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# Developing a colorimetric membrane sensor to detect trihalomethanes in water samples utilizing the Fujiwara reaction

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## Executive Summary

### Problem Statement

Trihalomethanes (THMs) are present in drinking supply water as a results of water cleaning processes and belong to a group of contaminants know as disinfection byproducts (DBPs). THMs present numerous health concerns, including carcinogenicity. Current testing methods are time consuming and expensive (Gas chromatography and Mass Spectroscopy). Any testing of water samples for THM contaminants cannot be done in the field, and must be sent off to laboratories with appropriate equipment. It is the goal of this work to create a device which allows for colorimetric detection of THMs in water samples which drastically reduces the time and cost of testing and eventually to create a prototype useable for immediate field testing.

### Results and Conclusions

The results of this work were to create a colorimetric sensor capable of detecting THMs in environmentally relevant concentrations, set by the Environmental Protection Agency (EPA). A sensor was produced using an electrospun polypropylene (PP) membrane created using a 2.6 weight % PP solution in cyclohexane, acetone, and dimethylformamide (DMF). Colorimetric reaction utilized was the Fujiwara reaction using pyridine and a supersaturated solution of ethanol and sodium hydroxide as reactants. The ideal operating conditions of this reaction were found to be 70 °C with volumetric ratios of reactants of 9:1 pyridine : ethanol/sodium hydroxide. This reactions produces a pink/red color change that was measured using imageJ software. The intensity of the color change was measured as a quantitative measure of the concentration present in the water sample. A calibration curve was then created to correlate the color intensity and

THM concentration (bromoform) over a range of 1-1000 ppb. The data was fit logarithmically and the color intensity found to predict the concentration with 97.7 % confidence.

**Broader Implications**

The work presented will ideally allow for a portable sensor to be created for the quick, cheap, and easy detection of THM concentrations in drinking water samples to determine if the sample is within EPA limits for safe drinking water. It would allow for immediate and accurate results to be obtained with very little work involved. If the eventual prototype can be made cheaply enough it could be provided to consumers to provide for home testing of water quality.

Benefits I have received from this project include and increased understanding of the research process and an appreciation for all the small details that must be accounted for in creating a functioning prototype. Exposure to relevant background materials has also given me a greater appreciation and understanding of environmental and water quality as well as the advanced research that goes into ensuring the safety of the public.

**Future Work**

Future work for this project will ideally result in a cheap portable device capable of provide immediate and accurate testing of water quality. The major steps needed to reach that point include further calibration of intensity and concentration curves for other compounds, and working to provide a reliable and scaleable way of producing membranes for this sensor. There must also be eventual work into how to turn this laboratory setup into a portable device able to be used by untrained individuals.

## Introduction

Trihalomethanes (THMs) are a class of chemicals with the general formula of $CHX_3$, with X being some combination of chloroform, bromoform, fluorine, or iodine. These compounds are known to have adverse health effects in humans, including carcinogenicity. THMs are found in drinking water supplies most commonly as a result of disinfection by chlorination, falling into a group of chemicals referred to by the EPA as disinfection byproducts (DBPs). In the context of drinking water quality, four THMs are monitored by the EPA: chloroform ($CHCl_3$), bromoform ($CHBr_3$), chlorodibromomethane ($CHClBr_2$), and bromodichlomethane ($CHBrCl_2$) [2]. The EPA has set a maximum allowable annual average level of 80 parts per billion of total THMs [1].

The most common method of testing for THMs in water samples is gas chromatography, with detection by flame ionization, electron capture or mass spectroscopy. This method of measurement can produce very accurate results even at low concentrations (detection limit ~0.5 ppb), however the equipment required is expensive and cannot be used in field testing as samples must be sent to a lab for testing [2]. To reduce both the cost and time required to test water samples for THM contamination, the work presented in this paper aims to create a colorimetric sensor that can provide fast, cheap, and portable testing of water samples.

The work presented focuses on detection of bromoform and chloroform in water samples, utilizing an electrospun hydrophobic polypropylene membrane and Fujiwara reaction chemistry. Images were taken of the observed color changes and analyzed to create calibration curves based on the intensity of the observed change. The use of the membrane functions as a concentration mechanism to allow the Fujiwara chemistry to be used at lower concentrations than in previous work.

**Background**

The Fujiwara reaction was initially discovered in 1916. The chemistry involved in this reaction involves a mixture of pyridine and a strong base to produce a pink/red intermediate chromophore in the visible spectrum in the presence of a THM [3, 4]. Experimental work has shown this reaction to be effective in detecting concentrations of THMs in water at concentrations down to 100 ppm [4]. Some research has been done to allow the use of the Fujiwara reaction to be used at lower concentrations closer to the EPA limit of 100 ppb, however this requires a separate step to preconcentrate the sample to achieve these results [4, 6, 11]. Research has also identified a limiting factor in utilizing the Fujiwara reaction for water quality testing to be the breakdown of the relevant chromophore when water is present [9, 10]. Effectively separating the contaminant from the water is likely key to achieving Fujiwara products at concentrations low enough to meet EPA standards of an 80 ppb limit [1].

## Experimental Methods

### Electrospinning

To create the membranes used for testing, polypropylene was dissolved into a solution of cyclohexane (12 g), acetone (1.5 g), and dimethylformamide (1.5 g). Solutions of various concentrations (1- 4 wt % PP) were prepared and placed in a heating element at 70 °C overnight prior to spinning. The prepared solutions were electrospun in a setup depicted in figure 1 below. The solution was pumped using a syringe pump at 25 ml/hr, and a voltage of 25 kV applied between the tip of the needle and foil substrate placed 25 cm apart.
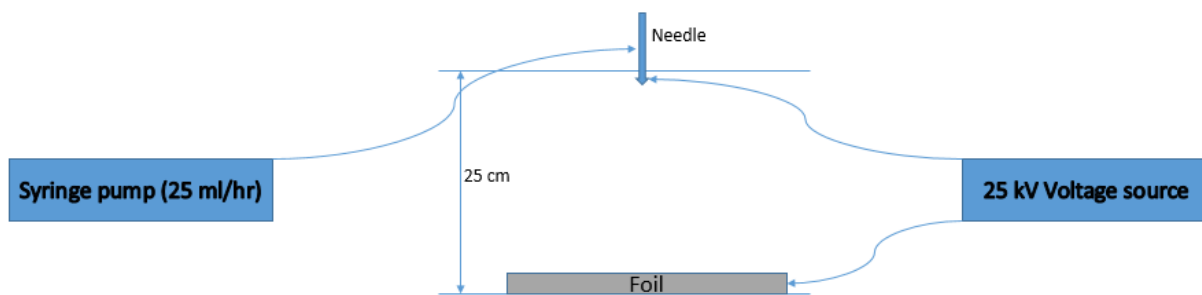


*Figure 1: Experimental setup for electrospinning polypropylene membranes*

### Testing

To run the prototype experiments, a testing membrane was cut from the sample spun onto the foil sheets, and placed between two Teflon ® blocks, which had been cut to allow the Teflon blocks to be placed snugly over a 20 mL scintillation vial, and allowing an exposed section of membrane above the vial to have the testing solutions added using pipets. The solutions used for testing consisted of pyridine, and a supersaturated solution of sodium hydroxide dissolved in ethanol. The solutions were added in a ratio of 9:1 pyridine:ethanol solution on a volumetric basis (ratio determined through experimentation) during testing at a rate frequent enough to provide constant wetting for the membrane. A known concentration of THMs was prepared in a

20 mL scintillation vial, the Teflon/membrane setup fitted on top of the vial, and then the vial

was placed in a water bath at 70°C. The testing solutions were then added periodically

throughout the experiment, with pictures being taken periodically to track the progress of the

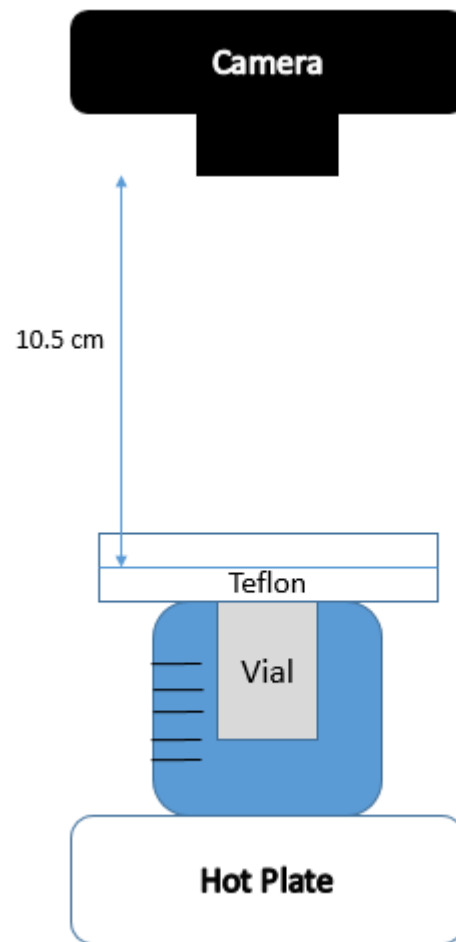color change. A diagram of the testing setup is shown in figure 2



Figure 2: Physical setup used for prototype testing

**Image Analysis**

Images were taken every five minutes throughout the experiment using a Nikon Coolpix

S9100 placed so that the lens was 10.5 cm away from the top of the membrane. These images

were analyzed using ImageJ software available from the National Institute of Health (NIH). The

area were the color change had taken placed was selected and its mean color intensity was determined using the software. This is the value that was used to quantify the results obtained from testing. This method was based off of earlier methods utilized in colorimetric sensors [6, 7, 8]

**Scanning Electron Microscopy**

Scanning electron microscopy (SEM) is performed on a JOEL JSM-6510LV operating at an accelerated voltage of 5 kV and high-vacuum mode. All samples were sputter-coated in a gold/palladium (Au/Pd) alloy for 1.5 minutes.

**Contact Angle Measurements**

Contact angle measurements were done using the sessile drop technique, conducted on a drop shape analyzer (DSA20E, KrüssUSA, Mathews, NC). The deionized water drops were applied using a micro-syringe pointed vertically downward onto the membrane surfaces and the instrument-provided image analysis software was used for contact angle determination.

## Data and Results

The first step in the experimental work was to determine an ideal composition (weight % PP) of the electrospinning solution. Solutions were prepared from 1-4 weight % PP and electrospun, and subsequently underwent SEM and contact angle measurements. Contact angle measurements were performed to determine the hydrophobicity of the membrane, which was seen to peak at the concentration of 2.6 weight % (figure 3)
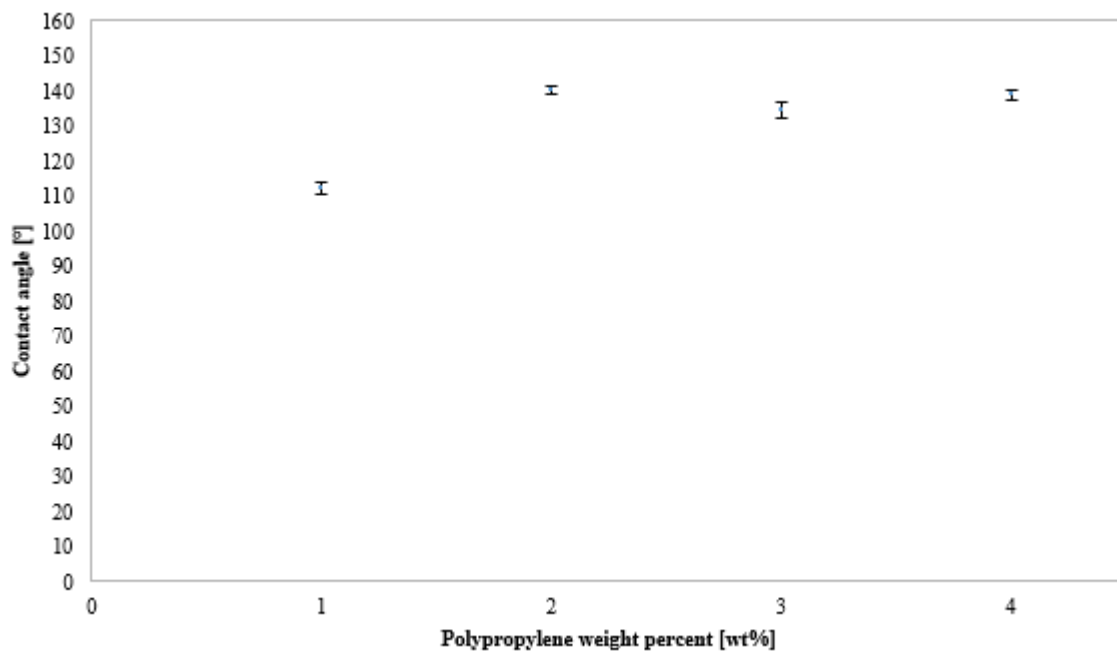


*Figure 3: Contact angle of electrospun membranes spun from solutions 1-4 weight % PP (Sessile drop technique))*

The results of the SEM imaging are shown in figures 3 and 4 below. SEM testing agreed with the contact angle measurements that 2.6 weight % PP was the ideal solution concentration.
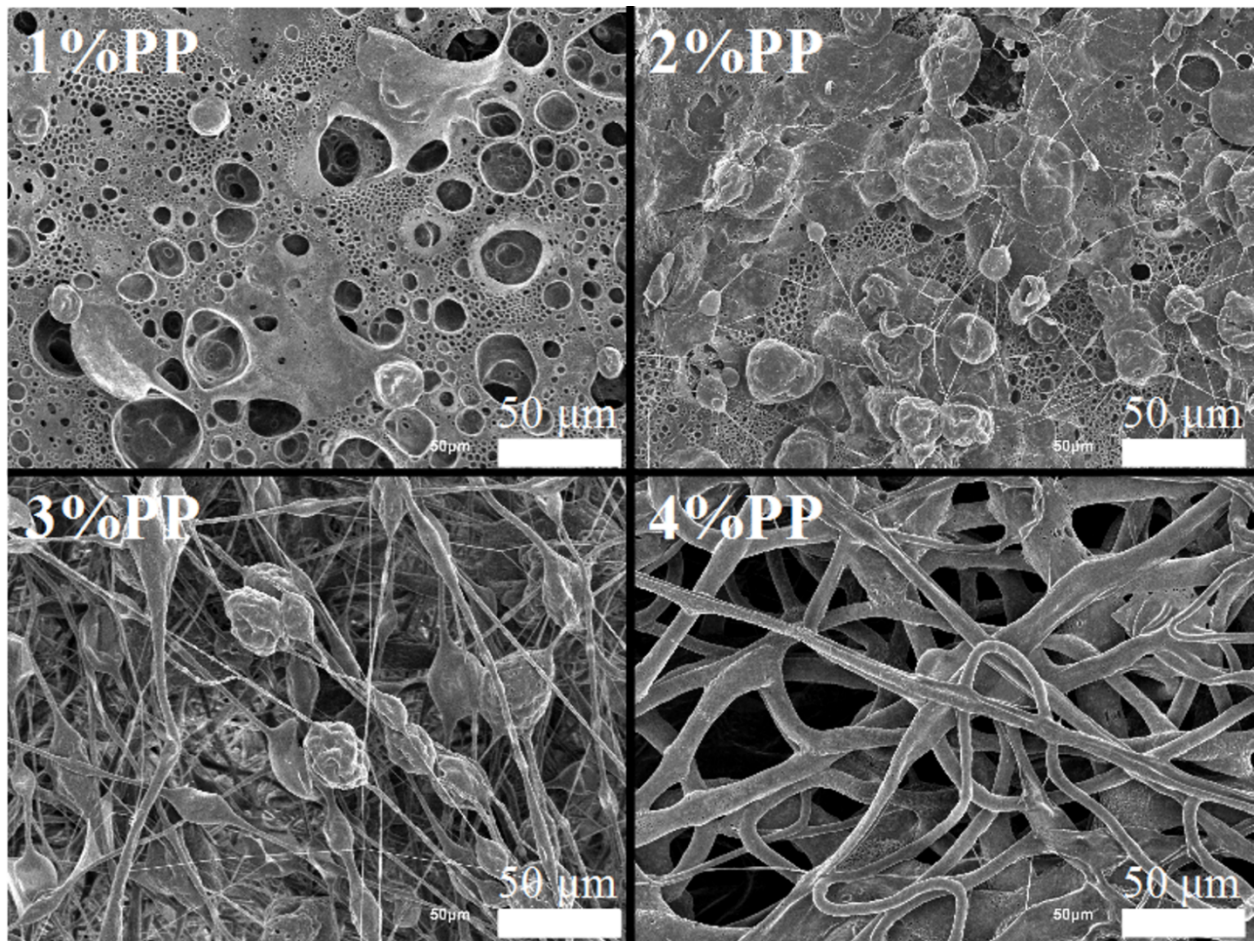
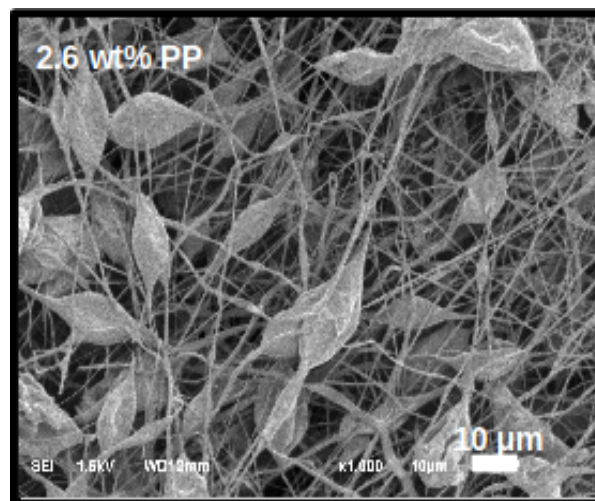Figure 4: SEM images of membranes electrospun using 1-4 weight % PP solution



Figure 5: SEM image of membrane electrospun with 2.6 weight % PP solution

All testing was then performed using membranes prepared with 2.6 weight % PP. The next step was to determine ideal operating conditions for the prototype testing; specifically the temperature, and ratios of pyridine and ethanol/NaOH solution used for testing. A design of experiments was conducted to determine these conditions, the results of which are shown in figure 6. The ideal operating conditions were found to be a 9:1 volumetric ratio of pyridine to ethanol/NaOH solution, and an operating temperature of 70 °C.



*Figure 6: Results of operating condition optimization, Temperatures tested at 70 and 90 °C, pyridine : ethanol/sodium hydroxide solution volumetric ratio of 9:1, 1:1, and 1:9*

An example of the type of results achieved using these testing method is shown in figure 7 below.
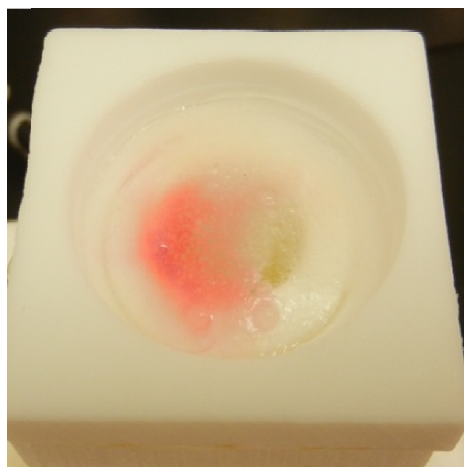
*Figure 7: example of final result of prototype testing, pink color change on membrane. Test conditions: 80 ppm bromoform, 90 °C, 9:1 pyridine : ethanol/sodium hydroxide solution*

The chosen operating conditions and membrane composition were then used to create a calibration curve comparing the color intensity to the concentration of the solution. Known concentrations of bromoform were prepared at 8, 80, and 250 ppb and tested using the membrane setup. The results of this calibration are shown in table 1 and figure 8.

*Table 1: Bromoform calibration curve results, (70 °C, 9:1 ratio, 2.6 weight % PP)*

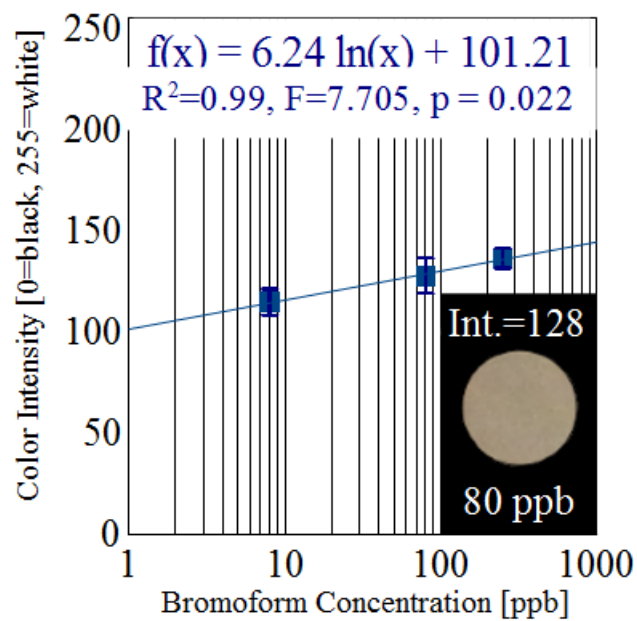| Bromoform Concentration | Color intensity | Std Dev |
|---|---|---|
| 8 | 114.5 | 6.4 |
| 80 | 127.6 | 8.7 |
| 250 | 136.3 | 4.9 |

*Figure 8: Calibration curve for detection of bromoform using the color intensity analysis of colorimetric sensor (2.6 weight % PP, 70 ° C, 9:1 pyridine : ethanol/sodium hydroxide*

## Discussion/Analysis

The main purpose of the membrane was to try to minimize the amount of water present in the reacting mixture, as the pink/red product has been seen to quickly decompose when water is present [9, 10]. To achieve this, a highly hydrophobic membrane was desired. Contact angle using the sessile drop technique was used to measure the hydrophobicity of the different membranes. Of the four concentrations initially tested (1, 2, 3, and 4 weight % PP), the highest contact angle was found to be at 2 % PP, followed by a significant drop at 3% PP, shown in figure 3. A concentration of intermediate composition, 2.6 % PP was then tested to identify a potential maximum between the two points, and was found to have the highest contact angle (most hydrophobic) of 150 °. The results of the contact angle testing indicated that 2.6 weight % PP solution was ideal.

The various membranes were then imaged using SEM to try to gain an understanding of the morphology of the different compositions, and confirm the predictions of the contact angle testing. The SEM images are shown in figures 4 and 5. In figure 4, it can be seen that as the weight % of PP increases, the membrane transitions from a porous sheet to a mesh of fibers. It was also observed that in the intermediate concentrations, nodules began to form along the lengths of some of the fibers. These nodules contributed to an increased hydrophobicity of the membrane, and are most clearly and abundantly observed in the 2.6 weight % PP solution shown in figure 5. The results of the SEM investigation of the morphology of the different membranes agreed with the contact angle measurement in predicting the 2.6 weight % PP solution to be ideal. This concentration was chosen and used for the remainder of the experiments.

Operating temperature and concentration (ratios) of reacting solution were the next parameters to be optimized. Tests were conducted at 70 °C and 90 °C, and at volumetric ratios of

9:1, 1:1, and 1:9 of pyridine : ethanol/sodium hydroxide solution using concentrations of 80 ppm and 1 ppm of bromoform. The results of this testing, shown in figure 6, indicate 70 ° C and 9:1 volumetric ratio to be the ideal operating conditions. These conditions were used for the remainder of the testing.

A calibration curve was then created, with the purpose of being able to reliably predict the concentration of a solution based on the color intensity observed on the membrane. Bromoform concentrations of 8, 80 and 250 ppb were used for this testing Shown in figure 8, the data was fit logarithmically and found to accurately predict THM concentration. The results of an analysis of variance (ANOVA) found a p value = 0.022 ($<\alpha$=0.023), meaning the calibration curve was found to predict the concentration of the solution using the color intensity with a confidence of 97.7 %.

The results of these experiments have created a colorimetric sensor system that has allowed for detection of THMs in water samples at much lower concentrations than previously achieved. This allows for the Fujiwara reaction to be used to test for THMs at concentrations relevant to environmental regulations (on the order of parts per billion). This type of testing is cheaper and faster than the methods currently used for these applications. In the future, the goal is to create a cheap portable device that can be used for field testing of water samples to provide quick and accurate predictions of THM contamination.

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