Instantaneous Microbial

Real-time bioburden monitoring promotes risk reduction and process control.

Water is utilized abundantly to process, formulate, and manufacture pharmaceutical products. Traditional culture-based methods used to ensure water quality, however, are ill-suited in providing a robust assessment of risk and control. These methods are plagued by limitations in sensitivity, episodic sampling, and retrospective results. New technologies based on laser-induced fluorescence (LIF) detect intrinsic fluorescence instead of growth, can operate continuously, and deliver real-time results. As applied to pharmaceutical water quality, LIF-based, instantaneous microbial detection technologies enable real-time bioburden monitoring, risk reduction, and process control.

The pharmaceutical industry continues to recognize a need to leverage modern technologies to advance the course of risk reduction and process control. This forward thinking has been captured in industry relevant guidance such as the FDA’s 2004 “Guidance for Industry” document on Process Analytical Technology (PAT), ICH Guidelines Q8, Q9, and Q10, and the FDA’s “Pharmaceutical CGMPs for the 21st Century,” which encourage the adoption of quality by design (QbD) principles and new technologies. More recently, working groups composed of representatives from key pharmaceutical companies have also joined forces to help articulate their needs in water quality assessment and encourage the development and use of new technologies best suited to today’s tasks.

Need for an online pharmaceutical water assessment tool

The currently accepted and primarily practiced method for assessing water quality throughout a pharmaceutical water loop is through samples obtained at points-of-use (POU), utilizing traditional culture-based methods. The goal of such testing is to ensure the quality of an entire water system; however, POU testing can occur as infrequently as once every two weeks at each sample point. This limited sampling frequency, combined with the retrospective nature of culture-based methods, make a robust and timely assessment of risk and control difficult. Furthermore, there is the potential for sample contamination during collection (a false positive), and for a false-negative result due to limitations in sensitivity of culture-based methods. While growth-based methods offer the opportunity for identification, a number of organisms go undetected, such as viable but non-culturable organisms, due to the chosen medium and incubation parameters. A complementary technology capable of real-time and continuous monitoring of water system bioburden, based on a different method of detection, could alleviate such limitations and aid in risk reduction and process control.

Online pharmaceutical water bioburden analyzer

With an aim to improve the tools being applied to pharmaceutical waters, an Online Water Bioburden Analyzer (OWBA) Workgroup recently outlined user requirements, a testing protocol, and business benefits to guide the development of an OWBA system. This workgroup, composed of representatives from seven major pharmaceutical companies, has a mission to aid instrumentation vendors in the creation of an online water bioburden analyzer that satisfies both industry and regulators. They believe, “an online water bioburden analyzer has the potential to eliminate sampling and testing errors via reduced manipulations while providing increased product safety and process control through the availability of statistically significant data.” According to the group, such an OWBA system is not primarily designed to eliminate compensatory water testing, but should be used as a risk reduction tool. Potential business benefits are shown in Table 1 and include energy savings, labor reduction (resource allocations), and increased product quality and process understanding.

Table 1: Business benefits summarized in the OWBA Business Benefit Estimates document.

<table>
<thead>
<tr>
<th>Online Water Bioburden Analyzer Business Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Savings</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Labor Reduction</td>
</tr>
<tr>
<td>Product Quality &amp; Process Understanding</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Technical system requirements are provided, which include specifications for bioburden sensitivity, calibration, chemical compatibility, operating parameters, and needed consumables. Also included is a requirement for a limit of detection (LOD) equivalent to that set forth for culture-based methods (10 CFU/100mL) and analysis modes that include continuous sampling, time-based sampling, and daily operation at designated times. Overall, the system should be capable of continuous and periodic monitoring of critical control points (CCP).
Detection for Water

and POU, with sufficient sensitivity to detect microorganisms in water and limited susceptibility to potential interferents such as rouge, residual sanitizer, and gasket materials.

**Laser-induced fluorescence**

One technique capable of satisfying the OWBA requirements is laser, or light, induced fluorescence (LIF). LIF is a spectroscopic technique capable of high sensitivity in the detection of compounds that fluoresce. Fluorescence is the luminescence that occurs with the absorption of radiation at one wavelength followed by the emission of radiation at a different wavelength. Substances that typically fluoresce may be referred to as fluorophores. Quinine is a familiar fluorophore due to its presence in tonic water.

The application of LIF to detect microorganisms has been leveraged in flow cytometry, capillary electrophoresis, solid-phase cytometry, adenosine triphosphate bioluminescence, and growth-based auto fluorescence. In a number of these techniques, microorganisms are dyed to increase the measurable fluorescence. Measuring the intrinsic fluorescence of a microorganism removes the requirement for dyes and sample preparation, but requires an instrument with significant sensitivity. As lasers of additional wavelengths at higher power levels have become commercially available, LIF has become very relevant in applications requiring detection of low levels of microbial intrinsic fluorescence.

A light source such as a laser is the excitation source in LIF. A laser of appropriate wavelength and intensity is capable of inducing intrinsic fluorescence emission from microbes due to constituent fluorophores such as tryptophan, nicotinamide adenine dinucleotides (NADH), and flavins that are present in microorganisms. The target excitation wavelength is based on the excitation spectra of target fluorophores such that sufficient fluorescence intensity is induced for measurement and a greater number of non-biologic materials may be excluded. Yet, non-biologic materials such as plastics, rubbers, and paper can also fluoresce pointing to the importance of software discrimination algorithms.

**OWBA: Instantaneous microbial detection technology for water**

An OWBA system based on LIF enables the instantaneous detection of microbes in water, without the need for consumables and the limitations presented by traditional testing methods. Commercially available systems for water employ a 405nm laser to simultaneously induce Mie scatter and intrinsic fluorescence, on a particle-by-particle basis, as a sample travels along a flow path and traverses this excitation source. Detection and correlation of the Mie Scatter and fluorescence signals provide real-time information on the presence and biologic status of particles. Detection based on the intrinsic fluorescence of microorganisms removes requirements for sample preparation. Furthermore, this fundamental method of detection is inherently different from traditional growth-based methods, and is not susceptible to the growth-based limitations resulting from improper media selection and incubation.

In LIF-based systems, intrinsic fluorescence is captured on a photomultiplier tube (PMT), a detector highly sensitive to light. Both one-PMT and two-PMT designs are available. In water, two-PMT designs provide better discrimination of non-biologic fluorescing materials such as rouge, as requested in the OWBA requirements. Each material and microorganism has a different excitation and emission spectrum. Once an excitation wavelength has been chosen, some materials show a broad fluorescence emission and others a narrow emission spectrum. Similarities in the emission spectra of biologic versus non-biologic materials can be used advantageously. Figure 1 contains the emission spectra of certain biologic and non-biologic materials with 405nm excitation. Two notional PMT detection regions have been highlighted on either side of a Raman band in this figure. The Raman band represents fluorescence produced from the interaction of the laser light with water. Therefore, in order to detect particulate within the interrogated water stream, this band must be avoided in the detection regions utilized by the system. With 405nm excitation, the Raman band for water has a maximum at approximately 469nm.

![Figure 1: Emission spectra of two microorganisms and eight materials with 405nm excitation. An approximate Raman band for water and two example detection ranges for an instantaneous microbial detection system with two PMTs are labeled.](image-url)
With two PMT detection regions, the differences in non-biologic versus biologic emission spectra can be utilized to aid in the classification of non-biologic materials as inert. As shown in Figure 2, a particle’s scatter and fluorescence signals can be combined to create a three-dimensional map of interferent and biologic particles. Advanced algorithms can then be utilized to aid in the discrimination of biologic and interferent materials.

Figure 2: With a scatter detector and two fluorescence detectors (PMTs), an instantaneous microbial detection system for water can create a three-dimensional plot of biologic and interferent particles. Through assessment of the three different signals and an advanced processing algorithm, such a system offers enhanced interferent discrimination capabilities.

Real-time bioburden monitoring, risk reduction, and process control

The use of an instantaneous microbial detection system for pharmaceutical water provides the ability to monitor bioburden continuously and in real-time, resulting in an increased potential for risk reduction and process control. Figure 3 shows representative data from the IMD-W™, a system designed with the OWBA requirements in mind, comparing IMD-W biologic counts to culture plate results for three OWBA suggested organisms. This data covers a wide dynamic range and speaks to the potential sensitivity and ability of such systems to monitor bioburden.

Figure 3: Representative data from the IMD-W system showing IMD-W biologic counts as compared to colony forming unit culture results obtained using the traditional method with TSA plates.

The continuous data offered by these systems creates a robust historical dataset that is ideally suited for trending, particularly when compared to episodic sampling with traditional methods. Sampling considerations set forth in "USP<1231> Water for Pharmaceutical Purposes" recommends monitoring pharmaceutical water systems at a frequency "sufficient to ensure that the system is in control and continues to produce water of acceptable quality." The general information chapter states it is best to operate monitoring instrumentation in a continuous mode such that a large volume of in-process data can be generated, and suggests the use of trend analysis as an alert mechanism for loop maintenance.

A combination of historical trending data and real-time results enable users to identify an out-of-specification event or deterioration in microbiological control significantly earlier than with traditional sampling methods. By continuously monitoring the state of control, timely loop maintenance can be performed if bioburden data trends upward, permitting further risk reduction and an increased level of loop control. A real-time and historical knowledge of control can also be important during a POU testing deviation. If POU testing is positive for microbial contamination, knowledge and data to support a state of control may narrow the root-cause investigation to the POU as opposed to contamination in the entire loop.

Conclusions

Regulatory guidance and calls from industry work groups support the need for better tools for pharmaceutical water monitoring. New instantaneous microbial detection systems based on LIF enable real-time bioburden monitoring, increased risk reduction, and process control for pharmaceutical waters. Through continuous monitoring, these systems provide significant historical data for robust trending and assessment of water loop bioburden levels, providing the means to monitor the level of control and react to out-of-specification events in a much more timely manner than with traditional methods alone. Users stand to benefit through increased product quality and process understanding, energy savings, and risk reduction.

References


Allison Scott is a senior applications engineer at BioVigilant, where she has worked since 2010. She is now part of dedicated team of technicians and scientists comprising Azbil North America Research and Development, Environmental Particle Solutions. She received her Ph.D. in Materials Science and Engineering from the University of Arizona and in Materials Chemistry from the University of Rennes.