

**DEVELOPMENT OF NOVEL TURBIDITY CALIBRATION
STANDARDS AND METHODOLOGIES USING APPROPRIATE
TECHNOLOGY**

by

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ABSTRACT

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Title: Development of Novel Turbidity Calibration Standards and Methodologies Using
Appropriate Technology

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The measurement of turbidity is currently conducted using a variety of methodologies for different applications, primarily using calibration standards composed of formazin, a material which can be difficult to obtain, short-lived, and toxic. The discrepancies between the various measurement methods lead to unreliability in sharing meaningful data across applications, and the deficiencies of the primary calibration standard make it difficult to conduct measurements in appropriate-technology environments. This research focused on examining the current methodologies and synthesizing new materials to use as calibration standards. The selection criteria for these materials were such as to ensure longevity, stability in solution, and ease of creation. Results showed that dilutions of dissolved sodium chloride yielded parabolic regression curves of comparable accuracy to formazin, which could be used for device calibration. These standards can be easily and safely synthesized in appropriate-technology settings. The use of fluorescent materials as calibration standards was also investigated, and the implications of the characteristic curves of turbidity versus solute concentration are discussed.

INTRODUCTION

Overview

Turbidity is the measurement of the relative optical clarity or cloudiness of a fluid (Myre & Shaw, 2006; Mullins, 2018). The measurement of turbidity is known as turbidimetry or nephelometry (from the Greek word *nephélē* meaning cloud), although there is dispute on whether these terms refer to slightly different aspects of measurement. The specific interpretation of turbidity measurements depends upon the application for which they are being used, and turbidity is often used as a surrogate parameter for other water quality metrics. It is usually measured by passing light through a small fluid sample and measuring the beam attenuation or light scattering at various angles in a device known as a turbidimeter. The unit of measurement most commonly used is the Nephelometric Turbidity Unit (NTU), although some sources refer to this unit by other titles.

Turbidity is related to total suspended solids (TSS), however, there are important differences between them. Generally, for a given material in suspension, increasing solute TSS will increase turbidity; however, it is not a linear correlation (Kitchener et al., 2017). Turbidity does not provide definitive information about the composition of the substance being studied; rather, it is a perceived optical phenomenon – fluid samples with suspended materials varying widely according to size, shape, color, etc. can have the same turbidity, making it difficult to draw meaningful inferences from the results of turbidity sampling.

In the United States, turbidity is a USEPA-regulated parameter. All public potable water sources must adhere to the National Primary Drinking Regulations contained in the 1974 Safe Drinking

Water Act. The USEPA Surface Water Treatment rule mandates that treatment systems using conventional or direct filtration must maintain turbidity under 1 NTU at all times and no higher than 0.3 NTU in 95% of samples in a given month. Turbidities under 10 NTU are not distinguishable to the unaided eye, yet water of this clarity may contain dangerous levels of pathogens. This furthers the need to regulate turbidity, since a person without access to water quality testing materials might consume clear (and possibly dangerous) water rather than turbid water (which may not be hazardous). Turbidity in water can also disrupt functions such as ultraviolet light treatment or chlorination.

Problem Description

The study of turbidity in water is conducted worldwide in a variety of fields, and yet a single, robust method for measuring turbidity does not exist. Various organizations prescribe their own methodology to determine and categorize turbidity (Hongve & Akesson, 1998), and many incongruities exist between these methods. Methodologies are often tied to specific devices, each with a unique configuration, and may be relevant only for a particular intended application (e.g. measuring agricultural runoff or studying aquatic life interactions), rendering shared definitions or data on turbidity unreliable.

Furthermore, rigorous analysis of the fundamental optical phenomena tied to light scattering in turbidity has not been conducted. This may be due to the fact that many of the actual applications of turbidimetry are industry-driven, not scientific, and therefore practical solutions may be preferred over analytical theories. It is acknowledged that in many contexts where turbidity is being measuring, in-use technologies achieve sufficient functional performance to meet their

goals. Notwithstanding this, there is opportunity for scientific advancement in this area that can yield more reliable, accessible, and meaningful results in the field of turbidity measurement.

Much of the recent scientific literature in this area has focused on identifying the inconsistencies in the various methodologies and making a case for greater standardization. Only a few (Kelley et al., 2014) recommend strategies that can be implemented to resolve these problems. As the trend of greater data management and internet-of-things connectivity continues, there will likely be more decentralized devices collecting data (such as turbidity) and an increased reliance this data, perhaps for as-yet-undiscovered applications. Specifically in austere environments, it is important to consider appropriate technology in developing solutions that provide scientific or industrial advantages at an affordable cost, while not sacrificing reliability. This research is being conducted with such outcomes in mind.

Research Objectives

Based on a review of the literature covered in Chapter 2, the following goals for this research are:

1. Develop a methodology for measuring turbidity of water in low-scale contexts, utilizing appropriate technology.
2. Create a reliable calibration standard from alternative material to ensure consistency in measuring and reporting turbidity
3. Document and report characteristics of the use of these methods and materials to advance the science of turbidimetry and provide recommendations for further studies.

LITERATURE REVIEW

History of Turbidimetry

Turbidity measurement as a science has been conducted for about a century (Rice, 1976). Simple observation has been used worldwide as a basic heuristic, although the unaided human eye cannot detect even dangerously high levels of pathogens. Various instruments and techniques have been developed to allow for precise and microscopic measurements of turbidity, but similar precision of the parameter definition or assessment methodology has not followed.

In 1900, the Jackson Candle Turbidimetry method was described in *Technology Quarterly* by Daniel Jackson and George Whipple. This now-outdated historical device involved the basic set-up of a candle beneath a transparent graduated cylinder, which would be filled with water of a given turbidity until light from the candle could no longer be seen when looking down through the water from above the cylinder. The water level in the cylinder at that point would be measured against the graduated markings, which were inscribed based on the depth required to completely obscure a thin metal wire when water mixed with specific parts-per-million amounts of silica were mixed in. As is easily understood, this method was calibrated to an arbitrary set of standards and lacked the rigor of an absolute scale.

Modern turbidimeters have been created and continually improved (Khairi et al., 2018; Wang et al., 2018). Most have a similar basic configuration, using a light source(s) to shine a beam through a sample and detect the light attenuated (in a straight line) and/or scattered (at various angles) with sensors. Differences in the methodologies prescribed by various regulatory organizations have

resulted in the tailoring of commercial devices for these purposes. Regardless of the exact configuration, since light extinction through a medium cannot be measured or compared meaningfully in an absolute sense, substances with precisely defined characteristics are used as standards. Samples of these substances are used to calibrate turbidimeters, providing a scale against which to meaningfully measure turbidities of unknown materials.

Methodologies

The basic set-up of most commercial turbidimeters is very similar. A device uses precise optical sensors to detect light transmitted through a small sample and measure it against source light emitted. Some devices only measure the amount of scattered light (as required by USEPA Method 180.1), while others measure light reflected at both 90 and 180 degrees. Some devices compare the values of 90-degree scattered light and attenuated light (known as the ratio method) and use that numeral proportion in their analyses. In all these configurations, the basic property being examined is the tendency of the dissolved and suspended particles in fluid to scatter or absorb light, reducing the overall transparency of the medium and causing it to become cloudy (turbid).

Other methods of measuring turbidity have been introduced, including low-technology devices such as the Secchi disk (a black-and-white disk, lowered into stable water by a marked string) and turbidity tube (a transparent, graduated cylinder with a black-and-white disk at the bottom). These types of equipment are often useful for field surveys but cannot examine water quality to the degree required to ensure proper sanitation.

Standard Materials

In 1926, formazin (a milky white polymer suspension with chemical structure $C_2H_4N_2$ synthesized from a suspension of hydrazine sulfate and hexamethylenetetramine in water) was recommended by F.B. Kingsbury et al. for use as a turbidity standard. By the 1950s, it had gained acceptance as the most commonly used standard, and in 1979 the U.S. Environmental Protection Agency (USEPA) codified its use in its “Method 180.1: Determination of Turbidity by Nephelometry,” (last revised August 1993). Other organizations followed suit, including the International Standards Organization (ISO 7027, 1999), Great Lakes Instruments, Inc. (GLI Method 2, 1992), and the American Society for Testing of Materials (ASTM Method D1889, 2000). Each of these organizations provide methodologies for the preparation of formazin standards and calibration techniques for its use. The units of turbidity are referred to by several titles: Nephelometric Turbidity Units (NTU), Formazine Turbidity Units (FTU), Formazine Nephelometric Units (FNU), Formazine Attenuation Units (FAU), and others.

Formazin (also spelled formazine) remains the primary calibration standard (PCAL) material prescribed by the USEPA, although a suspended microspheres polymer known as AMCO-AEPA-1 Styrene Divinylbenzene was added as another PCAL standard in 1982 (Hart et al., 1992). All USEPA turbidity monitoring and studying must use one of these two solutions to calibrate their devices, with a new stock solution prepared monthly and new dilute samples created daily. Secondary calibration standards (SCAL), defined as “commercially prepared, stabilized sealed liquid or gel” which are calibrated to one of the primary standards, may also be used for daily calibration checks of turbidimeters, as long as these are monitored against the primary standard “on a routine basis”. Little to no data publically exists comparing the stability of such secondary standards against primary standards.

Since the adoption of formazin as a primary standard, companies have created commercial products to meet regulatory, academic, and industrial demands. Stabilized formazin solutions are commercially available from many vendors, along with numerous proprietary secondary standards.

Literature-Identified Issues

Although scientific measurements of turbidity have been utilized for over one hundred years, surprisingly little progress has been made into researching its fundamental light-scattering properties. According to Munzberg et al. (2016), “Despite the importance of this calibration standard...there is no information available about the optical properties of formazine in the literature.” Additionally, the use of formazin (which is toxic, due to the carcinogenic character of hydrazine sulfate) as an arbitrary standard leads to further issues. The variety of methodologies prescribed by standards organizations are mutually inconsistent, and “are not based on the optical properties of light absorption and scattering by suspensions in water,” (Kitchener, 2017).

Literature sources agree that, although each of the various methodologies detail the steps to measure turbidity, these processes are not identical and in some cases are contradictory, including differences in type of light source used, wavelength measured, and optical sensor locations (Sadar, 2004). One source noted: “Turbidity methods, standards, reporting of units, and instruments are not identical...color can affect the measurements... Therefore, measurements of the same water by different methods and different instruments are not likely to yield similar values,” (Ziegler, 2001). Reviews of this issue promote further depth into the optical dynamic of the suspended particles (“Without a comprehensive understanding of the complex manner by which particle size, shape, and concentration affect the absorption and scattering of light, it will not be possible to interpret what a turbidity measurement actually means,” Kitchener, 2017).

Unfortunately, in many informal contexts, turbidity is used interchangeably with other parameters such as suspended sediments concentration (SSC) (Finlayson, 1985). Although an increase in turbidity often correlates with an increase in SSC, nevertheless, these two factors are neither identical nor linearly related. As an example, a water sample with reflective or translucent particles would be measured as less turbid than a sample with darker particles, even though the former sample might have a greater SSC concentration. Davies-Colley et al. (2001) show that there exists only a loose correlation between turbidity and suspended sediment concentration.

One important consideration when choosing a material to use as reference standard is whether its composition is similar to that of material which will commonly be tested. Turbidity is used in a wide variety of applications, from measuring agricultural runoff to drinking water sanitation, and in each use case, particle size is crucial in ensuring accurate measurements (Baker & Lavelle, 1984). It has been shown that that formazin has a particle size at least an order of magnitude larger than the wavelength of light used in turbidimeters, and several orders larger than the average particle size of particulates remaining in treated water (Papacosta, 2002). The EPA-prescribed turbidimetry methods are optimal for submicron particles, whereas formazin particles are approximately 6.0 microns in size, on average.

One issue that has received surprisingly little attention throughout most of the literature is the tendency for turbidimeters to produce false-low readings when their maximum detection level is reached. Voichick et al. (2018) describe this in detail in the context of in-line stream gauges, and show that after reaching their maximum ability to detect turbidity in streamflow, these turbidimeters will sometimes record a drop in turbidity even while other devices show an increase in SSC. This can be explained by the meters only recording incident light scattered (not attenuated) in the water. Light scattering will

increase in SSC until the concentration reaches a level where the suspended sediment will absorb most of the light, rather than scatter it. The literature did not give a definitive solution to this issue.

Finally, formazin is toxic, with explicit EPA warnings against contact or exposure. Each formazin standard prepared has a nominal shelf-life of only one year, making preparation of calibration standards time-consuming and/or expensive.

Research Approach

Since many of the problems in the literature related to the use of formazin, this study's experimentation will begin there. Attempts will be made to synthesize and evaluate it for a set of optical data, which can be used to confirm the reliability of any novel alternative material. This analysis will also be used to validate the methodology for measuring turbidity in low-scale contexts. Material selection for creation of surrogate turbidity calibration standards will focus on stability, longevity, and ease of synthesis. These surrogate standards, composed of alternate materials, will be subjected to similar validation testing to ensure reliability and accuracy. It is not the goal of this research to develop an overall model for how turbidity should be evaluated in all contexts, although some of the results may have implications for such a model.

METHODS

Experimentation Methodology Introduction

As is shown in the literature, it is difficult to rigorously define a methodology of turbidimetry in order to achieve precise and consistent results. This is owing to numerous factors including the definition of turbidity (whether it is simply a comparison of light-scatter or a ratio of light attenuation coefficients) and the techniques of its measurement, which in turn affect the design of equipment and measurement set-up. It is therefore necessary to be clear in defining the chosen methodology and taking large data sets, over the course of time.

Initially, before any experimentation-proper was conducted for this project, an attempt was made to understand and calibrate the equipment & materials being used. Commercial formazin standards were purchased at 1 and 10 NTU levels (Hach, Product #2659942 and # 2660242). Portions of the 10 NTU standard were diluted to various smaller levels (2, 3, 4, 5, 6, and 8 NTU). The commercial-grade turbidimeter (LaMotte 2020e) was calibrated using the 10 NTU standard solution, and each of the smaller level solutions were measured. The measured turbidity values of these dilutions often varied significantly from their predicted values; this may have implications for the characteristics of the formazin at very low concentrations, as well as for the precision of the turbidimeter in consistently measuring light scattering through near-pure water. In the majority of the experiments performed, significant calibration of the equipment to the standard material was required, and in many cases the expected turbidity value (even of commercially purchased, diluted formazin) was out of the calibration range of the equipment (i.e., a solution expected to be measured as 10 NTU would be read by the turbidimeter as below 8 NTU, with the turbidimeter only allowing calibration to +/-20% of its reading).

As noted in the Introduction, one research objective was to develop a methodology for measuring turbidity of water in low-scale contexts, utilizing appropriate technology. This was conducted using a device which was designed and created at Purdue University by Dr. Chad Jafvert and his research group, called a ‘colorimetry-turbidity meter’ (henceforth referred to as the CT Meter). Use of the CT Meter did not require extensive calibration, as its configuration is similar to a commercial turbidimeter. It consists of two light sources (situated at 90 degrees with respect to each other) aimed into a space where a small sample cell can be loaded. The device can measure both light scattering (at 90 degrees) and attenuation (at 180 degrees), however, these functions cannot be performed simultaneously. The configuration utilized for this study was that of light-scattering (detected light at 90 degrees to the light source). This research aimed in part to validate the functionality of the CT meter for turbidimetry.

Alternate Calibration Standards

Another objective of this research was the creation and testing of suspensions of materials in water to determine if these could operate as surrogates for formazin as a standard material for turbidity calibration. Recommendations from the relevant literature were used as a starting point. Primary considerations included solubility and stability in water, ease of creation, and appropriate technology suitability, since the immediate intended application for such a surrogate turbidity standard material was in low-scale, non-industrial contexts.

Once an alternative material was selected, a given amount was weighed and mixed into a clean container, then diluted with deionized water. The initial masses of material and volume of water were essentially arbitrary, as the measured turbidity of that mixture would be recorded and the mixture ratio adjusted iteratively in order to achieve a stock solution at a normal turbidity value (usually 100 NTU). This stock solution then served as the basis for dilutions of the suspended material, measured with both the turbidimeter and CT Meter for regression trendlines.

Experiment Execution

The first phase of experimentation aimed to create a lab-synthesized stock solution of formazin from its components. Quantities of hydrazine sulfate (HZS) (100 g, Hach, Product #74226) and hexamethylenetetramine (HMT) (500 g, Hach, Product #187834) were obtained and diluted into water according to the Hach-Lange 2100N Laboratory Turbidimeter Manual: 5 g of HZS were dissolved into 400 mL of deionized (DI) water, while 50 g of HMT were dissolved into a separate 400 mL of DI water. These solutions were then combined and diluted to 1 L. Over a period of roughly 12 hours, this mixture turned into the milky-white aqueous substance known as formazin, with a turbidity level officially determined to be 4000 Nephelometric Turbidity Units (NTU). This mixture formed a stock solution from which samples of other turbidity levels could be created. A 400 NTU sample was immediately then created, using a 1:10 dilution of formazin-to-DI-water, followed by a 100 NTU sample (1:4 from the 400 NTU sample) and a 10 NTU sample (1:10 from the 100 NTU sample).

From the 10 NTU solution, small samples of low-turbidity solution (1, 3, 5, and 8 NTU) were created and measured using the LaMotte 2020e turbidimeter as well as the CT meter. The initial results of this trial (see Results section) proved to be inconclusive, either as a result of low-meter sensitivity at that range or difficulty in maintaining precision over multiple iterations of dilution. Further analyses used samples of greater turbidity and achieved well-defined results.

In the process of using alternative materials to create surrogate standards, precise measurements (on the order of 0.01 grams) of solute were taken prior to mixing with DI water. As previously stated, the initial concentration was essentially arbitrary, then adjusted iteratively until it reached a level which yielded a turbidity of approximately 100 NTU. From that point, the solution was diluted linearly (usually at intervals of 10%) and its turbidity was recorded.

RESULTS

Formazin Synthesis and Testing

The first objective in this research was to create and test the established materials and processes currently in use in academic and industrial settings. The procedure for creating formazin (see Rice, 1976) has not substantively changed since it was proposed by Kingsbury et al. in 1926, and is still prescribed by the USGS National Field Manual for the Collection of Water-Quality Data (Section 6.7.2, Version 2.1, Sept 2005). Since this material is primary standard for turbidity and the basis for all secondary turbidity standards, it was deemed relevant to create and evaluate first-hand.

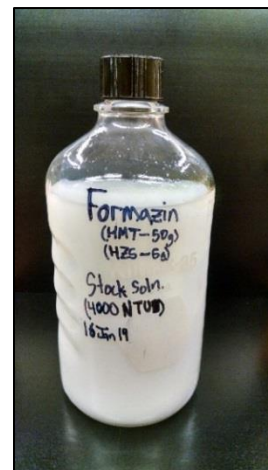


Figure 1: Formazin Stock Solution

As described in Chapter 3, quantities of hexamethylenetetramine and hydrazine sulfate were combined to create a stock solution of formazin, nominally defined as 4000 Nephelometric Turbidity Units (NTU). This solution was initially a shimmering, transparent liquid, but became highly opaque within 24 hours (see Figure 1). Once thoroughly mixed, a small quantity was diluted from 20 mL to 200 mL using deionized water as the diluent to create a 400 NTU solution, which was similarly diluted to create a 100 NTU solution, which became the basis for lower-NTU samples. Each of these suspensions proved highly unstable, however (see Figure 2); within several days of their creation, each would separate completely into two roughly equal portions: A transparent top portion on top and an opaque bottom portion. Briefly shaking the solution



Figure 2: Phase-separated formazin stock suspensions (4000 NTU, 400 NTU, and 100 NTU)

would reconstitute the original mixture, at which point it would once slowly separate again, on the order of hours (see Figure 3).

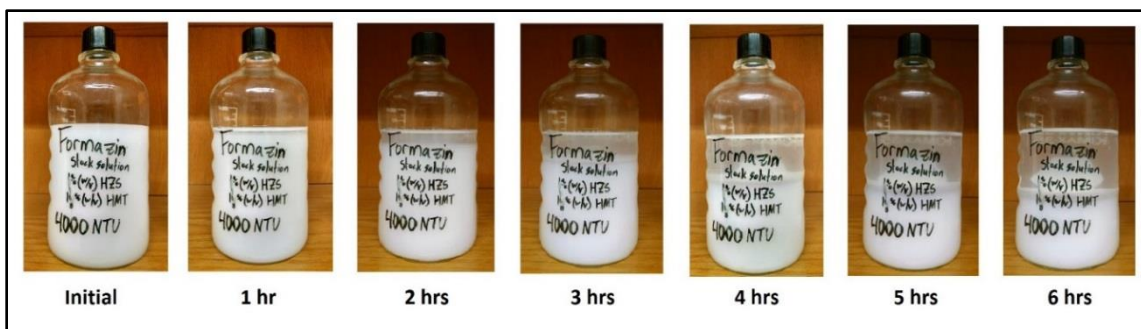


Figure 3: Timeline of formazin stock suspension phase-separation

Creating a stock solution of formazin in the laboratory permitted testing not only of its physical and chemical properties, but also of its usage in contemporary methodologies of turbidimetry. Much of the relevant literature assumed that a linear dilution of stock solution would result in a similarly linear reduction in turbidity of the standard, despite that “Formazin concentration does not in fact have a linear relationship to measured light attenuation,” (Kitchner, 2017). Since turbidity is a perceived optical property of a given fluid, an increase or decrease in its measured value does not necessarily mean a correlated change in its constituent material. In this project’s experimentation using formazin, linearity was achieved proportional to dilution on the 1-10 and 10 -100 NTU scales (see Figure 4).

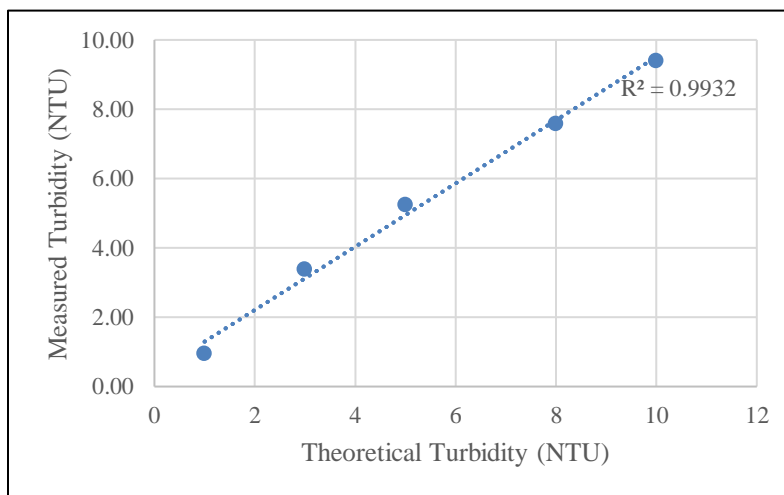


Figure 4: Turbidity of Lab-Made Formazin, Trial #1

First, five samples of formazin solution (1, 3, 5, 8, and 10 NTU) were created from a 10 NTU stock. The turbidity of these samples was evaluated using the LaMotte 2020we. Several turbidity values for each sample were recorded in quick succession, with the glass sample vial being rotated between measurements to reduce the impact of any imperfection in the glass vial, and the results were averaged. A second set of ten samples was created, at regular intervals from 10 NTU to 100 NTU, from the 100-NTU lab-made stock solution (along with 150 and 200 NTU samples created from the 400-NTU stock).

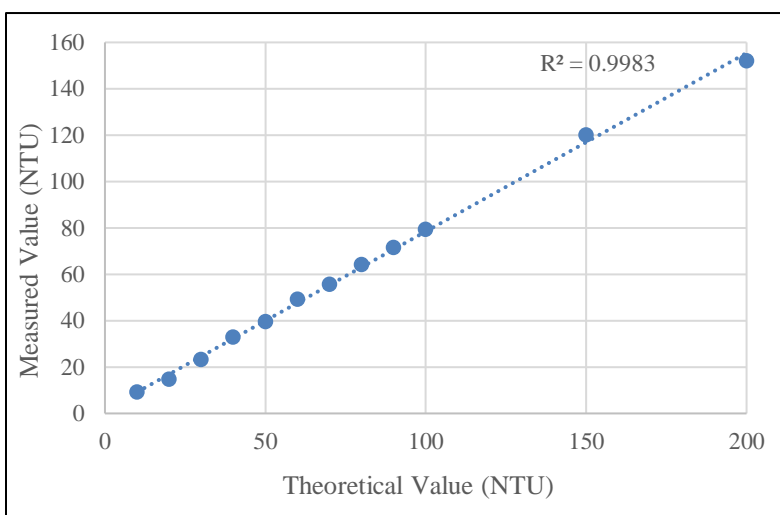


Figure 5: Turbidity of Lab-Made Formazin, Trial #2

Despite careful calibration of the turbidimeter using commercially-purchased formazin standards (Hach Stablcal® 10 and 100 NTU standards), as well as careful dilution of the samples, the measured turbidity value was consistently below the theoretical value based on dilution criteria. The LaMotte 2020we commercial turbidimeter could not be adjusted to allow the measured values to be set to their theoretical values, as its calibration range maxed out at $\pm 20\%$. Nonetheless, these charts seem to indicate a high degree of correlation for well-mixed dilute formazin in the 1-10 NTU ($R^2 = 0.9932$) and 10-200 NTU ($R^2 = 0.9983$) ranges.

CT Meter Validation

Having proven the capability of creating formazin with reliable linear characteristics in the laboratory, the next step was testing the methodology for measuring turbidity with a commercial device against the CT meter developed in-house. As described in the Methods section of this report, the design of the CT meter matched the typical configuration of a nephelometric turbidimeter, with a collimated light source aimed at a sample-housing chamber and a sensor located at 90 degrees to the incident beam. The main difference in design was the use of a white-light LED bulb instead of a Tungsten lamp (color temperature 2200-3000 K) required by USEPA Method 180.1 (Par. 6.2.1) for official testing. The light sensor in the CT meter was connected to an electrical circuit, giving a basic output in kHz which can be easily converted into light intensity. The data gathered show that the CT meter was capable of highly accurate ($R^2 = 0.9971$) turbidity measurement in the range of 10-150 NTU (see Figure 6).

This experimentation was repeated with an increase in CT meter voltage (from a 2V configuration to 5V), producing a gain on the amount of current (detected as frequency). It was anticipated that this would achieve greater linear correlation by offsetting any imprecision in detection capabilities. Although this may be true at very low turbidity levels over the course of many trials, it did not prove significant in the 10-150 NTU range and resulted in a slightly lower accuracy ($R^2 = 0.9966$, see Figure 7), although functionally almost identical to the 2V configuration and still extremely high.

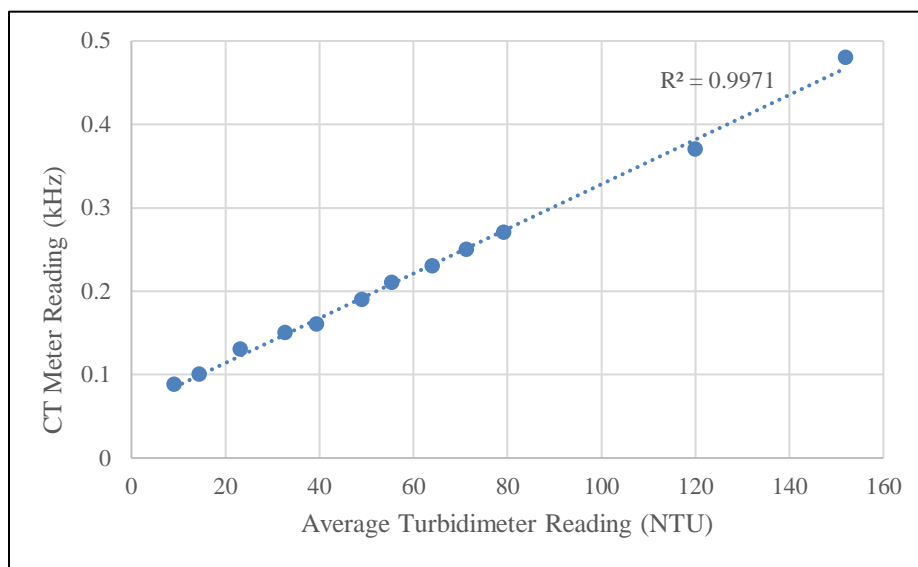


Figure 6: Commercial Turbidimeter vs CT Meter (Trial #1)

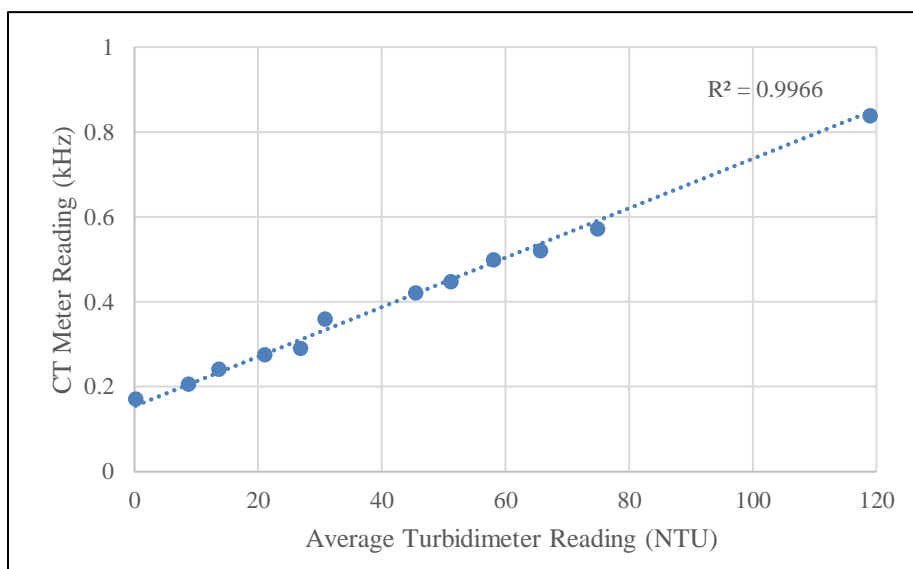


Figure 7: Commercial Turbidimeter vs CT Meter (Trial #2)

From this point onwards in experimentation, the CT meter was used in conjunction with the turbidimeter to analyze sample turbidity. This was owing to two primary reasons: First, a major objective of this research being the creation of a turbidimetry methodology using this specific device, it was necessary to utilize it to explore such a methodology after having shown it to be accurate comparable to commercial equipment. Secondly, since the CT meter was custom-built by Dr. Jafvert's research group, the schematics were well-understood and allowed for clear understanding of the effects being studied. Other than the voltage-toggling described above, no significant design changes were made to the configuration of the device while this research was being conducted. The 5V configuration was maintained for all trials described hereafter. In order to differentiate between the two devices, data collected from the commercial turbidimeter is listed in charts and graphs as "Turbidity," with units of NTUs, while data from the CT meter is listed as "Light Scattering," with units of kHz.

Sodium Chloride Standards Creation and Testing

The selection of alternate turbidity standards for the purposes of this research was based on criteria described earlier in this report, representing conclusions from a review of literature regarding weaknesses of contemporary standards. Some literature sources recognized the inherent shortcomings of current primary standards, and a few have attempted to address these deficiencies via suitable secondary standards or advanced methodologies. None recommended a definite replacement for current primary turbidity standards or methodologies.

Although the topic of moving to a new primary turbidity standard, along with a novel approach to turbidimetry as a science, is explored in this report (see Discussion section), more specifically, this research has aimed to develop and analyze materials and methods for suitability as alternate (i.e. secondary) turbidity standards in low-scale settings. As with the CT meter, the design constraints of these surrogate materials included a focus on appropriate technology level; that is, achieving

functionality for a specific purpose without unnecessary complexity. For this reason, along with the development criteria of longevity, stability, and safety, the additional factors of simplicity and ease/cost-of-development were considered. The specific application for turbidity sampling determines the range of turbidity examined and the method used, and so it is with this research: The use of turbidity sampling to ensure water quality in appropriate-technology settings requires a focus at turbidity in the 1-100 NTU range. While a more generalized solution to the problem of turbidity calibration standard would be desirable, such a solution lies outside the scope of this report.

As such, the ideal tentative material for use in experimentation would be not only extremely stable in solution, but also have a typical turbidity in the 1-100 NTU range based on graduated solute concentration. Some of the literature focused on materials such as silica gels or styrene divinylbenzene beads (SDVB). To begin experimentation for this research, however, it was decided that a simpler, more plentiful material would be used to test the creation of calibration standard curves: sodium chloride (NaCl), both iodized and non-iodized.

To begin testing, a stock of regular non-iodized table salt (Morton Salt brand, containing trace amounts of calcium silicate) was procured and brought to the lab. For an initial conjecture, 1 g of solute was carefully measured and dissolved into 10 mL of deionized water. This solution was measured to have a turbidity of roughly 100 NTU. Samples were diluted at 10% intervals, from 100% of original solution concentration to 10%. The results are shown in Figures 8 and 9.

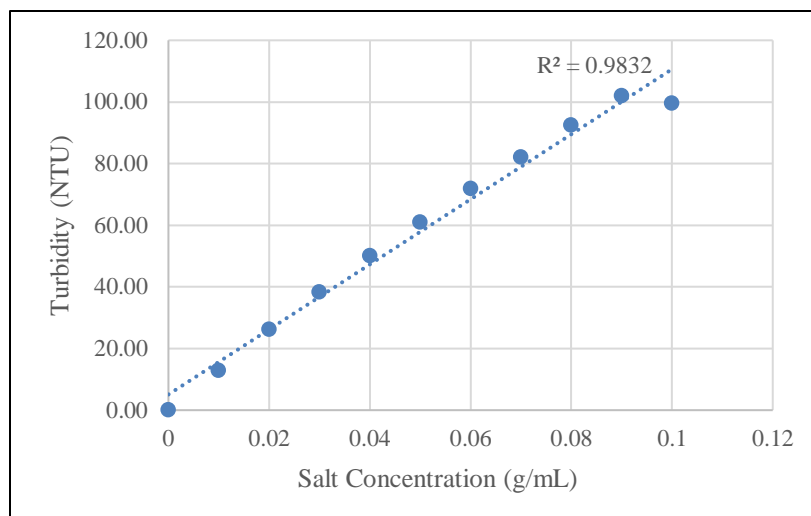


Figure 8: NaCl Concentration vs Turbidity

It can be easily noticed that, while the data follow a roughly linear trend with a high regression coefficient ($R^2=0.98$), the widest outlier is the point of highest concentration. This was initially thought to be insignificant; however, further experiments revealed an explanation. Additionally, although the data seemed to match a linear trendline, and although most literature sources assume a linear correlation between sample turbidity and calibration standard dilution, when the outlier of this data set was removed and a polynomial (i.e. parabolic) trendline assumed, the regression coefficient approached unity ($R^2=1$).

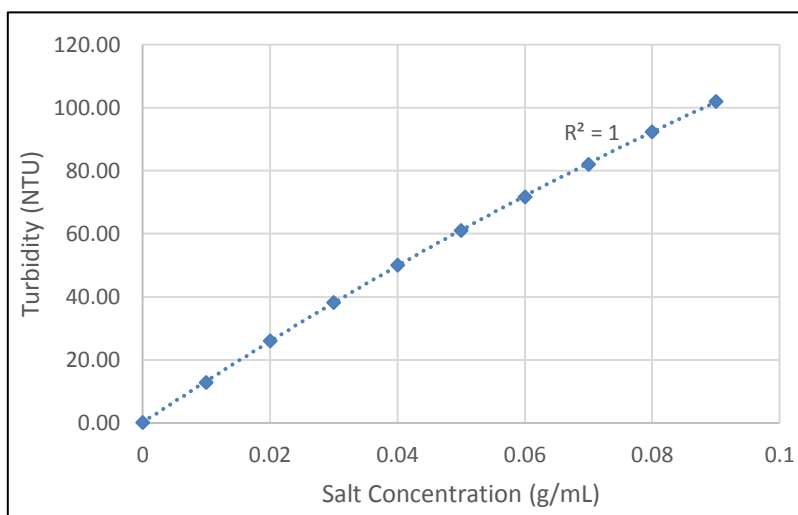


Figure 9: NaCl Concentration vs Turbidity (with parabolic trendline)

The same process was repeated for a solution of iodized sodium chloride (Gold Emblem brand, containing 45 μg of iodine per gram of NaCl and trace amounts of sodium silicoaluminate, dextrose, potassium iodide, and sodium bicarbonate). This trial was performed to control for any additives of the sodium chloride, and to examine the range of viable alternative material sources. For an initial conjecture, 1 g of solute was carefully measured into 4 mL of deionized water. This solution was measured to have a turbidity of roughly 105 NTU. Samples were diluted at 10% intervals, from 100% of original solution concentration to 10%. The results are shown in Figures 10 and 11.

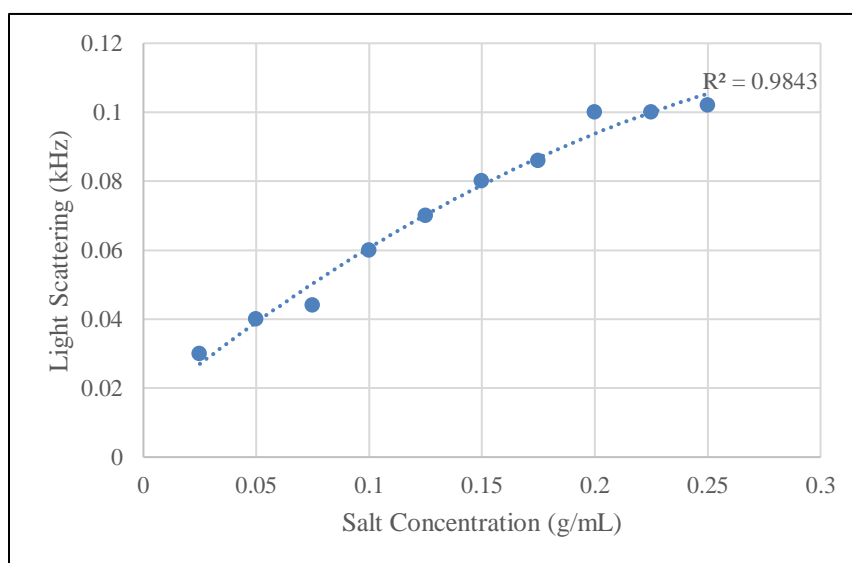


Figure 10: Iodized NaCl Concentration vs. Light Scattering

Noticeable again is the characteristic parabolic curve, which matches the data set with a more accurate regression coefficient than does a linear trendline. Figures 10 and 11 provide further evidence that the CT meter is a device functionally equivalent to a commercial turbidimeter.

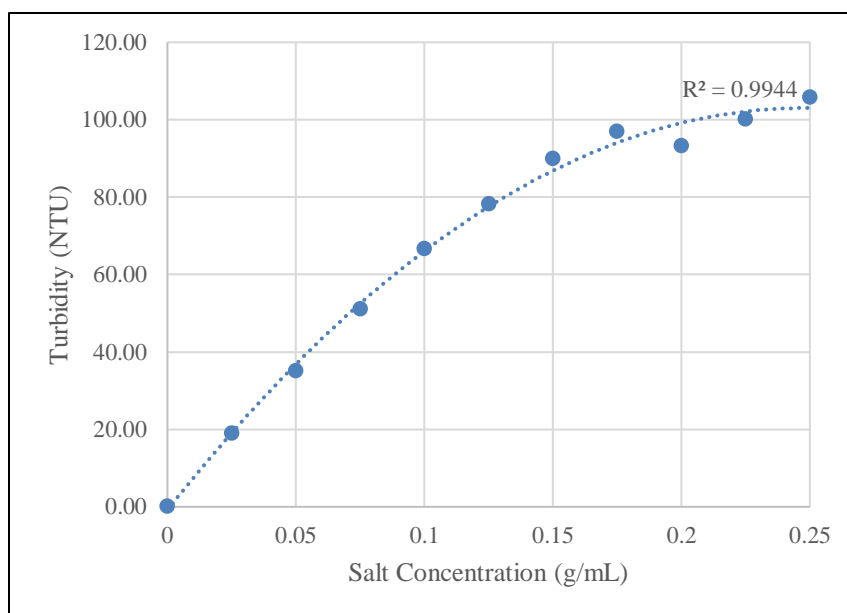


Figure 11: Iodized NaCl Concentration vs. Turbidity

Fluorescein Standards Creation and Testing

The final material tested for use in suspension as a surrogate turbidity calibration standard was fluorescein, a fluorescent material with a central emission peak in the visible green portion of the electromagnetic spectrum (520 nm, in water). This material was selected for trial based on the solubility of fluorescein salts in water, as well as the novelty of using a fluorescent material as a turbidity standard. It was speculated that the absorbance/reemission property of fluorescein might be useful in overcoming difficulties, since the emitted light conforms to a standard curve for a given material, which would help control for differences in light source used. Additionally, since the turbidity sensor would detect not only light scattered by suspended particles in the sample, but also light absorbed & reemitted by the fluorescein, this material would act to boost light detection for low-turbidity calibration standards. Fluorescein was selected as a common, representative fluorescent material.

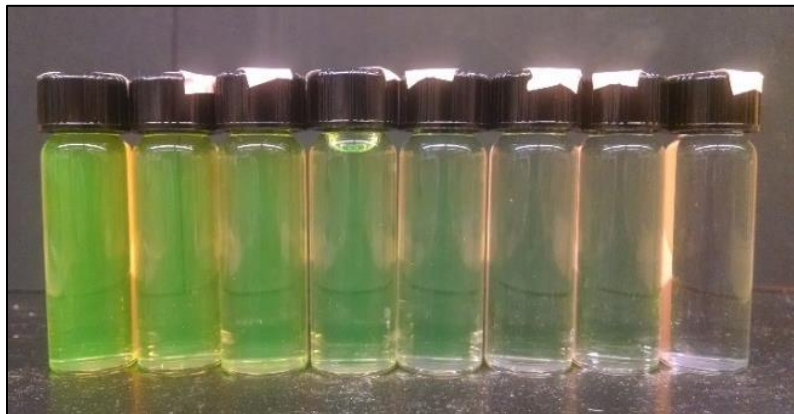


Figure 12: Fluorescein dilutions (ranging 0.01 to 0.00025 g/L)



Figure 13: Stock (left) and initial fluorescein suspensions

A quantity of fluorescein sodium salt was acquired (100g, Sigma-Aldrich) and stored in a fume hood until use. Its appearance was an orange-red fine powder. To create an initial suspension, 1 g was carefully weighed and placed in clean glassware, and 50 mL of deionized water was added. This resulted in a dark red-brown liquid, which would appear orange-red when in the form of a thin film. From this solution, 1 mL was drawn and added to 200 mL of deionized water, creating a phosphorescent green fluid. This solution, nominally having a concentration of 0.1 g fluorescein per liter of deionized water (equating to 0.0001% w/v), was used as a stock solution for further experiments.

Initially, the fluorescein stock suspension was diluted at equal intervals from 10-100%. Immediately upon visual inspection, however, it was seen that these samples were not noticeably different from one another, as had been the case with the NaCl alternate standards. The samples were evaluated in both the LaMotte turbidimeter and the CT meter, and upon experiment completion, it was decided to continue diluting the suspension with DI water at a 1:1 ratio, from 10% to 0.675%, to evaluate the material at very low concentrations. The results are shown in Figures 14 and 15.

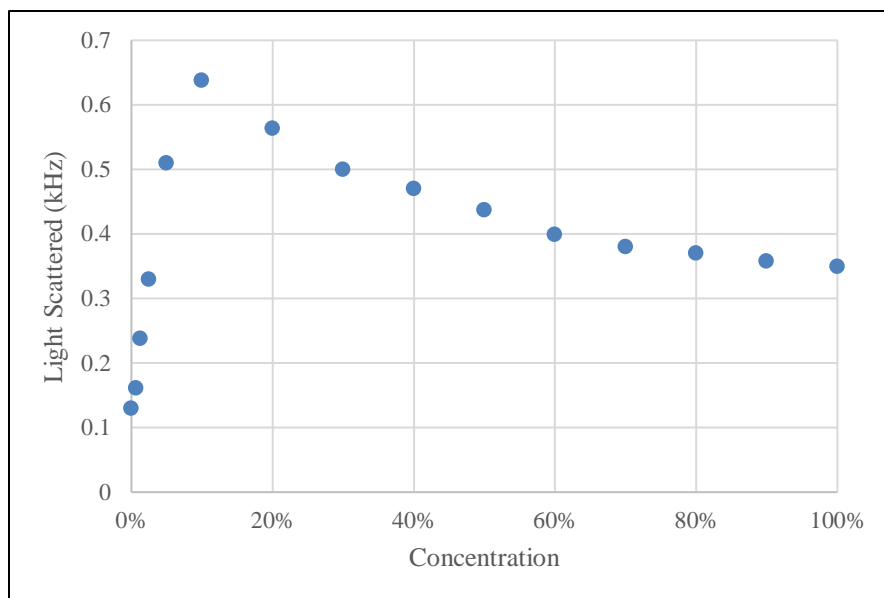


Figure 14: Fluorescein Light Scattering vs Dilution Percentage (CT Meter)

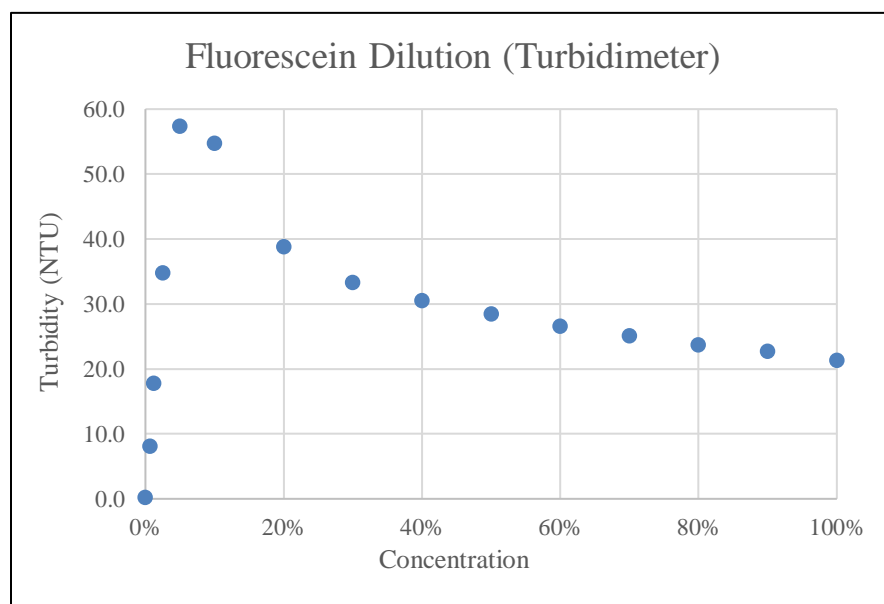


Figure 15: Fluorescein Turbidity vs Dilution Percentage (Turbidimeter)

Easily noticeable is that these data follow a similar characteristic curve, showing maximum response at roughly 5-10% concentration of the stock suspension (0.000005-0.00001% w/v). This corresponds to the concentration that causes the highest amount of light emission, aided in this case by absorption and reemission. Although no single trendline can fit these curves, it can be broken into two

sections which can be approximated with two different types of functions. The first section, from the lowest value to the peak, is parabolic ($R^2=0.99$). After the peak, it becomes an inverted logarithmic function ($R^2=0.99$). This dichotomy increases the difficulty in characterizing the light scattering function of the sample, although it is what would be expected based on optical physics. These two sections are shown in Figures 16-19 (measured via CT Meter and turbidimeter).

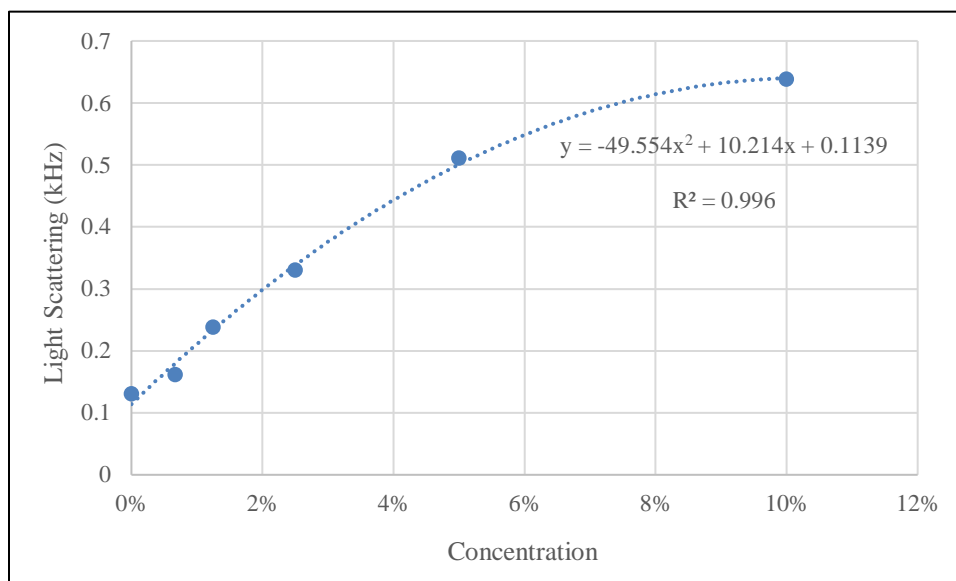


Figure 16: Fluorescein Light Scattering vs Dilution Percentage (Parabolic Section)

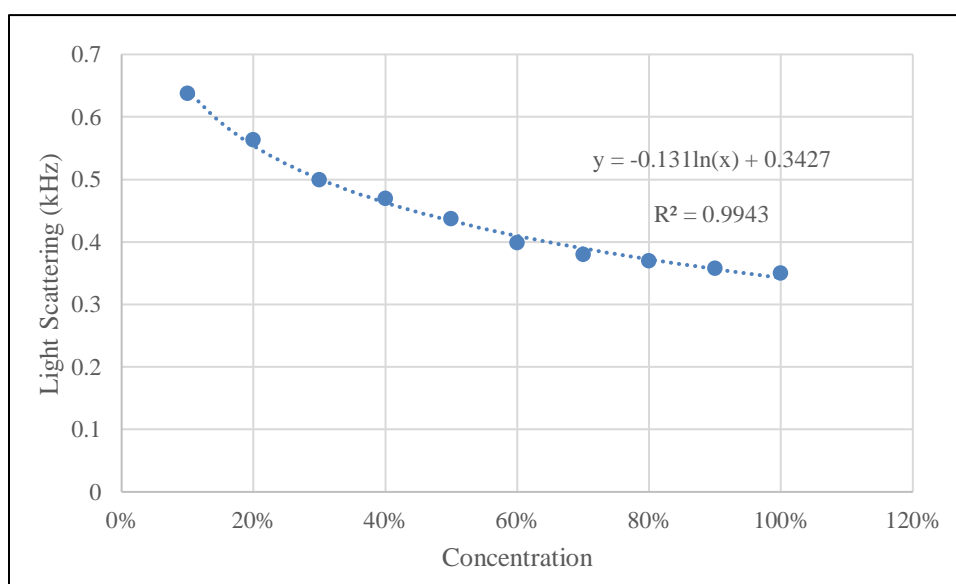


Figure 17: Fluorescein Light Scattering vs Dilution Percentage (Logarithmic Section)

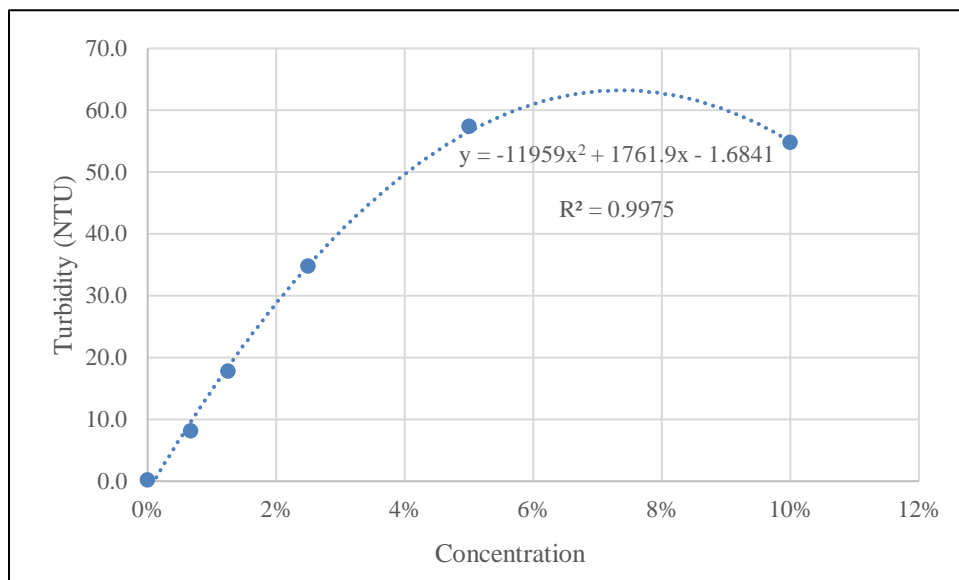


Figure 18: Fluorescein Light Scattering vs Turbidity (Parabolic Section)

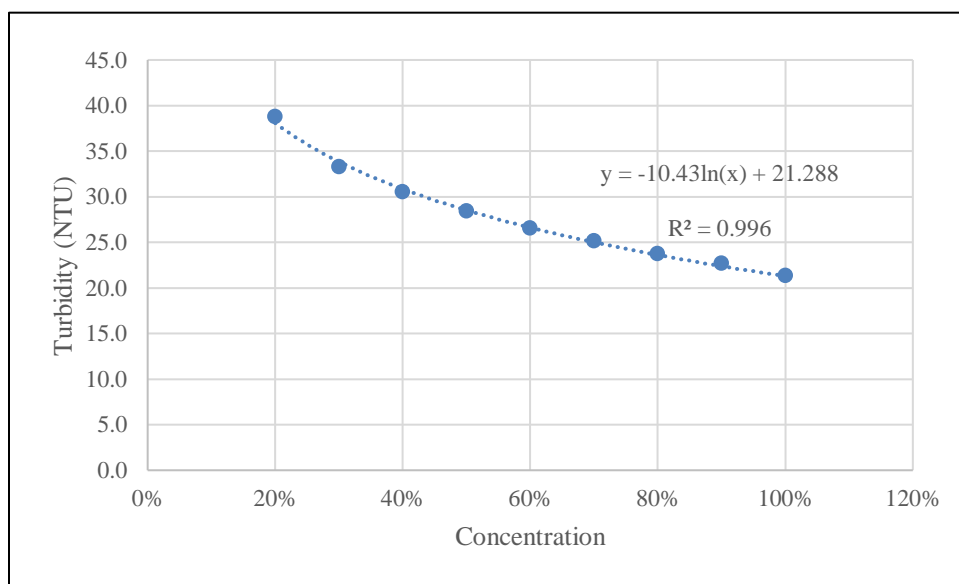


Figure 19: Fluorescein Light Scattering vs Turbidity (Logarithmic Section)

DISCUSSION

Data Analysis

One of the first things that should be clearly seen from the data presented here is that the industry-standard formazin is not totally unique in its capability to act as a calibration material for turbidimeters. Indeed, although it was adopted as the primary standard almost a century ago, a search of the relevant literature did not reveal any serious inquiries into replacing it with a different primary standard (perhaps excepting Kitchner, 2017) or rethinking turbidity measurement methodologies entirely. Additionally, no substantial literature was found to give reasoning for the selection of formazin in the first place to support its use as such.

The three materials (of two distinct varieties) examined here demonstrate both a novel-use and appropriate-technological perspective on substituting a different material for formazin in order to calibrate a turbidimeter. As previously mentioned, the specific application for which turbidity samples are collected dictates the measurement and calibration methodologies. In the case of appropriate-technology contexts, the optimal material for ensuring accurate turbidimeters calibration will be either simple and easy to synthesize, or possess a longevity that justifies its use in a setting where regularly replacing the calibration standards is not an option. In the former case, a material as simple as non-iodized sodium chloride may be reliable enough to calibrate a turbidimeter; this may only be applicable to settings not governed by EPA or similar regulations that require regular recalibration using a primary standard (formazin, AMCO-AEPA-1® Microspheres, or Hach Stablcal®). In the case that laboratory access is readily available, this study demonstrates that the reliability user-prepared formazin is comparable to that of commercial formazin, at a much lower cost. See Table 1 for cost breakdown.

Table 1: Cost Analysis of Lab-Made Turbidity Calibration Standards

Material	Amount	Cost
Hexamethylenetetramine (HMT)	500 g (purchased)	\$67.50
	50 g (used)	\$6.75
Hydrazine Sulfate (HZS)	100 g (purchased)	\$40.40
	5 g (used)	\$2.02
4000-NTU formazin stock solution	1 L	\$8.77
100-NTU calibration standard	1 L	\$0.22

If the entire amount of materials purchased was utilized, it would allow 10 liters of 4000-NTU stock solution to be created, which could be diluted to 400 L of 100-NTU suspension. If it were split optimally, this would be enough to create 72,727 sets of 10- and 100-NTU calibration standards. At a total material cost of \$87.70, each set of two standards would cost approximately \$0.0012.

Therefore, if cost is the only concern, user-prepared formazin is likely the best solution for turbidity calibration; alternatively, if simple, repeatable measurements in appropriate-technology contexts are desired, then the identification and evaluation of sodium chloride (or another easy-to-obtain substance), in the precise mixture, could yield reliable calibration results.

The assessment of fluorescein in suspension as a turbidity calibration standard shows that it could also be a viable alternate standard. However, since its peak emission occurs at such a low solute concentration (~0.01 g of fluorescein sodium salt per liter of water), the possibility of error in formulation is higher than with other surrogate materials. Fluorescein may prove useful if it can be shown to minimize or eliminate the effects of differences in light sources in turbidimeters. It could also be explored as an additive to very low-turbidity samples, in order to achieve a response gain.

All these results raise the question: Is formazin obsolete as a turbidity standard? Although it may not have been verified through this study whether a material such as sodium chloride is robust, stable, or versatile enough to be considered a primary standard, it should be noted that the uniqueness and idealness of formazin is in question. The deficiencies of formazin as previously described (toxicity, instability, particle size) ought to be sufficient to promote inquiry into whether a more suitable calibration standard or set of standards can be identified, analyzed, and established. While it may seem unlikely that the USEPA or other regulatory bodies will remove formazin from the list of acceptable turbidity standards altogether, it is possible in the future that more primary or secondary standards will be authorized, which may be cheaper or simpler to synthesize without compromising reliability.

Even beyond the question of what material to use to calibrate turbidimeters, the question of whether such a relative measurement methodology is optimal cannot be avoided. Few scientific fields rely so heavily on comparison to an arbitrary standard as does turbidimetry, and although device calibration may be a perennial consideration, it must be considered whether a different definition for turbidity could mitigate the reliance on a narrow set of materials. This definition must consider the reality of the non-linearity of turbidity as a function of solute concentration, which nearly all current studies and methodologies do not. The fact that turbid suspensions have a peak light scattering value, bounded by parabolic and logarithmic functions of solute concentration, could be used to define a new turbidimetry methodology, such as measuring sample turbidity as a percentage of maximum light scattering, or as a percentage of incident light. At a minimum, consideration should be given to simplifying the numerous methodologies and device configurations that exist, which all purport to measure the same parameter.

Ultimately, the specific application will continue to determine the methods and materials used to calibrate and operate turbidimeters. In laboratory and clinical settings, high precision and accuracy

may outweigh factors such as cost or ease of synthesis. In agricultural settings, a cheap and simple methodology targeting a particular range (e.g. 0-10 NTU for drinking water; 100-1000 NTU for storm runoff) could be optimal. In these cases, as in many, function precedes and determines form.

Conclusions

This study has endeavored to determine whether other materials besides formazin may be used to calibrate turbidimeters for the purpose of measuring the quality of water samples in low-scale, appropriate-technology contexts. It was found and is shown herein that multiple materials, including sodium chloride and fluorescein sodium salt, can be used in suspension to generate standard calibration curves with regression coefficients close to one. Thus, the hypothesis that reliable, alternate turbidity calibration standards can be synthesized was validated.

Recommendations

Many further questions in this subject remain, however. Detailed study into the optics and particle dynamics of fluid suspensions could enhance the ability to describe and define the relationship between suspended/dissolved particles (including particle size, shape, color, and concentration) and turbidity. The ability to characterize the contents of a suspension based on light attenuation or scattering could yield many practical benefits, such as streamflow content assessment and management. One recommended area of research is regarding the parabolic-logarithmic reflectance curve (shown for fluorescein in Figures 16-19). Since turbidity is related to the characteristics of the particles in suspension, it may be possible to identify the components (categorically, at least) of a turbid fluid simply by measuring turbidity vs. dilution and comparing with known characteristic curves. It is recommended that other materials, including those that could be used as surrogate turbidity calibration standards, be studied to determine their maximum-scattering function, and that all such materials tested be catalogued for analysis and comparison of such characteristic curves.

From a mathematical standpoint, analysis of the parabolic and logarithmic segments of these curves may be valuable, particularly in explaining the boundary between the two. From a practical standpoint, it is suggested that other individuals and organizations formulate and analyze their own alternate standards to determine the efficacy and versatility of alternative materials in suspension to act as surrogate turbidity standards.

One specific area of research could be a further study of fluorescent materials such as fluorescein to determine their robustness for use as a turbidity calibration standard; if demonstrated, these could potentially become reliable secondary calibration standards. Because their optical properties function differently than traditional calibration standards (following an absorbance-and-reemission phenomena versus a typical light-reflectance phenomena), these could avoid the drawbacks of other materials, including long-term instability (due to particle settling or flocking) and low-light imprecision. A small amount of fluorescent solute could therefore ensure turbidimeter calibration and proper operation over a long duration, at a much lower cost, than formazin. It is therefore recommended that the longevity of diluted fluorescein in water or other solutions be studied in greater detail.

In the case of this specific research's application, further validation of the CT meter is recommended to demonstrate full capability to function reliably as a turbidimeter. Various configuration changes could be tested (such as higher voltage, smaller or large sample cell size, or different LED wavelengths), as well as various analysis techniques (such as the ratio method of scattered and transmitted light). These optimizations, along with a stable, accurate calibration material, could support reliable, easy turbidity measurements to ensure water quality in appropriate-technology environments.

REFERENCES

- Baker, E., & Lavelle, J.W. (1984). "The Effect of Particle Size on the Light Attenuation Coefficient of Natural Suspensions." *Journal of Geophysical Research*, 89(C5), 8197-8203.
- Davies-Colley, R.J., and Smith, D.G. (2001). "Turbidity, Suspended Sediment, and Water Clarity: A Review." *Journal of the American Water Resources Association* 37(5): 1085–1101
- Finlayson, BL (1985) "Field Calibration of a Recording Turbidity Meter." *Catena* 12(2–3): 141–147.
- Hart, V., Johnson, C., & Letterman, R. (1992). "An Analysis of Low-Level Turbidity Measurements." *Journal, American Water Works Association*. 84(12), 40-45.
- Hongve, D., and Akesson, G. (1998) "Comparison of Nephelometric Turbidity Measurements using Wavelengths 400–600 and 860 nm." *Water Research* 32(10): 3143–3145
- Kelley, C., Krolick, A., Brunner, L., Burklund, A., Kahn, D., Ball, W., & Weber-Shirk, M. (2014). "An Affordable Open-Source Turbidimeter." *Sensors*, 14, 7142-7155.
- Khairi, M.T.M., Ibrahim, S., Yunus, M.A.M., and Faramarzi, M. (2015). "A Review on the Design and Development of the Turbidimeter." *Sensor Review* 35(1), 98-105.
- Kitchener, B.G.B., Wainwright, J., and Parsons, A.J. (2017). "A Review of the Principles of Turbidity Measurement." *Progress in Physical Geography*, 41(5) 620–642.
- Mullins, D., Coburn, D., Hannon, L., Jones, E., Clifford, E., & Glavin, M. (2018). "A Novel Image Processing-Based System for Turbidity Measurement in Domestic and Industrial Wastewater." *Water Science & Tech* (2018). 77.5:1469-1482.
- Munzberg, M., Hass, R., Khanh, N.D.D., and Reich, O. (2017). "Limitations of Turbidity Process Probes and Formazine as their Calibration Standard." *Analytical and Bioanalytical Chemistry* (2017) 409:719-728.
- Papacosta, K. (2002). Turbidity Calibration Standards Evaluated from a Different Perspective. Turbidity and Other Sediment Surrogates Workshop, 30 April – 2 May 2002, Reno, NV.

- Rice, E.W. (1976). "The Preparation of Formazin Standards for Nephelometry." *Analytica Chimica Acta*, 87 (1976), 251-253.
- Sadar, M. (2004). Making Sense of Turbidity Measurements – Advantages in Establishing Traceability Between Measurements and Technology. Hach Company.
- US EPA (1993) Method 180.1: Determination of Turbidity by Nephelometry. Available at:
https://www.epa.gov/sites/production/files/2015-08/documents/method_180-1_1993.pdf
- Voichick, N., Topping, D., & Griffiths, R. (2018). "Technical Note: False Low Turbidity Readings from Optical Probes During High Suspended-Sediment Concentrations." *Hydrol. Earth Syst. Sci.* 22, 1767-1773.
- Wang, Y., Shariar Morshed Rajib, S.M., Collins, C., & Grieve, B. (2018). "Low-Cost Turbidity Sensor for Low-Power Wireless Monitoring of Fresh-Water Courses." *IEEE Sensors Journal*, 18(11), 4689-4696
- Ziegler, A.C. (2002) Issues Related to Use of Turbidity Measurements as a Surrogate for Suspended Sediment. Turbidity and Other Sediment Surrogates Workshop, 30 April – 2 May 2002, Reno, NV.

APPENDIX

Tables of Experiment Data

Table 2: Data for Figure 4: Turbidity of Lab-Made Formazin, Trial #1

Theoretical Turbidity (NTU)	1	3	5	8	10
Measured Turbidity (NTU)	0.95	3.38	5.25	7.58	9.4

Table 3: Data for Figure 5: Turbidity of Lab-Made Formazin, Trial #2

Theoretical Turbidity (NTU)	10	20	30	40	50	60	70	80	90	100	150	200
Measured Turbidity (NTU)	9.17	14.55	23.23	32.8	39.48	49.15	55.5	64.05	71.38	79.3	120	152

Table 4: Data for Figure 6: Commercial Turbidimeter vs CT Meter, Trial #1

Turbidimeter (NTU)	9.17	14.55	23.23	32.8	39.48	49.15	55.5	64.05	71.38	79.3	120	152
CT Meter (kHz)	0.088	0.1	0.13	0.15	0.16	0.19	0.21	0.23	0.25	0.27	0.37	0.48

Table 5: Data for Figure 7: Commercial Turbidimeter vs CT Meter, Trial #2

Turbidimeter (NTU)	0.2	8.7	13.6	21.1	26.8	30.9	45.5	51.2	58.1	65.7	74.9	119.0
CT Meter (kHz)	0.17	0.205	0.241	0.275	0.29	0.359	0.42	0.447	0.498	0.52	0.571	0.837

Table 6: Data for Figure 8: Sodium Chloride Turbidity vs. Dilution

Concentration of Sodium Chloride (g/mL)	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01	0
Turbidity (NTU)	99.5	101.98	92.45	82.02	71.83	61.03	50.13	38.3	26.15	12.95	0.12

Table 7: Data for Figure 10: Iodized Sodium Chloride Light Scattering vs Dilution (CT Meter)

Concentration of Iodized Sodium Chloride (g/mL)	0.25	0.225	0.2	0.175	0.15	0.125	0.1	0.075	0.05	0.025
Light Scattering Detected via CT Meter (kHz)	0.102	0.1	0.1	0.086	0.08	0.07	0.06	0.044	0.04	0.03

Table 8: Data for Figure 14: Fluorescein Suspension Light Scattering vs Dilution (CT Meter)

Fluorescein Concentration (g/L)	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
Light Scattering Detected via CT Meter (kHz)	0.35	0.358	0.37	0.38	0.399	0.437	0.47	0.5	0.564	0.638

Table 8 Continued

Fluorescein Concentration (g/L)	0.05	0.025	0.0125	0.00675	0
Light Scattering Detected via CT Meter (kHz)	0.51	0.33	0.238	0.161	0.13

Table 9: Data for Figure 15: Fluorescein Suspension Turbidity vs Dilution (Turbidimeter)

Fluorescein Concentration (g/L)	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
Turbidity (NTU)	21.3	22.7	23.7	25.1	26.5	28.4	30.5	33.3	38.7	54.7

Table 9 Continued

Fluorescein Concentration (g/L)	0.05	0.025	0.0125	0.00675	0
Light Scattering Detected via CT Meter (kHz)	57.3	34.8	17.7	8.08	0.15