive than a nonscanning one because in the scanning mode, a particular signal is measured for only a small part of its total time of generation. However, in a restricted environment where not much interference exists, one characteristic mass peak, possibily in high m/z range, could be selected for the particle, and a quadrupole instrument could still be used for the detection. The problem associated with the use of a quadrupole mass spectrometer could be overcome by replacing it with a focal-plane mass spectrograph (nonscanning); then all the ions of different masses arising from a single particle could be measured simultaneously.

A miniaturized mass spectrograph (Mattauch-Herzog type) with an electronic detector known as electro-optical ion detector[79] covering the entire focal plane has been developed and built at the Jet Propulsion Laboratory. Figure 12 shows a photograph of this instrument. It has a 5-in. long focal plane and covers a mass range of 28 to 500 u. This instrument has demonstrated sensitivity at the femtogram (10^{-15} g) level, which, for a unit-density material, is equivalent to a spherical particle of 0.1 μm in diameter. The mass spectograph with the particle beam generator is being assembled. This system will provide a complete mass spectrum from individual particles. A computerized pattern-recognition technique of analysis will help differentiate the mass spectra of different microorganisms.

Since lasers have been found to provide a controllable and efficient method of ionization,[80] they may help generate characteristic mass spectra (fingerprints) of different particles. Preliminary results on the application of laser-induced volatilization and ionization of individual particles in the beam on the PAMS system have been obtained. A beam of KBP particles was generated, and individual particles in the beam were hit by high energy Nd-YAG laser pulses for their volatilization and ionization.[64] Mass spectral measurements show the production of ions at masses 39, 28, and 23. The signal at m/z 39 originates from K^+, and its combined efficiency is found to be $\sim 10^{-6}$. Signals at mass 28 and 23 were weaker in intensity. The former is believed to arise due to mass fragments CO from the carboxylic groups and the latter from sodium present as an impurity. The lower ionization efficiency and the absence of other mass signals from the organic part of KBP molecules are attributed to the low laser power density ($\sim 2 \times 10^5$ W/cm^2) as well as to the incomplete vaporization of the particle.

Work is continuing for the study of laser-induced ionization with increased power density on the particles while in the beam.

A library of mass spectral data of the different microorganisms should be compiled for identification by comparision. It is expected that biological particles in air may be mixed with nutrients, attached to dust particles, or mixed with other substances deliberately to obscure their identity; sometimes more than one kind of particles might be mixed together. Such interferences should also be studied.

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Figure Captions

Fig. 1. Schematic of PAMS systems. Reproduced with permission.[14]

Fig. 2. Overall particle transmission efficiency as a function of capillary-skimmer separation (Δz) and particle aerodynamic diameter (d_a) where $d_a = \sqrt{\rho} \cdot d_g$; d_g, the geometric particle diameter; and ρ, the density of the particle. For larger Δz, the skimmer opening becomes the limiting aperture that decreases the transmission. In the case of PAMS, Δz = 3 mm. Reproduced with permission,[62] after Estes.

Fig. 3. Design of Re V-type filament, $\theta \approx 10°$. The cylindrical elements support the filament and carry the heating current. Reproduced with permission.[14]

Fig. 4. Oscilloscope traces of: (a) the pulses showing the burst of ions produced from the volatilizing and ionizing of individual dioctyl phthalate particles of 1 μm diameter; (b) the fast scan of a single pulse. The horizontal scale is 100 ms per division for (a) and 20 μs for (b). Mass peak at 149 u is monitored. Reproduced with permission.[14]

Fig. 5. Average intensity measurement scheme for a mass peak.

Fig. 6. Mass spectrum obtained from potassium biphthalate particles of 1.7 μm diameter.

Fig. 7. Variation of the mass spectrometer (MS) signal with the particle volume; ▽ denotes mass peak at 86 from glutaric acid; ○ denotes mass peak 55 from from adipic acid; □ denotes mass peak 80 from ammonium sulfate; △ denotes mass peak 149 from dioctyl phthalate. Reproduced with permission.[14]

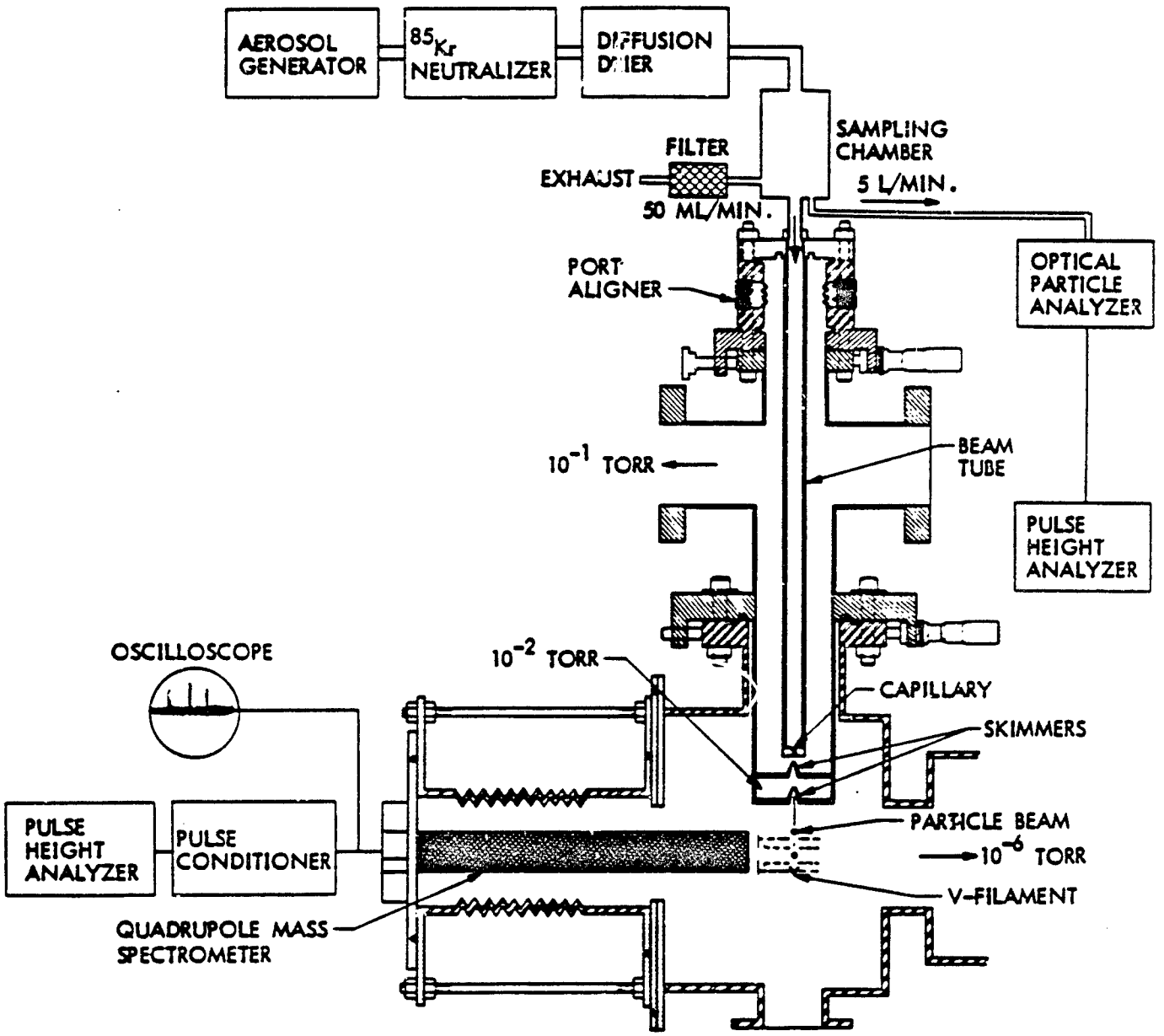
Fig. 8. Electron micrographs of Bacillus subtilis: (a) and (b) show the cells taken directly from their suspension whereas (c) and (d) are the cells collected from their aerosols after the diffusion drier.

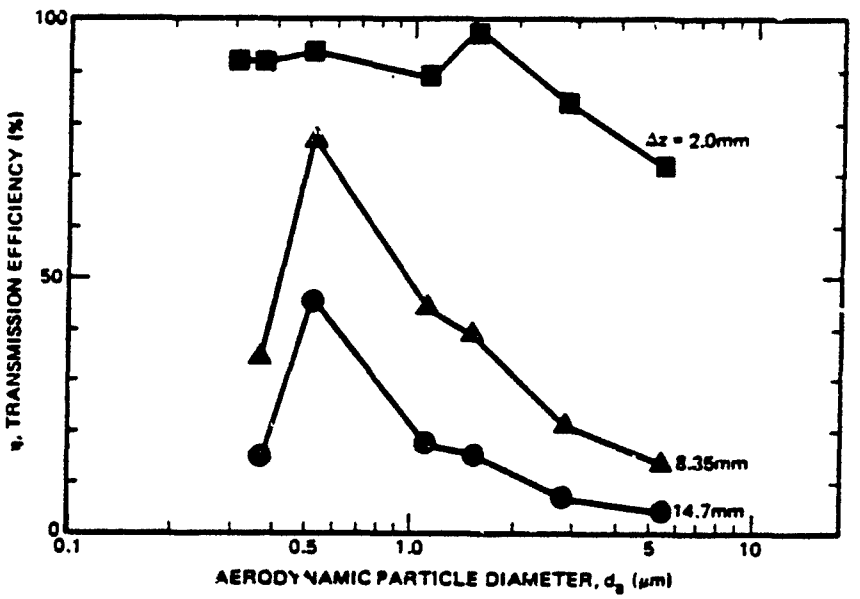
Fig. 9. Deposits of Bacillus subtilis particles from a beam on a glass slide coated with Apezion grease in the mass spectrometer chamber.

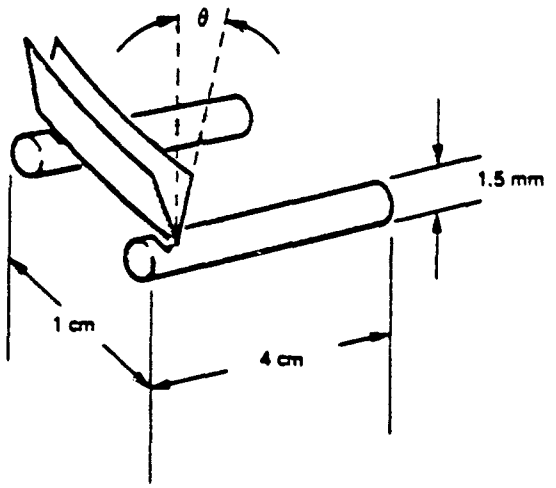
Fig. 10. Microscope photograph and electron micrograph of Bacillus subtilis from beams collected on Apezion grease-coated slides: (a) light microscope photograph; (b) electron micrograph.

Fig. 11. Mass spectra of bacteria particles: (a) Pseudomonas putida; (b) Bacillus cereus; (c) Bacillus subtilis. Reproduced with permission.[69]

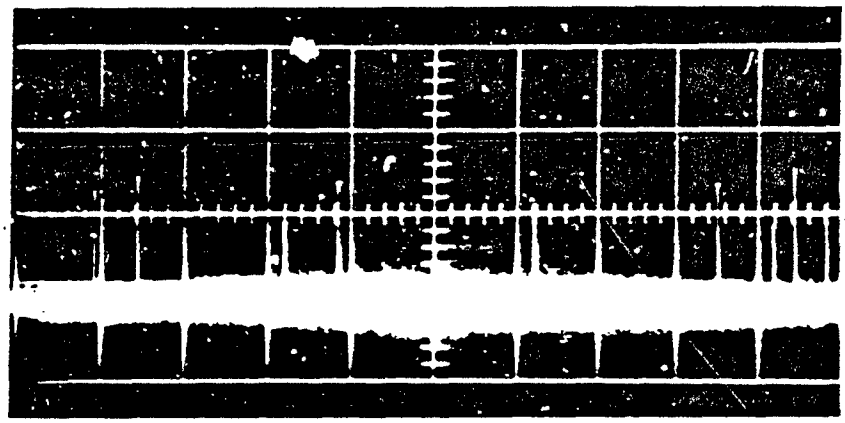
Fig. 12. Photograph of a miniaturized mass spectrograph with the electro-optical ion detector. Courtesy of C. E. Giffin.


AEROSOL GENERATOR
^{85}Kr NEUTRALIZER
DIFFUSION DRIER
SAMPLING CHAMBER
5 L/MIN.
FILTER
EXHAUST
50 ML/MIN.
PORT ALIGNER
OPTICAL PARTICLE ANALYZER
10^{-1} TORR
BEAM TUBE
PULSE HEIGHT ANALYZER
OSCILLOSCOPE
10^{-2} TORR
CAPILLARY
SKIMMERS
PULSE HEIGHT ANALYZER
PULSE CONDITIONER
PARTICLE BEAM
10^{-6} TORR
V-FILAMENT
QUADRUPOLE MASS SPECTROMETER

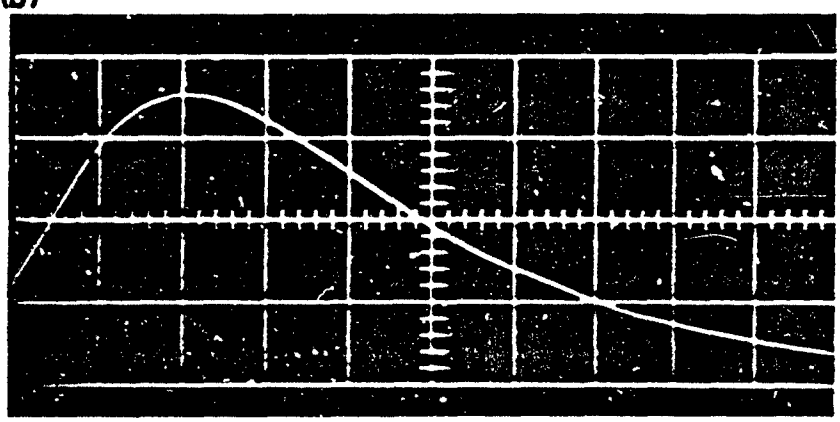

100
50
0
0.1
0.5
1.0
5
10
η, TRANSMISSION EFFICIENCY (%)
AERODYNAMIC PARTICLE DIAMETER, d_a (μm)
Δz = 2.0mm
8.35mm
14.7mm

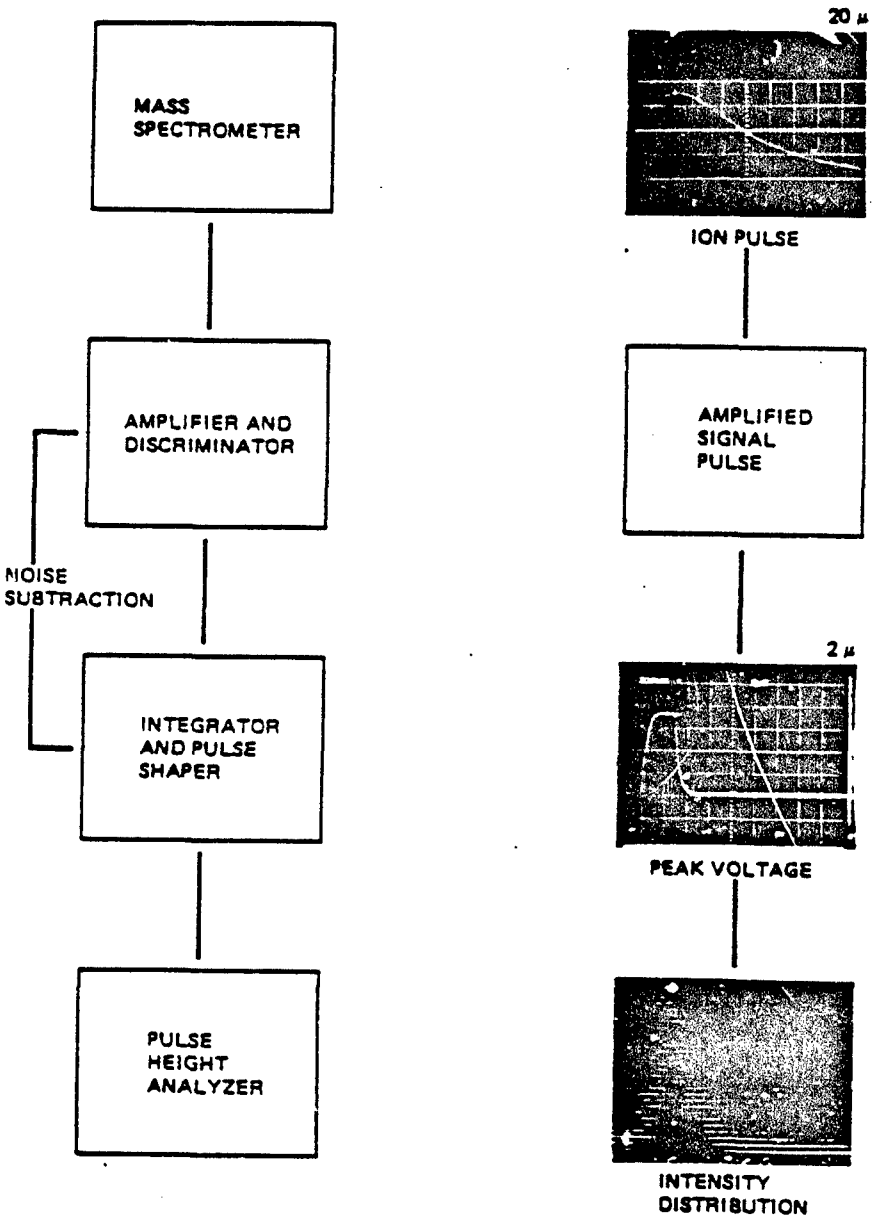

θ
1.5 mm
1 cm
4 cm

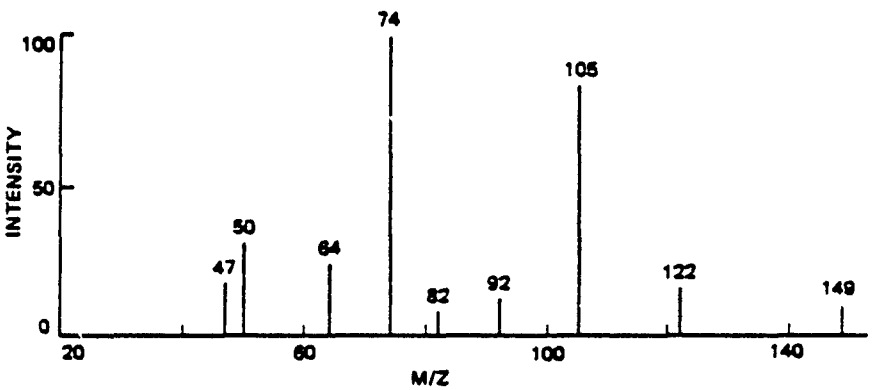
(a)

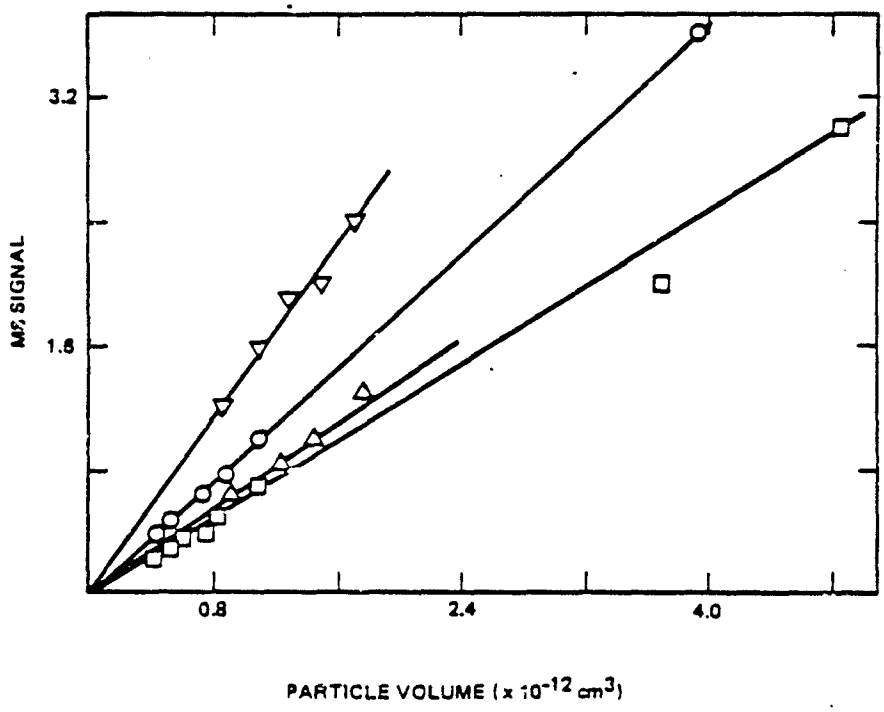


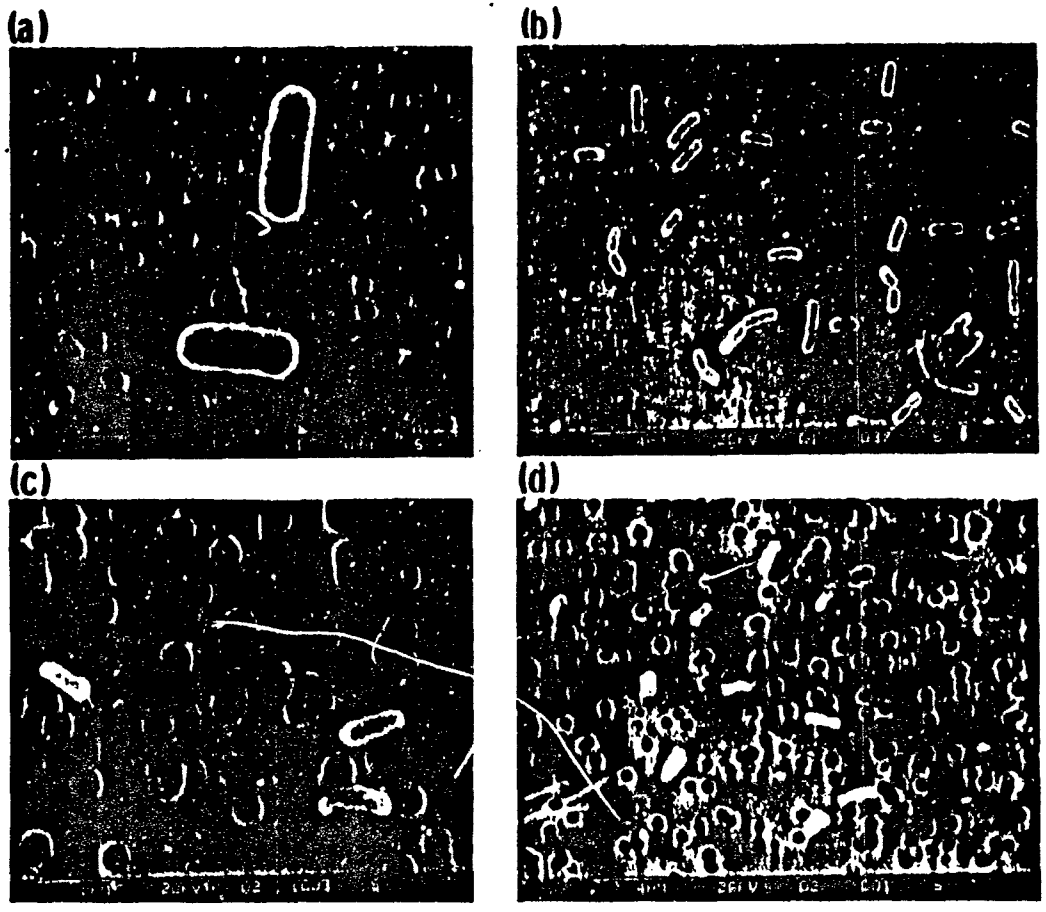
(b)

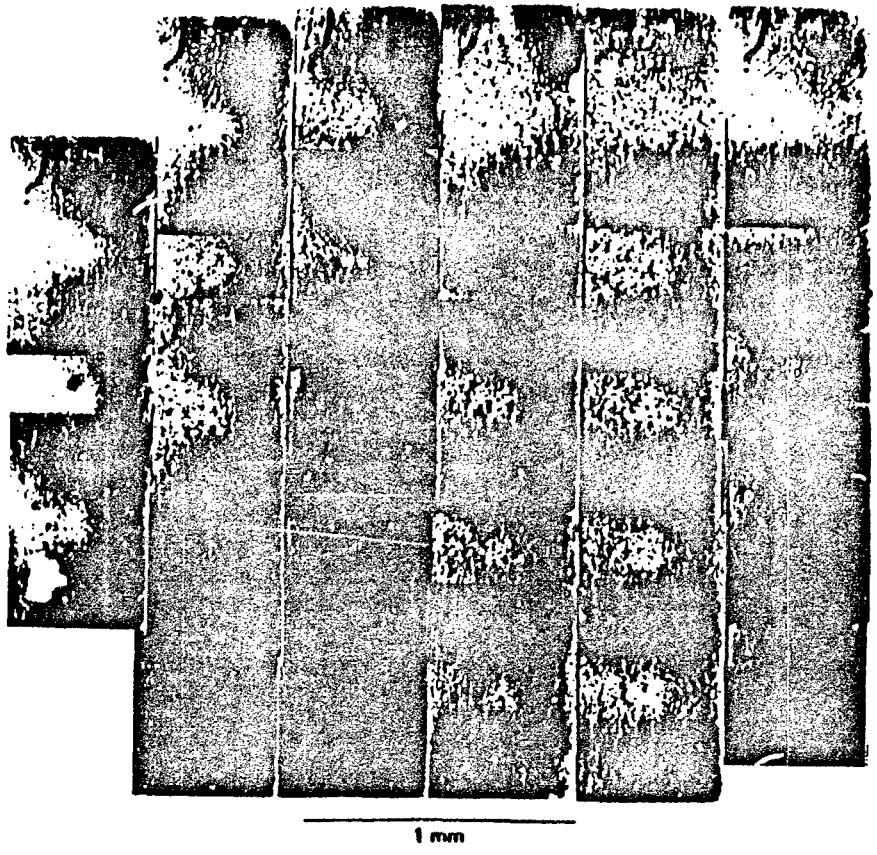



MASS SPECTROMETER
AMPLIFIER AND DISCRIMINATOR
NOISE SUBTRACTION
INTEGRATOR AND PULSE SHAPER
PULSE HEIGHT ANALYZER
20 μ
ION PULSE
AMPLIFIED SIGNAL PULSE
2 μ
PEAK VOLTAGE
INTENSITY DISTRIBUTION

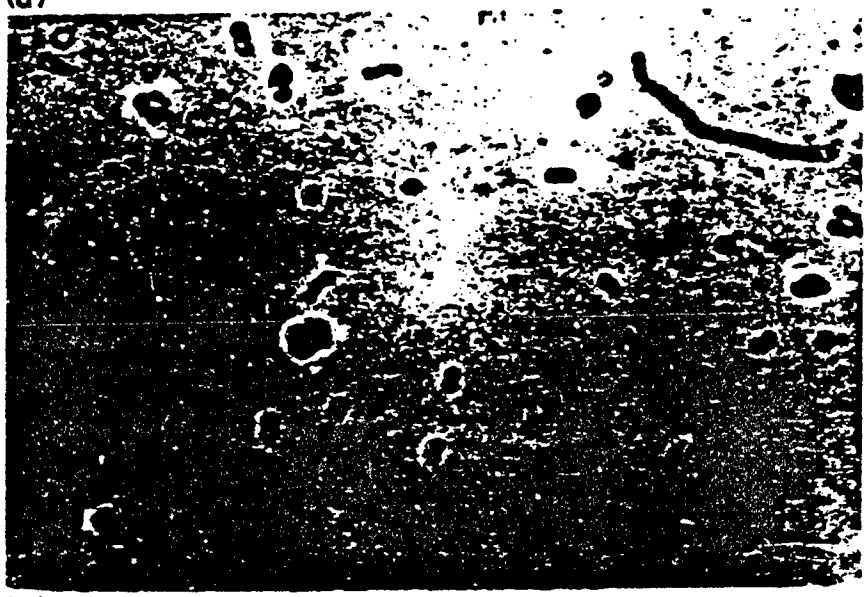

100
50
0
INTENSITY
74
105
50
64
47
82
92
122
149
20
60
100
140
M/Z


3.2
1.6
MF SIGNAL
0.8
2.4
4.0
PARTICLE VOLUME (x 10⁻¹² cm³)

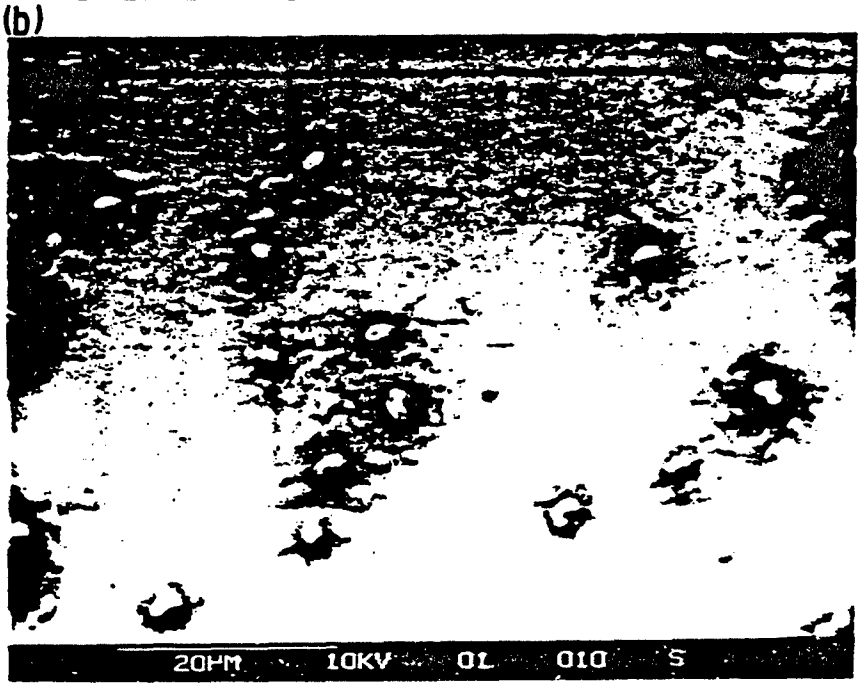

(a)
(b)
(c)
(d)

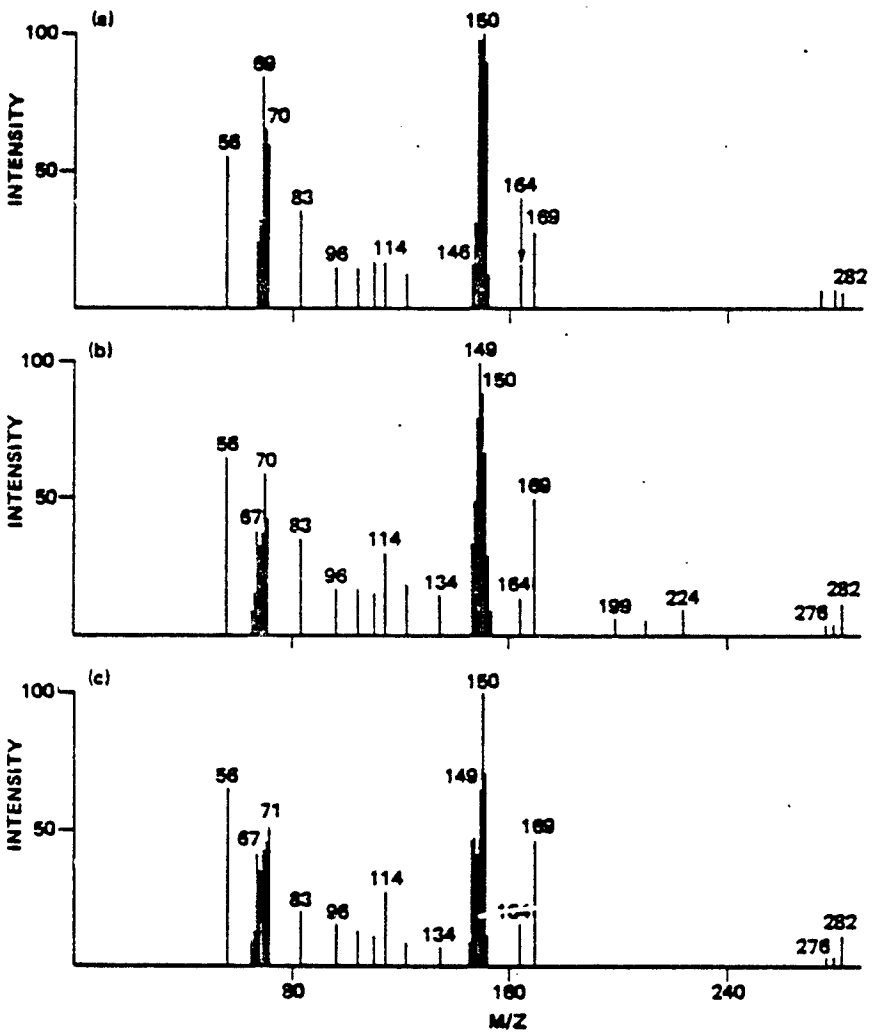

1 mm

(a)



(b)




(a)
100
50
INTENSITY
150
69
70
56
83
164
169
96
114
146
282
(b)
149
150
56
70
169
67
83
114
96
134
164
199
224
282
276
(c)
150
56
149
71
67
169
114
83
96
134
282
276
80
160
240
M/Z