

# DIRECT TURBIDITY MEASUREMENT USING PHOTOPETTE®

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- Photopette® makes measurement of turbidity efficient.
- Simple step-by-step measurements following USEPA measurement at 570 nm and ISO 7027 at 850 nm.

## OBJECTIVE

The objective of this application note is to demonstrate how Photopette® can be used for direct turbidity measurement of unknown liquid samples. Furthermore, it acts as a handy guide to get you started with Photopette®, and outlines application-specific parameters for reference.

## INTRODUCTION

Determining Turbidity of an aqueous solution is a frequently used procedure to estimate the quality of water, or even concentration of suspended particles such as cells, bacteria and mud.

Turbidity can be defined as a decreased in the transparency of a solution due to the presence of suspended and some dissolved substances, which causes incident light to be scattered, reflected and attenuated rather than transmitted in straight lines; the higher the intensity of the scattered or attenuated light, the higher the value of turbidity [1].

Nephelometric turbidity units (NTU) are often used to express turbidity. NTU can be defined as the intensity of light at a specified wavelength scattered or attenuated by suspended particles or absorbed at a method-specific angle (90 degrees), from the path of the incident light compared to a synthetic chemically prepared standard [1].

Following the USEPA method and ISO 7027 method, the turbidity is measured at wavelengths of 570 nm and 850 nm respectively. This application note highlights the measurements of OD at these two wavelengths using the Photopette®.

## MATERIALS AND APPARATUS

### Instruments:

- Photopette® custom with 570 nm and 850 nm wavelengths.

### Reagents:

- Turbidity 4000 NTU Calibration Standard - Formazin (Sigma Aldrich #TURB4000)
- De-ionized water

## METHOD

### EXPERIMENTAL PROCEDURE

Turn-on the Photopette® device and connect to the Photopette iOS/Android app. Refer to the “Photopette User Manual” for operating and safety precautions [2]. Select ‘Turbidity’ as the measurement type. Select dataset and set additional settings (if needed) before selecting ‘Start Measurement’. Please follow the video-tutorials available at [www.tipbiosystems.com](http://www.tipbiosystems.com) to get familiar with the measurement process.

Place a CuveTip® firmly onto the Photopette® device probe and insert into the blank sample to perform auto-zero. Ensure that there is no air-bubble trapped in the CuveTip® cavity. Presence of air bubbles may disrupt the optical path and cause errors. A guide to use the CuveTip® correctly is available for download [3]. Use the same CuveTip® to measure the turbidity standard solutions from low to high concentrations. Ensure that there is no liquid transferred between samples by contacting the CuveTip® with a tissue paper and remove any liquid by capillary action. The prepared formazin solutions with varying turbidity formazin solutions were vortexed to ensure a uniform mixing before dilution or measurement. The formazin solutions were measured immediately after vortex-mixing using Photopette® device. The same procedure was repeated for the sample with unknown turbidity (NTU).

### DETERMING CONCENTRATION OF AN UNKNOWN SAMPLE

Depending on the estimated unknown turbidity (NTU), prepare several dilutions. Use the dilutions as duplet or triplet samples as explained above. The turbidity (NTU) is calculated by substituting the OD value obtained into the equation of the standard curve and factoring in the dilution factor.

## RESULTS AND DISCUSSIONS

Table 1 below shows the obtained OD values at 570 nm as a function of the turbidity standard solutions.

Formazin Standard (NTU)	Average OD at 570 nm (OD)	Standard Deviation (SD)
4000	3.50	0.000
3000	3.44	0.010
2000	2.67	0.005
1000	1.54	0.008
800	1.26	0.005
500	0.86	0.009
400	0.68	0.005
300	0.49	0.003
200	0.35	0.002
100	0.16	0.003
50	0.05	0.001

Table 1: OD values taken at 570 nm with turbidity standard solutions of average of 5 repeats.

Table 2 below shows the obtained OD values at 850 nm as a function of the turbidity standard solutions.

Formazin Standard (NTU)	Average OD at 850 nm (OD)	Standard Deviation (SD)
4000	2.49	0.010
3000	2.06	0.010
2000	1.48	0.012
1000	0.77	0.004
800	0.62	0.002
500	0.41	0.020
400	0.29	0.004
300	0.23	0.004
200	0.13	0.006
100	0.09	0.010
50	0.00	0.001

Table 2: OD values taken at 850 nm with turbidity standard solutions of average of 5 repeats.

The data obtained in Table 1 and 2 were used for generation of standard curves, and to determine experimental parameters such as upper measurement-limit, limit of detection and linear range. The OD values in Table 1 were plotted in Figure 1 and the data from Table 2 were plotted in Figure 2.

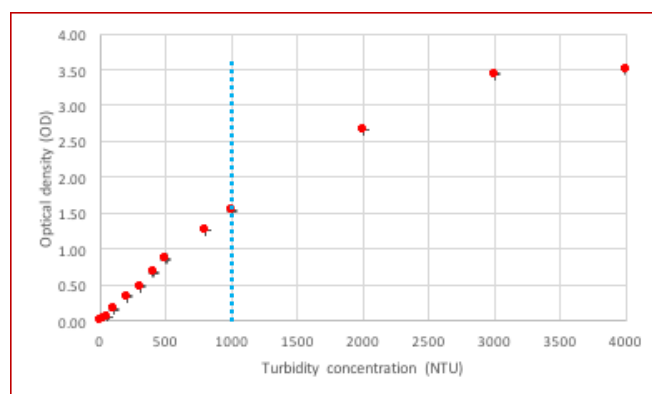


Figure 1: OD at 570 nm as a function of turbidity standards.

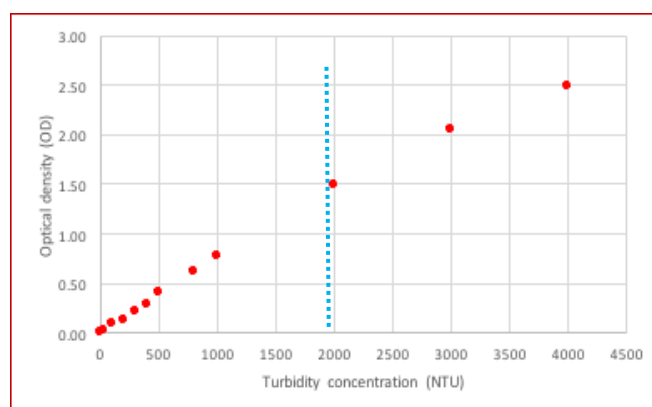


Figure 2: OD at 850 nm as a function of turbidity standards.

## EXPERIMENTAL PARAMETERS

### Upper measurement-limit and Linear Range

From Figure 1, we can deduce that the readings tend to saturate beyond 1000 NTU (indicated by the dotted line). Therefore, it is not recommended to include data beyond 1000 NTU in the standard curve as the measurement accuracy will be lower. Regression analysis indicates the linear range to be 50 NTU to 1000 NTU solution. The OD of 50 NTU prepared standard is below the recommended specification of the Photopette® Device. Thus, it was not included for further preparation of standard curve.

From Figure 2, we can deduce that the readings tend to saturate beyond 2000 NTU. Regression analysis indicates the linear range to be 200 NTU to 2000 NTU solution. The OD of 200 NTU prepared standard is below the recommended specification of the Photopette® Device. Thus, it was not included for further preparation of standard curve.

## STANDARD CURVE

A standard curve was plotted in Figure 3 for the measurements obtained at 570 nm within the linear range of

50 NTU to 1000 NTU. Similarly, a standard curve was plotted in Figure 4 for the measurements obtained at 850 nm within the linear range of 200 NTU to 2000 NTU. A linear regression was performed on the data using Microsoft Excel® software. The equation of the standard curve in Figure 3 has a R-squared value of 0.9954 and in Figure 4, a value of 0.9982. The slope obtained was 0.0016 AU/NTU and 0.0007 AU/NTU in Figure 3 and 4 respectively.

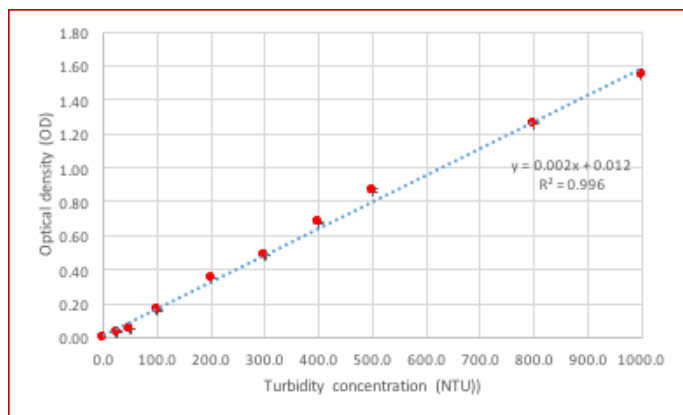


Figure 3: Standard curve at 570 nm for Turbidity standard solutions using Photopette®.

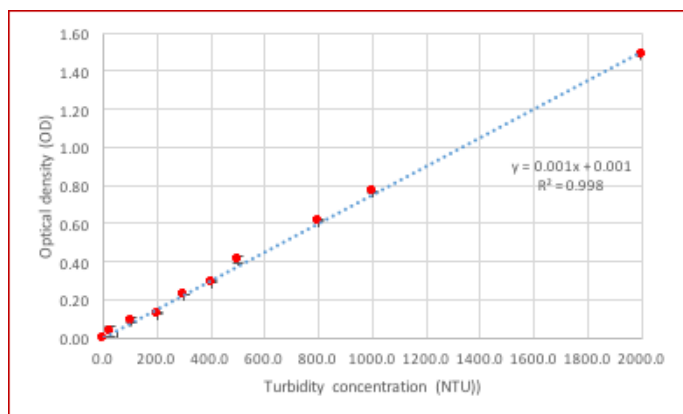


Figure 4: Standard curve at 850 nm for Turbidity standard solutions using Photopette®.

### Limit of Detection

The Limit of Detection (LOD) for this assay using Photopette® is determined by factoring in the standard-deviation for blank measurements as well as experimental data using the equation given below:

$$\text{LOD} = 3 \times \text{SD}_{\text{blank}} / \text{Slope}_{\text{(Standard Curve)}}$$

Standard Deviation for blank measurements with 50 repeats using same CuveTip was found to be 0.003 AU and 0.001 AU for 570 nm and 850 nm respectively.

$$\text{For 570 nm: LOD} = 3 \times (0.003 \text{ OD}) / (0.002 \text{ OD/NTU})$$

$$\approx 4.5 \text{ NTU}$$

$$\text{For 850 nm: LOD} = 3 \times (0.001 \text{ OD}) / (0.001 \text{ OD/NTU})$$

$$\approx 3 \text{ NTU}$$

### DETERMINING UNKNOWN TURBIDITY OF SAMPLES

For 570 nm, a water sample was collected and the average of triplets resulted in an OD of 0.591. The linear regression of the turbidity standards of Figure 3 is:

$$y = 0.002x + 0.0221$$

Re-arranging the “x” in the equation results in:

$$X = (y - 0.021) / 0.002$$

Inserting the OD of the unknown sample results in

$$X = (0.591 - 0.021) / 0.002$$

$$X = 285 \text{ NTU.}$$

For 850 nm, a water sample was collected and the average of triplets resulted in an OD of 0.257. The linear regression of the turbidity standards of Figure 4 is:

$$y = 0.001x + 0.001$$

Re-arranging the “x” in the equation results in:

$$X = (y - 0.001) / 0.001$$

Inserting the OD of the unknown sample results in

$$X = (0.257 - 0.001) / 0.001$$

$$X = 256 \text{ NTU.}$$

### LIMITATIONS

Suspended particles in a turbid solution may tend to settle down. Therefore, it is very important the solution is well-mixed and the readings are taken as soon external agitation is removed.

### SUMMARY

The application note guides the user for determining turbidity of an unknown aqueous samples. It highlights experimental parameters such as limit of detection and upper measurement-limit and linear range for such measurements as well using Photopette® device.

### REFERENCES

- [1] A. C. Ziegler, "Issues Related To Use of Turbidity Measurements As a Surrogate for Suspended Sediment," *Water*, no. Iso 7027, pp. 2001–2003, 2002.
- [2] Tip Biosystems Pte Ltd, "Photopette® User Manual V1.0.0", Singapore 2016.
- [3] Tip Biosystems Pte Ltd, Technical Note "How to use Photopette®'s CuveTip correctly"

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