Lovibond® Water Testing

Understanding the Limit of Detection
Lovibond® TB Series of Portable Turbidimeters

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September 2021

Introduction:

For analytical method development, the limit of detection (LOD) and the limit of quantitation (LOQ) are important parameters that need to be determined during method development and validation for any analytical procedure. LOD is commonly defined as the lowest value measured by a method that is greater than the uncertainty associated with it[1]. In other words, the measured analyte concentration is distinguishable from the method blank value with confidence. LOQ is the lowest value that can be reliably identified and quantified with a certain degree of confidence.

An established practice for determining the LOD / LOQ is to utilize the signal-to-noise ratio (SNR) close to the baseline measurement. Herein, a SNR of 3:1 is used to calculate the LOD and a SNR of 10:1 is used to calculate the LOQ. The LOD is a good estimate of the lowest possible change in turbidity that the instrument can detect. LOD and LOQ are method dependent and must be considered when comparisons are made between different instruments. As an example, an instrument which does not allow baseline subtraction as part of the method cannot be compared to one that does simply by calculating the standard deviation at an incremental turbidity value above the zero or baseline measurement. Sample preparation, handling, and matrix will also affect the LOD and LOQ results, in addition to the instrument noise and stray light.

There are multiple acceptable approaches of calculating LOD and LOQ. One common recommendation is to use sample blanks. The LOD is determined as the mean of a sample blank plus 3 times standard deviation of sample blank. The LOQ is determined as the mean of a sample blank plus 10 times standard deviation of sample blank, or 3 times LOD. In turbidity measurement however, the blank value is never “zero” because of molecular light scattering at the measurement wavelength. An alternate method must be used to determine the lowest incremental change in turbidity that can be detected.

When calculating LOD for a turbidimeter, a standard of very low, but known, turbidity value is used as the means for determining the standard deviation close to the baseline. The LOD is thus defined herein as 3 times the standard deviation of the very low turbidity standard. The LOQ is similarly defined herein as 10 times the standard deviation of the of the very low turbidity value.

LOD & LOQ: Best Case Scenario

For turbidity measurement, many variables can affect LOD such as stray light, molecular scattering of a turbidity free sample blank, the defects and cleanliness of the glass vial, as well as vial orientation. A best case LOD and LOQ turbidity measurement can be achieved in the laboratory using a single vial that is cleaned and surface treated with silicone oil, with no rotation of the vial for subsequent replicate measurements.

For comparison purposes and to best match the real-world applications and usage, various other conditions in addition to the best-case condition were tested for:

1) Sample vials that are not cleaned before measurement
2) Sample vials that are rotated between measurements

Experiment

The instruments under test are the Lovibond® TB350 IR (Infrared Emitting Diode) and TB350 WL (White Light Emitting Diode) turbidimeters. First, data was collected for filtered DI water blanks from the online purification system that ensures the water has a turbidity value of at least 5 times less than the expected LOD., This is used to establish measurement error due to the condition of the vial. Ten glass vials were filled with the filtered DI water and capped. Both TB350 instruments were calibrated and verified using vials of known quality before collecting the vial variation test data on the filtered DI water. Ten repetitive measurements of each sample vial were recorded.

With this set of 10 vials filled with DI, data was collected in the following order:

1) Take 10 measurements on each vial. The vials were not cleaned with silicone oil, there were no rotations between measurements.
2) Take 10 measurements on each vial. The vials were not cleaned with silicone oil, rotated the vial to a slightly different position between measurements.
3) Take 10 measurements on each vial. The vials were cleaned with silicone oil, there were no rotations between measurements.
4) Take 10 measurements on each vial. The vials were cleaned with silicone oil, rotated the vial to a slightly different position between measurements.

The inside of all vials were cleaned consistently throughout this study. This experiment specifically tests the effects of cleaning or polishing the outside of the vial with silicone oil.

After collecting data on the filtered DI water, it was spiked with a known amount of 5 NTU formazin turbidity standard, in a 0.010 NTU increment interval, using a precision pipette. This was repeated four more times to reach a 0.05 NTU level. This will provide an evaluation of sensitivity at the very low turbidity level.

Data Summary

Shown in Table 1 and Table 2 are the average readings on DI water under each test condition using white LED and IrED turbidity instruments, respectively. Table 3 and Table 4 are the corresponding standard deviations. Based on these sets of data, the effects of different vials, vial orientation and vial cleanliness on the low-level turbidity measurements can be evaluated.

Effect of Vial Cleanliness:

It is recommended in the manual that the user clean the vial using silicone oil before each use to minimize the effect of scratch marks and surface dirt. The vials should be stored a general laboratory conditions when not in use.

When the turbidity measurements under the conditions of the same orientation and varying orientation were compared, it was found that the uncleaned vials had an average NTU value 0.002 to 0.004 NTU higher for the white LED turbidimeter and 0.004 NTU higher for the IrED turbidimeter when compared to results obtained with vial that were surface treated with silicone oil. The variation in the measurement value was also slightly higher in the condition of uncleaned vs. cleaned and surface treated vials when tested in various rotated orientations. Under these conditions the NTU standard deviation for the white LED turbidimeter is 0.0003 NTU higher and for the IrED instrument, 0.0008 NTU higher.

Effect of Vial Orientation:

Because of the nature of the turbidity measurements, any variation in the glass vial can affect light scattering and the subsequent resulting turbidity measurement. Lovibond® TB Series turbidimeters utilize the patent-pending MultiPath90˚ BLAC™ optical design to minimize this effect.

In the white LED turbidimeter, the turbidity reading has an average 0.002 NTU lower with the rotation compared to no rotation for the uncleaned vials, and 0.0007 NTU lower with the rotation compared to no rotation for the cleaned vials. This may be due to the starting orientation of the non-rotation vial having a slightly higher scattering or the difference could likely be within the error of measurements. In terms of the variations caused by the vial orientation, the standard deviation of rotation has an average of 0.00031 NTU higher than non-rotation for the uncleaned vials and 0.00004 NTU higher for the cleaned vials.

In the IrED turbidimeter, the turbidity readings have an average 0.0002 NTU lower with the rotation compared to no rotation for both cleaned and uncleaned vials. Again, this may be due to the starting orientation of the non-rotated vial having a very slightly higher scattering or the difference could likely be within the error of measurements. In terms of variations caused by the vial orientation, the standard deviation of rotation has an average of 0.0009 NTU higher than non-rotation for the uncleaned vials and 0.0001 NTU higher for the cleaned vials.

Effect of Sample Glass Vial:

Similar to the effect of vial orientation, each vial affects light scattering differently. From the data collected, the variation among the vials can be calculated. With one reading from 10 replicas of each vial under "clean, no rotation" test condition, the standard deviation is 0.002 NTU among the set of 10 vials for the white LED turbidimeter and 0.003 NTU for the IrED turbidimeter.

White LED Turbidimeter, Average Readings of Filtered DI Water, NTU										
Vials	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	Vial 9	Vial 10
no clean, no rotation	0.0395	0.0378	0.0386	0.0395	0.0392	0.0417	0.0364	0.0378	0.0378	0.0371
no clean, rotation	0.0398	0.0368	0.0358	0.0364	0.0345	0.0390	0.0354	0.0347	0.0346	0.0349
clean, no rotation*	0.0378	0.0341	0.0348	0.0372	0.0342	0.0382	0.0327	0.0338	0.0336	0.0332
clean, rotation	0.0374	0.0328	0.0343	0.0369	0.0346	0.0381	0.0330	0.0330	0.0310	0.0312

Table 1: Average of turbidity measurements using a white LED turbidimeter under 4 different test conditions.
*= best test condition of cleanliness with no vial rotation

IrED Turbidimeter, Average Readings of Filtered DI Water, NTU										
Vials	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	Vial 9	Vial 10
no clean, no rotation	0.0143	0.0115	0.0137	0.0127	0.0134	0.0157	0.0126	0.0123	0.0116	0.0109
no clean, rotation	0.0161	0.0125	0.0133	0.0125	0.0112	0.0147	0.0123	0.0114	0.0112	0.0111
clean, no rotation*	**0.0135**	**0.0071**	**0.0099**	**0.0114**	**0.0087**	**0.0127**	**0.0064**	**0.0071**	**0.0058**	**0.0068**
clean, rotation	0.0143	0.0082	0.0094	0.0123	0.0087	0.0107	0.0066	0.0062	0.0050	0.0060

Table 2: Average of turbidity measurements using an IrED turbidimeter under 4 different test conditions.
*= best test condition of cleanliness with no vial rotation

White LED Turbidimeter, Standard Deviation Readings of Filtered DI Water, NTU										
Vials	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	Vial 9	Vial 10
no clean, no rotation	0.0143	0.0115	0.0137	0.0127	0.0134	0.0157	0.0126	0.0123	0.0116	0.0109
no clean, rotation	0.0161	0.0125	0.0133	0.0125	0.0112	0.0147	0.0123	0.0114	0.0112	0.0111
clean, no rotation*	**0.0135**	**0.0071**	**0.0099**	**0.0114**	**0.0087**	**0.0127**	**0.0064**	**0.0071**	**0.0058**	**0.0068**
clean, rotation	0.0143	0.0082	0.0094	0.0123	0.0087	0.0107	0.0066	0.0062	0.0050	0.0060

Table 3: Standard deviation of turbidity measurements using a white LED turbidimeter under 4 different test conditions.
*= best test condition of cleanliness with no vial rotation

IrED Turbidimeter, Standard Deviation Readings of Filtered DI Water, NTU										
Vials	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	Vial 9	Vial 10
no clean, no rotation	0.0008	0.0007	0.0005	0.0006	0.0006	0.0013	0.0009	0.0006	0.0006	0.0008
no clean, rotation	0.0023	0.0016	0.0020	0.0015	0.0017	0.0019	0.0021	0.0010	0.0016	0.0010
clean, no rotation*	**0.0006**	**0.0007**	**0.0007**	**0.0010**	**0.0006**	**0.0009**	**0.0008**	**0.0010**	**0.0007**	**0.0005**
clean, rotation	0.0004	0.0010	0.0011	0.0014	0.0006	0.0012	0.0009	0.0010	0.0007	0.0005

Table 4: Standard deviation of turbidity measurements using an IrED turbidimeter under 4 different test conditions.
*= best test condition of cleanliness with no vial rotation

Thus, from all the data shown above, the variation from vials is much greater than that of vial orientation and cleanliness. Especially after the cleaning, the variation in the vial orientation is insignificant.

LOD Summary

For typical water analysis methods, LOD is derived from the formula: mean of sample blank plus 3 times standard deviation of sample blank. This method was based on methods in which a true blank value can be subtracted as part of the measurement protocol for determining a given result. In turbidity measurement, the blank value is typically not subtracted. The differences in baseline NTU values reported above for the two wavelength emitters are primarily the result of elastic light scattering from water molecules (molecular scatter), even in the absence of any turbidity. When an LOD value is determined for turbidity without blank subtraction, the practical use of the LOD is the sensitivity of the method. That is the smallest turbidity change above a turbidity free baseline that a given instrument can detect and the change is due to a change in turbidity above the instrument noise level that was part of the turbidity free baseline.

A referenced test method for LOD determination is found in the International Organization for Standardization (ISO) 15839[2]. This test instructs to spike an analyte to a concentration that is approximately 5% of the working range of the instrument. The variance of the measurement of that spike is ultimately used to derive the LOD. Since turbidimeter designs can have an overall range that often covers several orders of magnitude, the test can be modified to a spike level at 5% of the lowest applicable range. Since the lowest applicable operating range is typically 0-1 NTU for drinking water applications, then the spike should be approximately 0.05 NTU above the sample turbidity free baseline.

The DI water passing through a series of filters, including a 0.03-micron filter, was collected as the sample blank for baseline. The turbidity of this sample blank is a function of many factors, such as wavelength, water molecular scattering, glass sample vial. The theoretical value for the sample blank is 0.028 NTU on white LED and 0.012 NTU on IrED turbidimeters. This DI water sample was collected in ten different sample vials and ten baseline turbidity readings were taken on each vial. Then each vial was spiked with known amount of 5 NTU turbidity standard in a 0.01 NTU increment. With each spike, the turbidity measurements were repeated. These steps continued until a total 0.05 NTU was spiked and measured. Shown in Table 5 and 6 are the standard deviation of each vial at each spike using white LED and IrED turbidimeters. The standard deviation of each vial at the 0.05 NTU spike level and average standard deviation data from all 10 vials were calculated. This value was multiplied by 3 to derive the LOD, the value was multiplied by 10 to derive LOQ.

White LED: LOD = 0.0111 NTU, LOQ = 0.037 NTU
IrED: LOD = 0.0135 NTU, LOQ = 0.042 NTU

White LED Turbidimeter, Standard Deviation Readings of Low Levels Turbidity, NTU											
	Spiking, NTU	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	Vial 9	Vial 10
Filtered DI		0.0009	0.0008	0.0007	0.0005	0.0006	0.0008	0.0010	0.0009	0.0006	0.0006
Spike #1	0.010	0.0013	0.0017	0.0063	0.0011	0.0011	0.0037	0.0016	0.0017	0.0015	0.0015
Spike #2	0.020	0.0061	0.0018	0.0008	0.0015	0.0020	0.0023	0.0013	0.0016	0.0011	0.0013
Spike #3	0.030	0.0020	0.0021	0.0018	0.0028	0.0020	0.0049	0.0032	0.0017	0.0018	0.0029
Spike #4	0.040	0.0032	0.0009	0.0027	0.0015	0.0023	0.0023	0.0017	0.0048	0.0032	0.0025
Spike #5*	**0.050**	**0.0036**	**0.0062**	**0.0072**	**0.0027**	**0.0062**	**0.0032**	**0.0026**	**0.0021**	**0.0016**	**0.0016**

Table 5: Standard deviation of turbidity measurements using a white LED turbidimeter at each turbidity spike level for each vial.
*= standard deviation data at spiking of 0.05 NTU is used for calculating LOD and LOQ

IrED Turbidimeter, Standard Deviation Readings of Low Levels Turbidity, NTU											
	Spiking, NTU	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	Vial 9	Vial 10
Filtered DI		0.0006	0.0007	0.0007	0.0010	0.0006	0.0009	0.0008	0.0010	0.0007	0.0005
Spike #1	0.010	0.0013	0.0015	0.0011	0.0023	0.0015	0.0024	0.0032	0.0014	0.0013	0.0027
Spike #2	0.020	0.0039	0.0018	0.0038	0.0014	0.0014	0.0025	0.0022	0.0013	0.0011	0.0036
Spike #3	0.030	0.0049	0.0026	0.0025	0.0030	0.0018	0.0025	0.0016	0.0024	0.0034	0.0104
Spike #4	0.040	0.0057	0.0016	0.0032	0.0014	0.0015	0.0022	0.0013	0.0017	0.0028	0.0030
Spike #5*	**0.050**	**0.0040**	**0.0063**	**0.0035**	**0.0022**	**0.0056**	**0.0059**	**0.0031**	**0.0044**	**0.0056**	**0.0046**

Table 6: Standard deviation of turbidity measurements using an IrED turbidimeter at each turbidity spike level for each vial.
*= standard deviation data at spiking of 0.05 NTU is used for calculating LOD and LOQ

Conclusion

Turbidity is an important parameter for water treatment plants where the optimized filtration system is employed. For example, many water plants will establish filter effluent limits that are below 0.1 NTU, which is significantly lower than the USEPA 0.30 NTU regulatory limit for drinking water filtration[3]. Note: At levels below 1.0 NTU, US EPA typically allows the measurement to be rounded to the nearest 0.05 NTU[4]. Turbidity LOD/LOQ is a measurement strongly dependent on the method. It may vary between labs or within the same lab from time to time because of different instruments, samples matrix, techniques, and operators. When used according to the manufacturer's recommendations, the TB Series of turbidimeters deliver LOD and LOQ values that exceed the established criteria in existing regulatory requirements and demonstrate the detection and quantification sensitivity that is required for low-level turbidity measurements.

References

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