White Paper: Evaluating Real Time Viability Detectors

Optical Spectroscopy Instruments Can Analyze Particles In Real Time. Understanding Performance Parameters Can Help In Instrument Selection.

Rapid microbiological methods (RMM) have been around for many years but have yet to make a significant impact on pharmaceutical manufacturing processes despite the advantages they offer in terms of cost savings and process improvement. The majority of RMM's are laboratory-based instruments that significantly reduce the time to obtain quantitative, or qualitative, microbiological results when compared to traditional culture plate methods. Most of these techniques require the collection of sample that must be prepared for analysis by the RMM device. A relatively new RMM entry is optical spectroscopy, which analyzes aerosol particles for viability on a particle- by-particle, real time basis.

Real time aerosol optical spectroscopy instruments use laser-induced fluorescence (LIF) to determine if a particle is viable or inert. The operational theory is based on the fact that viable cell metabolites fluoresce when excited by ultraviolet light. The principal metabolites targeted by aerosol LIF- based instruments are tryptophan, NADH, and flavins (riboflavin). LIF instruments have been produced for more than 20 years and were originally developed for military and homeland defense applications. Only recently has this technology been adapted for use in the pharmaceutical industry.

Several optical spectroscopy based real-time viable particle detectors are currently available on the market. While based on similar fundamental operating principles, they have different engineering approaches that require consideration when evaluating them for use in pharmaceutical manufacturing environments. A basic understanding of these critical operating parameters allows for the development of more effective instrumentation evaluation criteria, provides a better foundation for identifying applications that offer the largest potential benefit, and supports development of effective verification and validation programs.

As with most instrumentation design, there are tradeoffs associated with implementation of core technologies. LIF-based instruments can be characterized by four key operational parameters:

- Flow Rate
- Aerosol Efficiency
- Sensitivity
- False Positive Performance (discrimination)

Sample flow rate and aerosol efficiency are interrelated, as are sensitivity and false positive performance.

FLOW RATE VERSUS AEROSOL EFFICIENCY

Due to the low intensity of emitted fluorescence, particles must remain in the optical interrogation region for a sufficient time to raise the fluorescence emission to detectable levels. This requirement limits the sample flow rate that an instrument can achieve in the fluorescence measurement section of the instrument. Depending upon the optical design, flow rates of up to 5 L/min can be achieved. Higher flow rate instruments must reduce the flow rate of the particles in the fluorescence detection region. Aerosol concentration is the enabling technology.

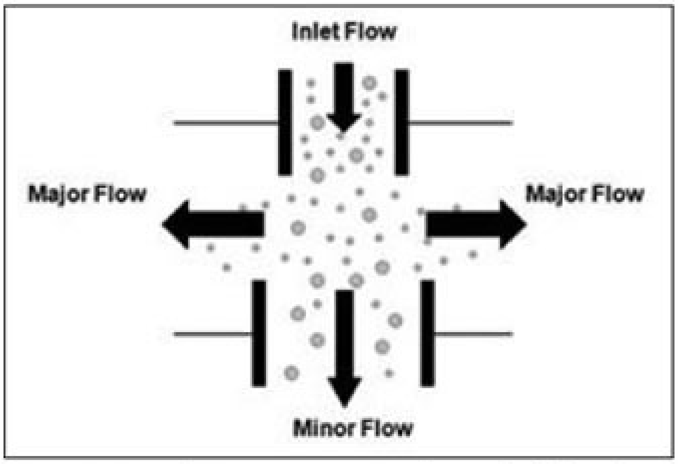

Figure 1: Aerosol Concentration: Small particles follow the major flow path and are not analyzed; large particles with greater inertia follow the minor flow and are subsequently analyzed.

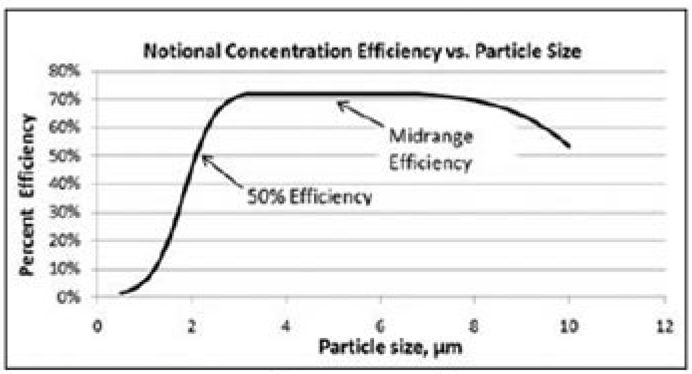

Figure 2: Concentrator Characteristic Efficiency Curve

Aerosol concentrators utilize particle inertia to concentrate larger particles of interest into a reduced flow volume at lower velocity. The incoming sample flow is separated into a high (major) volume flow that is exhausted from the system and a low (minor) volume flow that is analyzed. Smaller, low inertia particles follow the high volume flow path while larger, high inertia particles follow the low volume, analyzed flow path. Figure 1 illustrates the operating principles of a concentrator.

Two key parameters define concentrator performance: aerosol efficiency and D50 cut point. Figure 2 shows aerosol efficiency on the Y- axis and particle size on the X-axis. Aerosol efficiency is defined as the percentage of particles remaining in the minor (analyzed flow) flow versus the total number of particles present at the inlet of the concentrator.

The D50 is the particle size where 50% of the particles contained in the incoming flow are carried forward into the low volume analyzed flow. The midrange efficiency is the percentage of incoming particles above the D50 carried forward into the low volume analyzed flow. The diversion of smaller particles into the exhaust flow is not a major concern since the majority of biological particles range in size from 2 to 10 µm. The midrange efficiency is an important parameter since not all the particles in the incoming sample are analyzed; their numbers are reduced as described by the efficiency of the concentrator.

Aerosol efficiency is an important parameter to consider when evaluating high flow rate viability detectors. The user must be aware of how the analyzed particle distribution differs from the ambient particle distribution. For instance, if a concentrator is 70% efficient, the effective sampling flow rate of a 28.3 L/min instrument could be considered to be 19.8 L/min (0.70 efficiency x 28.3 L/min inlet flow). Active air samplers are characterized by the same operational parameters as concentrators and the pharmaceutical industry employs them extensively. The aerosol efficiency of high flow rate viable particle detectors should be considered in the same manner.

Understanding the aerosol efficiency of the active air sampler used when evaluating the performance of a viable particle detector is also important. In most cases, a company's active sampling protocol and agar plate culture methodology serves as the reference. It is important to be cognizant of the aerosol efficiency of both the viability detector under test, and the reference (active air sampler) to which it is being compared, as their relative efficiency can play an important role in drawing conclusions from the test.

Actual Particle	Classified As:	Result
Non-Viable	Non-Viable	True Negative
Non-Viable	Viable	False Positive
Viable	Viable	True Positive
Viable	**Non-Viable**	**False Negative**

Table 1 illustrates the four possible particle analysis outcomes

SENSITIVITY AND DISCRIMINATION

LIF-based techniques are non-specific and species information cannot be obtained due to the broad fluorescence response of viability metabolites. Due to the non-specific nature of the technique, non-viable fluorescing particles such as pollens and papers have the potential to be classified as viable particles. Table 1 illustrates the four possible particle analysis outcomes.

There is a tradeoff between false positive performance and false negative performance. The user must understand the tradeoff between sensitivity (ability to detect very low levels of biological particles) and discrimination (ability to limit the number of false positives). The pharmaceutical application environment has a large influence on this performance. In critical high-grade areas, a high sensitivity is required so any viable particles are detected (false negative). In lower grade areas that have larger acceptable levels of biological particles, a less sensitive setting might be preferred to limit the number of false positives. The user should understand how the environmental background conditions contribute to the false positive versus false negative performance tradeoffs. Multiple sensitivity settings offer the potential to optimize performance according to the instrument application space.

The critical performance parameters described influence the evaluation and validation of real time viable particle detectors. USP 1223 and EP5.1.6 contain guidance for evaluating RMM methods, but focus on evaluating alternative methods post sample collection.

Real time viable particle detector evaluation must consider not only the detection performance once the particles reach the fluorescence detection optics, but also the aerosol efficiency describing how many particle reach the viability detection region of the instrument. Additionally, the aerosol efficiency of the reference active air sampler affects conclusions when conducting comparability studies.

Finally, generation of biological test aerosols is challenging, especially when compared to the serial dilution methods referenced in current RMM guidance. The user must consider the aerosol generation and sample introduction path to the instrument. Vendor published test data should have the challenge aerosols introduced to the viability detector in a manner that mimics how the instrument will sample environmental aerosols. The false positive versus false negative performance must be considered in relationship to the operating environment of the instrument.

Instruments from different vendors incorporate different core parameters and algorithms to determine whether a particle is viable or non-viable. It is important to understand the impact on performance and tradeoffs made by the vendor during instrument development. Although there are many other factors to consider when evaluating real time viable particle detectors, aerosol efficiency and its relationship to sample flow rate and the false positive versus false negative tradeoff are important for the user to understand when evaluating real-time viable particle detectors

Darrick Niccum *is a senior global product manager-biotechnology for TSI Incorporated. He has been involved in development of particle detection instrumentation, including fluorescence based viability detections for over 12 years. TSI Incorporated, 500 Cardigan Road, Shoreview, Minn. 55126. Email: DNICCUM@TSI.com.*

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