ANALYSIS AND OPTIMIZATION OF COLORIMETRIC NANOSENSORS FOR RAPID DETECTION OF MICROBES IN WATER

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ABSTRACT

Analysis and Optimization of Colorimetric Nanosensors For Rapid Detection of Microbes in Water

Ruby Anne Lang

Access to safe water is a basic human right recognized by the United Nations General Assembly in 2010. Still, at least 2.2 billion people globally still are without safely managed water services meaning they use a drinking water source that can be contaminated with faeces . To improve water systems and prove they are safe water sources, water quality testing must occur. A solution to this issue is the development of rapid detection sensors for pathogens in water. The first chapter of this thesis aims to create an informed list of rapid detection sensors that should be focused on for future development. This is achieved by using multicriteria decision analysis techniques based on using two consecutive processes. The first is the Analytic Hierarchy Process (AHP), which was used to develop weightings for criteria being measured for different sensor alternatives. The second process is the Technique of Order Preference Similarity to the Ideal Solution (TOPSIS), which was used to perform the ranking of the sensors being reviewed based on the weighted criteria. The outcome of the multicriteria decision analysis was identifying the top 5 rapid detection nanosensors for future development. They can be further improved to include field scale applications while also achieving lower detection limits and shorter detection times. The cost for these sensors could possibly be reduced by changing the nanoparticles that the sensor is composed of.

Through improved methods, the goal of creating a cost effective, rapid-detection nanosensor for bacteria (e.g., Shiga-toxin producing *E. coli*) in drinking water can be achieved by prioritization of research on these promising nanosensors. The second chapter of the thesis focuses on optimizing a gold nanosensor developed in 2015 by Raweewab T. and Rawiwan L, hereafter called the "Original Method." The goal was to reduce the cost and improve the reusability of their indirect colorimetric gold nanosensor without compromising the simplicity of the detection platform. With a reusable and more cost-effective sensor, field applications for water quality testing in water system projects in impoverished areas can be more obtainable. The nanoparticle itself was the target of optimization in this study. The hypothesis was that the polyethylenimine (PEI) coating on the gold nanoparticle surface is the governing factor of how the sensor functions, meaning the core nanomaterial does not affect the function of the sensor. In this study, the results showed that sensor still maintained its function after replacing the PEI coated gold nanoparticle used in the Original Method with PEI coated silver nanoparticles. These findings indicated that with further development and future research, it will be possible to use less expensive nanoparticles for making the nanosensor. It will also be possible to make this sensor reusable through the development of PEI coated magnetite nanoparticles. Their magnetic quality could allow for recovering the nanosensors from the test media, then re-conditioned and used again.

Keywords: Nanotechnology, Nanosensor, *E. coli*, Detection, Water Quality

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1.1 INTRODUCTION

1.1.1 Background

At least 2.2 billion people globally use a drinking water source that can be contaminated with faeces . Of these 2.2 billion people, 435 million people are taking their water from unprotected wells and springs and 144 million people are collecting untreated surface water from lakes, ponds, rivers and streams. Contaminated water creates a burden of disease in the area such as diarrhoea, cholera, dysentery, typhoid, and polio . Contaminated drinking water is estimated to cause 485,000 diarrhoeal deaths each year even though diarrhoea is largely preventable . The deaths of about 297,000 children aged under 5 years each year could be avoided with improved water supply and services . Not only that, but better water sources reduce health expenditures and people are better able to remain financially secure. For children, who are the most at risk from water-related diseases, improved water systems means less of a risk for getting sick. This better health allows for better school attendance which later translates to better job opportunities and more possibility to stop the cycle of poverty.

With such a pressing global health issue, it is clear that improvement to water systems is important and required in the Agenda 2030 Sustainable Development Goals (SDGs). However, to improve water systems and prove they are safe water sources, water quality testing must occur. A common indicator of fecal contamination is the coliform

group of bacteria including *E. coli*. This bacterium, pathogenic or not, is known to occur concurrently with major water-borne pathogens that are hard to test for . Through detection of *E. coli* in water, the water can be deemed safe or not for recreation, drinking and cooking, or bathing. Environmental protection agencies all over the world have set standards for the number of *E. coli* present in water based on activities. For recreational activities, water should not contain above 500 colony-forming-units (CFU) per 100 mL ((European Directive 2006/7/EC) and 126 CFU/100 mL (USA EPA's Ambient Water Quality Criteria for Bacteria) . However, for drinking water, the global standard is <1 cell per 100 mL .

The most widely used methods for detection of *E. coli* include conventional plate counting techniques and biochemical tests. While these methods are reliable, they can take more than 2 to 3 days for results . In addition, electricity and advanced lab-based equipment are usually needed for analysis. With most of the people in need of improved systems being in impoverished areas, typically without access to labs and electricity, this is a major drawback. These limitations resulted in research investigations for developing alternative detection methods and have shown some promising results. Newly developed rapid detection methods eliminate the need for incubation and provide results in a matter of minutes rather than days. Some of these new platforms include: Polymerase Chain Reaction (PCR), Fluorescence/optical based methods, Electrochemical biosensors, and Surface Plasmon Resonance (SPR) techniques. However, these methods are relatively expensive, complicated, and need a trained/experienced operator .

A promising platform that overcomes these limitations is colorimetric bacteria sensing using engineered nanomaterials. This is a platform that has sensors that are both simple to perform the measurement and read the results. Results are deciphered through a visible color change (compared to a premade indicator scale) and/or a portable spectrophotometer for exact values. In addition, it is less expensive and more versatile than other methods. Extensive research has gone into different nanomaterial colorimetric methods. The platform has mainly developed into two main approaches, direct and indirect assays . The direct assay is based on the idea that some types of metallic nanoparticles change color as they increase in size (i.e., aggregate) in response to chemical/biological recognitions . The indirect assay on the other hand involves tracking an enzymatic reaction as it interacts with a nanomaterial-bacteria assay . Direct refers to directly tracking the nanoparticle characteristic change and indirect refers to the tracking of reaction change. Both produce a visible color change that indicates how much bacteria are in the assay.

1.1.2 Objective

Despite the emerging technology tackling the need for an effective and rapid detection of pathogens in water, there is a lack of a systematic evaluation of these technologies. This evaluation is important because it informs decisions on which sensor is more promising for meeting the global need for field scalable rapid detection. Recognizing this gap in information, the objective of this study is to conduct a multicriteria decision analysis to help prioritize research and development of these sensors.

To achieve this objective, a multicriteria decision analysis was performed using two consecutive processes. The first one is the Analytic Hierarchy Process (AHP), which was used to develop weightings for the criteria for evaluating the nanosensors alternatives. AHP was designed in the 1970s and is an analytic hierarchy process that is used for organizing and analyzing complex decisions involving multiple criteria . This method is based on a combination of mathematics and psychology . This allows the criteria to be analyzed individually and compared to each other in importance to the overarching goal. It is used herein because it eliminates decision bias through a systematic weighting procedure and generates weights for each criterion in relevance to the goal.

The second stages involved the use of the Technique of Order Preference Similarity to the Ideal Solution (TOPSIS) to perform the ranking of the sensors being reviewed. TOPSIS is a mathematical approach to decision making. This method is based on the idea that the best alternative should have the shortest Euclidean distance to the ideal solution. Meaning that the ranking is based on the distance each alternative is away from the ideal solution. This highly precise and systematic approach to ranking eliminated personal bias and purely focuses on ranking the alternatives in relation to the overall goal.

1.2 METHODOLOGY

1.2.1 Literature Review Process

To perform the multicriteria decision analysis, a review of the literature was conducted first to collect the publication relevant to the study goal. From the collected articles, a

detailed data extraction was performed from the article that focused on colorimetric nanosensors. The details of the literature review process are described below.

1.2.1.1 Database Search

Three databases were primarily used to search for sensors developed for pathogen detection in water: Compendex (Engineering Village), ScienceDirect, and ACS (American Chemical Society). Reports and peer-reviewed publications of developed sensors were the main target for literature collection. However, the type of publications searched was kept broad and included: research articles, book chapters, case reports, conference papers, correspondence, editorials, examinations, patent reports, practice guidelines, product reviews, replication studies, short communications, and video articles. This search approach was deliberate to gain as much of an overall understanding of what sensors are currently being studied and developed while also collecting recent advances on sensors for the multi-criteria decision analysis. Review articles on pathogen sensors were also collected and analyzed to gain a better understanding of the scope of these reviews and to retrieve any articles from their reference list that could have been missed during the literature search process conducted herein.

To collect articles, 4 keyword combination were used. The articles were chosen if they pertained to nanosensor, pathogen detection, water paired with detection, and nanotechnology paired with detection. See Figure 1.1 for the decision tree showing the inclusion and exclusion process of the articles found.

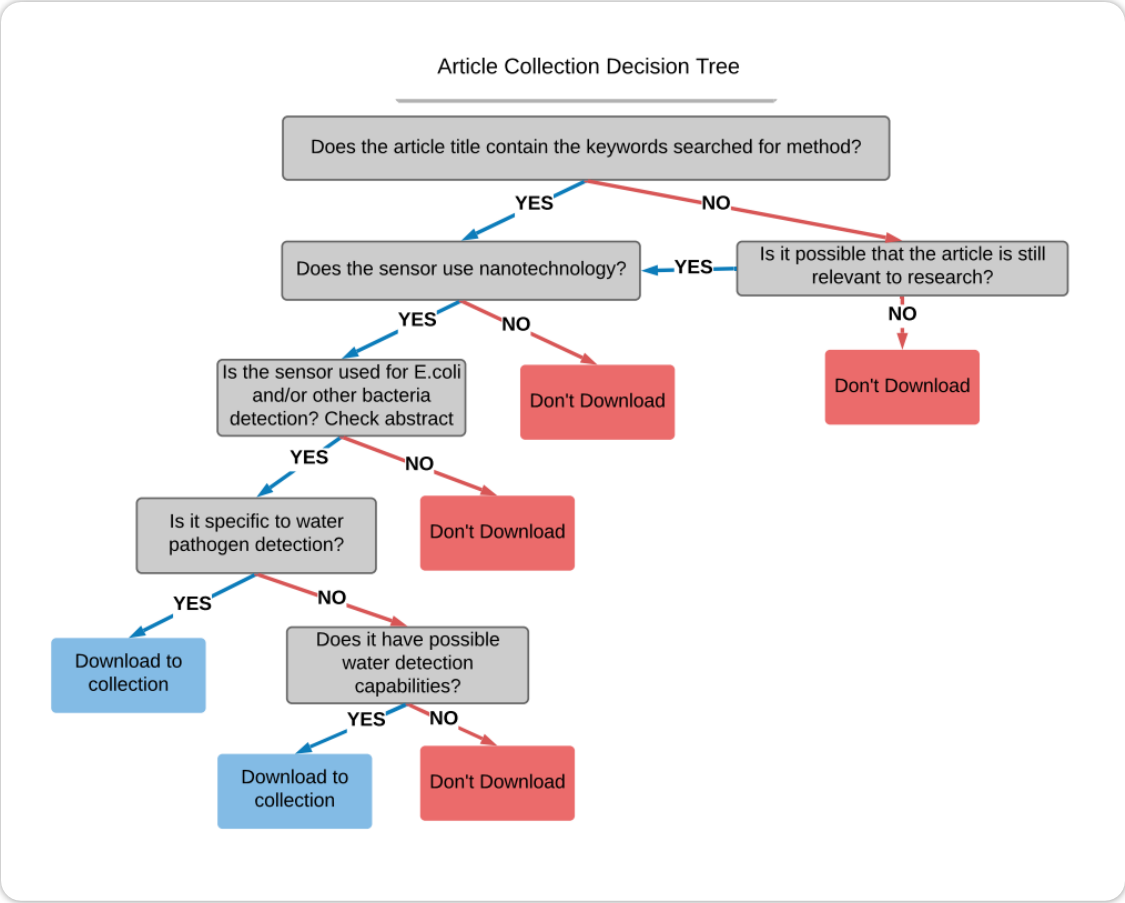

Figure 1.1: The Inclusion and Exclusion Process for Initial Collection of Articles.

The first keyword combination used for finding articles was to search the phrase "nanotechnology sensors for pathogens in water." Of the hundreds of publications that showed in the search, only 7 were deemed relevant to collect according to the process illustrated in Figure 1.1. The second keyword combination used was "alternative bacteria detection platforms" and the keyword "nanotechnology." This expanded the search to allow for possible sensors with water capabilities but used in the food industry. Within this search, 15 publications were found. The third keyword combination used the search term "colorimetric bacteria sensing" and the keyword

"nanotechnology." This focused the search on the type of specific sensors targeted in this study. This brought an additional 16 relevant publications.

In addition to the articles retrieved from the database search, 22 relevant articles were collected from the reference list of the study by Raweewan Thiramanas & Rawiwan Laocharoensuk (2015). This is the foundational study of a colorimetric nanosensor that this thesis aims to optimize. Lastly, an additional 9 articles were retrieved from reference lists of review articles on sensor development for detection of pathogens in water. In total, 69 different relevant publications that included peer-reviewed research articles, review articles, reports, and case studies were collected. From this collection, a second phase of filtering was performed.

The 69 collected articles were then further narrowed down using a more focused inclusion and exclusion process. This process looked at detection of water-based pathogens, the use of nanomaterials, and field scale capabilities or future potential for field scale capabilities. This was an interpreted understanding of each article based on the title and abstract presented. The 69 articles were weighted on a scale of 1-5 of relevance to the study objective; 1 being the most relevant and 5 being the least. This process resulted in the selection of 31 articles with a score of 1 for analysis.

1.2.1.2 Analysis of the Collected Literature

A systematic approach was used for extracting information from the collected articles. The first step consisted of pre-determining the important parameters and information that are critical for comparing and ranking the sensors. The target

parameters/information to extract from the articles and the rationale behind their selection are presented in Table 1.1.

Table 1.1: Criteria Used for Data Collection

Parameters	**Rationale**
Sensor Name	To track the sensor analyzed throughout the review process and sensor names were assigned based on title of publication
Method	To understand how the nanosensor works
Direct (D) OR Indirect (I) Colorimetric Sensor	To compare the two main types of colorimetric sensors
Color Changing Mechanism	To analyze if the mechanism affects other aspects of performance (detection limit, detection time, etc.)
Possible Pathogens Detected	To analyze the domain of applicability for the sensor
What Industry is this Sensor Used for	To gauge how these sensors are being used
Detection Limit	To rank the sensors best on lowest detection limits
Detection Time	To rank the sensors best on lowest detection time
Nanoparticle (NP) Type	To rank the sensors based on the nanoparticle type
Surface Coating	To analyze the role of surface properties on the functionality of the sensor
Surface Charge	To analyze the role of surface properties on the functionality of the sensor
NP Concentration	To determine the amount of nanoparticles needed for sensor function. Also, amounts of nanoparticles indirectly imply the sensor cost
Toxic (Y/N)	To analyze possible toxic effects of the sensor in order to account for the end-of-life disposal of the sensor (Y=yes and N=no)
Reusable (Y/N/F)	To analyze if the sensor has the potential for reuse/recyclability (Y=yes, N=no, F=Future referring to the possibility of reuse with future development)
Lab Scale (Y/N)	To understand how the sensor can be used in the lab setting (Y=yes and N=no)

Parameters	Rationale
Field Scale (Y/N/F)	To assess the potential for using the sensor for real-time detection in the field (Y=yes, N=no, F=Future referring to the possibility of field scale with future development)
Additional Info	Any additional information that made the sensor stand out or better understand its function

The second step was to retrieve this information from the articles and tabulate them for further analysis. Each article was reviewed in depth to collect the target data. Some articles did not report data for all criteria fields. In addition, some articles reported certain information vaguely or incompletely (mainly cost, toxicity and reuse data). For example, not a single article reported cost in a dollar amount, but rather as “cost effective.” To analyze the sensors and properly rank them, cost needed to be determined as it is a major limiting factor in most research and development. When conducting the multi-decision criteria analysis, assumptions and calculations were made for the unreported information based primarily on the materials used. All the data collected were placed in TableA1 (Appendix A) under the correct criteria. If a criterion was not reported in a publication and could not be reasonably determined, the criterion field in the table was left blank.

1.2.2 Multiple Criteria Decision Analysis (MCDA)

After the general literature review table was fully populated and analyzed, the next step was to perform a multi-criteria decision analysis (MCDA) with a goal to help prioritize research and development of sensors for detection of microbes in water. The MCDA focused solely on the colorimetric nanosensors (16 articles out of the 31 articles

reviewed). The evaluation criteria used for prioritization and the rationale for their selection are presented in Table 1.2.

Table 1.2: Criteria Used for Conducting MCDA

Category	**Reason**
Direct (D) OR Indirect (I) Colorimetric Sensor	To compare the two main types of colorimetric sensors
Color Changing Mechanism	To analyze if the mechanism affects other aspects of performance
Possible Pathogens Detected	To analyze the selectivity of sensor for microbes
Cost	To rank sensors based on cost effectiveness
Detection Limit (CFU/mL)	To rank the sensors best on lowest detection limits
Detection Time (min)	To rank the sensors best on lowest detection time
NP Type	To analyze effectiveness of nanoparticle type within the sensors
NP Concentration (nM)	To analyze the amount of nanoparticles needed for sensor function
Toxic (Y/N)	To analyze possible toxic effects of the sensor in order to account for the end-of-life disposal of the sensor
Reusable (Y/N/F)	To analyze if the sensor has the potential for reuse/recyclability
Lab Scale (Y/N)	To understand how the sensor can be used in the lab setting
Field Scale (Y/N/F)	To assess the potential for using the sensor for real-time detection in the field

Colorimetric nanosensors showed more versatility, cost-effectiveness, and application to water testing than other types of sensors . Because of this they became the focus and the criteria was used on each of them. The nanosensors were assigned names for easy reference in the thesis as shown in Table 1.3.

Table 1.3: Nanosensors Compared

Source	Sensor Name	Color Changing Mechanism	Nanoparticle Type
Miranda, 2011	Colorimetric Bacteria Sensor	Enzyme reaction	Gold: cationic gold nanoparticles
Banerjee, 2016	Magneto Nanosensors	Aggregation	Iron: iron oxide nanoparticles (IONPs), Ab-conjugated IONPs
Hossain, 2012	Multiplexed Paper Test Strip Sensors	Enzyme reaction	immunomagn-etic nanoparticles
You, 2020	AuNP Coated Starch Magnetic Beads Sensor	Aggregation	Gold: gold nanoparticle-coated starch magnetic beads (AuNP@SMBs)
Xie, 2019	UV-Induced Au Nanoclusters Sensor	UV and aggregation	Gold: gold nanoclusters (AuNCs)
Lim, 2012	Enhanced AuNP Sensor	Aggregation	Gold: AuNP, functionalized NPs (f-NPs) and streptavidin molecules (stAuNPs)
Russo, 2018	AuAg Nanoshells Sensor	Aggregation	Gold & Silver: AuAg nanoshells consist of a hollow structure composed of a gold−silver alloy shell, which encloses an inner cavity.
Raj, 2015	Cysteine Capped AuNP Sensor	Aggregation	Gold: Cysteine gold nanoparticles (CAuNPs)
Hayden, 2012	Cationic AuNP Sensor	Aggregation	Gold: Cationic monolayer-protected gold nanoparticles
Zheng, 2018	Simple Sensor	Aggregation	Silver: MPBA-AgNP
Jung, 2019	S-layer Protein AuNP Sensor	Aggregation	Gold: AuNP
Shrivastava, 2018	AuNP Biosensor	Florescence	Gold: aptamer-functionalized fluorescent magnetic nanoparticles
M.A.Ali, 2014	Conjugated AuNP Sensor	Aggregation	Gold: conjugated gold nanoparticles

Source	Sensor Name	Color Changing Mechanism	Nanoparticle Type
Thiramanas & Laocharoensuk, 2015	Competitive Binding Sensor	Enzyme reaction	Gold: cationic PEI-AuNPs
Robby, 2019	Polymer Dot Sensor	Florescence	Iron: PD-bCD-MMT /$Fe_3O4eCsWO_3$ nanocomposite
Su, 2011	AuNP Sensor	Aggregation	Gold: MEA-AuNPs

Because cost was not numerically reported, it was weighted on a scale of 1-5; 1 being the least expensive sensor and 5 being the most expensive. This scale was based on the type of nanoparticles used (gold, silver, Iron, or other) and the type of sensor being developed (direct or indirect colorimetric sensors). Based on general cost data collected through searching the websites of nanomaterials vendors (e.g., SkySpring Nanomaterials), the cost of gold nanoparticles per gram was substantially more expensive than that of silver and iron. Gold nanosensors were given a cost value of 4. Silver followed behind with a cost value of 2 and iron with a cost value of 1. Any sensor that used a different material was given a 3 because the two sensors that fell in this category involved complex particle composites. In addition to the nanoparticle type used, the overall sensor category played a role in the cost. Indirect sensors utilized enzymatic reactions which means they require the purchase of additional chemicals (e.g., enzymes and reagents) to operate. Through a general research of the cost of reagents, it was determined that indirect sensors will get an increase of 1 cost value to whichever nanoparticle type the sensor uses. For example, if an indirect nanosensor uses gold, the cost value would be 5 rather than 4.

Other factors such as toxicity and reusability were indirectly determined based on data collected from the articles, meaning it was not directly stated but determined based on additional information. Both of these factors were based primarily on the nanoparticle used as well as any additional materials used within the sensor. Silver and iron nanoparticles are more toxic to the environment than gold nanoparticles. So, the toxicity was stated not toxic if the sensor was gold based and toxic if it was not. Similarly, some iron oxide nanoparticles have magnetic capabilities, so if a sensor used these iron-based materials then it was deemed to have the potential to be reusable. Some sensors were already developed to be reusable, so they were clearly assigned "Y". Ones that used iron but did not clearly mention potential for reuse were assigned an "f" for future possibilities rather than a "no". It is important to note that both reusability and toxicity were not included in the ranking as there is not a clear way to confidently confirm if the determination given is true.

In addition, any missing information used for in the MCDA process were filled in using the average of the values that were reported for other studies. For detection limits and times given in a range, the lowest of the range was used within this analysis. A sensitivity analysis was preformed to assess the sensitivity of the final ranking. Since assumptions and criteria used to rank the sensors were largely based on personal decisions and judgment, it is important to verify that the ranks can withstand variations in the weighting compared to the originally assumed values.

After the focused table (Table 1.6, found in results Section 1.3.1) was populated with the information, it was then further reduced to just four criteria points for ranking. The

criteria determined for this more concise evaluation matrix were: Detection Time, Detection Limit, Cost, and Field Scale (i.e., usability in the field). These four criteria were chosen due to the importance each of them have on the performance of the sensor analyzed with respect to the overarching goal of this research, which is determining the most cost-effective sensors for in-field use in impoverished areas. Once the matrix (alternative sensors against evaluation criteria) was created, the MCDA was performed following two consecutive processes: 1) the Analytic Hierarchy Process (AHP) to determine criteria weights and 2) the Technique of Order Preference Similarity to the Ideal Solution (TOPSIS) to rank the sensors.

1.2.3 Analytic Hierarchy Process (AHP)

AHP begins with categorizing each aspect of the decision into hierarchies. The goal is placed at the top with the criteria in the descending level. Each one of the criteria stems from the goal. The third level included the alternatives. In this case, the alternatives are the sensors being ranked. Each alternative has their own value from each of the criteria. The hierarchy chart developed for the sensors is shown in Figure 1.2.

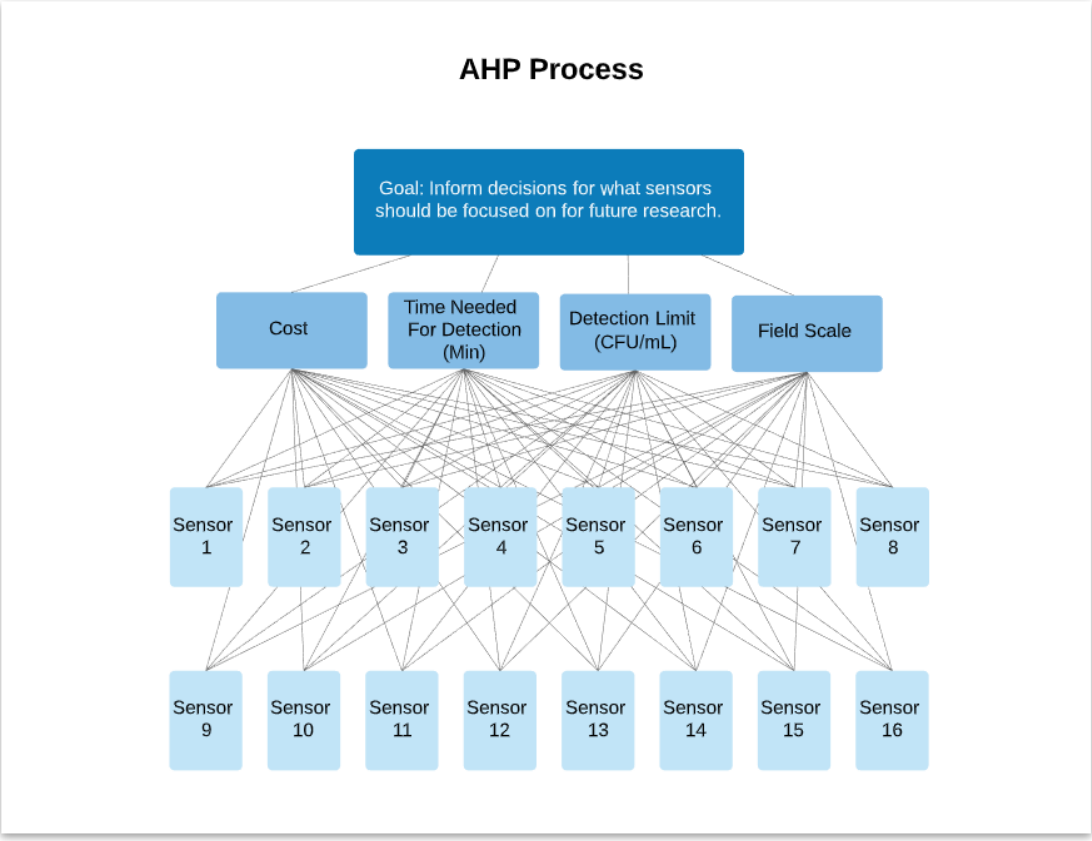

Figure 1.2: The AHP for Weighting the Criteria for MCDA.

Once the hierarchies are established, a pairwise matrix is made with the criteria weighted against each other. This matrix determines the relative importance of the different criteria with respect to the goal. This is created with the help of a "scale of relative importance" (Table 1.4) which depends on the decision maker.

Table 1.4: Scale of Preference Adapted from

Preference Factor	Degree of Preference	Explanation
1	Equally	Two factors contribute equally to the objective
3	Moderately	Experience and judgment slightly to moderately favor one factor over another
5	Strongly	Experience and judgment strongly or essentially favor one factor over another
7	Very strongly	A factor is strongly favored over another and its dominance is showed in practice
9	Extremely	The evidence of favoring one factor over another is of the highest degree possible of an affirmation
2,4,6,8	Intermediate	Used to represent compromises between the preferences in weights 1, 3, 5, 7 and 9
Reciprocals	Opposites	Used for inverse comparison

To decide this value, a question was asked as the pairwise matrix was being filled from left to right. The question asked is "how important is the horizontal criteria (row element) with respect to the vertical criteria (column element). This importance is given a value from the "scale of relative importance." For example: if cost is of equal importance to detection limit, it is assigned a 1 value. The column element was given a value of x and the row element is given a value of the relative importance times x (example: 1x). The value of the cell is equal to the $\frac{Row\ Element}{Column\ Element}$ (example: $\frac{1x}{x} = 1$). This method was repeated for each cell.

Once all the cells were assigned a value, the sum of each column was calculated. This was used to normalize the pairwise matrix. Each cell was divided by its column sum. Once the matrix was normalized, the criteria weights were determined by averaging all the values within each row.

The next step was to check for consistency within the matrix. This is important because it ensures the calculated values are correct. To do this, the non-normalized pairwise table was used and each cell is multiplied by the criteria weight for that column. The weighted sum value was then calculated by the summation of each row, leaving a new value for each criteria. This value was then divided by the original criteria weight to find the ratio of the two for each row. The ratio values were then used to find λ_{max} by averaging them. Next, the consistency index (C.I.) was calculated by using the Equation 1.

$$C.I. = \frac{\lambda_{max} - n}{n - 1} \qquad (1)$$

Where n is equal to the number of criteria. In this test n = 4 as there were 4 criteria used in this evaluation.

Then the Consistency Ratio (CR) was calculated by dividing the CR by the Random Index (R.I.). The R.I. is the consistency index of a randomly generated pairwise matrix. This value is a constant depending on the n value and taken from Table 1.5.

Table 1.5: Random Index (R.I.) value table adopted from

n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
RI	0	0	0.58	0.90	1.12	1.24	1.32	1.41	1.45	1.49	1.51	1.53	1.56	1.57	1.59

Within this test, the R.I. for n = 4 was used herein. Overall, the value of the consistency ratio needs to be less than 0.10, which is the standard for this weighting method.

1.2.4 Technique of Order Preference Similarity to the Ideal Solution (TOPSIS)

Once the weighting of each criteria is determined using AHP, the ranking of the alternative sensors was determined using the TOPSIS method. TOPSIS is a mathematical approach to decision making. This method is based on the idea that the best alternative should have the shortest Euclidean distance to the ideal solution. Meaning that the ranking is based on the distance each alternative is away from the ideal solution. To perform TOPSIS, the process illustrated in Figure 1.3 was followed.

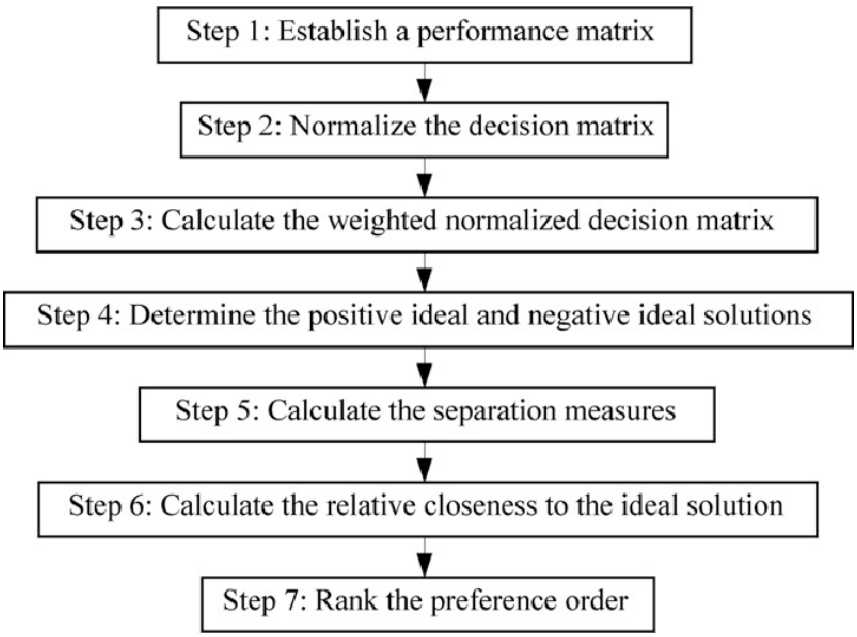

Figure 1.3: TOPSIS Flow Chart Adopted from .

Before the TOPSIS method can begin, all the information being tested were converted into numerical values. To do this, the field scale criteria were converted to a scale from 1-3 in value with 1 showing no field capabilities and 3 meant fully field capable. Some sensors showed potential for future field capabilities with further optimization. These sensors were given a value of 2. Once all values in the table became numerical, the

performance matrix was established. The matrix was then normalized. In TOPSIS this is performed by using vector normalization according to Equation 2.

$$\overline{X_{ij}} = \frac{X_{ij}}{\sqrt{\sum_{j=1}^{n} {X_{ij}}^2}} \qquad (2)$$

Where, X_{ij} is the original value of each cell and $\overline{X_{ij}}$ is the final value calculated for each cell to create a vector normalized matrix.

All values within each column were squared and then added together. Once the sum was determined, the square root was calculated. The original value in each cell was then divided by the final value of the square root for each column. This gives the vector normalized decision matrix. The value in each cell is known as the normalized performance value. The normalized performance values were then multiplied by the weights of each criteria. This weighting was determined by the AHP method earlier. This creates a weighted normalized decision matrix.

Once complete, the next step was to calculate the ideal best value (${V_j}^+$) and the ideal worst value (${V_j}^-$). To do this, each criteria needs to be deemed either beneficial (highest value being most desired) or non-beneficial (lowest value being most desired). For example, cost is non-beneficial criteria because the lowest cost value would be the best solution. So, the minimum value for cost would be the ideal best value (${V_j}^+$) and the highest value for cost would be the ideal worst value (${V_j}^-$).

Once the ideal best value (${V_j}^+$) and the ideal worst value (${V_j}^-$) have been determined for each column, the Euclidean distance from each was calculated. The distance from the

ideal best is represented as S_i^+ and distance from the ideal worst is represented as S_i^-. The Euclidean distance was calculated using Equations 3 and 4:

$$S_i^+ = [\sum_{j-1}^{m}(V_{ij} - V_j^+)^2]^{0.5} \qquad (3)$$

$$S_i^- = [\sum_{j-1}^{m}(V_{ij} - V_j^-)^2]^{0.5} \qquad (4)$$

This formula was applied to each row of the decision matrix for both the distance from the ideal best (S_i^+) and distance from the ideal worst (S_i^-). Once done, the performance score (P_i) was calculated for each sensor. This is calculated by using Equation 5. Finally, the sensors were ranked based on their performance scores.

$$P_i = \left(\frac{S_i^-}{S_i^+ + S_i^-}\right) \qquad (5)$$

1.2.5 Sensitivity Analysis

Since the weightings for the criteria used to rank the sensors was largely based on personal decisions and judgment, it is important to verify that the ranks can withstand variations in the weighting compared to the originally assumed values. To achieve this, several sensitivity tests were performed. The goal was to test if drastic changes to the weightings obtained from AHP would have an impact on the rankings found from the TOPSIS analysis. The range of weightings evaluated herein are presented in Figure 1.4. These weightings were entered into the TOPSIS model and the rankings was determined based on the new weightings.

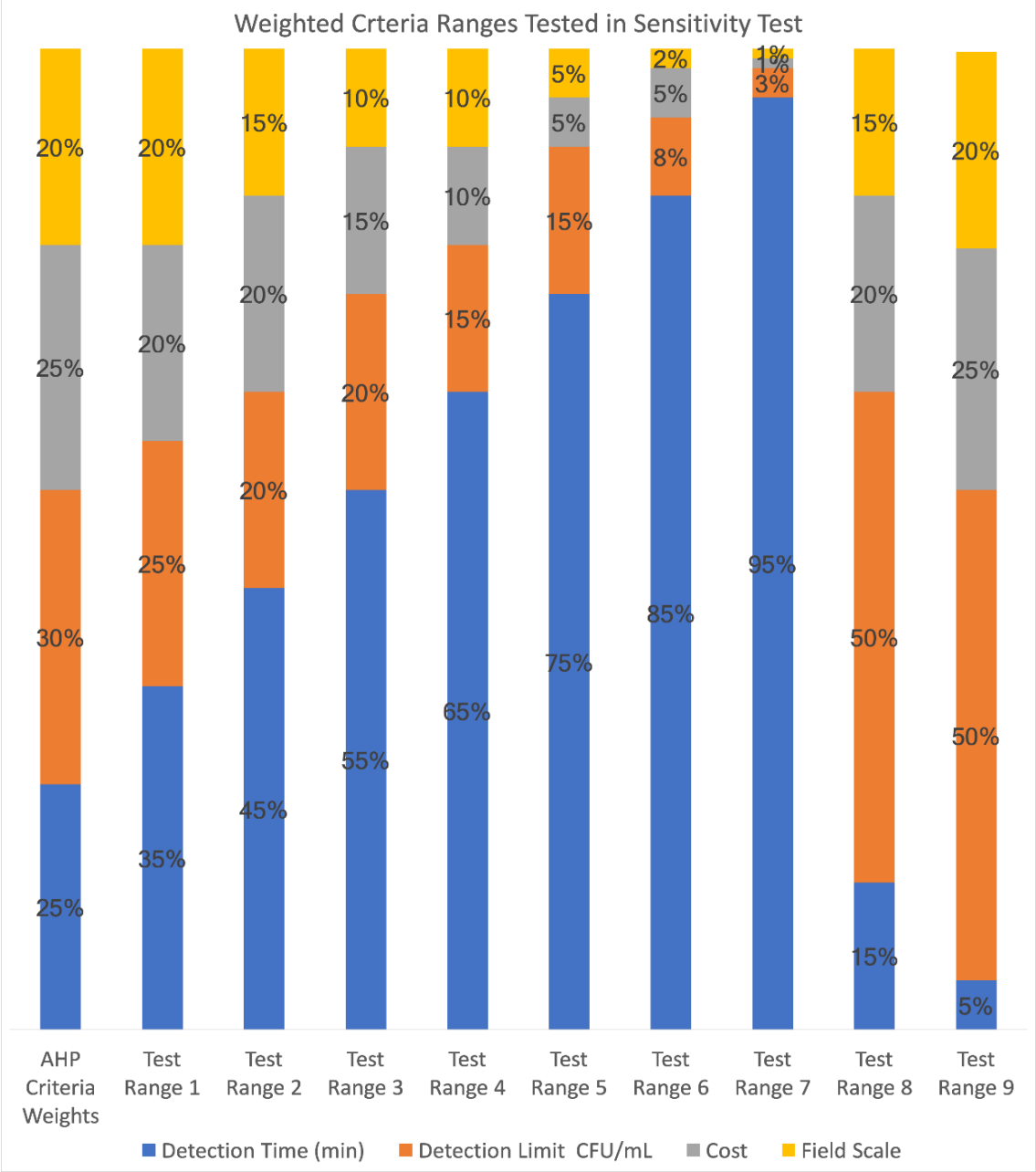

Figure 1.4: Sensitivity Test on Weightings for The Evaluation Criteria.

1.3 RESULTS AND DISCUSSION

1.3.1 Analysis of the Literature

The original 31 articles collected were summarized a as shown in Table A1 (Appendix A).

This table provides and overview of an important set of information such as the types

and characteristics of the nanosensors used, their mechanism of work, and their detection limits. Analsysis of the data in Table A1 shows that the nanosensor used the most in the literature was gold nanomaterials. Gold was in the majority of the studies as the main colorimetric detector (i.e., the nanoparticle changes color when it interacts with the microbe). In the rest of the studies, gold was paired with chemical and biological interactions so that other chemicals in the sensor mixture change color when microbes are present.

In addition to colorimetric methods, some nanosensors utilized fluorescence to detect microbes. An example of this is the Polymer Dot Sensor. This sensor's method of detection utilized the expression of alkaline phosphatase (ALP) from bacteria to design fluorescence ON/OFF system for bacteria detection . Rather than creating a visible change in color, the Polymer Dot fluoresce in the presences of bacteria. To enhance the sensor and make it recyclable, the method used a magnetic metal oxide .

The data collection from the 31 articles showed some major data and information gaps that are important to consider for future work within this field. It was difficult to quantify the cost of these sensors. Almost every article reviewed reported that their sensor was a cost-effective solution for rapid detection, but never provided an actual cost analysis. This made it challenging to compare sensors and forced a value scale to be created. While this value scale was based on the cost of nanoparticles, it is missing additional real-world factors such as labor, additional materials, and any additional items that could contribute to the expenses of the method. This was attempted to be

covered slightly using the information reported about the type of sensor used, but this was overall an educated guess on the cost value.

Additionally, it was difficult to find the concentration of nanoparticles used for each sensor as well as characteristics of the nanoparticles used (surface charge, size, shape, etc.) in some of the articles. Since nanoparticles react differently based on their characteristics, the type of nanoparticle alone is not enough information for a future researcher to best understand ways to optimize the sensor being reported. This also makes it challenging to understand the scalability of these methods. If a sensor is found to be the solution to the current testing limitations, but cannot be scaled up for manufacturing, it does not help the global need of water bacteria detection improvement. Real world application was a data gaps need to be filled also. Very few articles reported testing on real water samples from the field. These data gaps warrants the need for future research and development to optimize these sensors.

1.3.2 Analysis of Colorimetric Nanosensor Sensors

Of the 31 articles collected, 16 reports of colorimetric nanosensors were analyzed in a focused matrix shown in Table 1.6. From these 16 nanosensors, data was pulled form the articles to fill the matrix. Within Table 1.6, please note that Y= Yes the sensor has this quality, N=no the sensor doesn't have this quality, and F= with future development there is potential for having this quality.

Table 1.6: Focused Matrix for Colorimetric Sensor Review

Source	Sensor	Direct (D) or Indirect (I)	Mechanism	Cost	Detection Limit (CFU/ml)	Detection Time	NP Type	Toxic*	Reusable*	Lab scale*	Field Scale*
(Miranda, 2011)	Colorimetric Bacteria	I	Enzyme reaction	5	100	10	Gold	N	N	Y	Y
(Xie, 2019)	UV-Induced Au Nanocluster	D	UV and Aggregation	4	100	10	Gold	Y	N	Y	Y
(Lim, 2012)	Enhanced AuNP	D	Aggregation	4	100	10	Gold	N	N	Y	F
(Russo, 2018)	AuAg Nanoshells	D	Aggregation	5	100	10	Gold and Silver	Y	N	Y	F
(Raj, 2015)	Cysteine Capped AuNP	D	Aggregation	4	100	10	Gold	N	N	Y	N
(Banerjee, 2016)	Magneto	D	Aggregation	1	1	30	Iron	Y	Y	Y	N
(Hossain, 2012)	Multiplexed Paper Test Strip	I	Enzyme Reaction	3	5	30	Immunomagn-etic	N	N	Y	Y

Source	Sensor	Direct (D) or Indirect (I)	Mechanism	Cost	Detection Limit (CFU/ml)	Detection Time	NP Type	Toxic*	Reusable*	Lab scale*	Field Scale*
(You, 2020)	AuNP Coated Starch Magnetic Beads	D	Aggregation	4	1	18.5	Gold	N	Y	Y	N
(Heyden, 2012)	Cationic AuNP	D	Aggregation	4	11035	18.5	Gold	N	N	Y	N
(Zherg, 2018)	Simple	D	Aggregation	2	9000	20	Silver	N	N	Y	F
(Jung, 2019)	S-Layer Protein AuNP	D	Aggregation	4	167000	18.5	Gold	Y	N	Y	N
(Shrlvstev e, 2018)	AuNP Biosensor	I	Florescence	5	10	10	Gold	N	F	Y	Y
	Conjugated AuNP	D	Aggregation	4	10	10	Gold	N	N	Y	N
	Competitiv e Binding	I	Enzyme reaction	5	10	10	Gold	N	N	Y	F

Source	Sensor	Direct (D) or Indirect (I)	Mechanism	Cost	Detection Limit (CFU/ml)	Detection Time	NP Type	Toxic*	Reusable*	Lab scale*	Field Scale*
	Polymer Dot	I	Florescence	2	4.62	18.5	Iron	Y	Y	Y	F
	AuNP Sensor	D	Aggregation	4	1	10	Gold	N	Y	Y	N

**Y= Yes, it has this quality, N=no, it doesn't have this quality, and F= with future development there is potential for having this quality.*

Overall, most of the sensors used direct colorimetric methods (69%) and only 5 sensors analyzed used an indirect method as shown in Figure 1.5. Direct colorimetric methods were expected to be the majority of sensors analyzed as they preform rapid detection with very simple assay. Direct colorimetric methods typically utilize aggregation of the nanoparticle to change the color of the assy. This requires less materials and has less complex of a methodology then indirect sensor. However, the detection limit of indirect colorimetric sensors seems to have a more focused range based on the articles analyzed. Within the sensors analyzed herein (direct and indirect), the detection limit range of the direct and indirect sensors were 1 – 167,000 CFU/mL and 4.62 – 100 CFU/mL, respectively. It is also noted that aggregation of the nanoparticles can happen in the test media for reasons other than being induced by the presence of microbes. For example, some nanomaterials can aggregate under acidic pH conditions (< 4.0) and in relatively high ionic strength (100 mM) solution . This greatly depends on the

nanomaterial surface properties as electrically stabilized nanoparticles can aggregate in these conditions, but sterically stabilized will not .

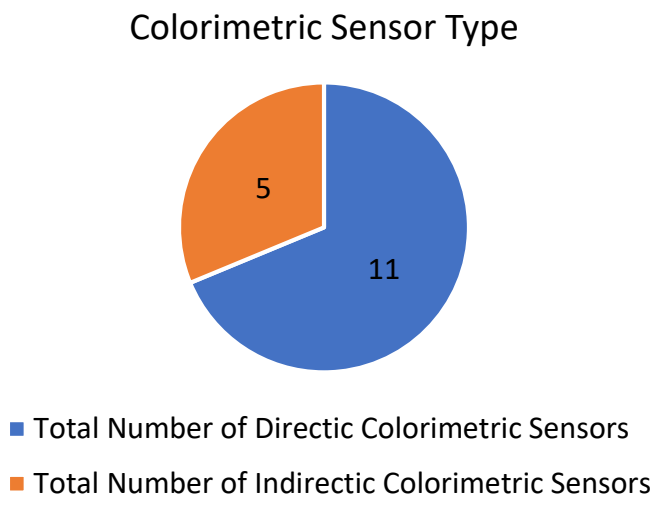

Figure 1.5: Distribution of Colorimetric Sensors Based on Mechanism of Color Change.

Indirect sensors, because of their higher sensitivity, seem to be an area that should be focused on in future research. They use different detection mechanisms as presented in Figure 1.6.

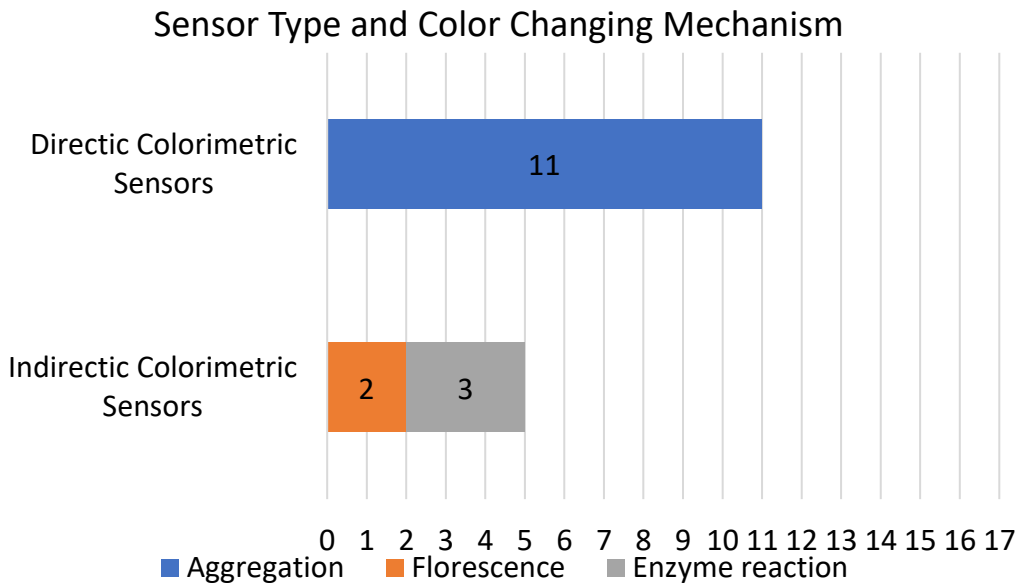

Figure 1.6: Mechanisms Used for Each Type of Colorimetric Sensor Investigated.

All 16 sensors had lab scale capabilities which means they are made to be utilized for testing water quality once samples are delivered to a lab. Only 4 out of the 16 sensors had field scale capabilities. These sensors were the Colorimertic Bacteria Sensor (indirect), AuNP Biosensor (direct), Multipexed Paper test Strip (direct), and the UV-Induced AuNC Sensor (direct) as seen in Table 1.6. However, an additional 5 sensors showed possibilities for future field applications. Simple assays that do not require the use of lab equipment (e.g., scales, incubators, hazardous chemicals, etc.) were deemed herein to be applicable for field use. For example, some methods showed potential for being optimized for use in the field by making the sensor into paper test strips (like litmus paper for pH testing) or a simplified test kits (similar to pool chemical tests).

Of the pathogens being detected, *E. coli* was the focus for 10 of the sensors analyzed. Three of the 16 sensors can test multiple types of bacteria (labeled on the chart as E. coli +) as seen in Figure 1.7. The remaining three articles targeted the detection of *S. aureus* and metal ions.

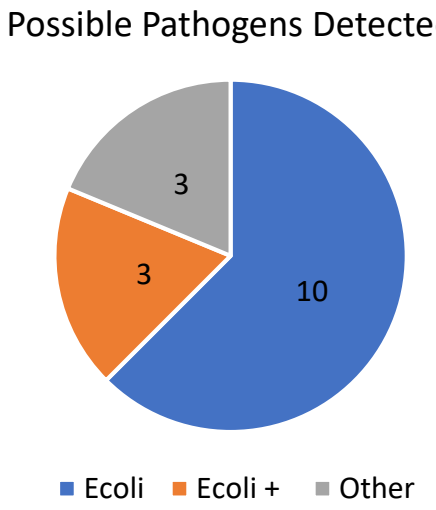

Figure 1.7: The Pathogens Detected by The Sensors Analyzed.

As mentioned in Section 1.2.1.2, cost was converted to a 5-point scale based on the sensor type and the nanoparticles used. While this scale is based on data collected and additional research into pricing, it still has gaps and uncertainties. However, to gain a general understanding of how these sensors compare to each other the 5-point scale was used for this review and the results are shown in Figure 1.8.

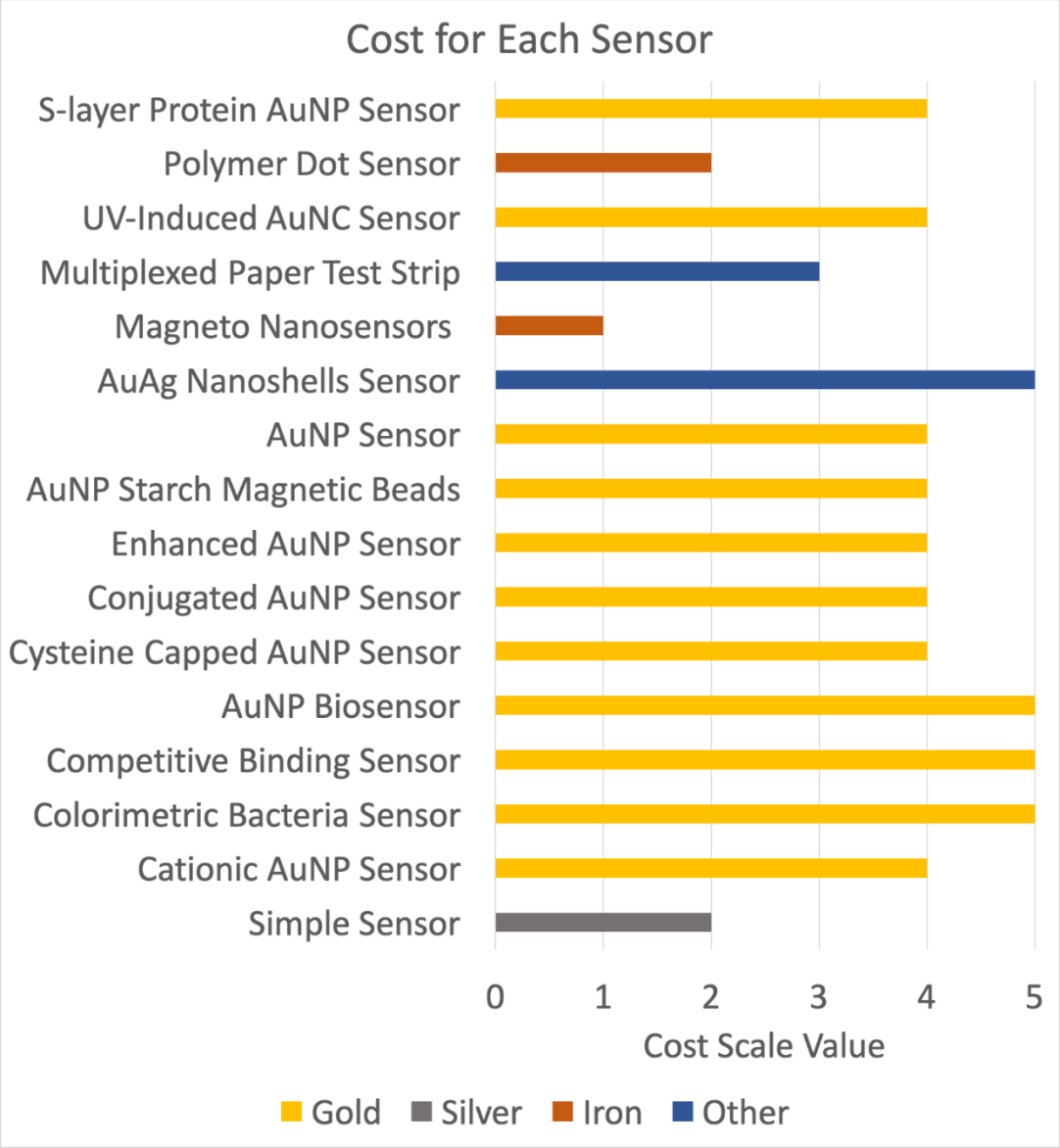

Figure 1.8: Cost of Each Colorimetric Sensor and The Type of Nanoparticle Used Based on a 5-Point Scale.

The cost of a sensor using gold nanoparticles is primarily more expensive then sensors using other materials. However, other materials and the sensor type can make the cost value supersede that of gold-based nanosensors because the reagents can be less expensive. Low cost for the sensors is desired since the goal application is to address the need for water quality testing in water system improvement projects in impoverished communities. Much of the need for water improvement systems are in low- and middle-

income countries. The cost needs to be low to be effective in this area. Again, to better analyze the cost effectiveness of a sensor more information are needed and sensor developers should quantitatively address the cost-effectiveness of their sensors. Still, most of the sensors used gold nanoparticles despite their relatively higher cost as seen in Figure 1.9.

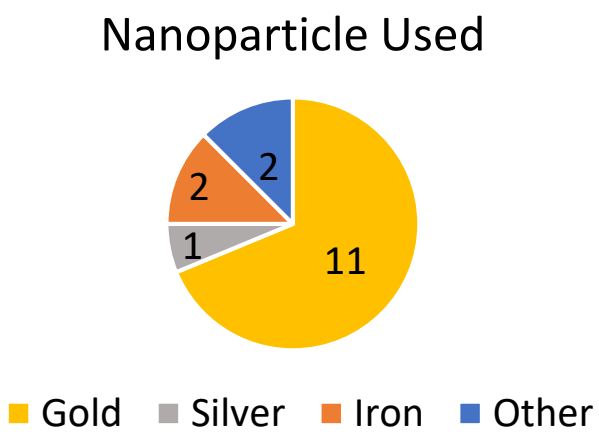

Figure 1.9: The Nanoparticles Used by The Sensors Reviewed.

Based on the nanoparticle type as well as mechanism of work of the sensors, most of them were deemed environmentally friendly (11 were non-toxic) while about 35% of the sensors were deemed potentially toxic. This important with regards to the options for disposal of these sensors after use. Only 4 sensors had reusable capabilities with an additional sensor of which had the potential with future development. This was expected as reuse application is a new concept for nanosensors. Reuse is expected to take more of an importance in the future development of nanosensors. Reusability is also a factor that could increase the cost of these sensors, however since it will have a longer life of testing this cost could be worthwhile.

No correlations were found between the nanoparticle type used and the detection limit of the sensor. For example, looking at only the lowest detection limits reported for the 16 sensors analyzed, it seems as though gold was used in sensors with very low detection limits, but also ones with much higher detection limits (Figure 1.10). Iron follows a similar trend. This points to the idea that detection limits are largely based on the mechanism of work of the sensor rather than the nanoparticle type. How the nanoparticle are used within the sensor and how they are made to react (a coating, bio-hybridization, etc.) seems to be more important.

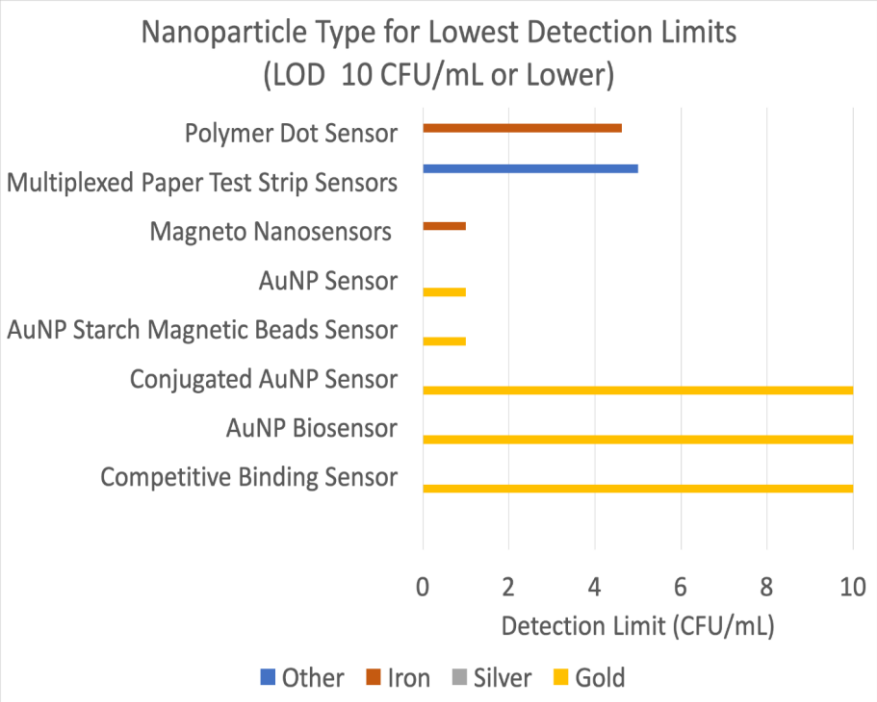

Figure 1.10: Type of Nanoparticle Used in Relation to The Detection Limit Less Than or Equal to 10 CFU/mL.

Overall, the majority of the sensors analyzed have relatively low detection limits as seen in Figure 1.11. Three of the 16 sensors analyzed had a detection limit of above 100

CFU/mL. Rapid detection with low detection limits is an important goal and aspect of all sensors. Because of this, the 3 sensors with high detection limits were excluded from further analysis as they were deemed ineffective. These sensors are the S-layer Protein AuNP Sensor, the Cationic AuNP Sensor, and the Simple Sensor (Table 1.6).

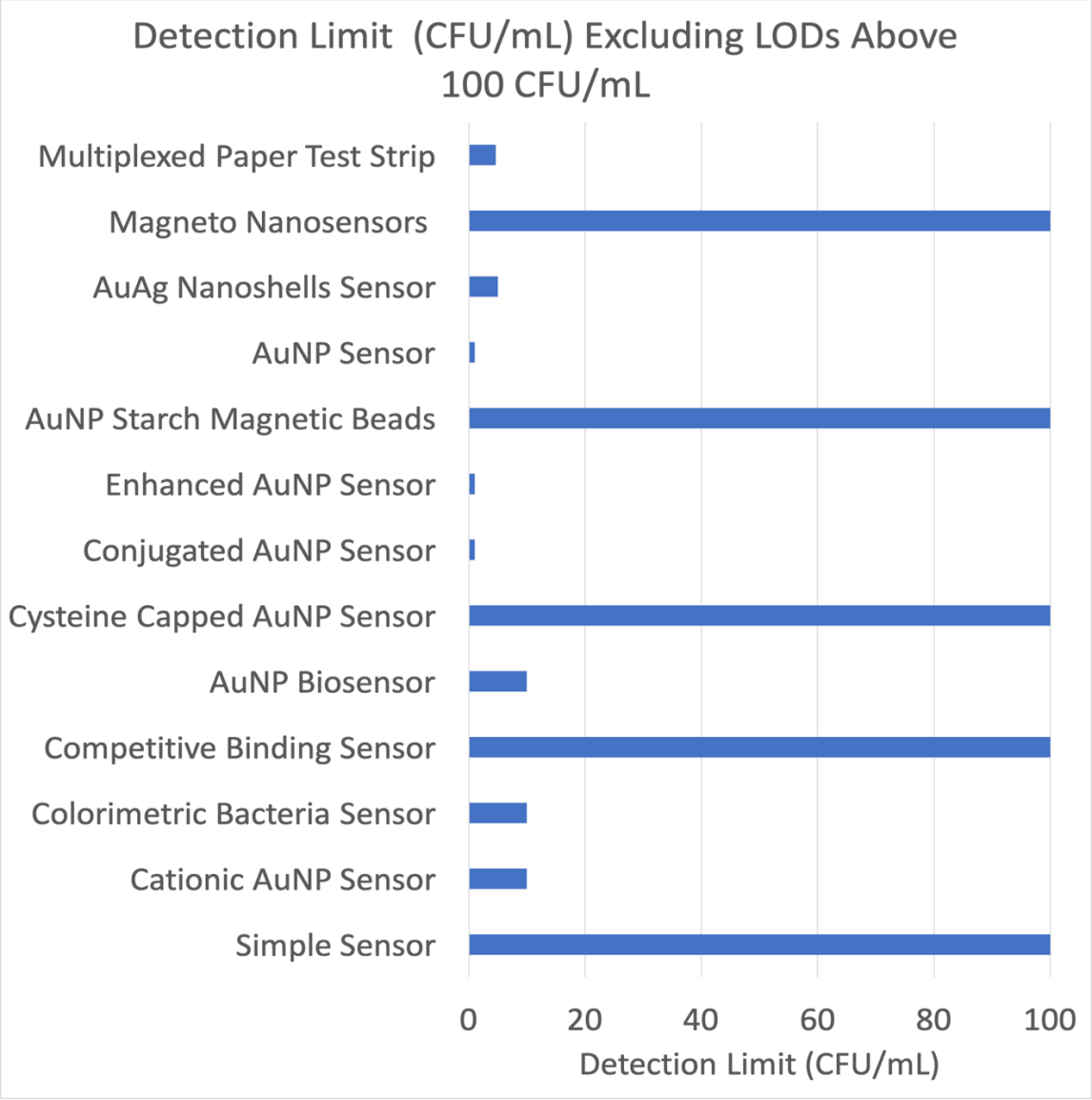

Figure 1.11: Low Detection Limit (LOD) of Sensors Excluding Values Above 100 CFU/mL.

Detection time was also shown to be very low. The maximum time needed was only 30 minutes at minimum. It is important to note that while detection can be found at a reported minimum (example: 30 minutes) some of the sensors run for much longer to ensure detection. For example, the Competitive Binding Sensor reports a minimum time needed for detection of 30 minutes, but has a method run of 3 hours to ensure full reaction.

1.3.3 Prioritization of Sensors using MCDA

The AHP resulted in a fairly equal weighting for all four criteria. This was expected as the criteria were selected due to its importance toward the goal of the review. Detection limit held the most weight at 30%. This is a critical aspect of a sensor since the recommended concentration of *E. coli* in drinking water is zero. Without a sufficiently low detection limit, there is a possibility for the sensor to provide a false sense of security when measuring pathogens in drinking water. As noted in Section 1.3.2, three of the original 16 sensors had very high detection limits and were deemed ineffective. Therefore, they were excluded from the MCDA process.

The time for detection and cost tie for the 2nd place position of importance at 25% weighting. Time for detection is an important aspect for a successful sensor with applications in the field (real-time detection without the need for incubation). A major goal for these sensors is to reduce the time needed for detection by eliminating the need for incubation. In addition, the faster a problem can be identified in the drinking water, the faster a solution can be implemented. Cost is an important aspect in any project, tool, or testing method. If the cost of a sensor is too high, it will not be a

suitable option to replace already practiced methods that are cost effective. The final criteria is field scale and has a weighting of 20%. This weighting shows that even though it has the lowest weighting it is still significant to the ranking. Field scale implies its application to in-field use. This is critical for water quality testing worldwide in areas and for people without easy lab access as mentioned. As noted previously, all sensors reviewed had lab scale capabilities. For use in non-field work (projects that can send samples to a lab), any of these sensors would work.

The TOPSIS process with the AHP weights resulted in a ranking of the sensors. The top 5 sensors are reported in Table 1.7. Of the 13 sensors evaluated (the original 16 became 13 once the 3 high detection limit sensors were taken out of the weighted ranking), the top 5 were analyzed. The Colorimetric Bacteria Sensor (top rank) used an indirect method. The following top 4 sensors used direct methods. The top 5 sensors had a detection limit of 100 CFU/mL and a minimum detection time of 10 minutes. The top 2 sensors have field scale capabilities with the next 2 sensors showing future possibilities for field use. The average cost score of the top five sensors was 4.4, which seemed higher than expected. However, all these sensors used gold nanomaterials, which plays a large role in having this high cost score. To make the TOPSIS weighting more precise in the future, more information about the exact cost of the nanosensors and we well as tests in real environmental samples. The direct colorimetric methods may work well in controlled aqueous media in lab setting; however, the chemistry of a true field water samples may lower their efficiency. True field water samples refer to samples that have been taken from a stream or water source and not generated in a lab. The impact of

environmental conditions such as varying pH and ionic strength may affect nanoparticle aggregation . As mentioned, some nanomaterials can aggregate under acidic (3.0) pH conditions and in relatively high ionic strength (100 mM) test media . This greatly depends on the nanomaterial surface properties as electrically stabilized nanoparticles can aggregate in these conditions, but sterically stabilized will not . Therefore, more research is needed to test and optimize the performance of such sensors to ensure that they will can detect microbes in real water samples.

Table 1.7: Description of Top 5 Sensors Ranked Using AHP and TOPSIS Methods

Ranking	Source	Sensor	Direct or Indirect	Mechanism	Cost	Detection Limit (CFU/ml)	Detection Time	NP Type	Toxic	Reusable	Lab scale	Field Scale
1	(Miranda, 2011)	Colorimetric Bacteria	I	Enzyme reaction	5	100	10	Gold	N	N	Y	Y
2	(Xie, 2019)	UV-Induced Au Nanocluster	D	UV and Aggregation	4	100	10	Gold	Y	N	Y	Y
3	(Lim, 2012)	Enhanced AuNP	D	Aggregation	4	100	10	Gold	N	N	Y	F
4	(Russo, 2018)	AuAg Nanoshells	D	Aggregation	5	100	10	Gold and Silver	Y	N	Y	F

Ranking	Source	Sensor	Direct or Indirect	Mechanism	Cost	Detection Limit (CFU/ml)	Detection Time	NP Type	Toxic	Reusable	Lab scale	Field Scale
5	(Raj, 2015)	Cysteine Capped AuNP	D	Aggregation	4	100	10	Gold	N	N	Y	N

1.3.4 Sensitivity Analysis

The sensitivity analysis looked at the AHP determined weighting and compared it to 9 additional weight percentages for the evaluation criteria. The additional weights tested were previously presented in Figure 1.4. The impact of change in weights of the evaluation criteria on the original sensor rankings is shown in Table 1.8. The sensitivity analysis did change the top five sensors, but the Colorimetric Bacteria Sensor stayed as the top sensor in the ranking. As seen in the last column of Table 1.8 titles “Stays in top 5” each sensor is given a percentage. This percentage is how much it shows up in the Top 5 sensors in all ten tests. From these percentages, the highest were used to create the sensitivity test top 5. This is based on the consistency of each sensor being ranked stays within the top five over the ten weighted tests (the AHP weights and additional 9 weight ranges).

Table 1.8: Sensitive Test Comparison of Weights

Sensor	AHP Weights	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8	Test 9	Stays in Top 5
Colorimetric Bacteria Sensor	1	1	4	5	5	5	6	5	1	1	90%
Magneto Nanosensors	6	4	2	2	2	2	2	2	8	11	70%
Multiplexed Paper Test Strip Sensors	7	5	1	1	1	1	1	1	10	12	70%
AuNP Coated Starch Magnetic Beads Sensor	8	8	3	3	3	3	3	3	9	8	60%
UV-Induced Au Nanoclusters Sensor	2	2	5	6	6	6	7	6	2	2	50%
Polymer Dot Sensor	13	9	9	4	4	4	4	4	13	13	50%
Enhanced AuNP Sensor	3	3	6	7	7	7	8	7	3	3	40%

Sensor	AHP Weights	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8	Test 9	Stays in Top 5
Cysteine Capped AuNP Sensor	5	7	7	8	8	8	5	8	5	5	40%
AuAg Nanoshells Sensor	4	6	8	9	9	9	9	9	4	4	30%
AuNP Biosensor	9	10	10	10	10	10	10	10	6	6	0%
Competitive Binding Sensor	10	11	11	11	11	11	11	11	7	7	0%
AuNP Sensor	11	12	12	12	12	12	12	12	11	9	0%
Conjugated AuNP Sensor	12	13	13	13	13	13	13	13	12	10	0%

The new top 5 ranked sensors resulting from the sensitivity analysis is presented in Table 1.9. All five sensors have a detection time of 30 minutes or less, with two of the sensors having a detection time of 10 minutes. Of the five sensors, only three have field scale capabilities. However, the average cost score of the top 5 sensors resulting from

the sensitivity analysis is 3.4 which is 1 value lower than the original TOPSIS ranking that was based on the AHP weighting. In addition, the detection limit values were overall lower. Two of the five sensors have a detection limit of 100 CFU/mL with the remaining 3 sensors having lower detection with the lowest being only 1 CFU/mL.

Table 1.9: Description of Top 5 Sensors Ranked Using Sensitivity Test

Ranking	Source	Sensor	Direct or Indirect	Mechanism	Cost	Detection Limit (CFU/ml)	Detection Time	NP Type	Toxic	Reusable	Lab scale	Field Scale
1	(Miranda, 2011)	Colorimetric Bacteria	I	Enzyme reaction	5	100	10	Gold	N	N	Y	Y
2	(Banerjee, 2016)	Magneto	D	Aggregation	1	1	30	Iron	Y	Y	Y	N
3	(Hossain, 2012)	Multiplexed Paper Test Strip	I	Enzyme Reaction	3	5	30	Immunomagn-etic	N	N	Y	Y
4	(You, 2020)	AuNP Coated Starch Magnetic Beads	D	Aggregation	4	1	18.5	Gold	N	Y	Y	N
5	(Xie, 2019)	UV-Induced Au Nanocluster	D	UV and Aggregation	4	100	10	Gold	Y	N	Y	Y

Overall, the sensitivity top 5 seem to be more cost effective as a group, have smaller detection limits and detection times, and more confirmed field capabilities. Within this improved ranking, it is positive to see that the top sensor was the same in both the sensitivity top 5 and the AHP top 5.

1.4 CONCLUSION AND FUTURE WORK

Of the 31 articles reviewed, it is clear there is advancements in detection methods for microbes in water. Still, there are still some large data gaps that are important for comparison of developed detection methods. Looking just at colorimetric nanosensors, indirect methods should be a focus moving forward. This approach, compared to direct colorimetric methods, can achieve on average a smaller detection limit without additional time needed. Further, the Colorimetric Bacteria Sensor developed by Marinda et al. (2011) should be the focus of future optimization. This rapid detection sensor is utilizing gold nanoparticles in an indirect enzymatic reaction as the methodology of the sensor. Through a field-friendly test strip format, bacteria bind to the gold nanoparticles used, which releases the β-Gal and restores its activity to them. This provides an enzyme-amplified colorimetric readout with the use of chlorophenol red β-D-galactopyranoside (CPRG) . Through this, it can achieve a low detection limit of 100 CFU/mL in only 10 minutes time. It is already field scale capable but has a high cost. Through future improvements, the cost could be reduced by possibly changing the nanomaterial used. While doing so, it is important to record and report as much information about the cost of the sensor as well as the characteristics of the

nanoparticles used. This will help continue the development of the sensors into the future.

2: OPTIMIZATION OF COLORIMETRIC NANOSENSORS FOR RAPID DETECTION OF MICROBES IN WATER

2.1 INTRODUCTION

2.1.1 Background

Contamination of drinking water by pathogens is a global health issue that is killing millions each year . Contaminated water creates a burden of disease in the area such as diarrhoea, cholera, dysentery, typhoid, and polio . Contaminated drinking water is estimated to cause 485,000 diarrhoeal deaths each year even though diarrhoea is largely preventable . The deaths of about 297,000 children aged under 5 years each year could be avoided with improved water supply and services . With better water systems in place, health is increased in the area leaving people with more time to enjoy and take part in their lives. This increases work and school attendance and productivity making the possibility to stop the cycle of poverty more possible.

Improvement to water systems is important. In the United Nations' Agenda 2030 Sustainable Development Goals (SDGs), clean water and sanitation (SDG 6) is a cross cutting goal that affects so many other aspects of the SDGs. As mentioned before, health, prosperity, industry, and education all are affected by water systems. However, to prove that these projects are safe water sources, water quality testing must occur. An important indicator for water quality to test for is E. coli. E. coli is an indicator of microbial contamination is the coliform group (fecal contamination) and is known to occur concurrently with major water-borne pathogens that are hard to test for . Through detection of E. coli in water, the water can be deemed safe or not for

recreation, drinking and cooking, or bathing. For drinking water, the global standard is 0 cells per 100 mL .

A major issue with testing for E. coli in water is that the conventional methods take a long time due to the need for incubation of the sample . This also means the method requires lab equipment and access to electricity which greatly limits field work applications. In addition, for low- and middle- income countries, typically these two requirements are not easily accessible . For areas/projects with connections to labs, transportation of water samples can cause unreliable readings without proper preservation protocols and equipment. Poor road conditions and transportation methods increase the time it takes to get from one point to the other, allowing more time for activity and changes to the microbial concentration in sample. A solution to this issue has been to make portable lab equipment such as a portable incubator for incubation of sample using traditional E. coli detection methods. These expensive and extremely heavy instruments are not only hard to transport to rural areas, but it also requires electricity. Without electricity, the portable lab equipment is also ineffective. If a remote and impoverished community overcome these issues and is able to accurately test their water it tends to take lots of connects, labor, and money which typically can only be done once. This means water quality may be tested at the completion of the project, but never again throughout the life of the water system.

Overall, the current methods for testing E. coli are extremely hard to be done in the field for low- and middle-income countries and places most in need of nonprofit organization (NGO) water and sanitation projects. The best NGOs can do is community surveys to

determine if people are getting sick or not and suggest additional steps such as boiling water .

2.1.2 Detection Methods for Waterborne Pathogens

The most commonly used methods for detection of *E. coli* include conventional plate counting techniques and biochemical tests. These methods are credible and produce reliable results, but they can take more than 2 to 3 days for results and require lab-based equipment and electricity . The limitations mentioned in Section 2.1.1 have urged the scientific community to investigate alternative detection methods. This research has shown some promising results. These new detection methods have eliminated the need for incubation. By doing so, these sensors provide results in a matter of minutes rather than days and so are referred to as rapid detection sensors. Some of these new platforms are still relatively expensive, complicated, and need a trained/experienced operator . These include: Polymerase Chain Reaction (PCR), Fluorescence/optical based methods, Electrochemical biosensors, Surface Plasmon Resonance (SPR) techniques . While these methods are producing promising results, they still hold limitations for application of water system projects in the “developing” world.

To tackle these limitations, but maintain rapid detection and reliable results, extensive research has investigated different nanomaterial colorimetric methods. Colorimetric bacteria sensing is both simple to preform and simple to read the results. Results are deciphered through a visible color change (compared to a premade indicator scale) and/or a portable spectrophotometer for exact values. It is less expensive and more

versatile than other methods . The platform has mainly developed into two main approaches, direct and indirect assays . Direct refers to directly tracking the nanoparticle characteristic change and indirect refers to the tracking of reaction change. Both produce a visible color change that indicates how much bacteria are in the assay.

An example of the direct assay is the study by Su et al. (2011) where the gold nanoparticle (AuNP) based colorimetric method was used. The method takes advantage of an interaction between amino-modified AuNPs and gram-negative bacteria. The aggregation of AuNPs in the presence of a certain number of bacteria results in an observable color change from red (dispersed AuNPs) to blue (aggregated AuNPs). This method demonstrated a linear detection range within 108–109 CFU/mL of E. coli O157:H7 .

An example of the indirect assay is a method from Miranda et al. (2011) and is a method that is based on pre-inhibited enzyme (AuNPs-enzyme complex). This complex regains its activity by the displacement of bacteria onto the surface of the cationic AuNPs which causes the visible color change. This method demonstrated detection of 1 × 102 and 1 × 104 CFU/mL in both solution and test strip formats, respectively .

Building on the second approach, indirect competitive binding between the nanoparticles, bacteria, and an enzyme is introduced in 2015 by Raweewab T. and Rawiwan L. This method, hereafter called “the Original Method”, achieved a detection limit approaching 10 CFU/mL without compromising the simplicity of colorimetric bacteria sensing. The Original Method was able to achieve this using polyethyleneimine coated gold nanoparticles (PEI-AuNP) and an enzymatic reaction. The detection

technique (illustrated in Figure 2.1) is based on creating a competitive binding reaction between the PEI-AuNP, the bacteria being detected, and an enzyme (β-galactosidase or β-Gal). Both the bacteria and enzyme are negatively charged, but the PEI coating gives the nanoparticles a positive charge. This difference in charge creates an attraction between the PEI-AuNP and negatively charged reactants. When there are just the PEI-AuNPs and the enzyme in this assay, the two bind together limiting the enzyme's ability to react with anything else. However, when bacteria are present, its slightly stronger negative charge starts to pull the PEI-AuNPs away from the enzymes which leaves free enzymes within the assay. This means a given number of PEI-AuNPs with various concentrations of bacteria will result in different levels of unbound enzymes . The competition between the reactants can be indirectly tracked using chlorophenol red β-D-galactopyranoside (CPRG) which reacts only with β-Gal in this method. As CPRG reacts with the free unbound enzymes, a visible readout from a yellow to chlorophenol red is produced .

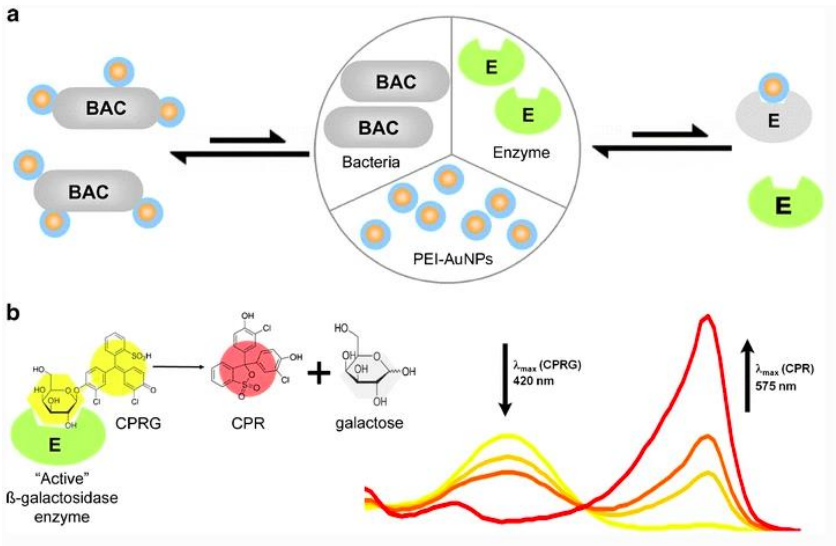

Figure 2.1: Original Method Competitive Binding Adopted form .

With a fixed concentration of nanoparticles and enzymes, different bacteria concentrations can be indirectly quantified based on color change. The higher the bacteria concentration, the more nanoparticles will be bound to bacteria. This leaves more β-Gal free to react with CPRG and creates a deep red/purple color. A small bacteria concentration means there is less opportunity for the PEI-AuNPs to bind to the bacteria, so they will bind to the β-Gal if bacteria cells are not present. This leaves less free β-Gal to react with CPRG which makes the color of the assay closer to yellow than red. The concentration of bacteria determines the color within the spectrum of yellow to red of the solution. Based on the absorbance of the solution's color change, the concentration of bacteria can indirectly be detected. It is important to note that *E. coli* under some conditions can produce β-Gal on their own. This could potentially increase the sensitivity of the sensor with further calibration of the method. Future investigation is warranted to address this possibility.

The Original Method shows a simple, rapid detection sensor with clean and easy to read results. Because of this, further optimization of the Original Method is appealing. By improving the technique, it is possible to make this sensor cost-effective, reusable, and have field scale capabilities.

2.1.3 Objective

The objective of this thesis is to optimize the Original Method. The specific goals are to 1) reduce the cost of the nanosensor by replacing nano-gold with a cheaper nanomaterial, 2) make it a recyclable and reusable sensor, and 3) maintain its detection

limit at 10 CFU/mL (or lower). These goals are to be achieved without compromising the simplicity of the colorimetric detection platform. With a reusable and more cost-effective sensor, field applications for development work can be more attainable. For simplicity moving forward, this optimized nanosensor work built on the Original Method has been called hereafter the “Modified Method.”

2.1.4 Hypothesis and Research Tasks

To accomplish the research objective, the target of optimization became the nanoparticle itself. The hypothesis is that the PEI coating is the governing factor of the competitive binding as it is the source of the positive surface charge on the nanoparticle surface. This hypothesis means it is predicted that the nanomaterial itself does not affect the function of the sensor, only the PEI coating. If this is true, it will be possible to use less expensive nanomaterials with the same PEI coating without changing the sensor function. It would also allow for the possibility of using a magnetic nanomaterial, which can be separated from the test media by a magnet (or a magnetic field). This will make the nanosensor reusable as the particles could be taken out of the assay, reconditioned, and then used again. All without compromising the original sensor’s detection limit and function.

To test the research hypothesis, three tasks were created. The first task (Task 1) was to verify and optimize the sensor assay reported in the Original Method. Task 1 involved: a) developing a method for synthesis of PEI-AuNPs because the synthesis protocol reported in the Original Method was not reproducible and b) replicating and optimizing the sensor testing protocol developed in the Original Method. Optimization herein

means finding the volume and concentrations of reagents needed to produce a functional sensor. Task 1 was performed in the absence of microbes to find the nanoparticle concentration required to inhibit the chemical reactions between a known amount of β-Gal and CPRG. Once the optimal protocol is found, different bacteria concentrations can be tested to determine the detection limit of the sensor. Task 2 was to replace gold with another nanomaterial type that have the same surface coating (PEI) to verify the research hypothesis (the coating is the governing factor for competitive binding). Task 2 was conducted using silver nanoparticles coated with PEI (PEI-AgNPs). The reason for choosing silver nanoparticles for this task are: a) it can be synthesized and coated using similar methods used for synthesis of gold nanoparticles and b) silver nanoparticles have been successfully coated with PEI (the coating of interest) in previous studies and PEI coated silver nanoparticles is also commercially available. If PEI coated silver nanoparticles were successful in inhibiting a color change, then the research hypothesis will be proven. Task 3 was to develop methods for coating magnetite nanoparticles with PEI to reduce the sensor cost and enable reuse through the magnetic properties.

2.2 METHODOLOGY

2.2.1 Synthesis of gold NPs

To achieve Task 1, two methods were investigated for synthesis of gold nanoparticles. The first synthesis method was reported by the Raweewab T. and Rawiwan L. The second was a modification to a method for synthesis of PEI coated silver nanoparticles . The Original Method reported a synthesis protocol that was simple and only involved

mixing a solution of PEI and $HAuCl_4$ for a period of time. First, 25 mL of a 1.4 mM aqueous $HAuCl_4$ solution was added to 0.5 mL of 1% PEI solution. Then the mixture was stirred vigorously at room temperature for 16 hours (or for 24 hours in another test batch).

The second synthesis method utilized a UV radiation to induce the formation of PEI-AuNPs. Originally created to manufacture PEI-AgNPs, the method was adjusted herein to create PEI-AuNPs. First, 0.182 g of HAuCl4 was added to 100 mL of DI water to obtain a 9.4 x 10-3 M solution. Then 0.085 g of HEPES was added to this mixture. In a separate beaker, a 1% branched polyethyleneimine (PEI) solution was created by adding 0.1 g of BPEI (MW=1.20 kgmol-1) into 10 mL of DI water. The 1% BPEI solution was then used to adjust the pH of the HAuCl4/HEPES mixture to 6.5. Once the desired pH was reached, the solution was exposed to UV radiation using a powerful UV light for about 10 minutes or until the color of the solution changed from yellow to deep red, which is an indication of the formation of the gold nanoparticles.

2.2.2 Commercial Silver Nanoparticles

A commercial silver nanoparticle coated in PEI was purchased from Nanocomposix Inc. (San Diego, CA). The concentration of the PEI-AgNP stock suspension was 1000 mg/L. As per the manufacturer, the size of the PEI-AgNP was 40 nm.

2.2.3 Surface Modification of Commercial Iron Nanoparticles

Iron nanoparticles are desired for testing because they are both a cheaper material than gold and silver and are magnetic. A simple method was tested herein to coat magnetite nanoparticles (Fe_3O_4 NPs), purchased from U.S. Research Nanomaterials Inc. (Houston,

TX), with PEI. This method was based on the assumption that contact with a PEI solution will provide a chance for PEI molecules to bond to the surface of the magnetite nanoparticles. The desired concentration of PEI- Fe_3O_4 NP solution was 1000 µM. To achieve this, 0.8 g of PEI was added to 40 mL of DI water to make a 2% PEI solution. Then 2.4 mL of stock Fe_3O_4 NPs suspensions was added to the PEI solution. This mixture was placed on a shaker for 24 hours. This method was tested twice. Once as is stated and second with a final step of centrifuging and washing the nanoparticles with DI water after it was shaken for 24 hours.

2.2.4 Characterization of NPs

It is important to find the concentration of the synthesized PEI-AuNPs as well to verify the formation of the nanoparticles. UV-Vis spectroscopy was used for determination of the surface plasmon resonance (SPR) peak of the synthesized gold nanoparticles. The wavelength of the SPR peak is a fingerprint of some types of metallic nanoparticles (e.g., gold and silver) . The SPR peak wavelength and value were used to verify the formation and determine the concentration of AuNPs, respectively. An SPR calibration curve (Figure B1, Appendix B) from the study by Spadavecchia et al. (2016) was used for calculating the concentration of the synthesized gold NPs.

2.2.5 Nanosensor Testing Assay

The testing protocol reported in the Original Method was followed to verify that the synthesized PEI-AuNPs can effectively bind β-Gal and inhibit its reaction with CPRG in the absence of microbes. The test mixture was comprised of 2 µL of 0.125 µM β-Gal, 30 µL of 0.75 µM CPRG, and 2 µL of 0.1 nM PEI-AuNP. However, the PEI-AuNP

concentration used was found to not work with the synthesized PEI-AuNPs. To optimize the mixture for this study's purposes, a range of nanoparticle concentrations were tested while fixing the amounts of β-Gal and CPRG used. This optimization process was performed for all the three nanoparticles investigated in this research (i.e., PEI-AuNPs, PEI-AgNPs, and PEI coated magnetite NPs).

First, various dilutions of the nanoparticle concentrations were prepared, and triplicate tests were conducted for each nanoparticle concentration. Then using a 96-well plate, the desired wells were filled with 2 µL of the nanoparticle dilution being tested, 2 µL of β-Gal, and 100 µL of phosphate buffer solution. The mixtures were left in the wells for one hour to allow for the β-Gal and nanoparticles to bind and reach equilibrium. After one hour, 30 µL of CPRG was added to each well and the well plate was immediately placed into a SpectraMAX plus plate reader to record the Optical Density (OD) values of the mixtures in the wells. The OD measurements were conducted at 575 nm every 10 minutes for 3 hours. Once inhibition of a color change was observed, the reactant volumes and concentrations that resulted in this inhibition were deemed optimal and could be used for further testing to detect microbes in water.

2.3 RESULTS AND DISCUSSION

2.3.1 Synthesis of Nanoparticles

The PEI-AuNPs synthesis method reported in the Original Method was not reproducible. A color change was not visible in two different attempts as shown in Figure 2.2.

Figure 2.2: Solution After Mixing for 16 Hours Showed No Color Change Indication and Stayed Yellow.

Even though there was no visible color change, the solution was tested for the gold nanoparticles' SPR absorbance peak using a UV-Vis Spectrometer. This was performed to verify the formation of AuNPs despite the lack of a visible color change. The SPR data indicated that a small amount of AuNPs likely formed but the conversion efficiency of ionic gold to nano-gold was low. This low concentration was not enough to result in noticeable color change. With some adjustments to this method, the efficiency may improve. However, this research switched to developing another approach for synthesis of AuNPs using another chemical reduction method assisted with UV-irradiation.

The UV radiation method for creating PEI-AuNPs was successful and produced a clear color change as shown in Figure 2.3. In this method, the solution was exposed to UV radiation for seconds at a time and the suspension color was checked after each short

exposure round. The desired color change happened after the 9th exposure making the total exposure time to be approximately 8 minutes.

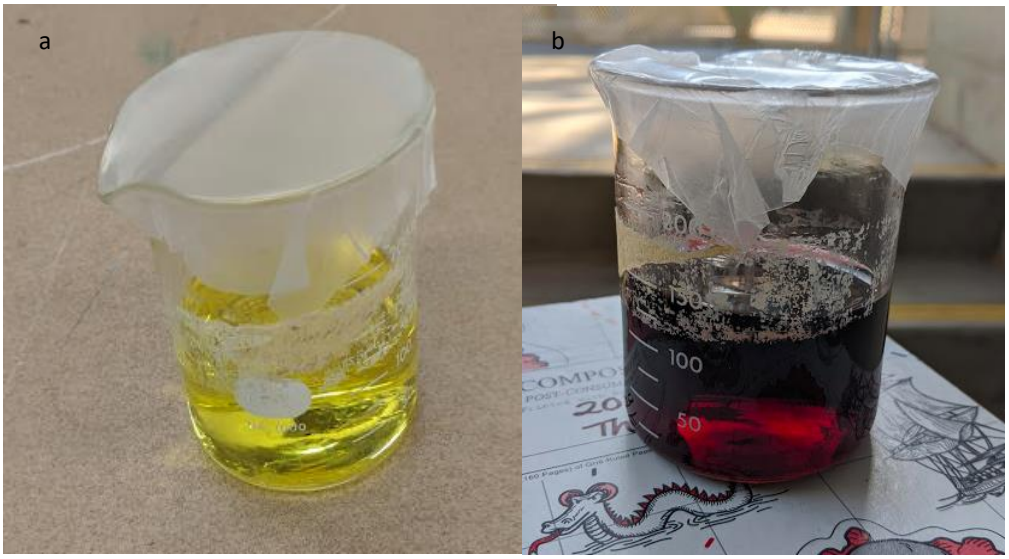

Figure 2.3: The Solution Color a) Before Exposure to UV and b) After Exposure to UV.

To verify the formation of the Au-NPs, the absorbance of a diluted PEI-AuNP suspension was measured at 21 different wavelengths and the absorbance peak was identified (Figure 2.4). The absorbance peak occurred at a wavelength of 520 nm, which is typical for gold nanoparticles .

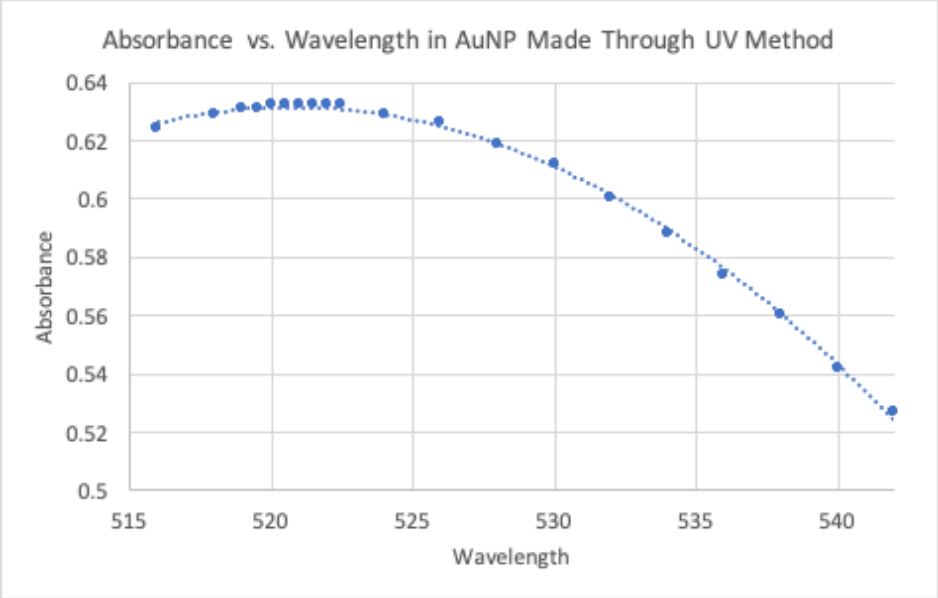

Figure 2.4: Absorbance Peak of the PEI-AuNPs. Wavelength is in The Unit of nm.

This showed that gold nanoparticles had formed. The concentration of the synthesized PEI-AuNP suspension was calculated based on the absorbance peak value using the calibration curve presented in FigureB1 (Appendix B). The concentration of the stock suspension was found to be 275 mg/L.

2.3.2 Coating of Commercial Iron Oxide Nanoparticles

To lower cost and increase reuse, surface modification of commercial magnetite nanoparticles with PEI was investigated. This was performed with the assumption that contact with a 2% PEI coating will results in coating the surface of the nanoparticles with PEI. After shaking the nanoparticle suspension in 2% PEI for 24 hours, the nanoparticles remained suspended. Then, the suspension was washed and centrifuged to remove any residual PEI. However, after centrifuging at 10,000 rpm for 8 minutes and washing with DI, the particles aggregated, and shaking did not result in fully re-suspending the particles.

2.3.3 Color Inhibition Assay Results

Inhibition of the color change is an indicator that there are enough nanoparticles in the test media to bind with the β-Gal enzyme and prevent its reaction with CPRG. Once inhibition was shown with PEI-AuNPs, PEI coated silver nanoparticles were tested to prove the hypothesis that the PEI coating is the governing factor and the gold nanoparticles can be replaced with cheaper ones.

For the PEI-AuNP assays, the OD was measured every 10 minutes over three hours to quantify color change of the PEI-AuNP/β-Gal/CPRG mixture. Change in color can also be observed by the naked eye. The PEI-AuNPs concentrations in the mixture ranged from 0 to 1400 µM. If the OD at 575 nm does not increase or the mixture color does not change over the course of the three hours, the sensor was deemed functional. PEI-AuNP concentrations ≥ 60.25 µM showed clear inhibition of β-Gal/CPRG reaction as evidenced by the lack of color change as seen in Figure 2.5.

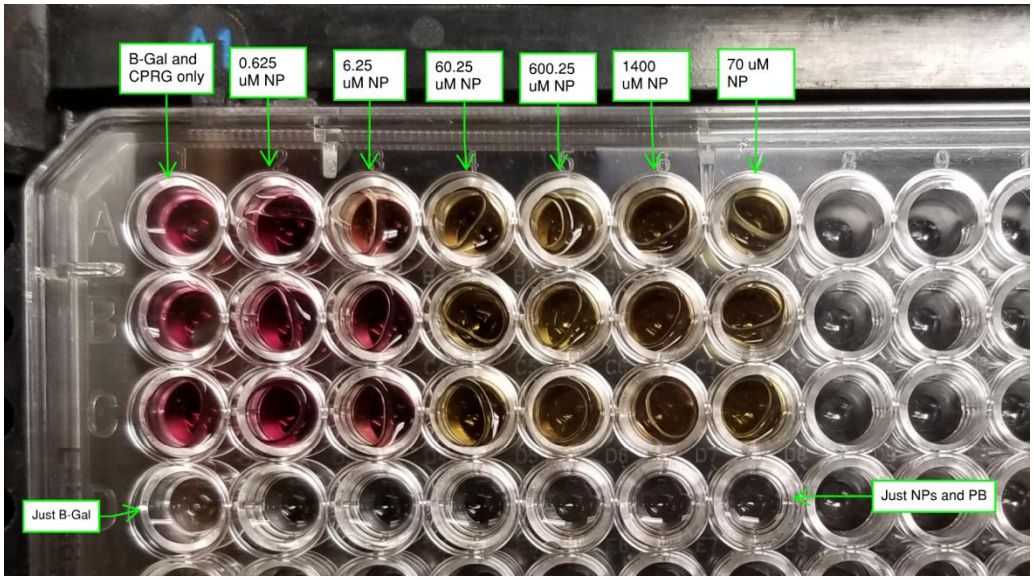

Figure 2.5: The Results After 3 Hours of Test Using PEI-AuNPs.

The OD results presented in Figure 2.6 align well with color change trends observed by the naked eye. At low nanoparticle concentrations, β-Gal was free to interact with CPRG causing color change. As the concentration of nanoparticle increased, the OD profile trended down. The smallest increase in OD over the 3 hours (less the 0.2) was observed when nanoparticle concentrations ≥ 60.25 µM were used. Full inhibition would occur if the OD values remained zero after the 3 hour testing period. However, the small OD values observed with the high nanoparticle concentrations maybe a result of experimental error or could be true reactions that caused these OD readings. Higher concentrations of nanoparticles should be tested in future research to determine if complete inhibition could be achieved. Nonetheless, this inhibition, while not complete, verifies the sensor is a good candidate for testing microbes.

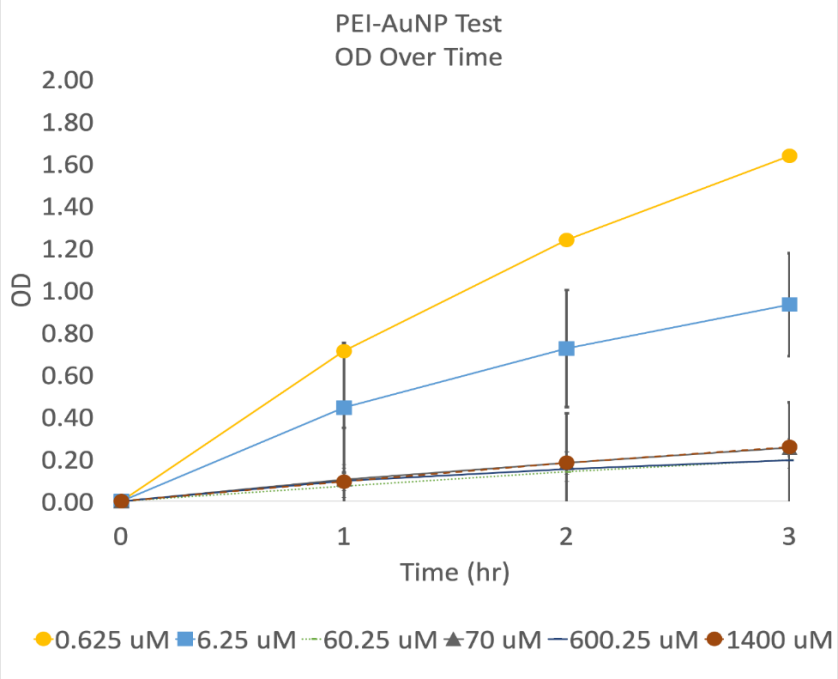

Figure 2.6: The Average OD of The Triplicates of Each Concentration Measured Over 3 Hours for PEI-AuNP.

The PEI-coated silver nanoparticles also showed inhibition of the β-Gal/CPRG reaction at all concentrations tested. This is indicated by the visual color change observations as well as the OD data presented in Figures 2.7 and 2.8, respectively. Furthermore, PEI coated silver nanoparticles inhibited the β-Gal/CPRG reactions at lower concentrations compared to PEI coated gold nanoparticles. This implies lower cost if silver nanosensors are used because lower amount of nanoparticles may be needed and silver is cheaper than gold.

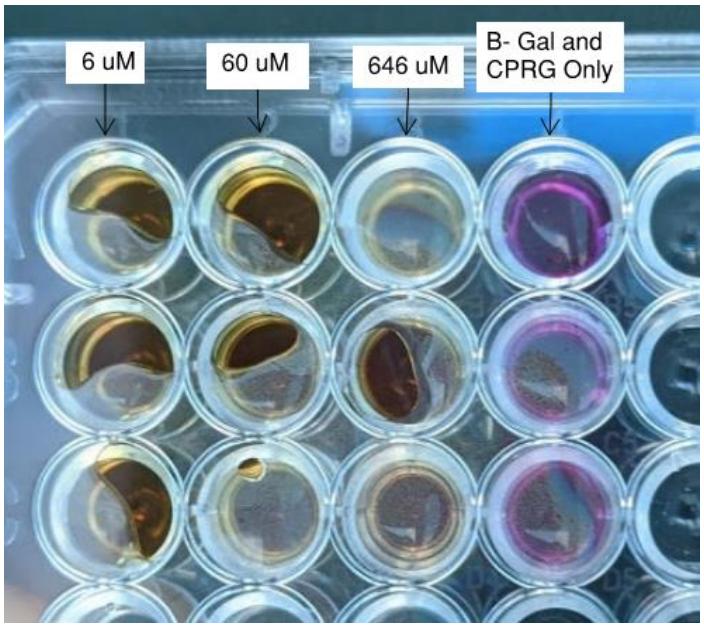

Figure 2.7: Photo of Results of PEI-AgNP Inhibition Test.

Similar to gold nanoparticles, relatively small OD readings were still recorded when silver nanoparticles were used. This warrants further research to understand the source of the OD readings. Possible sources could be experimental error or true reactions that generate the OD readings. Additionally, future experiments are needed to evaluate the testing period required. It may be also possible that microbes can be detected in less

than one hour. If this is the case, it will resolve issues with the observed increase in OD readings as the reaction time increased to 3 hours.

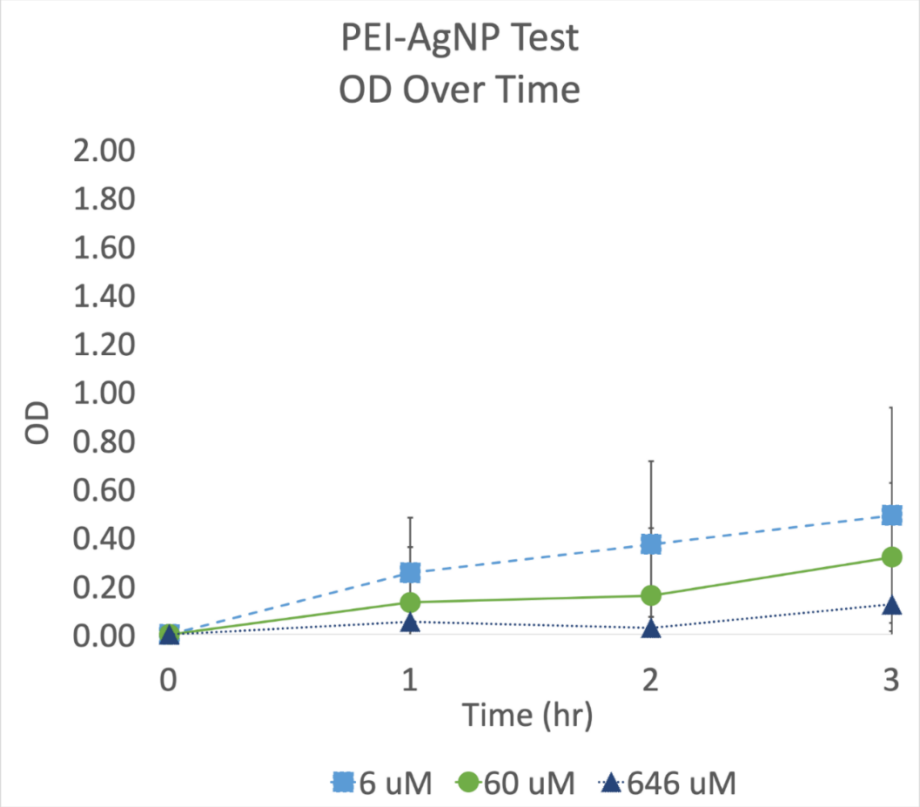

Figure 2.8: The Results of The Average OD of Each Concentration Measured at Time 0, 1, 2, and 3 Hours for PEI-AgNP.

The β-Gal/CPRG reaction inhibition achieved by both PEI coated gold and silver nanoparticles indicate that the PEI coating is likely the governing factor for how this sensing technique functions. This means that it may be possible to replace gold with less expensive materials and to make the sensor reusable if PEI coated magnetic nanoparticles can be synthesized.

To work towards reuse and cost-effectiveness improvements, a first attempt was made to coat magnetite nanoparticles with PEI and test their effectiveness in inhibiting the β-Gal/CPRG reaction. Unfortunately, this test gave negative results showing no inhibition for all concentrations tested (10-100 µM) as seen in Figure 2.9. The OD reached high values after one hour for all concentrations tested.

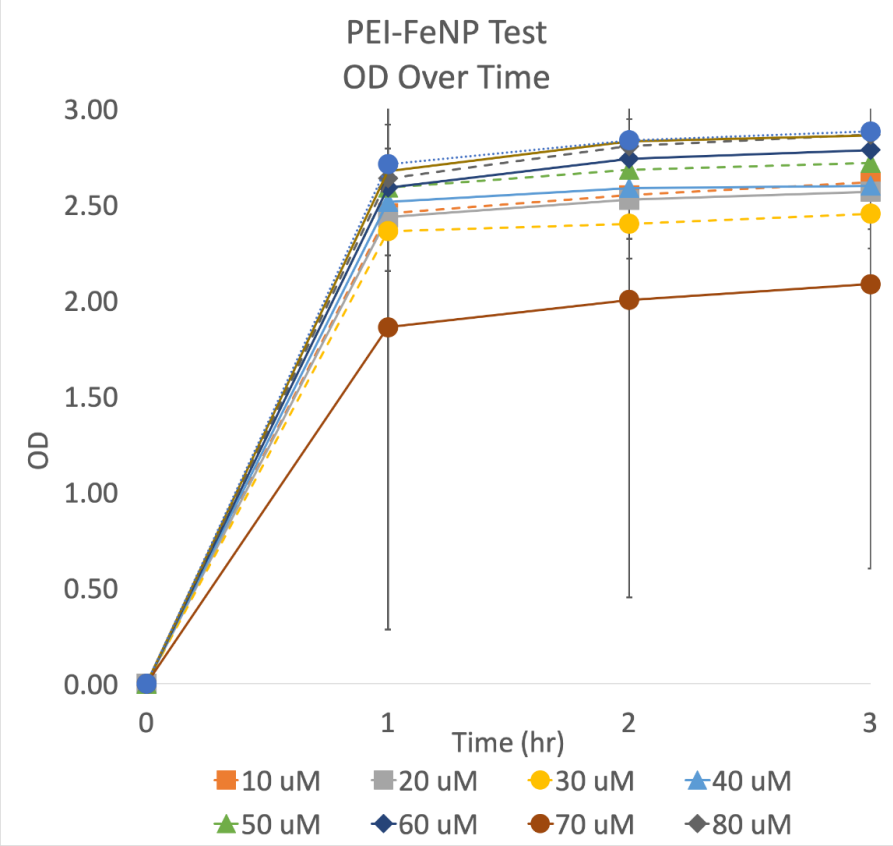

Figure 2.9: The Results of The Average OD of Each Concentration Measured at Time 0, 1, 2, and 3 Hours.

The lack on inhibition and overall reaction of the nanoparticles is most likely caused by a failed PEI coating approach and/or nanoparticle aggregation and falling out of the reaction mixture. The high standard deviation values shown in Figure 2.9 could be

another indicator that nanoparticle aggregation was taking place in the test media, which resulted in high variability in the data and made the sensor inefficient. Further research is needed to develop a method to effectively apply a PEI coating to the magnetite nanoparticles surface and to produce a stable nanoparticle suspension. Therefore, conclusions cannot be made regarding the possibility of using magnetite as a sensor for detection of microbes until stable PEI coated magnetite nanoparticles are produced and tested.

2.4 CONCLUSIONS AND FUTURE RECOMMENDATIONS

The objective of this investigation was to reduce the cost and improve the reusability of the indirect colorimetric nanosensor method developed in 2015 by Raweewab T. and Rawiwan L. (the Original Method) without compromising the simplicity of this detection platform. To improve field applications for water quality testing in impoverish areas, great importance is placed on optimizing methods to be both reusable and more cost-effective.

To accomplish this objective, the target of optimization became the nanoparticle itself. Optimizing the Original Method, the hypothesis was that the PEI coating is the governing factor of the competitive binding reaction. This meant the core nanomaterial does not affect the function of the sensor. This would also more cost-effective nanomaterials to be used with magnetic properties for reuse application. This hypothesis was proved by the inhibition of both gold and silver PEI coated nanoparticles using the Original Method. The PEI coating is the governing factor in this competitive binding method.

With further development and future research, it may be possible to use less expensive nanoparticles for this sensor. It may also be possible to make this sensor reusable through the development of PEI coated magnetite nanoparticles. Their magnetic quality could allow for recovery of the nanosensors from the test media followed by re-conditioned and then used again.

Comprehensive nanoparticle characterization is needed in future investigations. Identifying the characteristics of the nanoparticles can assist in interpreting the inhibition results and guide future work on improving the nanosensors. It is recommended to use Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for more accurate determination of the concentration of the nanoparticles. Fourier Transform Infrared Spectroscopy (FTIR) and Thermal Gravimetric Analysis are recommended to understand the surface properties of the nanomaterials and verify that the surface is sufficiently coated with the coating molecules. To determine the size and size distribution of the synthesized nanoparticles, Dynamic light scattering (DLS) measurements need to be conducted.

In addition, using the sensor for detection of microbes should be investigated. Non-pathogenic E. coli can be used as model microbes. The detection limit and time for the sensors need to be determined. Additional future work recommended is to test the senor in field water samples collected from different surface streams. In addition, it would be important to analyze the change in OD at both 420 nm and 575 nm throughout the assay to test the sensitivity at each wavelength. This could further improve sensitivity of detection.

Lastly, research is needed to develop methods for collecting the sensor from the assay media, detaching the microbes from the nanoparticle surface, and reconditioning the sensor to be re-used in new tests. Then collection can be performed using a magnet. By adjusting the pH of the samples to greater than 9, PEI will be significantly less protonated and will have minimal surface charge. This may help detaching the microbes from the nanoparticles. Once separated, it may be possible to reuse the nanosensors. In addition, future research should test the efficiency of other cationic polymers (in addition to PEI) as nanoparticles coating. This will further the research hypothesis and may result in more effective nanoparticle coatings.

Overall, the Original Method for colorimetric detection of E. coli in water shows promise for improvement. Its simplicity and rapid detection is a much-needed aspect for better water quality measurements. With the optimization of reusability and cost effectiveness, this sensor can be used in a wide range of applications.

REFERENCES

Ávalos, A. (2018). Effects of silver and gold nanoparticles of different sizes in human pulmonary fibroblasts. *Toxicological & Environmental Chemistry*, pp. 629-643.

Badawy, A. E. (2010). *Impact of Environmental Conditions (pH, Ionic Strength, and Electrolyte Type) on the Surface Charge and Aggregation of Silver Nanoparticles Suspensions .* ACS Publications.

Badawy, A. E. (2011). *The impact of stabilization mechanism on the aggregation kinetics of silver nanoparticles.*

Banerjee, T. (2016). *Multiparametric Magneto-fluorescent Nanosensors for the Ultrasensitive Detection of Escherichia coli O157:H7.*

Bhardwaj, N. (2019). *Optical detection of waterborne pathogens using nanomaterials.*

Bridle, H. (2014). *Nanotechnology for Detection of Waterborne Pathogens.*

Bridle, H., & Balharry, D. (2015). *Exploitation of Nanotechnology for the Monitoring of Waterborne Pathogens: State-of-the-Art and Future Research Priorities.*

Chhetri, S. K. (2015). *Manifestation of an Analytic Hierarchy Process (AHP) Model on Fire Potential Zonation Mapping in Kathmandu Metropolitan City, Nepal .* Geo-Information.

Da-Silva, E. (2018). *Biosensing platforms for Vibrio bacteria detection based on whole cell and nucleic acid analysis: A review.*

Driskell, J. (2009). Emerging Technologies in Nanotechnology-Based Pathogen Detection. *Clinical Microbiology Newsletter, 31*(18).

ELBadawy, A. (2019).

Fournier-Wirth, C., & Coste, J. (2010). *Nanotechnologies for pathogen detection: Future alternatives?*

García-Cascales, M. S. (2013). *Geographical Information Systems (GIS) and Multi-Criteria Decision Making (MCDM) methods for the evaluation of solar farms locations: Case study in south-eastern Spain.* Renewable and Sustainable Energy Reviews.

Hayden, S. C. (2012). *Aggregation and Interaction of Cationic Nanoparticles on Bacterial Surfaces.*

Hossain, S. M. (2012). *Multiplexed paper test strip for quantitative bacterial detection.*

Ivask et al., A. (2013). *Toxicity Mechanisms in Escherichia coli Vary for Silver Nanoparticles and Differ from Ionic Silver.*

Jung, J. (2019). *S-layer protein-AuNP systems for the colorimetric detection of metal and metalloid ions in water.*

Kang, E. B. (2018). *Alkaline phosphatase-responsive fluorescent polymer probe coated surface for colorimetric bacteria detection.*

Lim, S. (2012). *Enhancing Nanoparticle-Based Visible Detection by Controlling the Extent of Aggregation.*

Llaver, M. (2020). *MODERN ANALYTICAL NANOTECHNOLOGIES FOR BEVERAGES QUALITY CONTROL.*

M, K. (2018). *Portable Sensors for Water Pathogens Detection.*

M.A.Ali. (2014). *Detection of E.coli O157:H7 in feed samples using gold nanoparticles sensor.*

Mathew, M. (Writer), & Mathew, M. (Director). (2018). *Analytic Hierarchy Process (AHP)* [Motion Picture].

Miranda, O. R. (2011). *Colorimetric Bacteria Sensing Using a Supramolecular.*

Mustafa, F. (2017). *Multifunctional Nanotechnology-Enabled Sensors for Rapid Capture and Detection of Pathogens.*

Prajapati, S. (2020). *Nanotechnology-based sensors.*

Price, R. G. (2016). *E. coli as an Indicator of Contamination and Health Risk in Environmental Waters.*

Raj, V. (2015). *Cysteine capped gold nanoparticles for naked eye detection of E. coli bacteria in UTI patients.*

Robby, A. I. (2019). *Recyclable metal nanoparticle-immobilized polymer dot on montmorillonite for alkaline phosphatase-based colorimetric sensor with photothermal ablation of Bacteria .*

Russo, L. (2018). *Low-Cost Strategy for the Development of a Rapid Electrochemical Assay for Bacteria Detection Based on AuAg Nanoshells.*

Sanvicens, N. (2009). *Nanoparticle-based biosensors for detection of pathogenic bacteria.*

Scientific, T. (2020). *Coliform Incubator*. Retrieved from Thomas Scientific: https://www.thomassci.com/scientific-suplies/Coliform-Incubator

Shrivastava, S. (2018). *Culture-free, highly sensitive, quantitative detection of bacteria from minimally processed samples using fluorescence imaging by smartphone.*

Spadavecchia, J. (2016). *Targeted polyethylene glycol gold nanoparticles for the treatment of pancreatic cancer: from synthesis to proof-of-concept in vitro studies.*

Su, H. (2011). *Gold nanoparticles as colorimetric sensor: A case study on E. coli O157:H7.*

Sun, J. (2020). *Colorimetric sensor array based on gold nanoparticles: Design principles and recent advances.*

Thiramanas, R., & Laocharoensuk, R. (2015). *Competitive binding of polyethyleneimine-coated gold.*

UNICEF. (2017). *Progress on.* WHO Library Cataloguing-in-Publication Data .

USGS. (2020). *Bacteria and E. Coli in Water*. Retrieved from USGS.

Water Quality Archives. (n.d.). Retrieved from Engineers Without Borders USA.

WHO. (2020). *Drinking Water*. (World Health Organization) Retrieved from https://www.who.int/news-room/fact-sheets/detail/drinking-water

Xie, X. (2019). *UV-induced peroxidase-like activity of gold nanoclusters for differentiating pathogenic bacteria and detection of enterotoxin with colorimetric readout.*

You, S.-M. (2020). *Gold nanoparticle-coated starch magnetic beads for the separation, concentration and SERS-based detection of E. coli O157:H7.*

Yu, L. (2020). Progress of gold nanomaterials for colorimetric sensing based on different strategies. *Trends in Analytical Chemistry*.

Zheng, L. (2018). *A simple, rapid and cost-effective colorimetric assay based on the 4-mercaptophenylboronic acid functionalized silver nanoparticles for bacteria monitoring.*

Zor, E. (2018). *Silver nanoparticles-embedded nanopaper as a colorimetric chiral sensing platform.*

APPENDIX A: CHAPTER 1

Table A1: Data Collection from Articles

Source	(Zheng, 2018)	(Zor, 2018)
Type of Publication	Report	Report
Sensor Name	Simple Sensor	AgNP Nanopaper Senor
Direct OR Indirect	D	I
Mechanism	Aggregation	
Possible Pathogens	E. coli	chiral D-cysteine
Industry	Water	Water and food
Detection Limit	dynamic range from 5 × 104cfu mL−1to 1 × 107cfu mL−1with a LODof 0.9 × 104cfu mL−1	4.88 µM
Detection Time	20 min	
NP Type	Gold, MPBA-AgNP	silver nanoparticles
Surface Coating		Embedded transparent
Surface Charge		
NP Concentration	30 nM to 150 uM	
Toxic (Y/N)	N	N
Reusable (Y/N/F)	N	N
Lab Scale (Y/N)	Y	Y
Field Scale (Y/N/F)	F	Y

Table A1: Data Collection from Articles Continued

Source	(Xie, 2019)	(You, 2020)
Type of Publication	Report	Report
Sensor Name	UV-Induced Au nanosensor	AuNP Coated Starch Magnetic Beads Sensor
Direct OR Indirect	D	D
Mechanism	UV and aggregation	Aggregation
Possible Pathogens	S. aureus	E. coli
Industry	Water and food	Water
Detection Limit	100	as low as a single cell
Detection Time	10 mins or Less	
NP Type	gold nanoclusters (AuNCs)	gold nanoparticle-coated starch magnetic beads (AuNP@SMBs)
Surface Coating	chitosan composite	starch magnetic beads
Surface Charge		-32.8 to +46.8 mV
NP Concentration		
Toxic (Y/N)	Y	N
Reusable (Y/N/F)	N	Y
Lab Scale (Y/N)	Y	Y
Field Scale (Y/N/F)	Y	N

Table A1: Data Collection from Articles Continued

Source	(Su, 2011)	(Thiramanas & Laocharoensuk, 2015)
Type of Publication	Report	Report
Sensor Name	AuNP Sensor	Competitive Binding Sensor
Direct OR Indirect	D	I
Mechanism	Aggregation	Enzyme reaction
Possible Pathogens	E. coli	S. aureus and E.coli
Industry	Water	Drinking Water
Detection Limit		as low as 10 cfu·mL−1
Detection Time	Few Minutes	Few minutes to a few hours
NP Type	Gold, MEA-AuNPs	Gold, cationic PEI-AuNPs
Surface Coating	Mercaptoethylamine (MEA)	PEI
Surface Charge	positive	highly positive surface charge (+53.6 mV)
NP Concentration		0.1 nM.
Toxic (Y/N)	N	N
Reusable (Y/N/F)	N	N
Lab Scale (Y/N)	Y	Y
Field Scale (Y/N/F)	N	F

Table A1: Data Collection from Articles Continued

Source	(Shrivastava, 2018)
Type of Publication	Report
Sensor Name	AuNP Biosensor
Direct OR Indirect	I
Mechanism	Florecence
Possible Pathogens	S. aureus
Industry	Water and food
Detection Limit	as low as 10 cfu/ml
Detection Time	10 mins or Less
NP Type	Gold, aptamer-functionalized fluorescent magnetic nanoparticles: fluorescent magnetic nanoparticles (FMNPs)
Surface Coating	
Surface Charge	
NP Concentration	
Toxic (Y/N)	N
Reusable (Y/N/F)	F
Lab Scale (Y/N)	Y
Field Scale (Y/N/F)	Y

Table A1: Data Collection from Articles Continued

Source	(Russo, 2018)
Type of Publication	Report
Sensor Name	AuAg Nanoshells Sensor
Direct OR Indirect	D
Mechanism	Aggregation
Possible Pathogens	E. coli and S. typhimurium
Industry	Water
Detection Limit	down to 102 CFU/mL
Detection Time	Few Minutes
NP Type	AuAg nanoshells consist of a hollow structure composed of a gold–silver alloy shell
Surface Coating	poly(vinyl pyrrolidone) (PVP) layer adsorbed on their surface during their synthesis, a hydrosoluble polymer
Surface Charge	The charges were controlled and change inorder to preform test
NP Concentration	
Toxic (Y/N)	Y
Reusable (Y/N/F)	N
Lab Scale (Y/N)	Y
Field Scale (Y/N/F)	F

Table A1: Data Collection from Articles Continued

Source	(Miranda, 2011)		(Robby, 2019)
Type of Publication	Report		Report
Sensor Name	Colorimetric Bacteria Sensor		Polymer Dot Sensor
Direct OR Indirect	I		I
Mechanism	Enzyme reaction		Florecence
Possible Pathogens	E. coli		E. coli and S. aureus
Industry	Water		Water
Detection Limit	1X102 bacteria/mL in solution and 1X104 bacteria/mL		range of 101e107 CFU/mL (LOD 5.09 and 4.62 CFU/mL, respectively)
Detection Time	Few minutes		
NP Type	Gold, cationic gold nanoparticles		PD-bCD-MMT/Fe3O4eCsWO3 nanocomposite
Surface Coating			
Surface Charge	cationic		
NP Concentration	80 nM		0e10 mM
Toxic (Y/N)	N		Y
Reusable (Y/N/F)	N		Y
Lab Scale (Y/N)	Y		Y
Field Scale (Y/N/F)	Y		F

Table A1: Data Collection from Articles Continued

Source	(Raj, 2015)
Type of Publication	Report
Sensor Name	Cysteine Capped AuNP Sensor
Direct OR Indirect	D
Mechanism	Aggregation
Possible Pathogens	E. coli
Industry	Medical (Urine testing)
Detection Limit	linear relationship in the range of 1 103–4 103 cells/mL with a detection limit of 100 cells/mL.
Detection Time	Few minutes
NP Type	Gold, Cysteine gold nanoparticles (CAuNPs)
Surface Coating	modified AuNPs with cysteine
Surface Charge	– 30.8 mV which was reduced to 4.5 mV on interacting with E. coli 0157:H7.
NP Concentration	
Toxic (Y/N)	N
Reusable (Y/N/F)	N
Lab Scale (Y/N)	Y
Field Scale (Y/N/F)	N

Table A1: Data Collection from Articles Continued

Source	(Lim, 2012)	(M.A.Ali, 2014)
Type of Publication	Report	Report
Sensor Name	Enhanced AuNP Sensor	Conjugated AuNP Sensor
Direct OR Indirect	D	D
Mechanism	Aggregation	Aggregation
Possible Pathogens	E. coli	E. coli
Industry		Feed samples
Detection Limit	fewer than 100 CFU/mL	
Detection Time	Few minutes	Few minutes
NP Type	Gold, AuNP, functionalized NPs (f-NPs) and streptavidin molecules (stAuNPs)	Gold, conjugated gold nanoparticles
Surface Coating		citrate
Surface Charge		-32 mV
NP Concentration		
Toxic (Y/N)	N	N
Reusable (Y/N/F)	N	N
Lab Scale (Y/N)	Y	Y
Field Scale (Y/N/F)	F	N

Table A1: Data Collection from Articles Continued

Source	(Kang, 2018)
Type of Publication	Report
Sensor Name	Fluorescent Polymer Probe Sensor
Direct OR Indirect	I
Mechanism	
Possible Pathogens	E. coli and S. aureus
Industry	
Detection Limit	101–107 colony-forming units/mL
Detection Time	1 Hour
NP Type	phosphorylated fluorescent probe 2-hydroxychalcone (HCAP) conjugated with an adhesive cationic polymer
Surface Coating	
Surface Charge	
NP Concentration	
Toxic (Y/N)	Y
Reusable (Y/N/F)	N
Lab Scale (Y/N)	Y
Field Scale (Y/N/F)	N

Table A1: Data Collection from Articles Continued

Source	(Hossain, 2012)	(Jung, 2019)
Type of Publication	Report	Report
Sensor Name	Multiplexed Paper Test Strip Sensors	S-layer Protein AuNP Sensor
Direct OR Indirect	I	D
Mechanism	Enzyme reaction	Aggregation
Possible Pathogens	E. coli	metal andmetalloid ions
Industry	Water	Water
Detection Limit	the limit of detection was ~5 colony-forming units (cfu) per milliliter	1.67×10−5 mol/l
Detection Time	30 min	
NP Type	immunomagnetic nanoparticles	gold nanoparticles (AuNP)
Surface Coating		Eight Slayerproteins from different bacteria species were used for the functionalization of AuNP.
Surface Charge		
NP Concentration		
Toxic (Y/N)	N	Y
Reusable (Y/N/F)	N	N
Lab Scale (Y/N)	Y	Y
Field Scale (Y/N/F)	Y	N

Table A1: Data Collection from Articles Continued

Source	(Banerjee, 2016)	(Hayden, 2012)
Type of Publication	Report	Report
Sensor Name	Magneto Nanosensors	Cationic AuNP Sensor
Direct OR Indirect	D	D
Mechanism	Aggregation	Aggregation
Possible Pathogens	E. coli O157:H7	E. coli
Industry	Water and food	
Detection Limit	1 colony-forming unit (CFU)	
Detection Time	30 min	
NP Type	Iron oxide nanoparticles (IONPs), Ab-conjugated IONPs	Gold, Cationic monolayer-protected gold nanoparticles (AuNPs) size 6 or 2 nm
Surface Coating	poly(acrylic acid) (PAA)-coated	
Surface Charge		cationic
NP Concentration		
Toxic (Y/N)	Y	N
Reusable (Y/N/F)	Y	
Lab Scale (Y/N)	Y	Y
Field Scale (Y/N/F)	N	

Table A1: Data Collection from Articles Continued

Source	(Zheng, 2018)	(Zor, 2018)
Type of Publication	Report	Report
Sensor Name	Simple Sensor	AgNP Nanopaper Senor
Direct OR Indirect	D	I
Mechanism	Aggregation	
Possible Pathogens	E. coli	chiral D-cysteine
Industry	Water	Water and food
Detection Limit	dynamic range from 5 × 104cfu mL−1to 1 × 107cfu mL−1with a LODof 0.9 × 104cfu mL−1	4.88 µM
Detection Time	20 min	
NP Type	Gold, MPBA-AgNP	silver nanoparticles
Surface Coating		embedded transparent nano paper
Surface Charge		
NP Concentration	30 nM to 150 uM	
Toxic (Y/N)	N	N
Reusable (Y/N/F)	N	N
Lab Scale (Y/N)	Y	Y
Field Scale (Y/N/F)	F	Y

Table A1: Data Collection from Articles Continued

Source	(Xie, 2019)	(You, 2020)
Type of Publication	Report	Report
Sensor Name	UV-Induced Au Nanoclusters	AuNP Coated Starch Magnetic Beads Sensor
Direct OR Indirect	D	D
Mechanism	UV and aggregation	Aggregation
Possible Pathogens	S. aureus	E. coli
Industry	Water and food	Water
Detection Limit	100	as low as a single cell
Detection Time	10 mins or Less	
NP Type	gold nanoclusters (AuNCs)	gold nanoparticle-coated starch magnetic beads (AuNP@SMBs)
Surface Coating	chitosan composite membrane	starch magnetic beads
Surface Charge		-32.8 to +46.8 mV
NP Concentration		
Toxic (Y/N)	Y	N
Reusable (Y/N/F)	N	Y
Lab Scale (Y/N)	Y	Y
Field Scale (Y/N/F)	Y	N

Table A1: Data Collection from Articles Continued

Source	(Su, 2011)	(Thiramanas & Laocharoensuk, 2015)
Type of Publication	Report	Report
Sensor Name	AuNP Sensor	Competitive Binding Sensor
Direct OR Indirect	D	I
Mechanism	Aggregation	Enzyme reaction
Possible Pathogens	E. coli	S. aureus and E.coli
Industry	Water	Drinking Water
Detection Limit		as low as 10 cfu·mL−1
Detection Time	Few Minutes	Few minutes to a few hours
NP Type	Gold, MEA-AuNPs	Gold, cationic PEI-AuNPs
Surface Coating	Mercaptoethylamine (MEA)	PEI
Surface Charge	positive	highly positive surface charge (+53.6 mV)
NP Concentration		0.1 nM.
Toxic (Y/N)	N	N
Reusable (Y/N/F)	N	N
Lab Scale (Y/N)	Y	Y
Field Scale (Y/N/F)	N	F

Table A1: Data Collection from Articles Continued

Source	(Russo, 2018)	(Shrivastava, 2018)
Type of Publication	Report	Report
Sensor Name	AuAg Nanoshells Sensor	AuNP Biosensor
Direct OR Indirect	D	I
Mechanism	Aggregation	Florecence
Possible Pathogens	E. coli and S. typhimurium	S. aureus
Industry	Water	Water and food
Detection Limit	down to 102 CFU/mL	as low as 10 cfu/ml
Detection Time	Few Minutes	10 mins or Less
NP Type	Gold, AuAg nanoshells consist of a hollow structure composed of a gold–silver	Gold, aptamer-functionalized fluorescent magnetic (FMNPs)
Surface Coating	poly(vinyl pyrrolidone) (PVP) layer adsorbed on their surface during their synthesis	
Surface Charge	The charges were controlled and change	
NP Concentration		
Toxic (Y/N)	Y	N
Reusable (Y/N/F)	N	F
Lab Scale (Y/N)	Y	Y
Field Scale (Y/N/F)	F	Y

Table A1: Data Collection from Articles Continued

Source	(Miranda, 2011)	(Raj, 2015)	(Robby, 2019)
Type of Publication	Report	Report	Report
Sensor Name	Colorimetric Bacteria Sensor	Cysteine Capped AuNP Sensor	Polymer Dot Sensor
Direct OR Indirect	I	D	I
Mechanism	Enzyme reaction	Aggregation	Florecence
Possible Pathogens	E. coli	E. coli	E. coli and S. aureus
Industry	Water	Medical (Urine testing)	Water
Detection Limit	1X102 bacteria/mL in solution and 1	linear relationship in the range of 1 103–4 103 cells/mL with a	range of 101e107 CFU/mL (LOD 5.09 and 4.62 CFU/mL,
Detection Time	Few minutes	Few minutes	
NP Type	cationic gold nanoparticles	Cysteine gold nanoparticles (CAuNPs)	PD-bCD-MMT/Fe3O4eCsWO3
Surface Coating		modified AuNPs with cysteine	
Surface Charge	cationic	– 30.8 mV	
NP Concentration	80 nM		0e10 mM
Toxic (Y/N)	N	N	Y
Reusable (Y/N/F)	N	N	Y
Lab Scale (Y/N)	Y	Y	Y
Field Scale (Y/N/F)	Y	N	F

APPENDIX B: CHAPTER 2

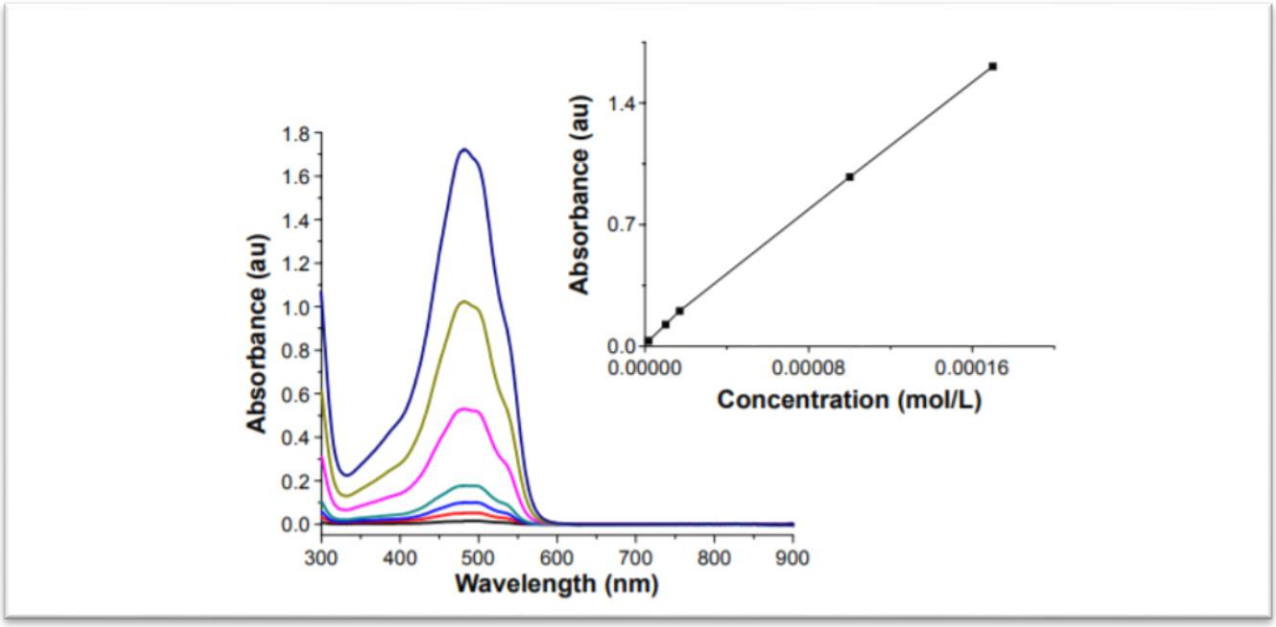

Figure B1: Calibration Curve Used to Determine Concentration of Gold Nanoparticles

Adopted from .

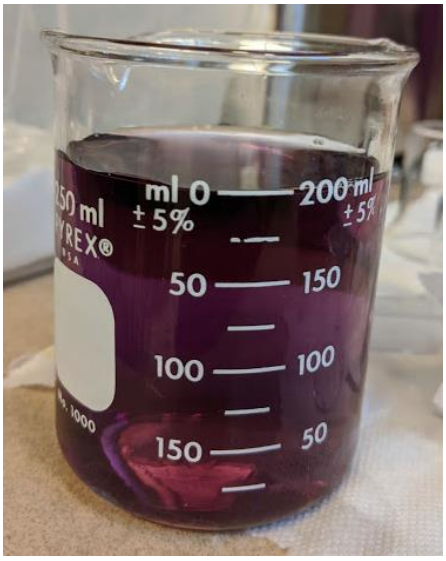

Figure B2: Dilution of Centrifuged PEI-AuNP Made by The UV Light Method.

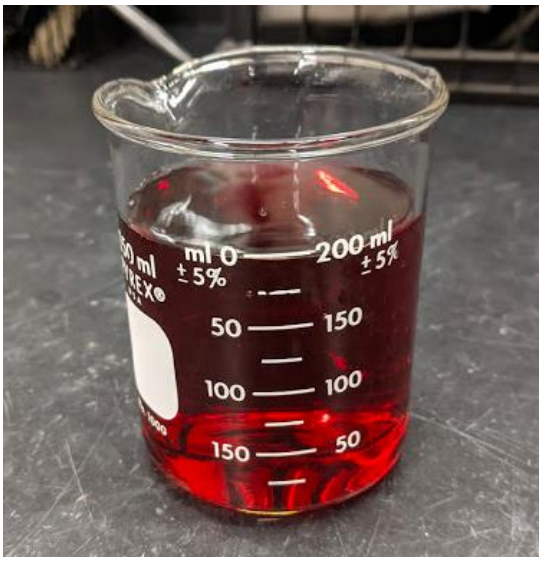

Figure B3: Dilution of Non-Centrifuged PEI-AuNP Made by the UV Light Method

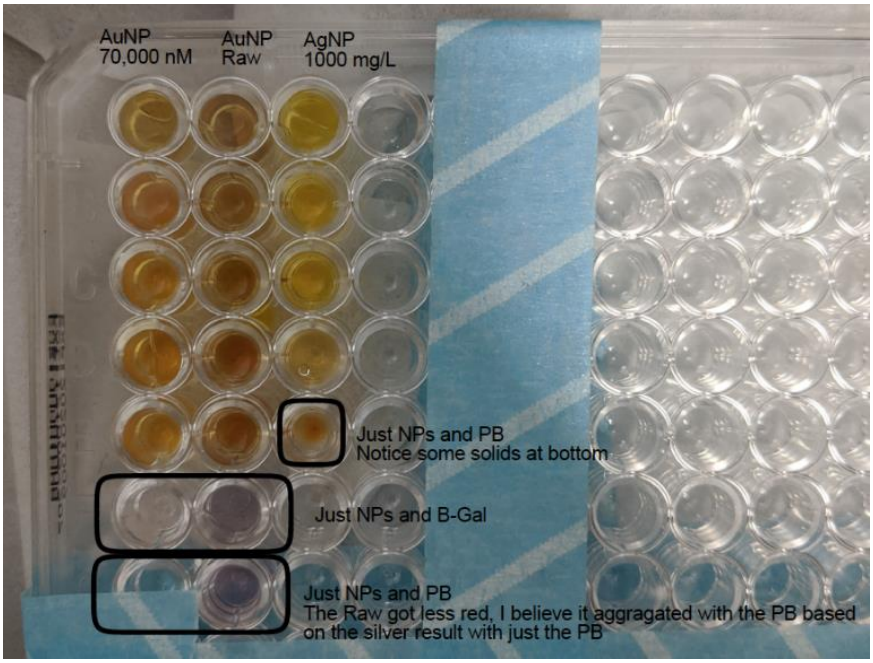

Figure B4: Photo of First Inhibition Test Done With PEI-AgNP. This was Just a Visual Test;

No OD Data was Collected.