Pharmaceutical Grade Water Investigation: What Are These Particles?

Additional water quality tests methods can deliver intelligent insight into pharmaceutical grade water systems. One such test method, only recently available, is online assessment of bioburden levels through use of online water bioburden analyzers (OWBAs). These systems offer real-time and continuous particulate and bioburden monitoring of the water being produced. Yet, because total particulate monitoring isn't performed during routine operations and the traditional culture-based method of microbial testing is unable to count all organisms potentially present, the count information provided by OWBA systems can lead to the question, *"What are these counts?"* Additional particulate analysis was performed on a pharmaceutical grade water system to reveal what current water quality testing did not find.

In-depth Water Analysis

An IMD-W™ OWBA system was installed on a pharmaceutical grade water loop return. Traditional, culture-based bioburden results were obtained and used for the comparison to OWBA data. Additionally, a water sample was given to an independent commercial laboratory for their standard water test analyses and included total organic carbon (TOC) values and viable bacteria counts. Scanning electron microscopy (SEM) particle count testing also was included to obtain additional water quality information. SEM/EDX (energy-dispersive X-ray spectroscopy) analysis provided the ability to enumerate biologic and non-biologic particles coupled with an elemental analysis of particulate in the sample.

Total Particulate and Bioburden

Total particulate and bioburden count information were obtained using the IMD-W system and, for the laboratory analysis, a Tescan Vega3 SEM. For the SEM sample, a 510 mL water sample was filtered using a 0.1 µm filter, and 338 total fields were counted. Biologic assignment was made based on the shape of the particle, with additional confirmation provided through EDX elemental analysis. The IMD-W particle and biologic count per mL results are based on a 100mL data sample, but the system was operated online and continuously sampled. A viable count was also obtained at the pharmaceutical facility during routine in-house point-of-use (POU) monitoring (labelled as *CFU/100mL* under IMD-W/POU in **Table 1**). The independent laboratory's *CFU/100mL* result represents the average of three 100mL replicates using the traditional culture-based method.

Table 1 provides IMD-W particle and biologic count/mL, and % biologic data for samples taken in November and March. Facility POU CFU/100mL data also is reported on these dates. The independent laboratory analysis was performed with water samples taken from the facility on the same day in March as the second IMD-W and POU samples were collected.

As can be seen in **Table 1**, biologic particles were found with the IMD-W system and in the laboratory's SEM/EDX analysis, while traditional culture results from the facility POU and independent lab tests consistently yielded zero CFU. The percentage of particle counts found to be biologic were 6% and 14.1% in the two IMD-W samples, and 8.3% in the laboratory's SEM/EDX analysis. As noted earlier, the IMD-W system was operated online while the independent laboratory and facility POU data are based on water samples collected from the water loop. Thus, the same water sample was not used for all analysis performed and, as a result, some variability is to be expected.

	IMD-W / POU (11/08/17)	IMD-W / POU (3/1/2018)	Laboratory (3/1/2018)
Particles/mL	2180	2319	904
Biologic/mL	131	327	75
% Biologic	6.0%	14.1%	8.3%
CFU/100mL	0	0	0

Table 1: *IMD-W and laboratory SEM total particle and biologic counts per mL reported with facility POU and independent laboratory traditional culture results. The IMD-W and SEM analysis both identify biologic counts in the water sample when the traditional method consistently reports 0 CFU.*

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SEM/EDX Particle Assessment

SEM also was used to gain visual images of particulate during counting, while EDX was used to obtain elemental information on these particles in an effort to identify the material. **Figure 1** shows images of two such particles. On the top is a rod-shaped particle that corresponds to the EDX elemental analysis data shown in the fourth row of data in **Table 2**. This particle is found to be composed of 80.6% carbon and 19.4% oxygen and, as a result, is one of the biologic particles included in the independent laboratory's biologic count per mL result shown in **Table 1**. The particle on the bottom in **Figure 1** corresponds to the third row of data in **Table 2**, and shows what is classified as a rouge particle, based on its composition of 61.8% carbon, 19.8% oxygen, 4.79% silica, 1.64% chromium and 12% iron. The first two rows of data in **Table 2** show the elemental composition of two other particles analyzed using EDX.

SEM/EDX Elemental Analysis					
	C	O	Si	Cr	Fe
SST	74.2	16.7		3.19	5.93
Si/SiC	80	11.9	8.03		
SST	61.8	19.8	4.79	1.64	12
Bacterium	80.6	19.4			

Table 2: *Laboratory SEM/EDX elemental analysis of four example particles within the water sample. SST indicates a stainless steel or rouge particle, Si/SiC a silica-based particle, and bacterium, a bacteria or biologic particle.*

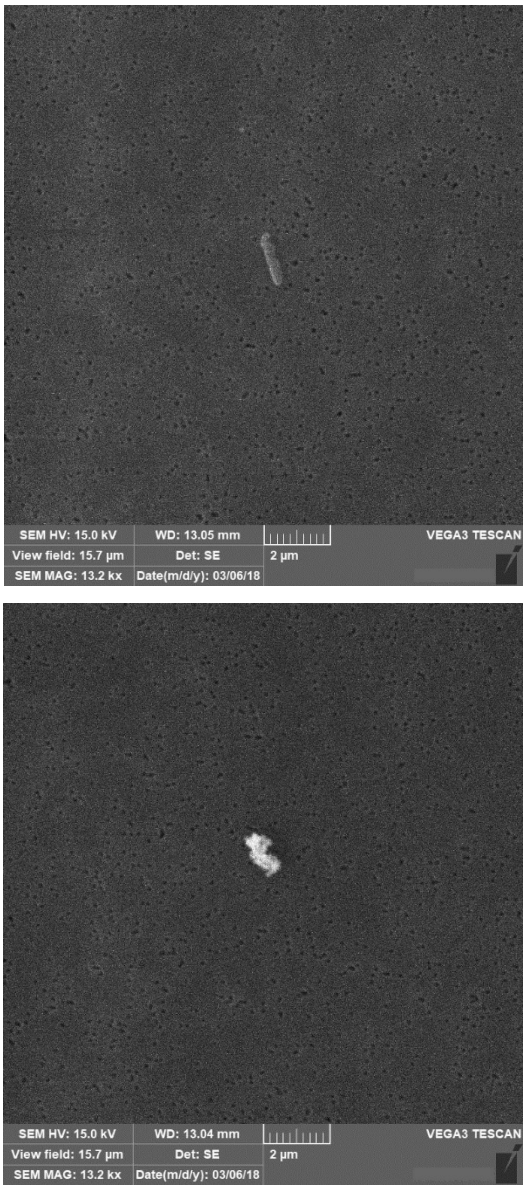

Figure 1: *SEM images of two particulate in the water sample. The rod-shaped particle on the top is identified as a bacteria (i.e. biologic particle) and the particle on the bottom as a rouge particle.*

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Conclusions

Additional water quality test methods can provide insight into particulate present in pharmaceutical water systems that are not detected by current routine water quality testing. The IMD-W system and SEM/EDX analysis both indicate the presence of biologics in the water system examined, despite consistent results of zero CFU with the traditional method, and TOC results of only 8.5 parts per billion. Although SEM/EDX analysis provides a spot check into particulate, the IMD-W system can be installed on a water system and operated continuously to obtain real-time bioburden and particulate counts. This added level of detailed information can enable intelligent insight into water system health and change, and provide support for maintenance decisions.

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