DEVELOPMENT OF A FORMALDEHYDE ANALYSIS METHOD FOR USE WITH PORTABLE SPECTROMETERS

A thesis presented to the faculty of the Graduate School of
Western Carolina University in partial fulfillment of the
requirements for the degree of Master of Science in Chemistry.

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November 2020

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

ABSTRACT

DEVELOPMENT OF A FORMALDEHYDE ANALYSIS METHOD FOR USE WITH PORTABLE SPECTROMETERS

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Formaldehyde testing in poor communities can be limited by the capital costs of laboratory grade instruments. Not having access to these instruments could result in the water supply not being tested, which can lead to health issues. The development of a rapid, field deployable measurement system would have wide application in these situations. Herein is described the development of a colorimetric reaction with phloroglucinol with a portable visible spectrometer-based method for formaldehyde detection. This method using a Vernier SpectroVis Plus provides an alternative to those who cannot afford the time or money to rely on laboratory-based instrumentation.

CHAPTER ONE: INTRODUCTION

Formaldehyde is a gas that is most commonly found with the photooxidation of hydrocarbons in the atmosphere.[1,2] A secondary source from combustion occurs naturally through forest fires or produced by humans through power plants and manufacturing.[3,4] Although the likelihood of finding formaldehyde in water is not common due to its degradation into other chemicals, there have been places where formaldehyde has been identified such as industry, chemical and oil processing, and municipal waste water discharges.[5,6] Scanning these effluents or drinking water sources require money and time which remote locations cannot afford. If a location that is hundreds of miles away from laboratory for analysis, time can cause issues with obtaining valuable data that may be too late.

There are two strategies for analysis of environmental samples from the field: bring samples to the laboratory or bring the laboratory to the samples. The first method entails deployment of a field technician to acquire the sample, transportation of the samples to the laboratory, which could include a shipping fee and a lengthy shipping time, sample preparation, and analysis via instrumentation. Because an instrument is required for an analysis, the capital investment is steep, and the time from sampling to results can be lengthy.

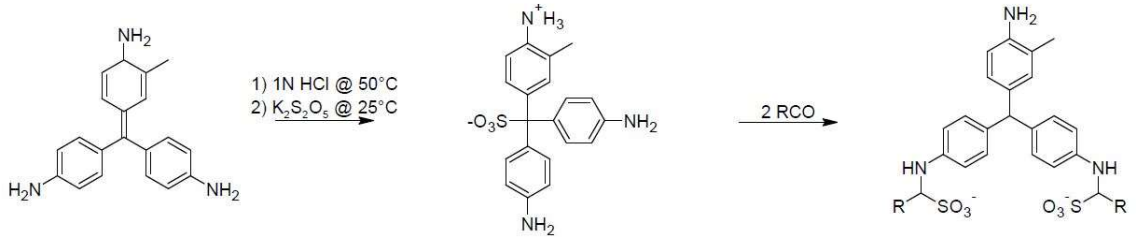
A different approach would be bringing the laboratory to the field. Development of devices that are light and portable is now becoming possible as technology advances. Common examples are portable spectrometers,[7] electrochemical probes,[8] or backpack

mass spectrometers[9] that can provide a signal directly inputted into a computer program for further data analysis.

The goal of this project is to develop a method for detection and quantification of formaldehyde using a portable spectrometer that produces results in the field within minutes and without the cost of using a laboratory instrument. The metric for success in the goal is based upon EPA recommended exposure limits. The EPA has stated that the limit of formaldehyde in water is 5 ppm for an exposure of ten days and 10 ppm for an exposure of one day.[10] By setting the target lower limit of quantitation of formaldehyde allowed in a water source to 5 ppm both of the EPA recommended exposure limits will be determinable. Deploying portable spectrometers to measure formaldehyde would be ideal for tracking chemical spills. If the location is isolated from a laboratory's access like a spill from a train accident, then this method can be deployed and have results in a matter of minutes instead of multiple hours or days. Tracking the concentration of formaldehyde above 5 ppm can be beneficial to deem the spill to be contained or still a potential health hazard.

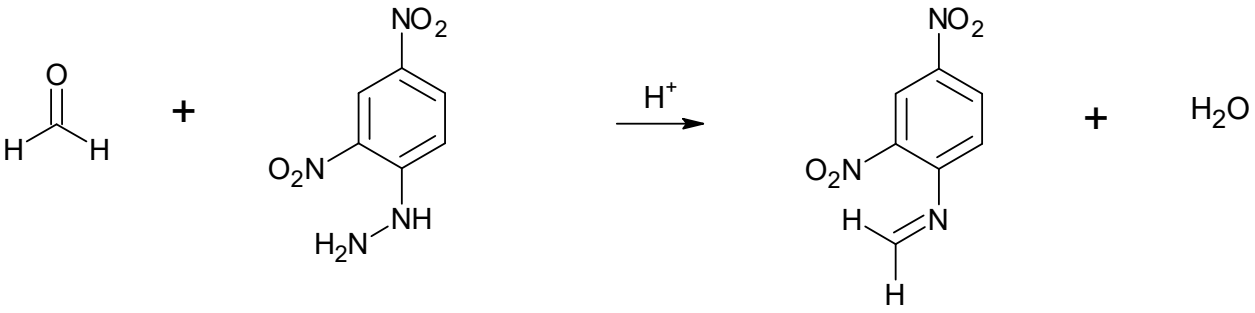
There have been numerous methods developed to quantify formaldehyde, but many rely on expensive instrumentation like HPLC or hazardous reagents.[11–13] Directly quantifying formaldehyde by use of UV-Vis spectrometers is not reasonable because the signal for this method is not sensitive enough to detect small changes of concentrations. Before spectrometers were invented, colorimetric reactions were a primary way to detect the presence of a chemical. With a reaction that results in a color change, a signal can be measured on a spectrometer.

A common colorimetric reaction for aldehydes is the Schiff reaction which entails an aldehyde and fuchsin dye resulting in a color change from colorless to a brilliant magenta as seen in Scheme 1.[11] A drawback with this reaction is that it is not selective to formaldehyde but rather reacts with many other aldehydes. When the fuchsin dye reacts with the aldehyde to form the magenta product, the product's absorbance does not obey the Beer-Lambert Law because the aldehyde can bind to the fuchsin reagent at multiple locations making it difficult to assign accurate concentrations.[11,14]



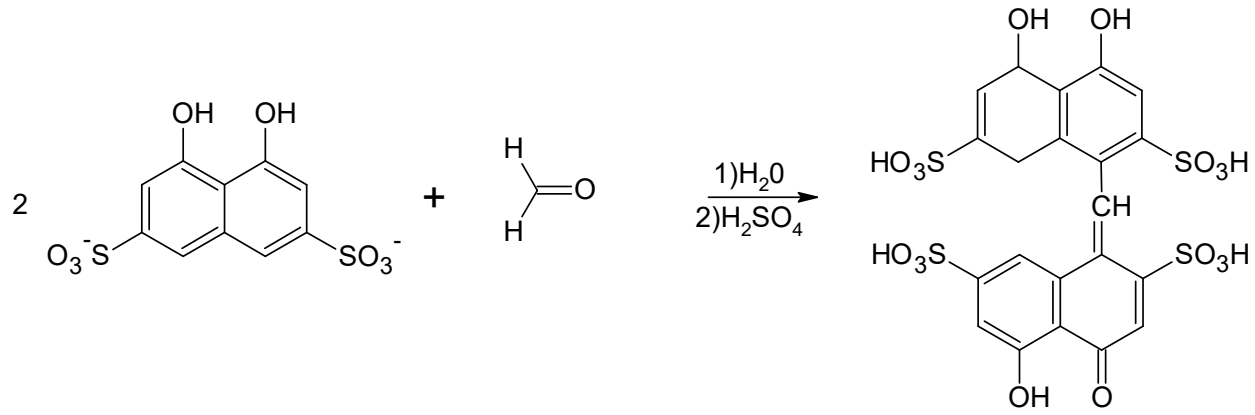
Scheme 1: Schiff reaction where fuchsin dye reacts with an aldehyde to produce a magenta color.

Another colorimetric reaction that can detect formaldehyde utilizes 2,4-dinitrophenylhydrazine (DNPH). DNPH is frequently used by national regulatory agencies like the EPA as a reagent to measure formaldehyde at low concentrations.[12] In Scheme 2 is shown the reaction of DNPH and formaldehyde to produce the DNPH derivative that is spectroscopically active.[12] A downside with this way of detection is that it needs to be measured using a HPLC to separate the formaldehyde product from other aldehydes. A HPLC is a large initial investment to purchase the instrument.[12]



Scheme 2: DNPH reaction with formaldehyde to produce the formaldehyde-DNPH derivative.

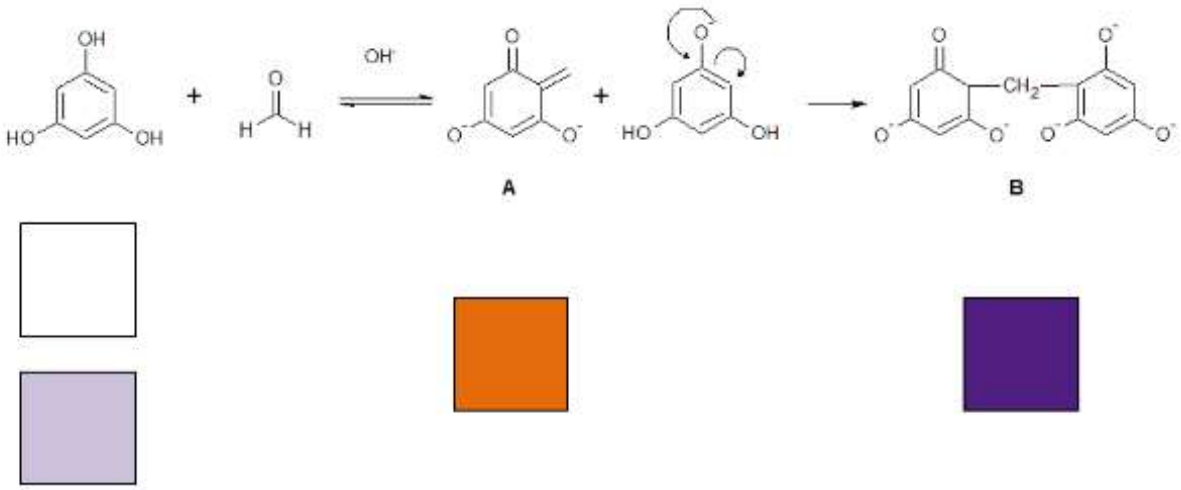
Chromotropic acid (CA) can also be used for detecting formaldehyde as a colorimetric reagent. When CA is reacted with formaldehyde with concentrated sulfuric acid, a product is formed that is spectroscopically active; however, the use of hazardous concentrated sulfuric acid makes fieldwork difficult for technicians due to safety.[15] A similar method uses CA with hydrogen peroxide, concentrated phosphoric acid, and concentrated hydrochloric acid provides the same safety issues as the original CA method.[13]



Scheme 3: Chromotropic acid reacting with formaldehyde to produce a spectroscopically active product.

Phloroglucinol gives a colorimetric method with good selectivity and sensitivity without the need for expensive instrumentation or harmful reagents.[16,17] Two previously conducted successful experiments have used phloroglucinol to react with a strong base to form a purple deprotonated phloroglucinol reactant which the reacts with

formaldehyde to form an orange intermediate and then a purple product.[16,17] Ramachandran et al. used 0.1 M sodium hydroxide as the reactions starting point in a volumetric flask which yielded a signal that can be monitored using a spectrometer; however, Li et al. altered the experiment utilizing a flow cell and 0.4 M potassium hydroxide (KOH).[16,17] A combination of the higher concentration KOH with similar standard and sample solutions proposed by Ramachandran et al., for the basis of the new method using a portable UV-Vis spectrometer that can be utilized for in-field testing that was developed in this work. Scheme 4 depicts the mechanism for the reaction of phloroglucinol and formaldehyde proposed by Li et al.[17]



Scheme 4: Proposed reaction of phloroglucinol and formaldehyde where structure A is the intermediate product and structure B is the final product. The colors correspond to each reactant and product above..

CHAPTER TWO: EXPERIMENTAL METHODS

Reagents

A solution of 37% w/v formaldehyde and solid calcium chloride were purchased from Sigma-Aldrich. Phloroglucinol was purchased from JT Baker. Potassium hydroxide, sodium chloride, sodium nitrate, sodium sulfate, magnesium chloride, and acetic acid were purchased from Fisher Scientific. Ethyl acetate, methanol, and propionaldehyde were purchased from Alfa Aesar. Deionized water was filtered through a carbon cation exchange fiberglass tank from Culligan which is exchanged semiannually.

Instrumentation

A Vernier SpectroVis Plus was used as the field deployable instrument for these experiments. The Vernier uses an incandescent white bulb and a LED bulb as the light sources. The detector is a charged-coupled device. The total time to scan each spectra was ~2 sec. All spectra were scanned from 380 – 900 nm resulting in 649 absorbance values per spectra. One blank was used per scan with deionized water. Cuvettes used had a 1 cm pathlength.

Potassium Hydroxide Solution

A 0.4 M solution was made by weighing out 5.618 g of KOH, which was transferred to a 250 mL volumetric flask. Then deionized water was added to the fill line and the solution was inverted to mix.

Phloroglucinol Solution

A 0.1 M solution was made by weighing out 1.259 g of phloroglucinol which was transferred to a 100 mL volumetric flask. The flask was filled ~75% full with 10% v/v ethanol and then placed on a wrist action shaker for 30 min. The solution was diluted to the line with 10% v/v ethanol and inverted to mix.

Formaldehyde Solutions

A 10,007 ppm formaldehyde solution was made by mechanically pipetting 6.757 mL of 37% formaldehyde into a 250 mL volumetric flask. The solution was diluted to the line with deionized water and inverted to mix. Serial dilutions were made by using the stock solution to make 4.8 ppm, 12.8 ppm, 24, ppm, 48 ppm, 74.9 ppm, 107 ppm, 205 ppm, and 394 ppm formaldehyde solutions.

Standard Solutions

Using analytical pipettes, 4 mL of 0.1 M phloroglucinol solution and 2 mL of formaldehyde solution were added to a 10 mL volumetric flask. The solution was diluted with 0.4 M KOH. The volumetric flask was inverted to mix and the cuvette was filled with the standard solution. The measurement was started at 1 min.

Validation Sample

The sample was prepared and measured in the same manner as the standards. The final concentration of formaldehyde was 74.9 ppm.

Interferent Samples

Solutions at 1% v/v of acetic acid, ethyl acetate, propionaldehyde, and methanol were prepared by transferring 1 mL of reagent into separate 100 mL volumetric flasks via analytical pipette. Each 1% solution was diluted to the line with deionized water and inverted to mix.

The formaldehyde interferent samples were prepared by pipetting 4 mL of each 1% solution and 10 mL of 394 ppm formaldehyde solution into separate 100 mL volumetric flasks. The volumetric flasks were diluted to the line with deionized water and inverted to mix resulting in ~200 ppm solutions of each interferent and 39.4 ppm of formaldehyde.

For interferents that are solid, 40 mg of $CaCl_2$, $MgCl_2$, NaCl, and $NaNO_3$ were added to separate 100 mL volumetric flasks. To those flasks, 10 mL of 394 ppm formaldehyde solution was added. The volumetric flasks were diluted to the line with deionized water and inverted to mix resulting in ~200 ppm solutions of each interferent and 39.4 ppm of formaldehyde.

CHAPTER THREE: RESULTS AND DISCUSSION

Analytical Wavelength Determination

When the standards were scanned, there were two spectral bands at 398 and 474 nm shown in Figure 1A. The peak at 474 nm is the orange intermediate product seen in Scheme 4 A, and the peak at 398 nm corresponds to the purple final product Scheme 4 B. A plot of both absorbances against the known formaldehyde concentrations to give a Beer's Law calibration curve, showed a significant difference in the slopes. In Figure 1B is shown the calibration curve at 474 nm and 394 nm. The 473 nm band has a slope of 0.0018 ppm^{-1} whereas the band at 398 nm has a slope of 0.0009 ppm^{-1}. The slope is twice as steep for the 474 than the 398 nm. By selecting the wavelength that has the steeper slope, it can be expected to increase the method's sensitivity as seen as

$$S_A = k_A C_A.$$

where S_A is signal of the analyte, k_A is the sensitivity (slope), and C_A is the concentration of the analyte. More signal per concentration is directly proportional to more sensitivity. Therefore, the band with a λ max at 474 nm was chosen for the analytical wavelength.

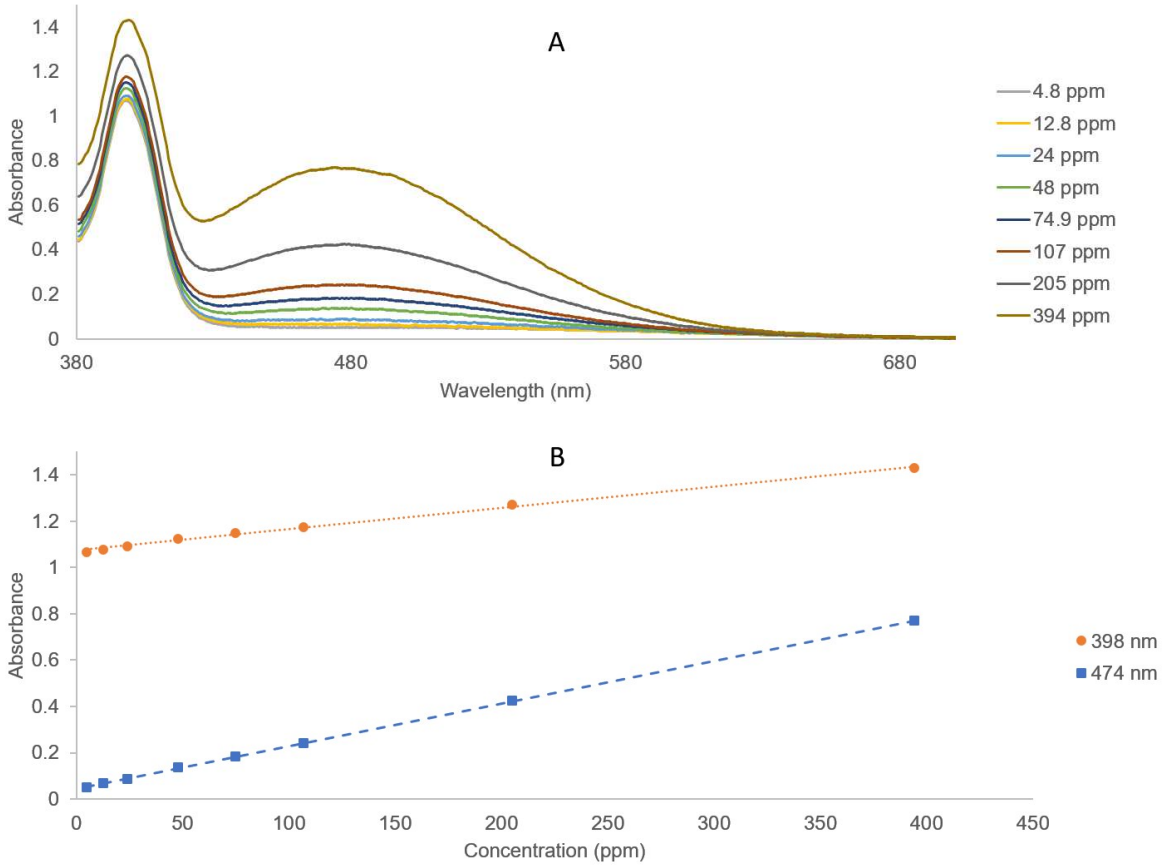


Figure 1: A) UV-Visible spectra of formaldehyde standards ranging from 4.8 ppm to 394 ppm. B) Beer's Law calibration curve at 474 nm and 398 nm.

Measurement Time Determination

In Scheme 4 is shown a proposed mechanism for the color forming reaction between phloroglucinol and formaldehyde from Li et al. A color change occurs when the base is added from colorless to purple. As the reaction continues, structure A in Scheme 4 is the orange intermediate product that is measured at 474 nm. Because the intermediate product is not stable, the intermediate product will then change to the final product as

seen in structure B in Scheme 4. The final product has a purple color. The more formaldehyde present, the more the solution transitions to orange. At lower concentrations of formaldehyde, the solution remained purple. The solution turned to a purple color after the reaction was complete. Because the compound changes from one color to another, a kinetic study was performed to determine when the absorbance at the analytical wavelength was maximized.

Diluting the solutions with the 0.4 M KOH took approximately 25 sec. In Figure 2 is shown the results of the time-based experiment. Due to the preparation time of the standards, the time on the x-axis was adjusted to when the solution started scanning. This means t=0 is when the KOH was added to the solution and t=25 represents when the scan on the instrument started. At the maximum absorbance, the slope will be the

smallest indicating the smallest amount of change of absorbance. The maximum absorbance was around 60 sec, and this is when all future samples were scanned.

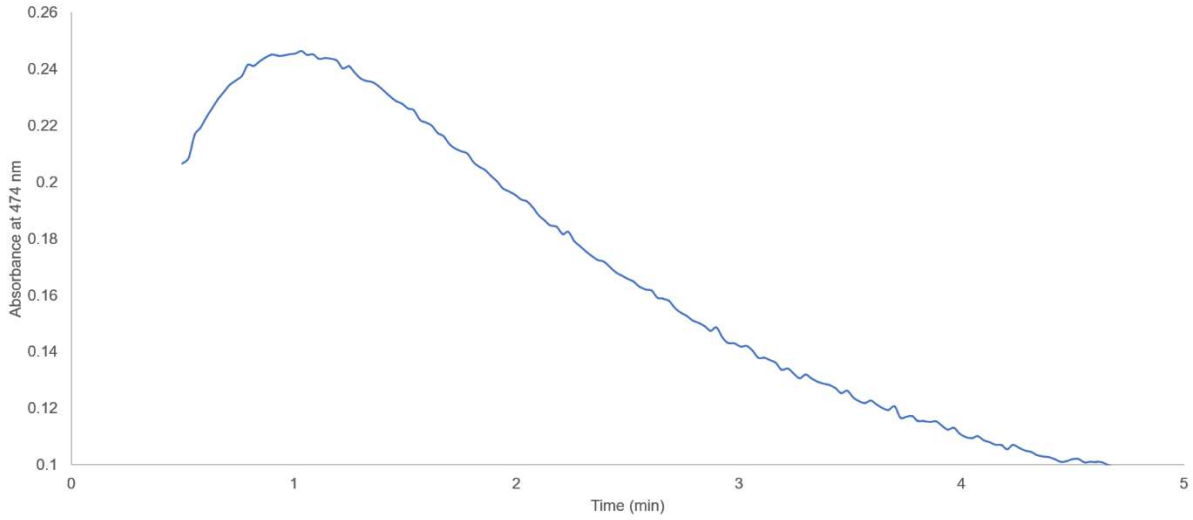


Figure 2: UV-Visible time-based reaction measurement at 474 nm.

Calibration Curve

The calibration curve was determined by using standards ranging from 4.8 ppm to 394 ppm, whose spectra are shown in Figure 3. As stated before, the analytical wavelength used was 474 nm.

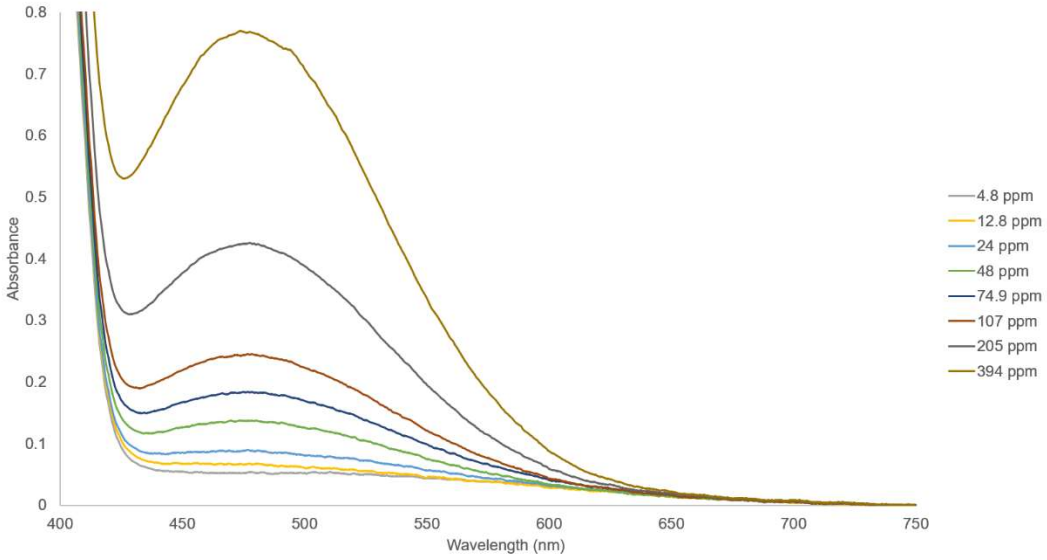


Figure 3: UV-Visible spectrum of calibration curve using standards ranging from 4.8 ppm - 394 ppm.

In Figure 4 is shown the calibration curve with linear fit of the absorbances versus the concentrations of the standards. The coefficient of determination from calibration curve was 0.9999. Because coefficient of determination relates to the variance of the dependent variable to the independent variable, the coefficient of determination is related to the method's precision with 1 being the most precise and 0 being the least precise. Based on the value of 0.9999 given, it can be ascertained that the method is precise. The slope of the calibration curve gives a value of 0.00184 ppm^{-1} cm^{-1} which is the molar absorptivity at 474 nm.

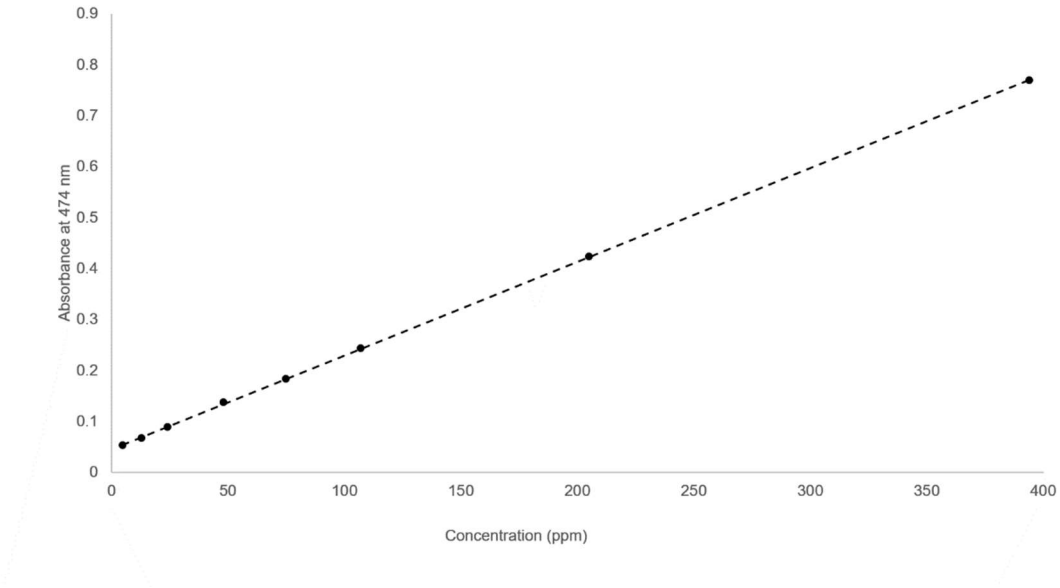


Figure 4: Beer's Law calibration curve measured at 474 nm.

Accuracy and Confidence Interval

The accuracy of the method was determined by a separate validation of a sample containing 74.9 ppm of formaldehyde. When the sample was measured, the absorbance detected was 0.1831. Once the absorbance was found, the concentration was determined by using:

$$Concentration = \frac{Absorbance - y\ intercept}{slope}$$

This resulted the measured concentration to be 74.94 ppm. The percent error produced from the results was 0.047%.

Measurements from another day were used to determine the confidence interval of the method using:

$$CI = \pm \frac{ts}{\sqrt{n}}$$

where t represents the t-critical value, s is the standard deviation, and n is the number of replicates. The t-critical value was used at 95% confidence level and a degrees of freedom of $n - 1 = 2$. The standard deviation between the three samples was 0.61, and the number of replicates was 3. The validation sample's concentration was found to be 74.9 ± 1.5 ppm.

Limit of Linearity

The upper linear limit of the calibration curve was determined. In Figure 5 A is shown the 4.8 – 856 ppm formaldehyde spectra overlaid with one another. In Figure 5 B is shown the absorbance at 474 nm versus concentration. By testing the linear fit of each data point's coefficient of determination, the calibration curve becomes non-linear at concentrations above 400 ppm, which was assigned as the upper limit of linearity. The arrow in Figure 5 A is indicating the higher concentration spectra are showing an additional band forming whose position is centered around 493 nm that is likely due to a complexation between intermediate products which may be the source of the nonlinearity at these higher concentrations.

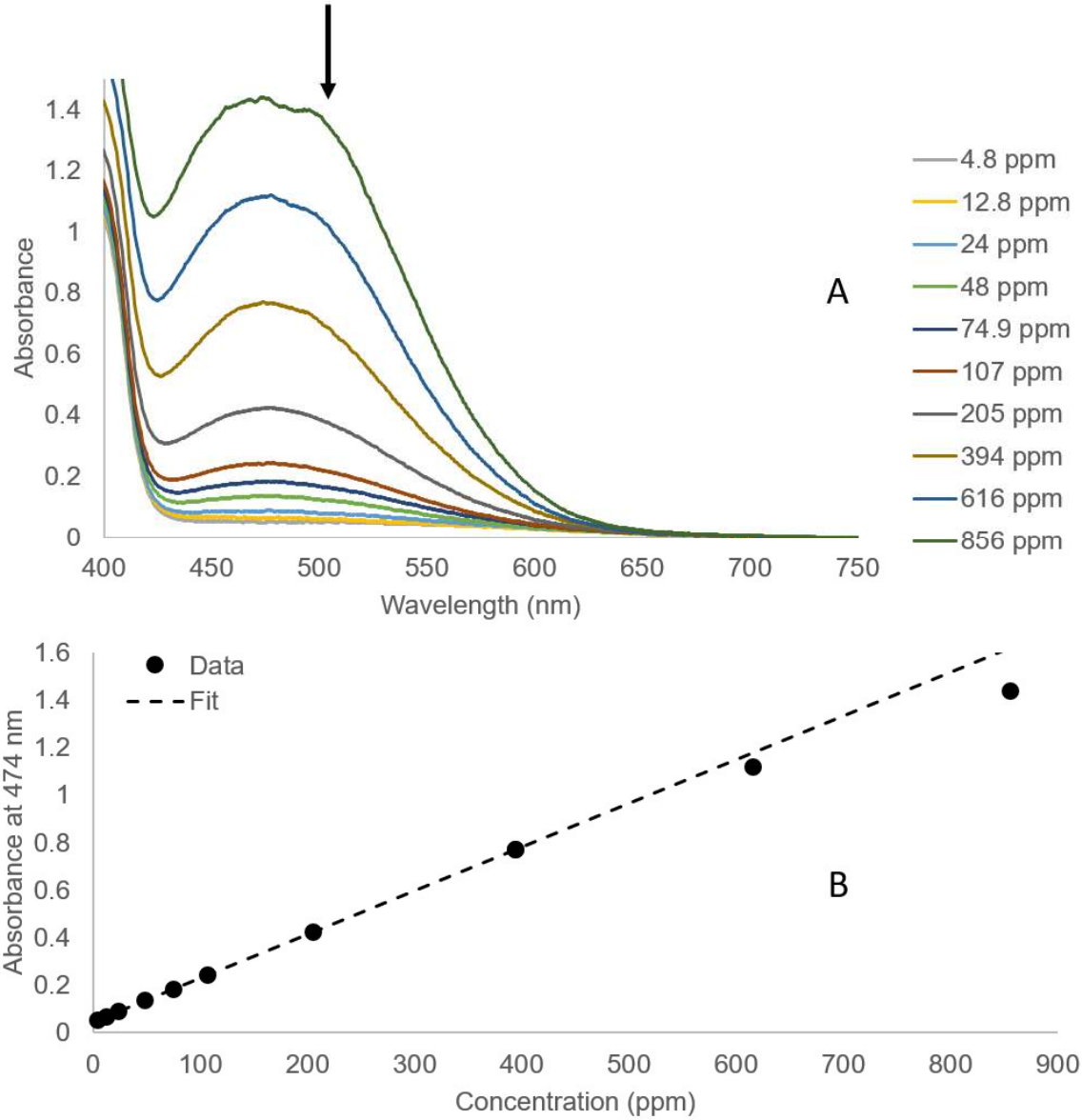


Figure 5: A) UV-Vis Spectra of linearity testing. B) Linear fit of absorbance at 474 nm vs. concentration of formaldehyde. Fit shown is for concentrations in the linear range, 0-400 ppm.

Lower Limit of Detection

The smallest amount of formaldehyde that can be measured and seen as not a signal from the blank is the lower limit of detection (LLOD).[18] The LLOD was determined by measuring a blank consisting of 2 mL of deionized water (this represents the formaldehyde solution with no formaldehyde), 4 mL of 0.1 M phloroglucinol, and diluted. The measurement was measured in triplicate. From these data, the LLOD was determined to be 1.72 ppm.[18] The equation used to find the LLOD is:

$$(S_A)_{LOD} = S_{mb} + 3\sigma_{mb}$$

where $(S_A)_{LOD}$ represents the LLOD, S_{mb} represents the signal of the media blank, and σ_{mb} represents the standard deviation of the media blank.

Lower Limit of Quantitation and Linear Range

The smallest concentration of formaldehyde that can be measured and reported with confidence is the lower limit of quantitation (LLOQ).[18] The LLOQ was also determined from the same measurements as the limit of detection. The limit of quantitation was determined to be 4.56 ppm. The equation used to find the LLOQ is:

$$(S_A)_{LOQ} = S_{mb} + 10\sigma_{mb}$$

where $(S_A)_{LOQ}$ represents the LLOQ, S_{mb} represents the signal of the media blank, and σ_{mb} represents the standard deviation of the media blank.

According to the EPA, 5 ppm is the 10 day exposure concentration that is deemed to cause health effects, so this method can be used to quantify values of formaldehyde concentrations larger than what is recommended.[10] Any sample with a

concentration lower than 4.56 ppm cannot be tested because it is lower than the LLOQ. Combining the LLOQ and the limit of linearity the linear range was determined to be 5 - 395 ppm.

Selectivity

In Table 1 is shown the percent recovery of a 39.4 ppm formaldehyde solution when spiked in solutions containing 200 ppm of contaminants commonly found in water or structurally similar compounds. Methanol contains a single carbon and bound to an oxygen, ethyl acetate and acetic acid contain carbonyls, and propionaldehyde is an aldehyde; therefore, these organic molecules are similar to formaldehyde. Ideally the interference should not play a role with samples taken in the field. The percent recovery is not equal to 100% in most samples, but these solutions represent extreme cases where the amount of ions are in excess of analyte by 500% should not interfere when under normal conditions. If the ionic compounds are tested separately from the formaldehyde, then an inflated percent recovery can be avoided or accounted for.

Table 1: Calculated formaldehyde concentration in the presence of 200 ppm potential interferents. Spike recovery is the normalized difference from expected formaldehyde concentration of 39.4 ppm.

Interferent	Calculated Concentration of Formaldehyde (ppm)	Recovery (%)
Methanol	44.2	112
Ethyl Acetate	40.1	102
Acetic Acid	53.2	135
Propanal	54.4	138
$CaCl_2$	46.0	117
$MgCl_2$	37.9	96
NaCl	43.6	111
$NaNO_3$	44.6	113

CHAPTER FIVE: CONCLUSIONS

Locations that are hundreds of miles away from the nearest lab will have issues gathering rapid results if a formaldehyde spill occurs. The EPA has stated that the limit of exposure from formaldehyde over a ten-day period is 5 ppm and one-day period is 10 ppm. It has been shown that formaldehyde concentration can be determined at or above these threshold concentrations with a portable visible spectrometer. The linear range was found for this developed method to be from 4.56 to 394 ppm; however, if the concentration of the analyte is outside that range, then further testing with another method will need to be done to determine the concentration of formaldehyde or with sample dilution if the concentration is found to be above the upper linear range.

Future work into this portable method will need to compare the figures of merit of this field deployable instrument and a laboratory instrument. This can help identify shortcomings relative to the conventional instrument. Investigation of other portable methods, that require less monetary and time investment, could shed light on other avenues on how to improve this method's limitations. If we use alternative portable spectrometers that have better parts, then better results could be within reach. Another possible next step could incorporate air sampling methods to determine the amount of formaldehyde the atmosphere or indoors.

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