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Electrospray-Assisted Ultraviolet Aerodynamic Particle Sizer Spectrometer for Real-time Characterization of Bacterial Particles

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The ultraviolet aerodynamic particle sizer (UVAPS) spectrometer is a novel, commercially available aerosol counter for real-time, continuous monitoring of viable bioaerosols based on the fluorescence induced from living microorganisms. For aerosolization of liquid-based microorganisms, general aerosolization methods such as atomization or nebulization may not be adequate for an accurate and quantitative characterization of the microorganisms because of the formation of agglomerated particles. In such cases, biological electrospray techniques have an advantage because they generate nonagglomerated particles, attributable to the repulsive electrical forces among particles with unipolar charges. Biological electrosprays are quickly gaining potential for the detection and control of living organisms in applications ranging from mass spectrometry to developmental microbiology. In this study, we investigated the size distribution, total concentration, and fluorescence percentage of bacterial particles in a real-time manner by electrospray-assisted UVAPS. A suspension containing *Escherichia coli* as a test microorganism was sprayed in a steady cone-jet mode using a specially designed electrospray system with a point-to-orifice-plate configuration based on charge-reduced electrospray size spectrometry. With the electrospray process, 98% of the total *E. coli* particle number concentration had a size of <1 μm and the geometric mean diameter was 0.779 μm, as compared with the respective values of 78% and 0.907 μm after nebulization. The fractions of fluorescence responsive particles and of particles that contained viable organisms in culture were 12% and 7%, respectively, from the electrospray process and 34% and 24% from nebulization. These results demonstrate that (1) the presence of agglomerated particles can lead to markedly overestimated fluorescence and culturability percentages compared with the values obtained from nonagglomerated particles, and (2) electrospray-assisted UVAPS can provide more accurate and quantitative real-time characterization of liquid-based microorganisms, owing to the generation of nonagglomerated particles.

Novel real-time methods for the rapid detection of airborne or waterborne microorganisms are promising techniques for addressing the public health problems and other biological and environmental effects associated with these microorganisms.[1−4] In situations of risk exposure, the involved biological particles must be detected immediately to allow for appropriate control methods. Delays in identification or enumeration of biological particles may have critical consequences for human health and the environment.[5]

Conventional methods for detecting biological particles involve two stages, sample collection and laboratory analysis. These methods are highly labor intensive and time-consuming. This often leads to a limitation of the number of samples, for practical reasons, and in low accuracy in the rate of detection.[6−8] A near real-time technique for the rapid detection of biological particles should eliminate the time-consuming processes in sampling and particle characterization. Where continuous monitoring of biological particles implies fast repetition of characterization, the stream of information may also yield more accurate results than those obtained by conventional sampling and analysis.

In recent years, there has been considerable interest in the development of methods for nearly real-time continuous detection and identification of biological particles. These methods can be broadly subdivided into three types: (1) single particle Raman spectrometry,[9–12] (2) single particle mass spectrometry,[13–17] and (3) fluorescence spectrum analysis.[18–26]

The ultraviolet aerodynamic particle sizer (UVAPS; model 3314; TSI Inc., St. Paul, MN) is an airborne particle counter and

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spectrometer capable of measuring the fluorescence emitted by living microorganisms. This instrument measures aerodynamic size, number concentration, and fluorescence of airborne particles having a size range of 0.5−15 μm. The fluorescence that is produced by exciting the particles at a wavelength of 355 nm using a pulsed UV laser beam is detected between 420 and 575 nm.[27] Under these conditions, the detected fluorescence signals result from molecules produced by all living cells, specifically nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate (NAD(P)H), and riboflavin. Thus, these fluorescence signals are considered specific for living microorganisms.[23,27,28] As NAD(P)H is involved primarily in the energy-producing respiratory process in actively growing cells and in protein biosynthesis,[29] the fluorescence signals from these molecules can be used to distinguish viable particles (i.e., those comprising microorganisms) from nonviable particles. A detailed description of the UVAPS can be found elsewhere.[23,30−34]

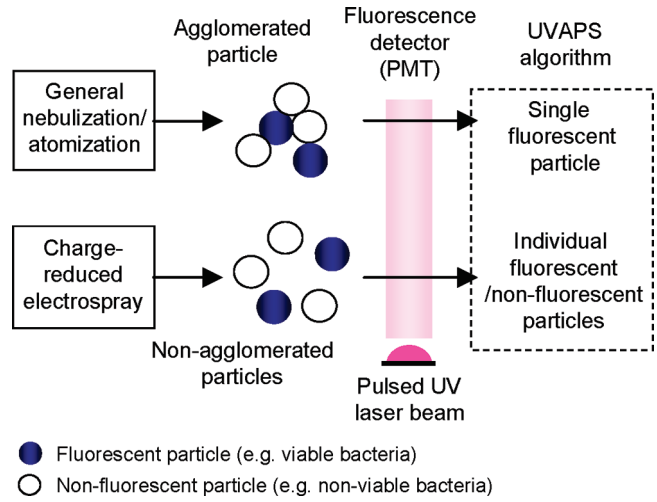

Figure 1. Response algorithm of the UVAPS with respect to particle agglomeration.

The observed fraction of fluorescent particles, as a proportion of the total particle population, can indicate the concentration of viable microorganisms in the environment being studied. Although the UVAPS cannot be used to identify biological particles at the genus and species level, it can be beneficial in characterizing viable microorganisms from various environments for purposes such as the control of infectious bacterial and fungal diseases, airborne/waterborne microorganism inactivation studies, and bioaerosol monitoring of indoor/outdoor environments.

The feasibility and performance of this instrument for the detection of biological particles have been previously demonstrated by several studies.[23,28,31] Nevertheless, the accurate and quantitative characterization of biological particles, including liquid-based microorganisms, has remained insufficient because the commonly used nebulization process produces agglomerated particles during aerosolization. Nebulization processes, representative of atomization technologies, are widely used to generate droplets from a liquid suspension via pneumatic orifice flow or through ultrasound-assisted atomization.[35−37] However, the sizes of the initial droplets produced by nebulization can be several tens of micrometers, which is larger than most microorganisms.

As shown in Figure 1, the pulsed UV laser beam in the optics of the UVAPS illuminates particles at a wavelength of 355 nm, and the particles excited by the UV laser emit fluorescence at characteristic wavelengths. The emitted fluorescence is detected between 420 and 575 nm by the photomultiplier tube (PMT) located behind a UV-blocking filter. With nebulization, a proportion of the resulting bacteria particles will be agglomerated because of the atomization mechanism even when the bacteria had originally been suspended in the liquid medium as nonagglomerated particles. The degree of agglomeration depends on the initial size of the droplets and the concentration of the biological material. Agglomerated particles composed of both viable (fluorescent) and nonviable bacteria (nonfluorescent) that enter the detection area will be illuminated by the pulsed UV laser and will emit a fluorescence signal that is then recorded as a single fluorescent particle, based on the UVAPS algorithm. Under these

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conditions, the fluorescence and culturability percentages, which are determined relative to the total particle concentration, can be overestimated.

The electrospray technique has become a popular method for creating nonagglomerated and uniform droplets and particles.[38,39] In the electrospray process, a liquid jet breaks into droplets when the local liquid surface tension is overcome by the influence of electrical forces exerted on the charged liquid surface.[40] Depending on the strength of the electric stresses at the liquid surface relative to the surface tension stress and depending on the kinetic energy of the liquid being pumped out of the nozzle, various functional spraying modes can be obtained: microdripping, spindle, cone-jet, and multijet modes.[41,42] The cone-jet mode is preferred because it produces monodisperse droplets in a stable manner.[43−46]

Among the analytical and biological applications of the electrospray technique, charge-reduced electrospray size spectrometry was recently used for the size analysis of macromolecules based on the electrical mobility spectra.[37,47−57] Kaufman and co-workers developed an electrospray size spectrometer for analyzing the size characteristics of biological organic materials, including globular proteins,[58] DNA,[51] protein complexes,[48] and hexagonal bilayer hemoglobin.[50] Biswas and co-workers used the charge-reduced electrospray technique to study various bacteriophages and elucidated the fragmentation phenomena at the virus capsid head−tail noncovalent interface during the electrospray process.[53] Siuzdak and co-workers evaluated whole viruses by electrospray ionization mass spectrometry.[59,60] Jung and co-workers used the charge-reduced electrospray technique to investigate the generation characteristics of nonagglomerated MS2 bacteriophages, compared with the characteristics obtained using a nebulization process.[37] Heuvel and Heck reported the general review about the analysis of noncovalently bound supramolecular structures using the electrospray ionization mass spectrometry.[61] Previous studies on size spectrometry, however, have focused mainly on nanosized biological materials. Although Kim and co-workers generated a bacterial bioaerosol using an electrospray technique, they only measured the size distribution of electrosprayed droplets with approximately 1−20 μm size (not single bacterial particles) using a phase Doppler particle analyzer.[62]

In the presently reported study, we demonstrated an electrospray-assisted UVAPS, of which the design was based on the charge-reduced electrospray size spectrometry system. As a first step toward the real-time characterization of biological particles using an electrospray-assisted UVAPS, pure *Escherichia coli* bacteria samples were tested. We investigated various physical/biological characteristics, including particle size distribution, total particle number concentration, fluorescence percentage, and recovery from culture using an electrospray-assisted UVAPS and conventional culture techniques. We also compared the results obtained with the electrospray to those acquired with pneumatic nebulization.

MATERIALS AND METHODS

Figure 2 illustrates the experimental configuration of the electrospray-assisted UVAPS system. The details of the charge-reduced electrospray system were discussed in our previous paper.[37] The spray chamber is in the point-to-orifice-plate configuration, with the capillary tube facing the orifice plate. The bacterial suspension was injected into a stainless steel (SS) capillary with a fused-silica capillary tip (inner diameter, 0.03 mm; outer diameter, 0.36 mm; SilicaTip; New Objective Inc., Woburn, MA). A positive voltage of a few kilovolts relative to the ground electrode (i.e., the orifice plate) was applied to the capillary. The SS orifice plate (inner diameter of 1 mm; outer diameter, 10 mm) was placed 10 mm below the capillary tip and connected to the ground. The sample suspension with flow rate of 10 μL/h was transported through a Teflon tube by a syringe pump (KDS220; KD Scientific Inc., Holliston, MA) with a 1 mL syringe (Gastight 81320; Hamilton Co., Reno, NV). A SS capillary connected at the end of a Teflon tube was electrified by a high-voltage DC power supply (Korea Switching Co., Seoul, South Korea).

Transparent viewing ports on opposite sides of the spray chamber were designed to monitor the system spray modes. One was for the introduction of light (MLC-150; Motic Instruments Inc., Richmond, BC, Canada), and the other was for observing the shape of the electrospray liquid meniscus using a microscopic system, including a lens (70XL; Thales Optem Inc., Fairport, NY), CCD digital camera (MARIN F-145C2; Allied Vision Technologies Inc., Stadtroda, Germany), and computer for monitoring and recording the spray phenomena.

The CO_2 and air flows were filtered through a HEPA filter and diffusion dryer and then introduced to the system uniformly along the capillary, where they served as a sheath surrounding the electrospray capillary tube to suppress possible corona discharge at the capillary tip.[46] The CO_2 and air flow

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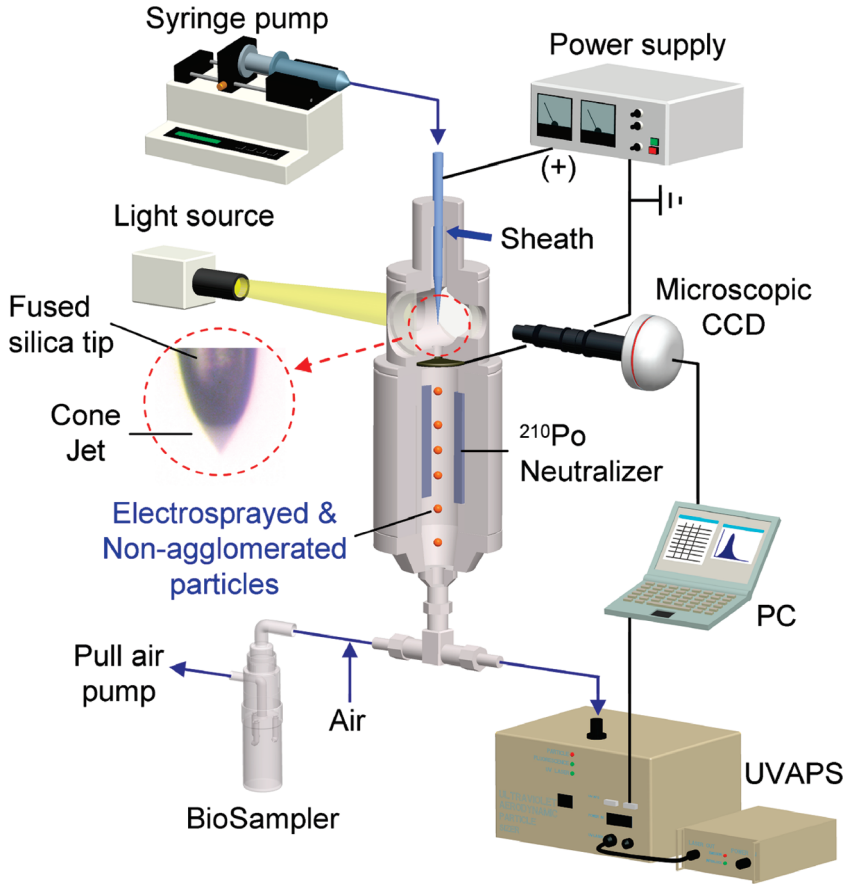

Figure 2. Experimental configuration of the electrospray-assisted UVAPS system. Electrosprayed bacterial particles were sampled by BioSampler and UVAPS in sequence.

rates were 0.25 L/min and 4.75 L/min, respectively, and were controlled by mass flow controllers (MFC; 1179A Mass-Flo; MKS Instruments, Andover, MA). After droplets were produced, they were transported immediately into the neutralization chamber, which space is located directly below the orifice plate. Two ^{210}Po radioactive sources, each with an activity of 500 μCi, were used to reduce the electrical charge on the droplets before they reached the Rayleigh size limit (i.e., particle charge limit).[49,63−65] This prevented Coulombic explosion of the electrosprayed droplets during evaporation of water. The size of the liquid droplets was further reduced by evaporation, and the characteristics of residual particles were measured using a UVAPS.

The UVAPS had a double crest optical system, to provide both aerodynamic and scattered light measurements of airborne particles. The aerodynamic diameter, D_a, of a particle is equivalent to that of a standard density spherical particle with the same gravitational settling velocity.[63,66,67] A pulsed UV laser beam provided fluorescence excitation at a wavelength of 355 nm, and fluorescence emission in the band from 420 to 575 nm was detected by a PMT. The particle aerodynamic diameters are represented in 52 size channels, with the respective concentrations recorded as the number of particles per cubic centimeter (particles/cm^3). The fluorescence emission of each individual particle is registered in one of the 64 channels of the UVAPS, where increasing channel numbers represent gradually increasing fluorescence intensities. Data on nonfluorescing particles are represented in the first channel, and data on fluorescing particles are in channels 2−64. The fluorescence percentage (%) can be calculated as the number ratio of total fluorescent particles (channels 2−64) to total particles (channels 1−64) as in eq 1.[30,68]

$$\text{Fluorescence percentage (\%)} = \frac{\text{Total fluorescent particles (channels 2−64)}}{\text{Total particles (channels 1−64)}} \times 100 \quad (1)$$

The fluorescence sensitivity is influenced by settings of the pulsed UV laser and PMT. At high fluorescence sensitivity, the UVAPS internal fluorescence baseline may include measurements of fluorescence from particles that do not contain a fluorophor. Thus, the fluorescence baseline should be checked occasionally using nonfluorescent test particles such as polystyrene latex (PSL) particles (Duke Scientific Corp., Palo Alto, CA). In the present study, monodisperse PSL particles with diameters of 0.720, 1.110, and 2.010 μm, all with a density of 1.05 g/cm^3, were used at an adjustment of PMT voltage setting of the UVAPS as shown in

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Table 1. Physical Properties of the Water and Test Bacterial Suspensions

sample	electrical conductivity, K, [S m^{-1}]	viscosity, n, [mPa·s]	surface tension, γ, [mN m^{-1}]	density, ρ, [kg m^{-3}]
pure water[a]	2.62×10^{-4}	1	73	994
E. coli suspension[b] (3.0×10^8 CFU/mL)	8.1×10^{-2}	1	74	994

[a] Distilled, deionized, and filtered water. [b] All bacterial suspensions were diluted with 10 mM ammonium acetate solution.

Figure S-1 (Supporting Information). Aliquots of each PSL particle suspension were diluted with sterile, distilled, deionized water, and then, each PSL suspension was electrosprayed in cone-jet mode at a suspension flow rate of 10 μL/h.

We used *Escherichia coli* (ATCC 8739), a Gram-negative bacterium, as a test microorganism. Gram-negative bacteria are commonly found in various environments, and many are pathogenic.[4,69–71] Cultures of *E. coli* were grown in tryptic soy broth (TSB) (Becton Dickinson, NJ) at 37 °C for 18 h and harvested by centrifugation at 5000*g* for 10 min (RC-5B; Sorvall Co., Newtown, CT). The pellets were carefully washed twice with sterile distilled water. Finally, the pellets were resuspended in 10 mM aqueous ammonium acetate (Sigma-Aldrich, St. Louis, MO) for consistent electrospraying.[37] A high-titer stock of bacteria was prepared as 3×10^8 colony-forming units (CFU) per milliliter.[4] Table 1 presents the physical properties of the water and bacterial suspension.

Bacterial culturability, termed recovery from culture, was determined by culturing samples of electrosprayed bacterial suspensions obtained under the various experimental conditions. A BioSampler (SKC Inc., Eighty Four, PA), operated by a pull vacuum pump with a flow meter (Gast IAQ pump; EMS Inc., Charleston, SC), was used for sample collection. Electrosprayed bacterial particles were collected for 10 min at a nominal flow rate of 12.5 L/min into 20 mL of phosphate-buffered saline (PBS, pH 7.4).[2,4] Aliquots of 0.1 mL of the BioSampler suspensions were serially diluted with PBS, plated on tryptic soy agar (TSA; Becton Dickinson, NJ), and incubated at 37 °C for 12 h. After incubation, the colonies that formed on the plates were counted. The concentration of culturable bacteria was determined by converting the number of CFUs into an aerosol concentration (CFU/cm$^3_{air}$). To calculate the bacterial culturability, the concentration of culturable bacteria was divided by the concentration of total bacterial particles measured using the UVAPS as in eq 2.

$$\text{Bacterial culturability (\%)} = \frac{\text{Concentration of culturable bacteria}}{\text{Concentration of total bacterial particles}} \times 100 \qquad (2)$$

RESULTS AND DISCUSSION

Voltages producing a stable cone-jet mode with various concentrations of bacterial suspension were determined by observing the shapes of the meniscus and liquid jet at the capillary tip and by measuring the variation in the number fraction of

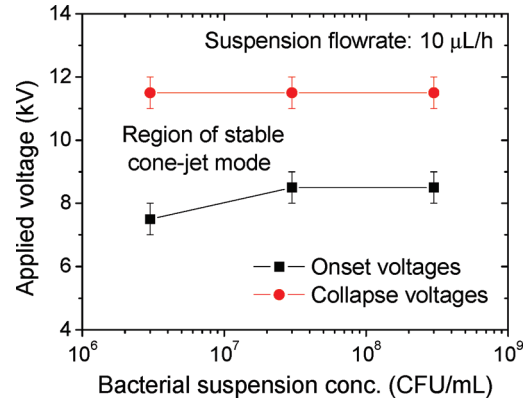

Figure 3. Stability domain for the cone-jet mode of the electrospray at various applied voltages and bacterial suspension concentrations. Error bars indicate standard deviations.

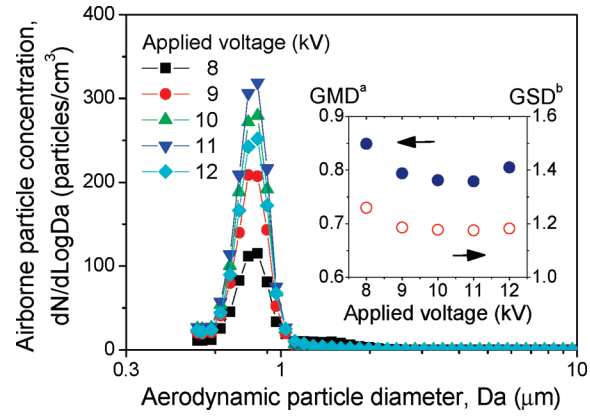

Figure 4. Particle size distribution, GMD, and GSD using the electrospray technique at various applied voltages. Each total generation time was ~90 min. [a]GMD: geometric mean diameter (μm). [b]GSD: geometric standard deviation.

generated particles. The stability of the cone-jet mode domain in terms of applied voltage and bacterial suspension concentration ranging from 3×10^6 to 3×10^8 CFU/mL is shown in Figure 3. The lower curve corresponds to an onset voltage of a stable cone-jet mode, and the upper curve shows an unstable cone-jet mode. An unsteady, broken electrospray pattern was observed in the region below the lower curve, and fluctuating and multijet modes were observed above the upper curve.[42] A stable domain was obtained at an applied voltage between 7.5 ± 0.50 and 11.5 ± 0.50 kV, with a slightly increased onset at higher concentrations.

Figure 4 shows the variation in the size distribution for *E. coli* particles generated from 3×10^8 CFU/mL at different applied voltages. As the applied voltage increased from 8 to 12 kV, which included unstable (8 and 12 kV) and stable (9−11 kV) cone-jet modes, the total particle concentration increased up to 11 kV. For the stable cone-jet domain, the particle size distributions were maintained almost uniformly, with a slight downward trend of the geometric mean diameter (GMD; 0.794−0.779 μm) and the geometric standard deviation (GSD; 1.186−1.175). However, in the unstable (8 and 12 kV) cone-jet mode, a relatively higher GMD (0.849 and 0.805 μm at 8 and 12 kV, respectively) and GSD (1.260 and 1.183 at 8 and 12 kV, respectively), compared to the stable mode, were observed because of irregular electrospray jets. In addition, the unsteady spray resulted in a decreased particle number concentration.

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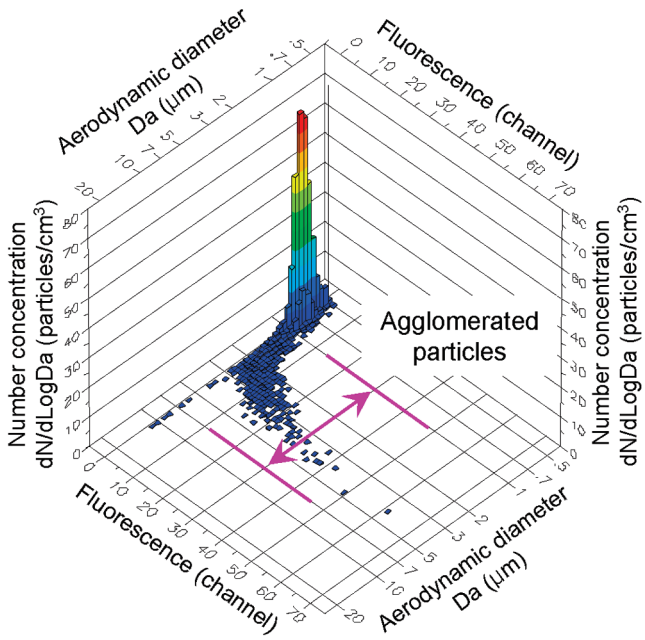
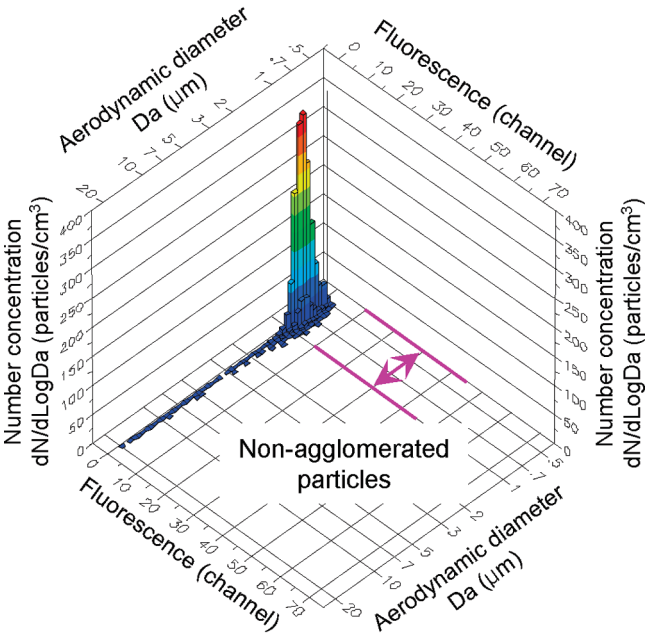


(a)



(b)

Figure 5. Size and fluorescence distributions of electrosprayed bacterial particles: (a) unsteady cone-jet (7 kV) and (b) steady cone-jet (11 kV). The total particle concentrations in (a) and (b) were 15.8 and 63.1 particles/cm^3, respectively.

Figure 5 shows the size and fluorescence distributions of electrosprayed *E. coli* particles in the unstable (7 kV) and stable cone-jet modes (11 kV). As above, the GMD and GSD were higher in the unstable cone-jet mode than in the stable mode. Particles over 1 μm in size produced relatively higher fluorescence intensities than those smaller than 1 μm (Figure 5a), suggesting that the particles over 1 μm were agglomerates of bacterial

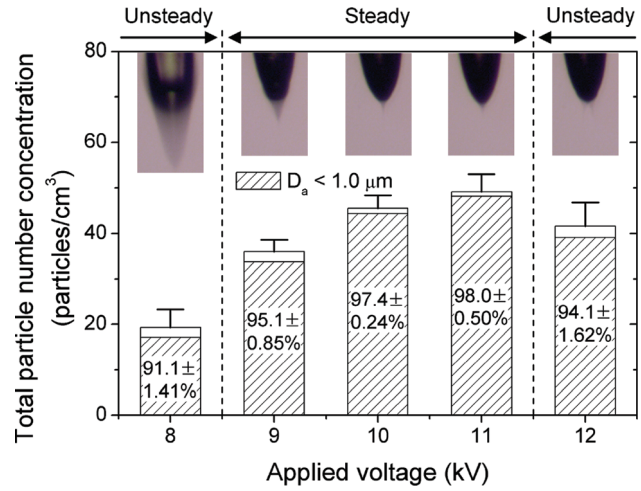


Figure 6. Variations in the cone shape of the electrospray and the total number concentration at various applied voltages. The shaded areas in the bars and the listed percentages correspond to the fraction of particles with aerodynamic diameter smaller than 1 μm.

particles because rod-shaped *E. coli* range from 0.6 to 0.8 μm in diameter and 0.8 to 1.2 μm in length.[4] Thus, the physical characteristics of the bacterial particles, including size distribution, GMD, and GSD, depended on jet stability in the electrospray process.

Figure 6 displays the variation in the total number concentration of electrosprayed bacterial particles at various applied voltages. Changes in the cone-shaped meniscus in the capillary tip are also shown for each experiment. As the voltage applied to the capillary increased from 8 to 11 kV, the liquid cone tip became slightly shorter, because of the increasing strength of the electric force on the liquid cone surface relative to the surface tension force. However, at 12 kV, an unsteady multijet mode was observed. As mentioned in previous studies,[44–46,49,72,73] the sharpness of the cone apex plays an important role in generating droplets of smaller size and that geometric property can be changed by control of the applied voltage and suspension flow rate. At lower flow rates or higher applied voltages, the liquid cone has a cusplike shape and the tip is thinner. This cusplike liquid cone is useful for generating monodisperse droplets.

The total particle number concentration and percentage of particles smaller than 1 μm increased as the applied voltage increased within the stable cone-jet domain. The percentage of particles smaller than 1 μm increased from 91.1% at an unsteady cone-jet mode voltage (8 kV) to 98.0% at a stable cone-jet mode voltage (11 kV). This was used as a direct index of the generation of nonagglomerated bacterial particles.

The fluorescence percentage and bacterial culturability varied with changes in the applied voltage within the stable cone-jet domain (Figure 7). As the voltage increased from 9 to 11 kV, the fluorescence percentage decreased, from 13.5 ± 3.02 to 8.4 ± 1.51%. In contrast, the bacterial culturability remained nearly constant at 6.5 ± 0.25% until 10 kV and decreased at voltages above 10 kV (4.2 ± 1.67 and 3.3 ± 1.49% at 10.5 and 11.0 kV, respectively). The decline of fluorescence percentage with increasing voltage was consistent with the reduction in bacterial culturability. There are

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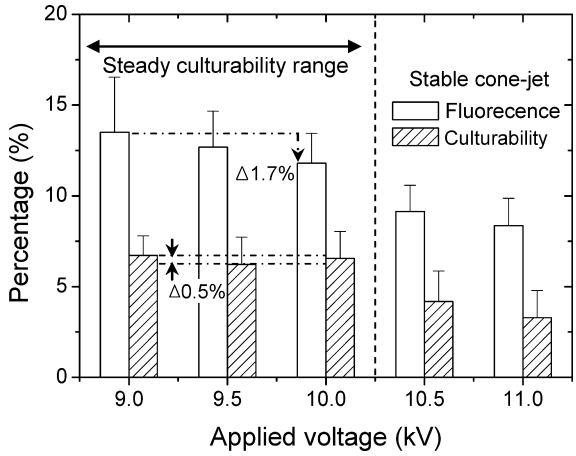

Figure 7. Percentages of fluorescence and culturability of electrosprayed bacterial particles at various applied voltages.

two possible explanations for these changes in fluorescence percentage and bacterial culturability. First, an increase in the applied voltage in the electrospray process might have led to an increase in the total particle number concentration (specifically, the percentage of particles below 1 μm) owing to greater electrical separation of agglomerated particles (Figure 6), which would have decreased the fluorescence percentage and bacterial culturability as percentages of the total particle number concentration. Second, the reductions in fluorescence percentage and bacterial culturability might have been caused by electrically induced mechanical stresses during jet formation and jet breakup. However, the precise mechanism of the variation in bacterial viability remains unknown. Detailed biological characterizations of electrosprayed biological materials, including living organisms ranging in size from micrometers to nanometers, are needed to broaden the application of this technique.

We suggest an applied voltage ranging from 9.0 to 10.0 kV for an adequate and stable operating setting of the electrospray-assisted UVAPS designed in this study (Figure 7). At this voltage range, the variations of the fluorescence percentage and bacterial culturability were only about Δ1.7% and Δ0.5% on average, respectively.

The difference between the fluorescence percentage and bacterial culturability was almost 4.2% (Figure 7). A BioSampler was used as a conventional method for sampling the test microorganism, whereas the UVAPS was used for real-time data. The biological efficiency, which is defined as the ability to maintain the viability of airborne microorganisms, may be diminished when sampling with a BioSampler.[74−77] Physical sampling efficiency can also play an important role in viability. Especially, given that only a small proportion of microorganisms are culturable,[78] enumeration of bioaerosol samples by culturing significantly underestimates the actual concentration. Therefore, the term culturability, instead of viability, more exactly reflects what is measured when sampled microorganisms are enumerated using the conventional culture plate technique.[79,80] In this case, measured culturability can be lower than fluorescence emitted by living microorganisms. Compared with conventional methods, real-time characterization using UVAPS can enhance not only the biological and physical sampling efficiency of airborne microorganisms but also the accuracy of the viable microorganism data. However, as shown in Figure S-1 (Supporting Information), it is critical to establish the fluorescence intensity baseline using nonfluorescent test particles (e.g., PSL particle), because the pulsed UV laser power and PMT gain voltage can dramatically affect the fluorescence percentage of biological particles.

Table 2. Comparison of *E. coli* Characterizations Generated by Charge-Reduced Electrospray and Nebulization Processes[a]

UVAPS 3314 parameter	charge-reduced electrospray[b]	1-jet collison nebulizer[c]
total number concentration (particles/cm^3)	45 ± 2.9	13 ± 1.3
total number concentration below 1 μm (%)	97 ± 0.2	78 ± 0.4
GMD[d] (μm)	0.779 ± 0.0013	0.907 ± 0.0001
GSD[e]	1.175 ± 0.0038	1.163 ± 0.0050
fluorescence (%)[f]	11.8 ± 1.65	34.0 ± 0.70
culturability (%)[g]	6.6 ± 1.49	24 ± 2.2

[a] All values are presented as average ± standard deviation. [b] Condition: bacterial suspension, 3.0×10^8 CFU/mL; suspension flow rate, 10 μL/h; sheath air, 5 L/min; and applied voltage for steady cone-jet mode, 10 kV. [c] Condition: bacterial suspension, 3.0×10^7 CFU/mL; supplied air, 0.8 L/min; sheath air, 7.2 L/min. [d] GMD: geometric mean diameter. [e] GSD: geometric standard deviation. [f] Ratio of total fluorescent particles (channels 2−64) to total particles (channels 1−64). [g] Ratio of culturable bacteria concentration to total number concentration.

We compared the particle characteristics obtained from the charge-reduced electrospray process with those obtained from a nebulization process using a 1-jet Collison nebulizer (BGI Inc., Waltham, MA), which has been widely used for the aerosolization of microorganisms from liquid suspensions.[35,36,81−83] One milliliter of a bacterial pellet, which was also used in the electrospray experiment, was added to 29 mL of sterile, distilled, deionized water. The bacterial particles were then aerosolized using a Collison nebulizer at an air flow rate of 0.8 L/min, passed through a neutralizer, and mixed with filtered sheath air of 7.2 L/min. Finally, the bacterial particles were also introduced into the UVAPS and sampled using the BioSampler. Table 2 compares the *E. coli* particles generated by the charge-reduced electrospray and nebulizer processes. With the electrospray process, 98% of the total *E. coli* particle number concentration had a size of <1 μm and the geometric mean diameter was 0.779 μm, as compared with the respective values of 78% and 0.907 μm after nebulization. The fluorescence and culturability percentages were 12% and 7%, respectively, with the electrospray process and 34% and 24%,

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respectively, with nebulization. In general, the nebulization process initially generated droplets measuring several tens of micrometers; when these water-based droplets evaporated in diluted air or by diffusion drying, the agglomeration of bacteria and/or their fragments proceeded naturally. On the other hand, the charge-reduced electrospray process produced a monodisperse size distribution of particles because the initial droplets were 100−200 nm in diameter.[47,82] In addition, as the particles generated by electrospray have electrical charges of the same polarity, mutually repulsive electric forces contribute to the creation of nonagglomerated particles.

The present study demonstrates that (1) the presence of agglomerated particles can lead to markedly overestimated fluorescence and culturability percentages compared with the values obtained from nonagglomerated particles (Table 2), and (2) electrospray-assisted UVAPS can provide more accurate and quantitative real-time characterization of microorganisms suspended in a liquid, owing to the generation of nonagglomerated particles.

CONCLUSIONS

Real-time in situ monitors of the biological particles not only enhance characterization efficiency but also improve the accuracy of data, as compared with conventional methods. In this study, we investigated the real-time characterization of *E. coli* particles using an electrospray-assisted UVAPS system. The charge-reduced electrospray technique can be used to produce stable aerosolization of bacterial particles without particle agglomeration, owing to the repulsive electrical forces generated with the system. Our results indicate that the agglomeration of particles resulting from nebulization can markedly affect the real-time characterization by UVAPS. The electrospray-assisted UVAPS system allows more accurate and quantitative characterization of bacterial particles in real-time and has potential applications in the control of bioaerosols and in bioengineering research. The results presented in this paper enhance the understanding of the UVAPS, particularly its performance with nonagglomerated particles and, thus, assists in developing methodologies for routine application in various environments, including air and liquid-based media. In addition, this recommended system may also be useful for designing new detection methods for biological materials in real-time. To broaden the application of the electrospray technique, the biological and physiological characteristics (e.g., culturability, injury, fragmentation, and gene expression patterns) of bacterial species of different sizes and reproduction rates should also be investigated.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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