Performance evaluation of the UVAPS in measuring biological aerosols: fluorescence spectra from NAD(P)H coenzymes and riboflavin

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ABSTRACT

This paper presents the results of the performance evaluation of the Ultraviolet Aerodynamic Particle Sizer (UVAPS, model 3312, TSI Inc., St. Paul, MN), the novel instrument for real-time monitoring of biological aerosols. The main objective of the study was to compare the UVAPS response in measuring aerosols containing the NADH, NADPH, or riboflavin particles. At the excitation and emission wavelengths at which the UVAPS operates, these compounds are the primary intrinsic fluorophores specific to biological particles. In addition, the study was focused on determining the detection limits of the UVAPS for these fluorophores. This information is important for the interpretation of UVAPS data while measuring bacterial aerosols. Fluorescence measurements were initially taken with a Varian Cary Eclipse Fluorescence Spectrophotometer for all three fluorophores. The samples were then aerosolized with the 6-jet Collison nebuliser. Riboflavin was found to be a stronger fluorophore than both NAD(P)H coenzymes. The fluorescence signals were considerably weaker for the NADPH samples compared to the NADH samples. The sensitivity of the UVAPS was found to be sufficiently high to detect the NADH and riboflavin at the concentrations characteristic to bacterial cells. The results of this study are discussed in a context of the results previously reported for the bacterial aerosols. It can be concluded, that the amount of fluorophores detectable in uniformly mixed particles is equal to or less than the fluorophores expected to be present in the individual bacterial particles.

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INTRODUCTION

Exposure to biological aerosols, or biologically derived airborne contaminants, may affect human health in various ways including infectious diseases, acute toxic reactions and allergies (Macher 1999; AIHA 1996; ACGIH 1994; Cox and Wathes 1995). The symptoms can range from eye, nose, and throat irritation to dizziness, lethargy, or fever (Burge 1990; Lacey and Dutkiewicz 1994). Bioaerosols may also cause animal and plant diseases, resulting in loss of agricultural productivity (Cox and Wathes 1995; Lighthart and Mohr 1994).

Monitoring of biological aerosols is required for controlling air quality, for exposure assessment in health risk evaluation studies, for identifying a source of bioaerosol emission, for investigating dispersion of bioaerosols in atmosphere, or other research purposes.

The conventional methods for monitoring viable bioaerosols are laboratory-based, laborious and time-consuming, requiring at least 24 hours to provide the results. In the situations when a risk from an exposure to airborne microorganisms has to be established urgently, in order to organize the adequate control measures, the delays with obtaining viable bioaerosol concentration data may be vital.

The Ultraviolet Aerodynamic Particle Sizer (UVAPS, Model 3312, TSI Inc., St. Paul, MN) is a novel aerosol counter for real-time, continuous monitoring of viable airborne microorganisms. It measures aerodynamic diameter and the intrinsic fluorescence of aerosol particles within a size range of 0.5-15 μm. The fluorescence is produced by exciting particles with a pulsed ultraviolet laser beam at an excitation wavelength of 355 nm and detected at an emission region between 420 and 575 nm.

Under such conditions, the measured fluorescence is characteristic to the reduced pyridine nucleotides (NADH and NADPH) and flavins, which present in all living cells. This property of viable airborne microorganisms to produce specific fluorescence is, therefore, used to distinguish them from the non-viable aerosol particles (Hairston et al. 1997; Ho et al. 1999; Lakowicz and Cherek 1981). A detailed description of the UVAPS can be found elsewhere (Agranovski et al., 2003a, Brosseau et al, 2000; Hairston et al, 1997).

Despite the fact that the feasibility of the UVAPS to detect viable bioaerosols has been demonstrated by the designers of the monitor in a number of studies (Hairston et al. 1997; Ho et al. 1999; Brosseau et al. 2000), the collection of meaningful data and their adequate interpretation require a thorough evaluation of its performance under range of experimental conditions.

The study presented in this paper is a part of a wider experimental program aimed towards comprehensive evaluations of the UVAPS performance. The initial tests, focused on evaluating counting efficiency of the UVAPS to measure airborne bacteria of a similar size (Agranovski et al. 2003a), have revealed that the instrument performance depends on the type of aerosolized bacteria. For example, while relatively strong fluorescence signals were detected for the aerosolized *Bacillus subtilis* viable cells, no fluorescence was detected in the case of *Pseudomonas fluorescens* aerosols containing at least 30 % of viable cells (Agranovski et al. 2003a). Considering the sensitivity of these two bacteria to environmental stresses (*B. subtilis* is a robust and *P. fluorescens* is a sensitive to environmental stresses bacterium), these results indicated that bacterial metabolic state has a strong influence on the fluorescence signals of the UVAPS. The subsequent study (Agranovski et al. 2003b) has confirmed this hypothesis

and indicated that the amount of characteristic intracellular fluorophores within viable bacterial cells may be altered due to aerosolization stresses, to the extent specific to the individual bacterial specie. To get better insight into the origin of the fluorescence signals of the UVAPS when monitoring biological aerosols, it is important to evaluate the contribution of the individual fluorophores to the signals. Thus, it was an objective of this study to compare fluorescence spectra of NAD(P)H[1] coenzymes and riboflavin, which are the primary intrinsic biological fluorophores under the UVAPS operating conditions (Hairston et al. 1997; Ho et al. 1999; Lakowics and Cherek 1981). In addition, the study was focused on determining the detection limits of the spectrometer for these three fluorophores.

MATERIALS AND METHODS

Starting materials

Pre-weighed β-NADH, NADPH, and riboflavin (RF) were purchased from Sigma Chemicals (St. Louis, MO, USA). The solutions, with the concentrations ranging from 0.5 to 1000 μg/ml, of each compound were prepared fresh daily. NADH and NADPH were diluted using Tris buffered saline (0.05M Tris, 0.138M NaCl, 0.0027M KCl), pH 8.0 (Sigma, St. Louis, MO, USA). Riboflavin solutions were diluted with 1% of acetic acid. These buffers were used in this study as they were reported by manufacturer to be optimal for ensuring fluorescence properties of the fluorophores under investigation, when dissolved in aquatic media. These buffers are also typically used in the

[1] NAD(P)H denotes both NADH and NADPH coenzymes.

spectrophotometric studies of these compounds (Nokubo et al. 1989; Jones et al. 1999; Schmidt et al. 1999; Rulifson et al. 2002; Zhang and Qi 2002). All chemicals and solvents were of analytical grade. Since riboflavin is light sensitive, its solutions were prepared in amber glass bottles and stored in a refrigerator (4°C).

Preliminary fluorescence measurements

Fluorescence intensity measurements were initially carried out on a Cary Eclipse Fluorescence Spectrophotometer (Varian Australia Pty Ltd, Victoria, Australia). To gain better understanding of the fluorescent properties of the samples, preliminary measurements were conducted to determine the optimum excitation and emission wavelengths for all three fluorophores. For the excitation spectra, emission was measured at 530 nm for riboflavin and 445 nm for NAD(P)H, with spectral slit widths at 10 nm (excitation) and 5 nm (emission). For the emission spectra, excitation wavelength was set at 450 nm for riboflavin and 340 nm for NAD(P)H, with slit widths at 5 nm (excitation) and 10 nm (emission).

To assess the linearity of the spectrophotometer, the measurements were taken at multiple fluorophore concentrations using the constant wavelength analysis of the samples. The excitation wavelength was set at 355 nm and the emission wavelength at either 450 nm (for HAD(P)H) or 530 nm (for riboflavin). The sample concentrations ranged from 5 to 1000 μg/ml. The measurements of the fluorescence intensity were then plotted against the concentrations.

To evaluate relative fluorescence properties of the three fluorophores under conditions similar to the UVAPS, the emission spectra were then scanned from 400 to

700 nm at excitation wavelength of 355 nm for each compound. The excitation and emission slit widths were set to 5 nm. The photomultiplier detector voltage was set at 700 V and the scan rate of the monochromators was maintained at 600 nm/min with data interval of 1 nm. Fluorescence spectra for the Tris and 1% acetic acid blank solution were obtained to correct for background fluorescence of the buffers. All experiments were performed at room temperature (27°C).

The UVAPS measurements

The experimental setup used in this study was similar to that reported previously (Agranovski et al. 2003a, b). It is schematically shown in Fig. 1. The aerosols were generated by a 6-jet Collison nebulizer (BGI Inc., Waltham, MA) at a flow rate of 12 L/min. The aerosol stream was then passed through an electrostatic charge neutralizer (10-mCi ^{85}Kr, model 3012, TSI Inc., St. Paul, MN) for minimizing electrostatic removal of particles by the inner surfaces of the test system. The aerosolized droplets containing test particles were then rapidly dried and diluted by filtered compressed air (30-40 L/min) before introducing into a mixing chamber. Given that the droplet lifetime is proportional to diameter squared (May 1973), the aerosolized aqueous droplets evaporated very rapidly as a result of mixing with dry air. Specifically, for a droplet size range generated by the Collison nebulizer (Gussman 1984), the droplet wet lifetime at a given temperature and relative humidity would be in order of 0.01 - 0.1 sec.

The experiments were performed at the air temperature in the chamber within 20-23 $^{\circ}$C and the relative humidity within 45-55 %. The entire setup was placed inside a

Class II, Type A, Biological Safety Cabinet (SG-400 SterilGARD, Email Westinghouse Pty Ltd, Australia).

Before starting the experiments, the UVAPS sensitivity was adjusted with non-fluorescent Polystyrene Latex (PSL) Particles (0.993 μm, Duke Scientific Corporation, Palo Alto, CA) using the procedure described in details elsewhere (Agranovski et al. 2003a). The spectrometer was afterwards calibrated with blue fluorescent (BF) microspheres (Duke Scientific Corporation, Palo Alto, CA) with a diameter of 0.91 μm. Both the PSL and the BF particles had a density of 1.05 g/cm^3, which corresponded to aerodynamic equivalent diameters of 1.2 and 0.93 μm, respectively (Hinds 2001). Since the counting efficiency of the UVAPS was found to depend on the particle concentration (Agranovski et al. 2003a), the aerosol concentrations were maintained at arbitrary level of approximately 3 x 10^7 particles/ m^3.

To assess the sensitivity of the UVAPS for the tested fluorophores, measurements were taken at multiple fluorophore concentrations. Initial measurements with the Tris or 1% acetic acid blank solutions were conducted to determine the level of background/blank signals. Afterwards, the aerosols were generated from the series of solutions with the fluorophore concentrations ranging from 0.5 to 500 μg/ml. Preliminary experiments with the NADH aerosols were performed with the solutions of up to 1000 μg/ml.

All measurements were taken with a sampling time of 3 seconds and were repeated at least five times.

Statistical analysis

Statistical analysis was conducted on the complete data set using the Student's *t*-test. Statistical significance was accepted at the $P<0.05$ level of probability.

RESULTS

Fluorescence spectra measured with the Cary Spectrophotometer

Both NAD(P)H coenzymes had the excitation and emission maxima at 366 ± 1 nm and 464 ± 2 nm, respectively. For riboflavin, the maxima were detected at 439 ± 1 nm (excitation) and 525 ± 1 nm (emission). The optimal excitation wavelengths for the materials used in this study were, therefore, 366 nm for NAD(P)H coenzymes and 439 nm for riboflavin.

The fluorescence intensity measurements taken at 355 nm excitation, which corresponded to the UVAPS operating conditions, revealed that while not optimal, this excitation wavelength was appropriate to produce relatively strong fluorescence signals for all tested compounds (Fig. 2). The measurements also revealed that riboflavin signals were much stronger than the fluorescence signals of both NAD(P)H coenzymes (Fig.2a; scanned from 400 to 700 nm). Comparison of the NAD(P)H emission spectra showed that the intensity of the NADH fluorescence was up to 20% higher than the intensity of the NADPH fluorescence, within the linearity region (concentrations below 30 μg/ml, Fig. 3) for these two compounds (Fig 2a). At higher concentrations, the difference between the NADH and NADPH intensities reached approximately 45% (Fig. 2b; scanned from 440 to 600 nm). The fluorescence intensity measurements were taken at multiple concentrations ranged from 0.5 to 500 μg/ml for each fluorophore.

Comparison was performed for the measurements obtained for the samples of the same concentrations.

The UVAPS measurements

The main two objectives of these tests were evaluation of the instrument response for the NADH, NADPH, and riboflavin aerosols and determination of the detection limits for these three fluorophores.

Since the UVAPS does not provide nominal values for the fluorescence intensity, the fluorophore intensities could only be evaluated relatively. The fluorescence detected for each particle was registered in one out of 64 channels, depending on the intensity of the signal. The increasing channel numbers corresponded to gradually increased fluorescence intensity of the particles. Thus, by analyzing the concentration of the particles recorded in each channel, it was possible to conclude on a relative fluorescence intensity of a particular fluorophore. The comparison of the UVAPS spectra was performed for the aerosols generated from the solutions of the same concentrations, which ranged from 0.5 to 500 μg/ml.

Examination of the UVAPS fluorescence spectra revealed that, similarly to the results obtained with the Cary spectrophotometer, the riboflavin aerosols produced stronger fluorescence signals than the NADH aerosols, which in turn produced stronger signals than the NADPH aerosols (Figs 4-5). For example, analysis of the spectra for the aerosols produced from 10 μg/ml solutions revealed that the fluorescence particles were detected within the first 24 channels for the NADH, the first 13 channels for the NADPH, and up to channel 31 for riboflavin (Fig. 5a). In the case of the aerosols

generated from 100 μg/ml solutions (Fig. 4), all aerosols produced relatively strong fluorescent signals with the particles detected in all 64 channels. However, the concentration of particles detected in the upper channels was the highest for riboflavin and the lowest for NADPH solutions (Fig. 5b). Specifically, approximately 10 % of riboflavin, 3% of NADH, and 0.5 % of NADPH particles were registered in the last channel (Fig. 5b).

Sensitivity of the UVAPS

The UVAPS sensitivity, or limit of detection, is defined here as that amount of a fluorophore within the aerosol particles which yields the instrument response of two standard deviations above the background signal. In other words, the limit of detection of the UVAPS for a particular fluorophore is expressed as the minimum detectable quantity of NADH, NADPH, or riboflavin in the fluorescent particles. For the NAD(P)H aerosols, the background signals corresponded to those measured for the aerosolized Tris blank solutions. In the case of riboflavin, the measurements obtained for the aerosolized 1% acetic acid were regarded as the background signals.

Both background aerosols produced signals in the first two channels (the first channel corresponds to zero fluorescence). In addition, a percent fluorescence (*e.i.,* the concentration ratio of the fluorescent particles to the total aerosol particles measured by the UVAPS, which was converted to a percentage) of the backrgound aerosols did not exceed 2 %. Specifically, the Tris blank solutions produced 0.7 ± 0.4 % fluorescence, while the acetic acid produced 1.0 ± 0.2 % fluorescence. Thus, for NAD(P)H aerosols, when the percent fluorescence values exceeded 1.5 % (*i.e.*, two standard deviations

above the background signal) and when the signals were registered in the channels above the second channel, the measured fluorescence was regarded as originated from the fluorophores rather than buffer. In the case of riboflavin, the measurements with percent fluorescence values above 1.4 % and with the fluorescent signals in the channels above the second channel were regarded as above the background level.

To determine the sensitivity, it was necessary to determine the minimal amount of a particular fluorophore in the particles which produce signals stronger than the background. Thus, the minimal concentrations of the solutions at which the signals were detected in the channels above channel two and at which the percent fluorescence was distinctively higher (two standard deviations) than the background level were initially determined for all fluorophores. These minimal concentration values, which appeared to be different for different fluorophores, were then used to calculate the amount of a particular fluorophore in the particles in accordance with the following rationale.

The size of dry aerosol particles (d) after water evaporates depends on: (1) the initial droplet size (d_D), (2) the amount of soluble compounds (solutes) within the droplet. The solutes originated from the Tris solution with concentration C_T, and from the fluorophores with concentration C_F. The mass of the dry particles after water evaporates will thus be given by:

$$m = C_T \frac{\pi}{6} d_D^3 + C_F \frac{\pi}{6} d_D^3$$

The mass concentration of fluorophores in the solutions, from which the particles were nebulised, was significantly smaller than the concentration of the solutes from the Tris buffer ($C_T >> C_F$) (see Materials). We can, therefore, assume that the major contributor

to the particle mass will be the solutes from the Tris buffer, or $m \approx C_T \frac{\pi}{6} d_D^3$. The diameter of the dry particles is then given by:

$$d = \sqrt[3]{\frac{6V}{\pi}} = \sqrt[3]{\frac{6m}{\pi \rho_T}} = \sqrt[3]{\frac{C_T}{\rho_T}} d_D \,, \qquad (1)$$

where ρ_T is density of the Tris buffer. The measurements have shown that the density of the Tris buffer was equal to 1.5 g/ml.

The amount of fluoropore that has been left over on the dry particle, after water evaporated, is proportional to the initial droplet size (d_D) and the concentration of fluoropores in the water solution (C_F) and is given by:

$$m_F = C_F \frac{\pi}{6} d_D^3 \qquad (2)$$

If the dry particle diameter is known then the diameter of the initial droplet can be estimated from Equation 1. Combining Equation 1 and Equation 2, the amount (mass) of fluorophores in a dry particle of diameter d is given by:

$$m_F = \rho_T \frac{C_F}{C_T} \frac{\pi}{6} d^3$$

The UVAPS measures the aerodynamic diameter of dry particles (d_a). The relationship between d and d_a is given by (Hinds 2001):

$$d = d_a \left(\frac{C_C(d_a)}{C_C(d)} \frac{\rho_0}{\rho_T} \right)^{1/2}$$

where $C_C(d_a)$and $C_C(d)$ are the respective slip correction factors for d_a and d, and ρ_0 = 1 g/ml.

Thus, under assumption that the particles are spherical, the aerodynamic diameters (d_a) measured by the UVAPS can be converted into the particle diameters (d) from which the amount (mass) of fluorophores can subsequently be calculated.

The characteristics of the aerosols which were used to determine the UVAPS detection limits for the selected fluorophores are summarized in Table 1. The minimal concentrations of the solutions which produced the fluorescent signals stronger than the background levels were 3 μg/ml for the NADH, 5 μg/ml for the NADPH, and 1 μg/ml for the riboflavin aerosols. The fluorescent particles were detected within the first four, ten, and five channels for the NADH, NADPH, and riboflavin aerosols, respectively. Taking into account the size of the smallest detectable fluorescent aerosol particles and the concentration of the solutions (Table 1), the detection limits for NADH, NADPH, and riboflavin are 1.44×10^{-14}, 2.23×10^{-13}, and 9.46×10^{-15} mg, respectively. The issue of how these values compare with the amount of the fluorophores in the bacterial cells is discussed further in detail.

DISCUSSION

The main objectives of this study were to examine the fluorescent properties of NADH, NADPH, and riboflavin and to determine the UVAPS detection limits for these three compounds. NAD(P)H coenzymes and riboflavin are considered to be the primary intrinsic fluorophores specific to biological particles, under operating conditions of the UVAPS (Hairston et al. 1997; Ho et al. 1999). Thus, evaluating the effect of each of these compounds on the fluorescence signals of the UVAPS may help to gain a better understanding of its performance while measuring microbial aerosols. In addition, this

information may be helpful in getting a deeper insight into the levels of these compounds in the airborne microorganisms.

Preliminary experiments carried out with the Cary Spectrophotometer revealed that the optimal excitation wavelengths are 366 nm for both NAD(P)H coenzymes, and 439 nm for riboflavin. Maximum emission of fluorescence was observed at 464 nm for NAD(P)H and 525 nm for riboflavin. These results are consistent with the literature (Li et. al. 1991; Coghlan et al. 2000; Brewer et al. 2002), within the limits of the experimental error. Taking into account the excitation wavelength of the UVAPS (355 nm) and the optimal excitation wavelengths for the materials used, it becomes obvious that the UVAPS operational conditions are not optimal for producing the strongest fluorescence signals for the tested aerosols. It has to be noted, however, that the fluorescent properties of these fluorophores are known to depend on the environment and may differ for bound and free compounds (Ichinose et al. 1991; Lakowicz et al. 1992; Huber et al. 2000; Zhang et al. 2002). For example, the excitation wavelength of 340 nm is often used as optimal when measuring the NAD(P)H fluorescence of biological materials (Ramanujam et al 1994; Huber et al 2000). Thus, the above statement on the effect of the UVAPS operational parameters on its sensitivity relates only to the results obtained in this study for laboratory prepared non-viable aerosols.

Both the Cary spectrophotometer and the UVAPS data showed that riboflavin is a stronger fluorophore than both NAD(P)H coenzymes. Similarly, both methods found that NADH produces stronger fluorescence signals than NADPH. Given that the amount of NADH is, in most bacteria, approximately five times higher than the amount of NADPH (London and Knight 1966; Bae and Rittmann 1996), it can be then suggested that the impact of the intracellular NADH on the fluorescence signals is considerably

greater than for the NADPH. This finding is important for correlating the UVAPS data with bacterial viability as a function of their metabolic state (Agranovski et al. 2003b).

The UVAPS detection limits were found to be 1.44 x 10^{-14} mg for the NADH, 2.23 x 10^{-13} mg for the NADPH, and 9.46 x 10^{-15} mg for the riboflavin. Intracellular NADH and NADPH concentrations in bacteria vary within the range of 0.1 - 11 μmoles/g and 0.04 - 0.7 μmoles/g of dry weight of organisms, respectively (London and Knight 1966; Bae and Rittmann 1996; de Graef et al 1999; Sipkema et al. 2000). Thus, the detection limit value for NADH appear to be below intracellular bacterial concentrations, whereas the amount of NADPH in bacterial cells may be below the UVAPS detection limit for this fluorophore. Specific values of the NAD(P)H concentrations within the microorganisms of a particular interest for the present study are shown in Table 2. As can be seen, the concentrations of the NADPH in both *P. fluorescens* and *B. subtilis* cells appear to be lower than the UVAPS detection limit for this compound. The intracellular NADH content in these bacteria is, however, higher than the detection limit obtained in the present study for NADH. Why then was the UVAPS unable to detect any fluorescence for the *P. fluorescens* aerosols (Agranovski et al. 2003 a, b)? A few possible reasons for this apparent inconsistency are discussed later.

In regards to riboflavin, it has to be noted that there are two other intrinsic microbial fluorophores, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (Weimar and Neims 1975; Ichinose et al. 1991; Munro & Noble 1999), which may contribute into the UVAPS fluorescence signals for bacterial aerosols. These flavin coenzymes together with riboflavin are better known by generic name of Vitamin B_2. Riboflavin is the precursor of both flavin coenzymes, which together with the NAD(P)H

coenzymes are essential players for electron transport functions of proteins involved in the energy metabolism of the cell (Neidhardt et al. 1990). As riboflavin has fluorescence properties similar to the flavin coenzymes (Weimar and Neims 1975; Ichinose et al. 1991; Munro & Noble 1999), it was appropriate to use it as a model for all microbial flavins in the context of the present study. Contrary to the NAD(P)H coenzymes, information on intracellular content of the flavins is very limited. It is only known that the total (i.e., FMN, reduced FMN, and FAD) intracellular pool of flavins may range from 0.2×10^{-12} to 65×10^{-12} mg/cell in a variety of bacterial species (Chappele 1975). These values appear to be significantly higher than the UVAPS detection limit for riboflavin.

Summarizing the results of the present study, the sensitivity of the UVAPS was found to be sufficiently high to detect NADH and riboflavin at the concentrations characteristics to individual bacterial cells. It has to be, however, noted that the data obtained in this study correspond to free compounds, whereas their fluorescence properties may differ from those within bacteria. The fluorescence may be quenched or masked for bound fluorophores within the cells. This may partially explain a difference between the fluorescence signals observed in this study and the signals detected for bacterial aerosols (Agranovski et al 2003a, b). In addition, the literature data on the intracellular coenzymes content of bacteria correspond to the cells in the batch cultures (Kaplan 1960; Takebe and Kitahara 1963; London and Knight 1966; Chappele 1975). Aerosolization may, however, affect the coenzymes content within the cells. As was discussed in the previous paper (Agranovski et al. 2003b), aerosolization stresses may cause metabolic changes in airborne cells which will be accompanied by a decrease of the amount of these compounds, in some cases (e.g., *P. fluorescens*) below the UVAPS

sensitivity level. To clarify the issue, further work has recently been initiated: (1) to estimate the fluorescence intensity of the bacterial solutions containing the cells before and after aerosolization using the Cary spectrophotometer; (2) to find a correlation between the fluorescence intensity and the bacterial viability. The results of this study will be reported soon.

SUMMARY AND CONCLUSIONS

The relative fluorescence intensities of the NADH, NADPH, and riboflavin were assessed to evaluate the potential of these fluorophores to contribute into the UVAPS fluorescence signals within the concentration range characteristic to bacteria. The sensitivity of the UVAPS was found to be sufficiently high to detect the NADH and riboflavin at the concentrations characteristic to bacterial cells in batch cultures. However, aerosolization stresses may diminish the amount of the fluorophores in airborne cells, in some cases below the sensitivity level of the UVAPS, which may have a dramatic effect on the UVAPS performance while measuring bacteria that are sensitive to the stresses.

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FIGURE CAPTIONS

Table 1. Characteristics of the aerosols used to determine the limits of detection.

Table 2. Concentration of NAD(P)H coenzymes within bacterial cells.

Fig. 1. Schematic diagram of the experimental set-up.

Fig. 2. Emission spectra measured with the Cary Eclipse Fluorescence Spectrophotometer (excitation at 355 nm): A) for 30 μg/ml solutions; B) for 300 μg/ml solutions. The fluorescence is expressed in arbitrary units.

Fig.3. Linearity of the Cary Eclipse Fluorescence Spectrophotometer: dependence of the fluorescence intensity on the NAD(P)H concentration.

Fig. 4. The UVAPS spectra of the aerosols generated from the 100 μg/ml solutions: A) NADH; B) NADPH; C) riboflavin. The concentrations (dW) are given in particles/cm^3 and display interval particle size distribution. The concentration in any channel represents the concentration within the particle size boundaries for that channel.

Fig. 5. Particle distribution between the UVAPS channels: A) for aerosols generated from 10 μg/ml solutions; B) for aerosols generated from 100 μg/ml solutions. The particles concentration expressed as a proportion of particles detected in a particular channel to the total number of particles on a percentage basis.

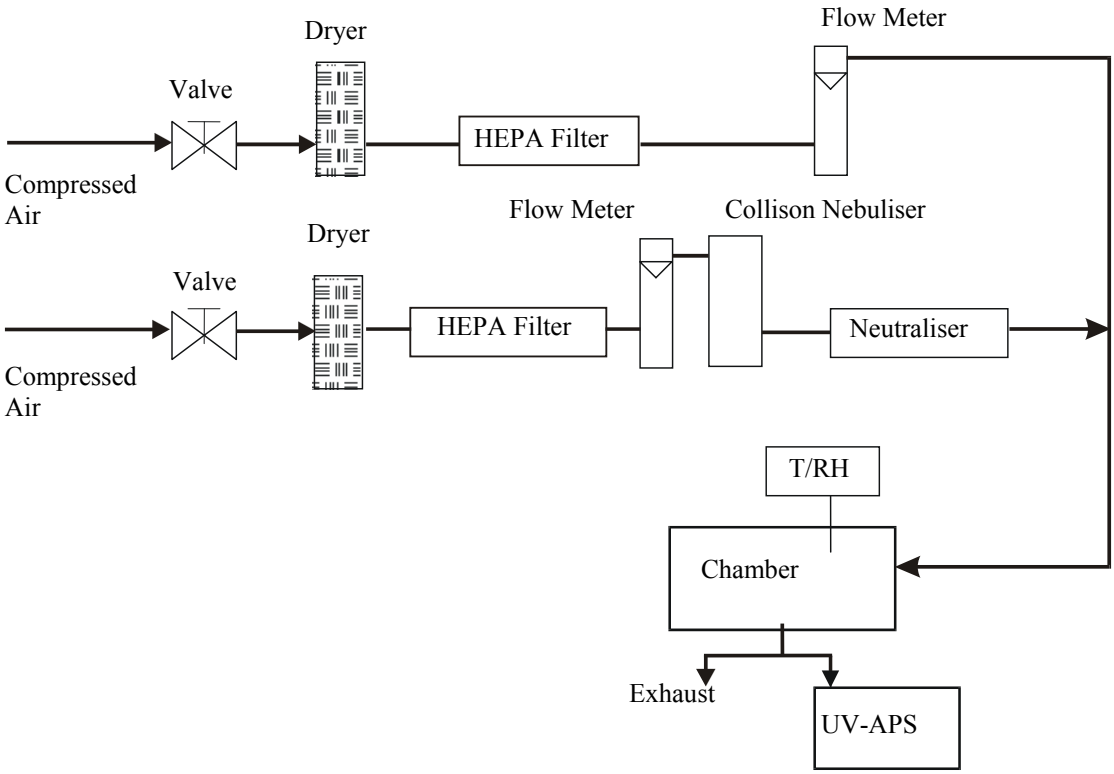
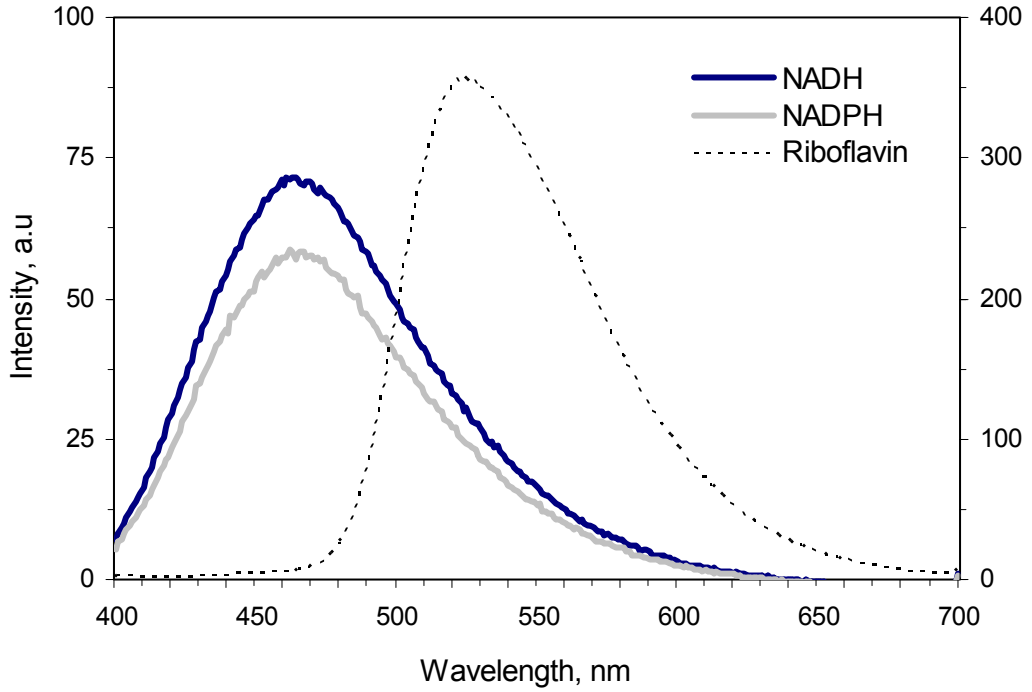


Fig. 1. Schematic diagram of the experimental set-up.

A)



B)

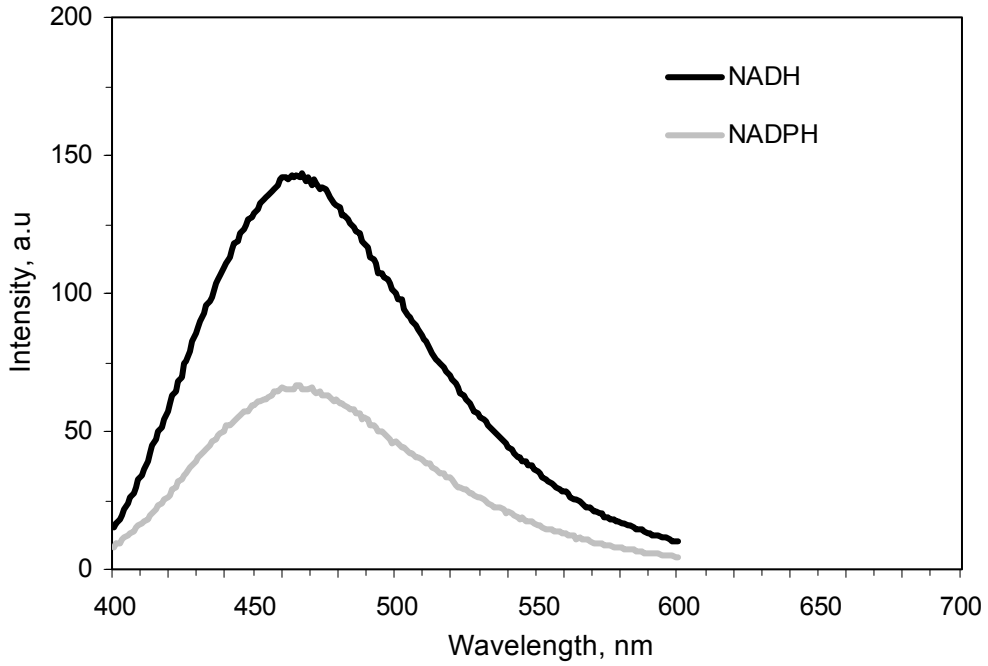


Fig. 2. Emission spectra measured with the Cary Eclipse Fluorescence Spectrophotometer (excitation at 355 nm): A) for 30 μg/ml solutions; B) for 300 μg/ml solutions. The fluorescence is expressed in arbitrary units.

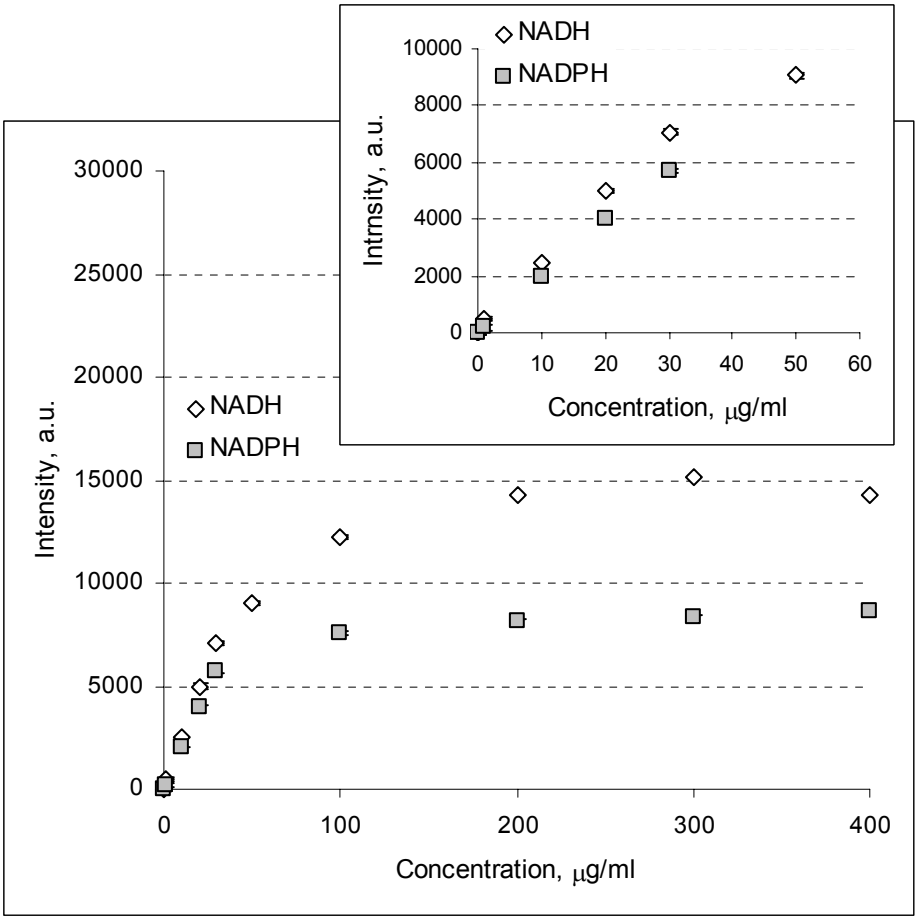
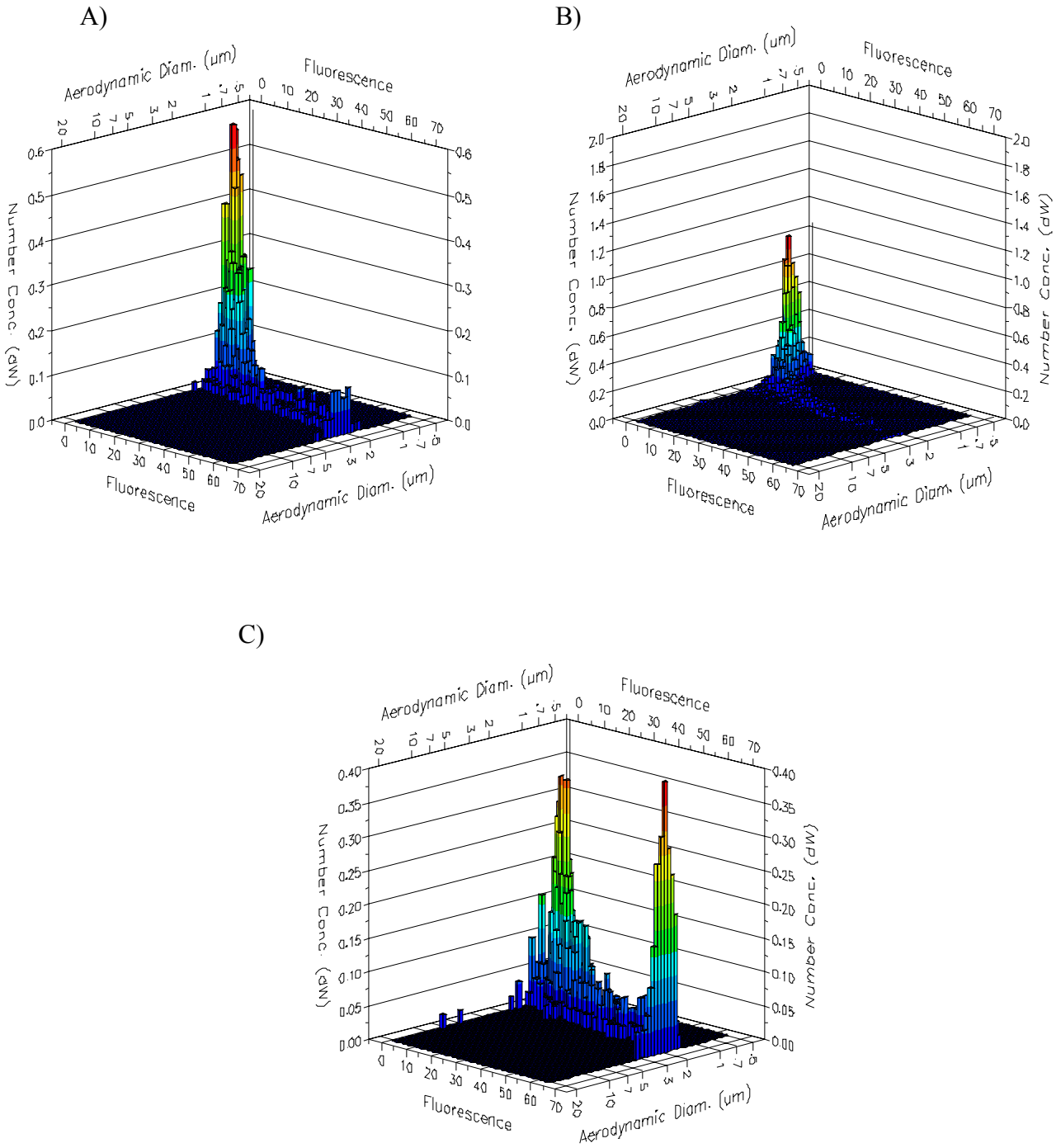


Fig.3. Linearity of the Cary Eclipse Fluorescence Spectrophotometer: dependence of the fluorescence intensity on the NAD(P)H concentration.



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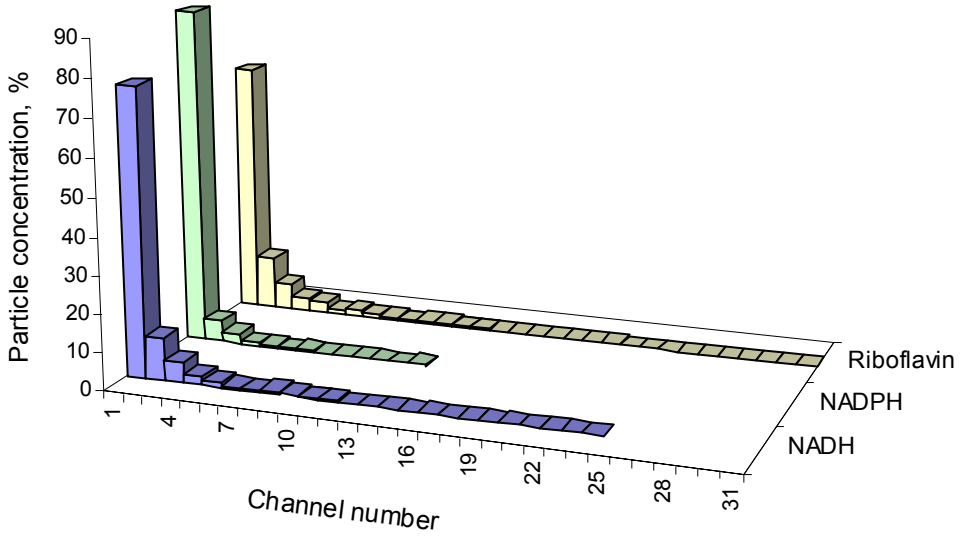
A)



B)

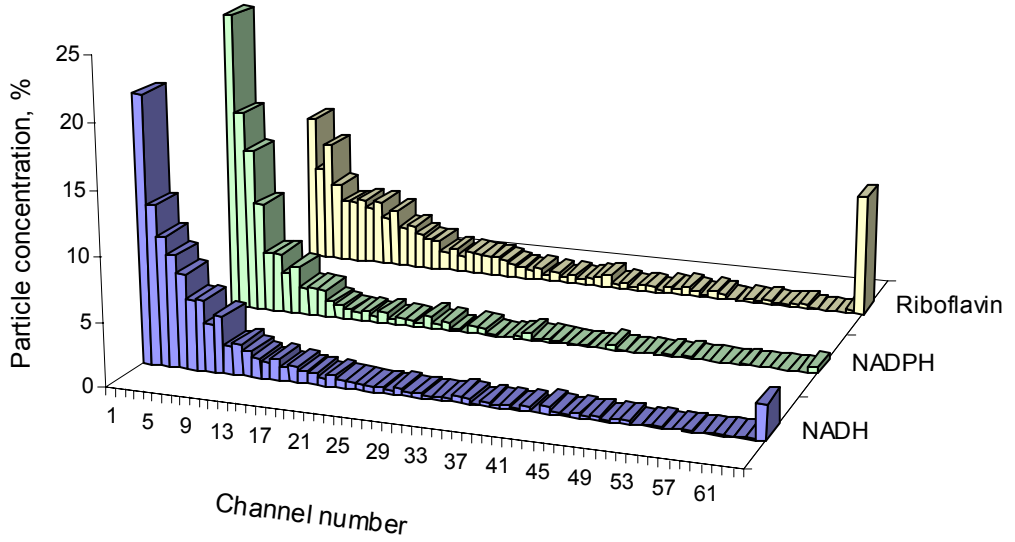


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Table 1. Characteristics of the aerosols used to determine the limit of detection.

Fluorophore	Molecular mass, g	Concentration of solution [a], μg/ml	CMD [b], μm	Concentration of fluorescent particles [c], %
NADH	709.4	3	1.68 (0.07)	2.8 (0.9)
NADPH	833.4	5	1.76 (0.07)	3.7 (1.2)
Riboflavin	376.4	1	1.64 (0.07)	3.9 (0.9)

[a] This is a minimal concentration of the solutions used to generate a particular aerosol, at which the fluorescent signals were higher than the background.

[b] Count Median Diameters of the fluorescent particles; standard deviation values are given in brackets.

[c] So called percent fluorescence (proportion of the fluorescent particles detected in the channels from 2 to 64 to the total particles detected in the channels from 1 to 64).

Table 2. Concentration of NAD(P)H coenzymes within bacterial cells.

Microorganism	NADH		NADPH	
	μmoles/g [a]	x 10^{-14}, mg [e]	μmoles/g	x 10^{-14}, mg [e]
P. fluorescens	0.19 (0.08) [b]	2.8	0.04 (0.01) [b]	0.7
	1.35 [c]	20.1	0.26 [c]	4.5
	0.2 [d]	3.0	0.3 [d]	5.2
B. subtilis	0.35 (0.14) [b]	5.2	0.70 (0.12) [b]	1.2
	1.5 [c]	22.3	0.17 [c]	3.0

[a] For a gram of dry weight of bacterial cells.

[b] London & Knight 1966.

[c] Takebe & Kitahara 1963.

[d] Kaplan 1960.

[e] Calculated taken into account molecular mass of the fluorophore, the size of bacteria, and assuming that 1 g of dry mycelium occupies approximately 2.5 ml (Brody 1972).