DESIGN AND DEVELOPMENT OF SILVER NANOPARTICLE BASED SENSOR FOR DETECTION OF MELAMINE IN MILK

Project report submitted in partial fulfilment of the requirement for the degree of

Bachelor of Technology

IN

ELECTRONICS AND TELECOMMUNICATION ENGINEERING

SUBMITTED BY

Asha Priya Bairagi (210610014009)

Chandrima Paul (210610014012)

Rhitwija Goswami (210610014030)

Sneha Borah (210610014038)

Under the guidance of

DR. RASHI BORGOHAIN

DEPARTMENT OF ELECTRONICS & TELECOMMUNICATION ENGINEERING

ASSAM ENGINEERING COLLEGE

JALUKBARI- 781013, GUWAHATI

DECEMBER, 2024

ASSAM ENGINEERING COLLEGE

CERTIFICATE

This is to certify that the project report entitled “DESIGN AND DEVELOPMENT OF SILVER NANOPARTICLE BASED SENSOR FOR DETECTION OF MELAMINE IN MILK” submitted by Asha Priya Bairagi (210610014009), Chandrima Paul (210610014012), Rhitwija Goswami (210610014030), and Sneha Borah (210610014038), in the partial fulfilment of the requirements for the award of Bachelor of Technology degree in Electronics & Telecommunication at Assam Engineering College, Jalukbari, Guwahati is an authentic work carried out by them under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

Signature of Supervisor

Dr. Rashi Borgohain

Associate Professor

Electronics and Telecommunication Engineering

Assam Engineering College

December,2024

DECLARATION

We declare that this written submission represents our ideas in our own words and where others' ideas or words have been included, we have adequately cited and referenced the original sources. We also declare that we have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in our submission. We understand that any violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

Rhitwija Goswami

210610014030

Date:

Sneha Borah

210610014038

Date:

Asha Priya Bairagi

210610014009

Date:

Chandrima Paul

210610014012

Date:

ACKNOWLEDGEMENT

We would like to thank our Dr. Rashi Borgohain, Associate Professor, and the Department of Electronics and Telecommunication, Assam Engineering College, for giving us the opportunity to do this project titled “DESIGN AND DEVELOPMENT OF SILVER NANOPARTICLE BASED SENSOR FOR DETECTION OF MELAMINE IN MILK.” This project has helped us enhance our knowledge about nanoparticles, nanoparticle-based sensors, and its implementation in detection of hazardous compounds. Also, this project has taught us an important lesion in time management and teamwork among the team members.

Asha Priya Bairagi (210610014009)

Chandrima Paul (210610014012)

Rhitwija Goswami (210610014030)

Sneha Borah (210610014038)

ABSTRACT

Milk is a vital dietary component, rich in essential nutrients such as calcium, proteins, and vitamins, crucial for human health. However, widespread milk adulteration, particularly with melamine, poses significant health risks. Melamine, a nitrogen-rich industrial compound, is often misused to artificially inflate protein content in milk, leading to severe health consequences like kidney stones, renal failure, and, in extreme cases, death. The 2008 Chinese melamine scandal highlighted the urgency of developing reliable detection methods to ensure food safety.

This project focuses on designing and developing a silver nanomaterial-based sensor for rapid, cost-effective, and sensitive detection of melamine in milk. Utilizing a colorimetric approach, the sensor visually indicates contamination levels through distinct colour changes upon reaction with melamine. This method offers simplicity, accessibility, and high efficiency compared to traditional laboratory-based techniques, which are often expensive and time consuming. Validation of results using UV-Visible spectroscopy further ensures accuracy.

The nanomaterial-based sensor employs advanced nanotechnology to detect melamine even at trace levels, demonstrating excellent specificity and sensitivity. Its portability and user-friendly design make it suitable for field applications, benefiting consumers, dairy producers, and food safety regulators alike. Beyond melamine detection, this approach can be adapted to identify other milk adulterants such as urea and hydrogen peroxide, paving the way for comprehensive quality monitoring in the dairy industry. This project not only enhances public health protection but also sets a new benchmark in combating food adulteration globally.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

Melamine, a nitrogen-rich organic compound, has garnered significant attention due to its involvement in food adulteration, particularly in dairy products. It is used to falsely enhance the protein content in milk and milk-based products, leading to potential health hazards. When melamine is combined with cyanuric acid, it can form harmful kidney stones, especially in infants and young children, leading to serious health issues such as kidney failure, ulcers, and digestion problems. The detection of melamine in food products, therefore, has become a crucial area of research in food safety and quality control. Conventional methods for detecting melamine, such as High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS), are often time-consuming, expensive, and require sophisticated instrumentation. These limitations have driven the need for simpler, rapid, and cost-effective detection methods that can be applied easily in both industrial and field settings.

1.2 PROBLEM STATEMENT

The presence of melamine in milk is a significant concern for food safety, requiring efficient and reliable detection methods. Traditional detection techniques, though accurate, are not always practical due to their complexity and high costs, making them unsuitable for widespread, real-time monitoring. Therefore, there is a growing demand for the development of alternative methods that are rapid, cost-effective, and suitable for on-site testing without requiring advanced laboratory setups. This project focuses on developing a simple, sensitive, and cost-effective method for detecting melamine in milk, using silver nanoparticles (AgNPs) and a colorimetric detection approach.

1.3 OBJECTIVE OF THE STUDY

The primary objective of this study is to design and implement a colorimetric sensor for the detection of melamine in milk, utilizing the properties of silver nanoparticles (AgNPs). The specific objectives of the project include:

1. Synthesis and characterization of silver nanoparticles (AgNPs) using a green and cost-effective approach.

2. Investigating the interaction between melamine and AgNPs to develop a colorimetric detection mechanism.

3. Optimizing the sensor for sensitivity and selectivity in detecting melamine in milk samples.

4. Evaluating the feasibility of the developed method for real-time, on-site melamine detection with low detection limits.

1.4 SIGNIFICANCE OF THE STUDY

The development of a silver nanoparticle-based colorimetric detection method for melamine offers several advantages. First, it provides a fast, low-cost, and simple alternative to conventional methods, making it ideal for use in resource-limited settings or as a quick on-site test for milk quality. The colorimetric nature of the detection enables easy visual identification of melamine contamination, eliminating the need for complex instrumentation. Additionally, the use of silver nanoparticles in this method ensures high sensitivity and selectivity, which are crucial for detecting melamine at low concentrations in complex milk samples. This method could serve as an essential tool in food safety, ensuring that milk products meet the necessary safety standards and preventing health risks associated with melamine contamination.

1.5 SCOPE AND LIMITATIONS OF THE STUDY

This study focuses on the development of a colorimetric sensor based on silver nanoparticles for detecting melamine in liquid milk and milk powder. The scope of the research is limited to the synthesis of silver nanoparticles, their characterization, and their application in the detection of melamine. While the method is expected to be highly effective in detecting melamine, it may not be suitable for detecting other types of adulterants or compounds in milk. Additionally, the performance of the sensor may vary with different milk compositions or environmental conditions, which could affect the accuracy and reliability of the detection method.

1.6 STRUCTURE OF THE REPORT

This report is structured as follows:

Chapter 2: Literature Review – Provides a detailed overview of the current research on melamine detection methods, particularly colorimetric and nanoparticle-based approaches.

Chapter 3: Methodology – Describes the materials, experimental procedures, and methods used for synthesizing silver nanoparticles and detecting melamine in milk.

Chapter 4: Results and Discussion – Presents the results of the experiments, including the characterization of the synthesized AgNPs, optimization of the detection method, and performance evaluation of the sensor.

Chapter 5: Conclusion and Future Work – Summarizes the findings, concludes the study, and suggests possible directions for future research.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The detection of melamine in milk has become a critical research area due to its severe implications for human health. Melamine, a nitrogen-rich chemical, is often illegally added to milk and other food products to falsely enhance protein content in quality tests. However, its ingestion can lead to serious health issues, including kidney stones and renal failure, especially when consumed over prolonged periods. This underscores the urgent need for reliable, sensitive, and cost-effective methods for detecting melamine in milk to ensure public health and safety.

Recent advancements in sensor technology have opened new possibilities for detecting chemical adulterants like melamine with high sensitivity and specificity. Among these, nanomaterial-based sensors have gained significant attention due to their unique properties, including high surface area, excellent conductivity, and strong catalytic activity. These attributes make them highly effective for detecting trace amounts of contaminants in complex matrices such as milk.

This chapter reviews the existing body of research that informed the development of our project: designing a nanomaterial-based sensor for melamine detection in milk. The review begins by examining the challenges associated with melamine detection and the shortcomings of conventional analytical techniques. It then explores the potential of nanomaterials as sensing elements and evaluates various sensor technologies employed in similar studies. The insights derived from these studies have played a crucial role in shaping the objectives and methodologies of this project.

By identifying gaps in the current literature and highlighting relevant advancements, this chapter establishes the context and rationale for the present study. It aims to demonstrate how our work contributes to addressing these gaps and advancing the field of food safety monitoring.

2.2 LITERATURE REVIEW

This section presents concise summaries of the key research papers referred to during the development of this project. Each study has been analysed for its objectives, methodologies,

and findings, with an emphasis on their relevance to designing a nanomaterial-based sensor for melamine detection. By highlighting the most significant contributions of these studies, this section provides a detailed understanding of the scientific advancements that informed our approach and helped identify gaps addressed by this project

The paper “Nanosensor platforms for detection of milk adulterants” [1] aims to address the increasing concern of milk adulteration by exploring the potential of nanomaterial-based sensing systems for detecting adulterants in milk products. The primary objective is to assess the benefits, drawbacks, and applicability of optical and electrochemical nano sensors, while also highlighting new developments in nano ensemble platforms for rapid and sensitive detection. The findings reveal that nanomaterials, such as nanoparticles, nanowires, and nanocomposites, significantly enhance sensor sensitivity and selectivity through techniques like fluorescence resonance energy transfer (FRET), surface-enhanced Raman scattering (SERS), and electrochemical methods. Despite the limitations of some sensors, such as poor selectivity or electrochemical inactivity of certain adulterants, nanomaterial-based sensors show promise for real-time and on-field detection. The study emphasizes the need for further optimization of nanostructures, such as nanocrystals and nanoflowers, to create efficient, portable, and scalable diagnostic tools for ensuring milk authenticity and safety.

The paper “Review on Nanomaterial-Based Melamine Detection” [2] focuses on the development and evaluation of nanomaterial-based sensing techniques for detecting melamine in food products, addressing the critical need for precise and real-time solutions to mitigate the risks of melamine contamination. The objective is to explore the potential of various nanomaterials, such as carbon dots, quantum dots, nanoclusters, nanorods, and nanotubes, for melamine detection using mechanisms like fluorescence resonance energy transfer (FRET), aggregation, surface-enhanced Raman scattering (SERS), and self-assembly. The findings highlight the effectiveness of Au and Ag nanoparticles for colorimetric sensing and SERS strategies as promising approaches for melamine determination. However, the study emphasizes gaps in research, such as insufficient innovation in carbon dot-based assays, limited exploration of Pt and bimetallic nanoclusters, and unclear mechanisms in some nanocomposite-based systems. Future research is encouraged to investigate novel nanostructures like nano cubes, nanoflowers, and nano stars to enhance sensing performance. Despite challenges in optimization and instrumentation, nanomaterial-based sensors offer significant potential to revolutionize food safety and health monitoring systems.

The paper “Visual and Optical Absorbance Detection of Melamine in Milk by Melamine-Induced Aggregation of Gold Nanoparticles” [3] aims to develop a simple, rapid, and cost-effective method for detecting melamine in milk powder products, focusing on the aggregation of bare 5 nm gold nanoparticles (AuNPs) as a reactant-free probe. The detection mechanism relies on the strong electrostatic interaction between melamine's positively charged exocyclic amine groups and the negatively charged ions on AuNPs, resulting in a visible colour change and measurable UV–Vis absorbance. The method demonstrates high sensitivity, with detection limits of 1×10^{-9} M for visual observation and 1×10^{-11} M for UV–Vis analysis, both well below the safety threshold for melamine ingestion in infant formula. Additionally, it achieves excellent recoveries (~95%) with low relative standard deviations (RSD), making it suitable for real-time melamine screening without advanced instruments. The findings suggest that this AuNP-based approach is a promising candidate for developing portable test kits for melamine detection in milk and dairy products.

The paper “A carbon-dot based biosensor for melamine detection by fluorescence resonance energy transfer” [4] aims to develop a novel fluorescence resonance energy transfer (FRET) system using amino-functionalized carbon dots (C-dots) as energy donors and gold nanoparticles (AuNPs) as energy acceptors for the sensitive detection of melamine. The system's fluorescence intensity of C-dots is influenced by the presence of melamine, disrupting the FRET process and enabling quantitative detection. Key parameters, including incubation time, AuNP concentration, and pH, were optimized to enhance detection efficiency. The method demonstrates high sensitivity with a detection limit of 36nM and a linear range of 50–500nM, providing satisfactory results in real milk sample analysis. Compared to previous methods, this system offers significant advantages, including high sensitivity, simplicity, cost-effectiveness, and short analysis time, paving the way for designing advanced optical sensors for melamine detection.

The paper “One-Pot Interference-Based Colorimetric Detection of Melamine in Raw Milk via Green Tea-Modified Silver Nanostructures” [5] aims to develop a simple, cost-effective, and one-pot colorimetric sensing technique for the detection of melamine in milk, using green tea extract as both a reducing agent for silver nanoparticle (AgNP) synthesis and a sensing element. The interaction between the $-NH_2$ groups in melamine and the polyphenols in green tea extract via hydrogen bonding inhibits nanoparticle formation, resulting in a distinct colour change. This innovative approach achieves a low detection limit of 1.44 ppm, within permissible safety levels, with a recovery rate of 93% and a dynamic sensing range of 0.1 to 15 ppm. The method

is validated using UV–Vis spectroscopy and high-performance liquid chromatography (HPLC), demonstrating its simplicity, efficiency, and potential for real-time melamine detection in milk.

The study “Design and development of electrochemical biosensor for the simultaneous detection of melamine and urea in adulterated milk samples” [6] aims to develop a highly sensitive acetylcholinesterase (AChE)-based electrochemical biosensor using a zinc oxide nanosphere-modified platinum electrode (Pt/ZnO/AChE/Chitosan bioelectrode) for the simultaneous detection of melamine and urea in cow milk. The optimized bioelectrode demonstrated excellent permeability and selectivity for melamine and urea mixtures, with a detection range of 1–20nM and ultra-low detection limits of 3pM for melamine and 1pM for urea. The system achieved high accuracy with recovery rates between 99.96% and 102.22%, unaffected by potential interferents in milk. Additionally, linear regression models were developed to enhance predictive accuracy using electrochemical parameters. This innovative sensor presents a promising analytical tool for monitoring melamine and urea contamination in milk and dairy products.

The study “Colorimetric detection of melamine in milk by using gold nanoparticles-based LSPR via optical fibres” [7] aims to develop a low-cost, simple biosensing system using optical fibres and localized surface plasmon resonance (LSPR) of unmodified gold nanoparticles (AuNPs) for colorimetric detection of melamine in liquid milk samples. The system uses a broadband light source, optical fibres, and a miniature spectrometer to measure the colour change of AuNPs in response to melamine, which shifts from wine-red to blue due to inter-particle coupling effects. The system showed a linear detection range from 0.0μM to 0.9μM with a high correlation ($R^2 = 0.99$) and a detection limit of 33nM. It demonstrated good recovery rates (99.2%–111%) and high sensitivity, making it a promising tool for melamine detection in milk, with potential applications for other molecules by modifying the ligands.

The study “Identification and Determination of Melamine in Milk by High Performance Liquid Chromatography – UV Detector” [8] aims to develop a method for detecting melamine and other nitrogen-rich compounds (such as ammeline, ammelide, and cyanuric acid) in milk products, which are often adulterated to falsely increase protein content. The method utilizes High Performance Liquid Chromatography (HPLC) with UV detection to analyse milk samples after suitable pretreatment. The findings demonstrate that melamine can be detected at very low concentrations (0.01 μg/mL) using reverse-phase HPLC with ion-pairing chromatography

and UV detection. The method is simple, sensitive, and robust, providing reliable results in less than 25 minutes with low costs, making it an effective tool for routine melamine monitoring in food safety testing.

The objective of the study "Visible and fluorescent detection of melamine in raw milk with one-step synthesized silver nanoparticles using carbon dots as the reductant and stabilizer" [9] was to develop a simple, sensitive, and selective method for detecting melamine in raw milk using a one-step synthesis of silver nanoparticles (AgNPs) with carbon dots (C-dots) as both the reductant and stabilizer. The findings show that the presence of melamine alters the formation and aggregation of AgNPs, causing colour and absorbance changes, as well as fluorescence enhancement, which can be used for detection. The method provides a low detection limit of 30nM (3.78 ppb) using UV-vis spectrophotometry, and 600nM (75.6 ppb) by visual detection, both of which are below the safety limits set by various authorities. The method is highly sensitive, selective, and offers the potential for real-time, on-site detection of melamine in milk, particularly in low-resource settings.

The objective of the study "One-step, room temperature, colorimetric melamine sensing using an in-situ formation of silver nanoparticles through modified Tollen's process" [10] was to develop a rapid, sensitive, and selective colorimetric detection method for melamine (MEL) in milk powder using an in-situ formation of silver nanoparticles (AgNPs) through a modified Tollens process at room temperature. The findings indicate that MEL interacts with Ag+ ions, forming a covalent Ag–N bond, which leads to the aggregation of AgNPs and a detectable yellow-to-brown colour change. The method is highly sensitive, with a limit of detection (LOD) of 10nM, and can detect MEL within 30 minutes. It also discriminates MEL from other milk-related compounds and does not require complex instrumentation or organic solvents, making it a simple and efficient detection tool for illegal additives in dairy products. Future work will explore the use of gold nanoparticles (AuNPs) for more noticeable colour changes.

2.3 CONCLUSION

In this study, we chose silver nanoparticles (AgNPs) coupled with a colorimetric detection method to identify melamine in milk products. Silver nanoparticles are highly effective in surface plasmon resonance (SPR), which enables them to display strong optical properties that are sensitive to environmental changes, such as aggregation. These nanoparticles are known to interact with various compounds through covalent bonding, particularly with nitrogen-rich substances like melamine. When melamine is present in the milk, it forms a bond with the

silver ions, causing the AgNPs to aggregate, which leads to noticeable changes in colour. This colour change, from yellow to brown, is easy to detect visually, making it a suitable approach for rapid, on-site testing. The simplicity of this method allows it to be performed without the need for sophisticated instruments or lengthy procedures, which is essential for real-time monitoring in resource-limited areas.

The colorimetric detection method was selected due to its efficiency in detecting melamine at very low concentrations. Melamine, being a small, nitrogen-rich molecule, interacts specifically with silver nanoparticles, influencing their aggregation and resulting in observable changes in colour. This provides a straightforward mechanism for detecting melamine without relying on complex analytical equipment. The process is rapid, requiring less than 30 minutes, and the detection sensitivity is high, with a limit of detection (LOD) as low as 10nM. Such attributes are vital for detecting melamine in milk products, where it is often added to falsely increase protein content, posing serious health risks. By utilizing UV-Vis spectrophotometry or even visual inspection, this method makes melamine detection both accessible and cost-effective.

Moreover, the colorimetric method using silver nanoparticles overcomes many limitations of conventional techniques. Unlike traditional methods that may require organic solvents, complex sample preparations, or enzymatic reactions, this approach is simple, environmentally friendly, and cost-effective. It also provides high selectivity, as the method discriminates melamine from other common milk-related compounds. The system can detect melamine in complex matrices like milk powder with minimal interference, providing a reliable and efficient tool for routine monitoring. Given these advantages, the use of silver nanoparticles combined with a colorimetric detection method offers a promising solution for melamine detection, with great potential for use in food safety and quality control across various industries.

CHAPTER 3

METHODOLOGY

3.1 CHEMICALS AND INSTRUMENTS USED

The following chemicals and materials were used throughout the experiments:

1. Silver Nitrate ($AgNO_3$) – A chemical compound used as a precursor for the synthesis of silver nanoparticles (AgNPs).
2. Tri-sodium Citrate Dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) – A reducing agent and stabilizer used in the synthesis of silver nanoparticles.
3. Milk Powder – Used as a sample for the detection of melamine.
4. Trichloroacetic Acid (TCA) – Used in the pretreatment of milk to eliminate fat and protein that may interfere with the detection mechanism.
5. Melamine – The target analyte, used to spike milk samples for detection.
6. Whatman Filter Paper – Used for filtering the milk supernatant.
7. Distilled Water (DI Water) – Used for dissolving chemicals and preparing solutions.

The following Instruments and Equipment were used during the experiments:

1. Magnetic Stirrer – Used for continuous stirring of the solutions to ensure uniformity during the preparation of silver nanoparticles and other reagents.
2. Micro-pipette – Used for precise measurement and transfer of small volumes of liquids.
3. Weighing Scale – Used for measuring the required amounts of chemicals.
4. Sonicator – Used to apply ultrasonic waves to the milk samples, ensuring proper mixing and breaking down of fat and protein particles during pretreatment.
5. Centrifuge Machine – Used for separating the solid particles from the liquid milk supernatant during the milk pretreatment process.
6. UV-Vis Spectrophotometer – Used to measure the absorption spectra of the solutions, which helps in analysing the colorimetric changes in the presence of melamine and milk with silver nanoparticle.

3.2 PREPARATION OF SILVER NANOPARTICLES

The silver nanoparticles were synthesized using a modified Tollens process. This process involved the reduction of silver ions (Ag^+) in solution to silver nanoparticles (AgNPs) by tri-sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), which also serves as a stabilizing agent.

1. **Preparation of 5mM Silver Nitrate ($AgNO_3$):** To prepare a 5mM solution of $AgNO_3$, the following calculation was performed: Amount of $AgNO_3$ = 5 x 10^{-3} x 10 x 10^{-3} x 169.87 = 0.0084gm. 0.0084 g of $AgNO_3$ was dissolved in 10 mL of distilled water to make the required concentration.
2. **Preparation of 5mM Tri-sodium Citrate ($Na_3C_6H_5O_7$):** To prepare a 5mM solution of tri-sodium citrate, the following calculation was performed: Amount of $Na_3C_6H_5O_7$ = 5 x 10^{-3} x 10 x 10^{-3} x 294.1 = 0.0147gm. 0.0147 g of $Na_3C_6H_5O_7$ was dissolved in 10 mL of distilled water.
3. **Synthesis of Silver Nanoparticles:** In a beaker, 90 mL of distilled water was heated to its boiling point. Once the water reached the boiling point, 10 mL of both the $AgNO_3$ solution and the $Na_3C_6H_5O_7$ solution were added to the boiling water. The solution was then kept under continuous stirring to ensure the formation of silver nanoparticles. The colour change from colourless to brownish-yellow was observed, indicating the reduction of silver ions and the formation of AgNPs. After the colour change was significant, the solution was allowed to cool to room temperature, resulting in a colloidal silver nanoparticle solution of yellow colour.

3.3 PRE-TREATMENT OF RAW MILK

Milk contains fat, protein, and other compounds that can interfere with the detection of melamine. To eliminate these potential interferences, the milk was pre-treated using a series of steps:

1. **Preparation of Pre-treatment Solution:** 1.8 g of milk powder was mixed with 12 mL of 61mM trichloroacetic acid (TCA) and 4 mL of acetonitrile. This combination helps to break down proteins and fat in the milk and prepare it for detection.

2. **Sonication and Shaking:** The milk solution was subjected to 15 minutes of sonication, followed by 15 minutes of shaking. Sonication helps to break down the larger fat and protein molecules, ensuring a uniform mixture.

3. **Centrifugation:** The solution was then centrifuged at 5000 rpm for 15 minutes. This process separated the solid particles from the liquid phase.

4. **Filtration:** The resulting supernatant was filtered twice using Whatman filter paper to remove any remaining solid impurities, leaving the liquid milk sample ready for melamine detection.

3.4 PREPARATION OF STOCK SOLUTION

The pre-treated milk was spiked with different concentrations of melamine (MEL) to create samples with varying melamine levels. The concentration of melamine was determined using the standard addition method.

1. **Preparation of 100 ppm Stock Solution:**

   To prepare the 100ppm melamine stock solution, we used the molecular weight of melamine, which is 126.12 g/mol. Since 1 ppm is equivalent to 1 mg/L, for 100 ppm, we need 100 mg of melamine in 1 litre of solution.

   To make 100 ppm, we weighed 0.0012 g (1.2 mg) of melamine and dissolved it in 100 mL of distilled water. This results in a 100ppm solution, as:

   Concentration (ppm) = Mass of solute (mg)/Volume of solution (mL) ×100

   Concentration = 1.2 mg/100 mL] × 1000 = 100 ppm

   Thus, we achieved a 100ppm melamine solution by dissolving 0.0012 g of melamine in 100 mL of distilled water.

2. **Preparation of 2.5 ppm from 100 ppm:**

   To prepare 2.5 ppm, we used the dilution formula C1V1 = C2V2

   C = concentration V = Volume

   100ppm × 1mL =2.5ppm×V2

   V2= (100 ppm × 1mL)/2.5 ppm = 40 mL

   Thus 1 mL of 100 ppm solution is mixed with 40 mL of water to achieve 2.5 ppm.

3. **Preparation of 1 ppm from 100 ppm:**

   To prepare 1 ppm, again we used the dilution formula C1V1 = C2V2

   100ppm × 1mL =1 ppm×V2

   V2= (100 ppm × 1mL)/1 ppm = 100 mL

   Thus 1 mL of 100 ppm solution is mixed with 100 mL of water to achieve 1 ppm.

4. **Preparation of 0.5 ppm from 100 ppm**

   To prepare 1 ppm, again we used the dilution formula C1V1 = C2V2

100ppm × 1mL =0.5 ppm×V2

V2= (100 ppm × 1mL)/0.5 ppm = 200 mL

Thus 1 mL of 100 ppm solution is mixed with 200 mL of water to achieve 0.5 ppm.

3.5 PREPARATION OF STANDARD SOLUTION OF MILK AND MELAMINE

The spiked milk samples were prepared by adding specific amounts of melamine to the pre-treated milk solution. The following concentrations of melamine were used to create different test samples:

1. First Beaker: 350 µL of pre-treated milk + 50 µL of 100 ppm melamine solution.
2. Second Beaker: 300 µL of pre-treated milk + 100 µL of 100 ppm melamine solution.
3. Third Beaker: 250 µL of pre-treated milk + 150 µL of 100 ppm melamine solution.

Similar preparations were carried out for 2.5 ppm, 1ppm and 0.5 ppm melamine concentrations to test the range of the detection method.

3.6 COLORIMETRIC SENSING OF MELAMINE

The colorimetric sensing of melamine was achieved by observing the changes in the colour of the silver nanoparticle solution upon the addition of melamine to the milk samples:

1. **Testing Procedure:** 10 mL of the prepared silver nanoparticle solution was added to each beaker containing the spiked milk samples. Significant colour changes, such as the formation of aggregates and a shift in the colour from yellow to brown, were observed in response to the presence and concentration of melamine.
2. **UV-Vis Spectrophotometric Analysis:** To confirm the colorimetric changes, the absorption spectra of the solutions were recorded using a UV-Vis spectrophotometer. The UV-Vis spectra allowed for the detection of changes in the absorption peaks, indicating the formation and aggregation of silver nanoparticles, which correlates with the concentration of melamine in the milk samples.

CHAPTER 4

RESULT ANALYSIS

4.1 SILVER NANOPARTICLE

The size of silver nanoparticles (AgNPs) is a critical factor influencing their physical, chemical, and optical properties, with their typical dimensions ranging from 1 nanometre to 100 nanometres. This size can be precisely controlled during synthesis by adjusting parameters such as precursor concentration, reducing agents, stabilizers, temperature, and reaction time. The size of silver nanoparticles directly affects their localized surface plasmon resonance (LSPR), which determines their optical behaviour and colour. Smaller nanoparticles, such as those in the range of 10–20 nm, exhibit sharper and more defined plasmon resonance peaks in the visible spectrum, whereas larger particles show broader peaks and wavelength shifts. This optical tunability makes silver nanoparticles highly versatile in applications like sensing and imaging.

Chemically, the smaller silver nanoparticles are more reactive due to their high surface area-to-volume ratio. This makes them highly effective in applications requiring higher sensitivity, such as detecting melamine or other adulterants. Conversely, the larger nanoparticles, though less reactive, often offer better stability under certain conditions. However, the larger particles are also more prone to aggregation due to van der Waals forces, while the smaller nanoparticles, if properly stabilized with ligands or surfactants, can maintain better dispersion and stability than the larger nanoparticles.

The size of AgNPs can be tailored during synthesis. For instance, higher concentrations of reducing agents and higher temperatures typically produce smaller nanoparticles, while longer reaction times can lead to the growth of larger ones or result in particle aggregation. Capping agents or stabilizers play a crucial role in controlling nanoparticle growth and preventing excessive aggregation.

Ultimately, the choice of nanoparticle size depends on the intended application of the nanoparticle. Smaller particles are preferred for biosensing and catalysis due to their enhanced reactivity, while larger particles are often chosen for optical, photothermal, or plasmonic applications. Controlling the AgNP size is essential for optimizing their performance in diverse scientific and industrial uses.

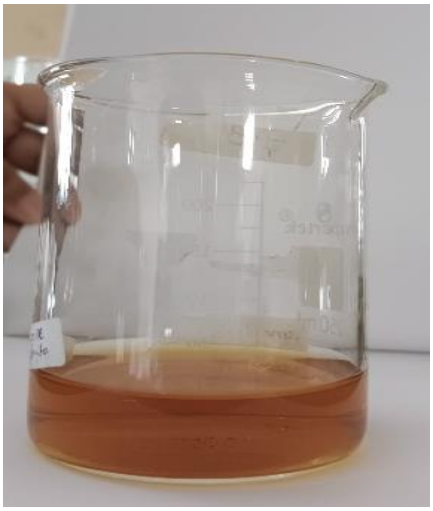

Fig 4.1: Bigger Silver Nanoparticle

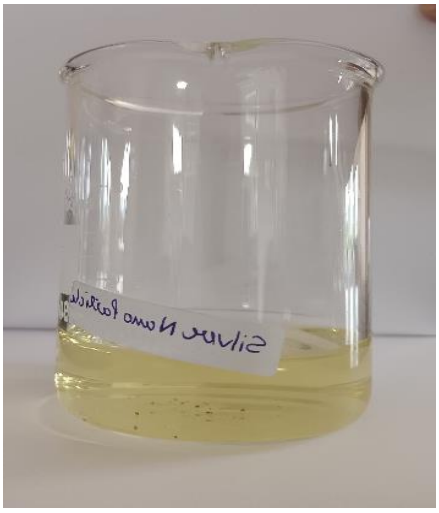

Fig 4.2: Smaller Silver Nanoparticle

4.2 PRE-TREATED MILK

Before testing for melamine, milk undergoes pre-treatment processes such as sonication and centrifugation to ensure accurate and reliable results. These treatments significantly alter the properties of milk, preparing it for precise analysis. Centrifugation is employed to separate milk into its components based on density, effectively removing the fat-rich cream layer and yielding skimmed milk. The removal of fat reduces the viscosity of the milk, making it thinner and less creamy, while also altering its optical properties by decreasing light scattering. This results in milk that appears less opaque and more translucent, ensuring minimal interference from fat during analytical testing.

Sonication, which uses high-frequency sound waves, is another crucial step in this pre-treatment process. It breaks down fat globules into smaller particles and disperses them uniformly throughout the milk. When used in conjunction with centrifugation, sonication enhances the efficiency of fat removal and homogenization. Additionally, it partially denatures proteins, improving their solubility and distribution within the milk. This creates a uniform mixture, ensuring that the milk sample is representative and consistent for testing.

Furthermore, sonication disrupts microbial cell walls, reducing the microbial load in the milk. This not only improves the milk's safety but also prevents microbial interference during melamine detection. By removing fats and reducing microbial content, the pre-treatment ensures that the milk's components, such as proteins and sugars, are uniformly distributed, minimizing variability during analysis.

These pre-treatment processes play a vital role in preparing milk for melamine detection by improving its homogeneity, reducing interference from fats, and ensuring that the sample is stable and consistent. As a result, the accuracy and reliability of the subsequent melamine testing are significantly enhanced, making these steps critical in the analytical procedure.

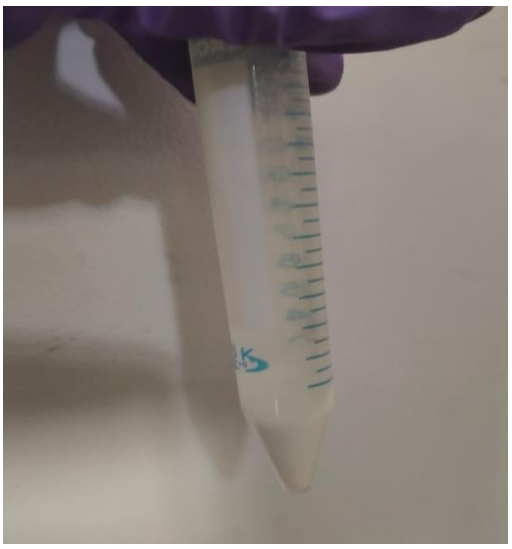

Fig 4.3: Pretreated Milk

4.3 MELAMINE STOCK SOLUTION

To evaluate the maximum concentration of melamine that can be detected in milk, a stock solution of melamine at 100 ppm was prepared. From this stock solution, working solutions of 2.5 ppm, 1 ppm, and 0.5 ppm were subsequently prepared through serial dilution. These concentrations were selected to cover a range of low-level melamine contamination, allowing for the determination of the sensitivity of the detection method.

Each of the prepared melamine solutions was mixed with pre-treated milk samples. The milk had undergone sonication and centrifugation to remove fat and homogenize its components, ensuring consistent sample quality and minimizing interference during testing. The diluted melamine solutions were added to the milk in controlled quantities, and the resulting mixtures were subjected to the analytical detection procedure.

This approach enabled the identification of the lowest concentration of melamine that could be reliably detected in the milk samples. By testing multiple concentrations, the method's sensitivity and effectiveness were assessed, providing valuable data on the detection limits of melamine in milk. These findings are critical for ensuring food safety and regulatory

compliance, as well as for optimizing the detection technique for potential applications in real-world scenarios.

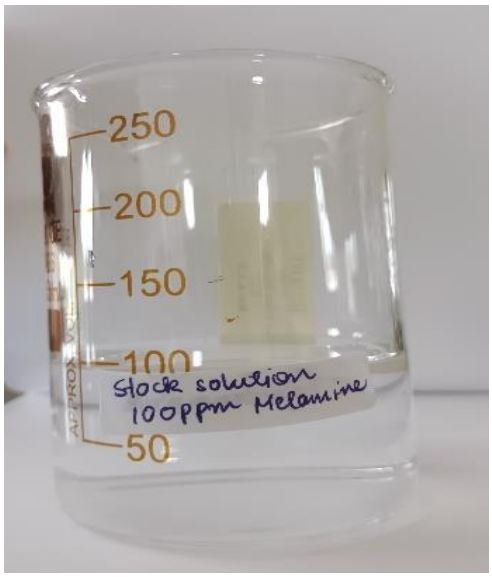

Fig 4.4: Stock Solution of 100ppm

4.4 STANDARD SOLUTION OF MILK AND MELAMINE IN PRESENCE OF SILVER NANOPARTICLE

After preparing standard solutions of milk and melamine with varying concentrations of melamine and incorporating silver nanoparticles, distinct results were observed, highlighting the effectiveness of the detection method. Silver nanoparticles were introduced as a sensing agent due to their sensitivity and colorimetric properties, which change in response to the interaction with melamine.

Fig 4.5: Standard solution of milk and melamine in presence of silver nanoparticle

In the experiment, milk samples were spiked with melamine solutions of varying concentrations, ranging from high to low. Upon mixing the silver nanoparticles with these samples, a noticeable variation in colour was observed, corresponding to the concentration of melamine present in the mixture. The beaker containing the highest concentration of melamine exhibited a dark brown colour, which indicates a strong interaction between the melamine molecules and the silver nanoparticles. This interaction likely caused significant aggregation of the nanoparticles, altering their optical properties, and resulting in a pronounced colour change.

As the melamine concentration decreased in subsequent samples, the colour of the mixtures progressively lightened, showing shades of brown that became increasingly less intense. This gradient in colour provides a clear visual indication of the melamine concentration in the samples. The change in colour occurs due to the concentration-dependent aggregation of silver nanoparticles induced by melamine. Higher melamine levels promote more extensive aggregation, while lower concentrations result in less pronounced changes.

While the samples with 100ppm, 2.5ppm and 1ppm melamine showed significant and detectable colour change to dark brown, the samples with 0.5ppm melamine showed no visible and detectable colour change. This gives us the detection limit of 1ppm melamine for our experiment.

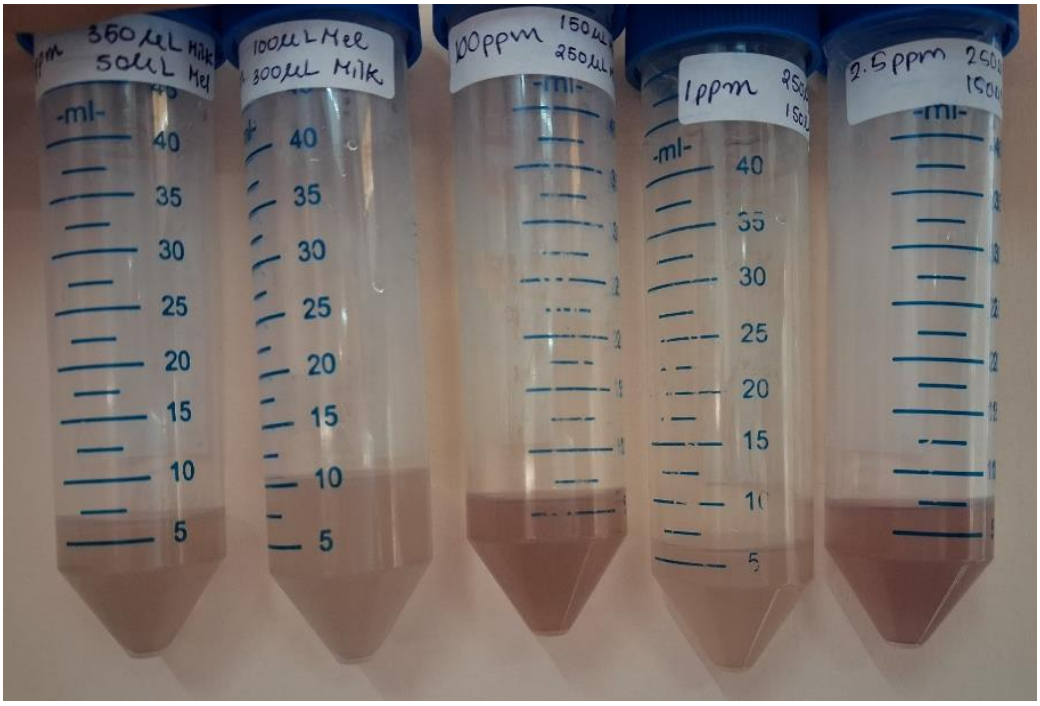

Fig 4.6: Samples after colour change

This experiment demonstrates the potential of silver nanoparticles as a reliable, colorimetric sensor for detecting melamine in milk. The observed colour variations provide a straightforward and effective method for both qualitative and semi-quantitative detection of melamine.

4.5 UV-VIS SPECTROPHOTOMETRIC ANALYSIS

To evaluate the colorimetric response of the silver nanoparticles (AgNPs) to varying concentrations of melamine in pre-treated milk samples, UV-Vis spectrometric analysis was performed. The absorbance spectra for the prepared samples were recorded to observe the optical changes corresponding to melamine concentrations of 100 ppm, 2.5 ppm, 1 ppm, and 0.5 ppm.

When the sample containing 250 µL of pre-treated milk and 150 µL of melamine solution was subjected to UV-Vis spectroscopic analysis, distinct absorbance peaks were observed for the melamine concentrations of 100 ppm, 2.5 ppm, and 1 ppm. The absorbance peak was found to be highest for 100 ppm, indicating a strong interaction between the silver nanoparticles and melamine, resulting in significant aggregation and a corresponding change in optical properties.

As the concentration of melamine decreased, the intensity of the absorbance peaks also diminished. At 2.5 ppm, a moderate peak was observed, while at 1 ppm, the peak was further reduced but still detectable. At 0.5 ppm, no significant absorbance peak was detected, suggesting that the concentration of melamine was below the detection threshold of the method using the current setup.

The results demonstrate a clear trend in the UV-Vis absorption spectra. The absorbance intensity correlates with the concentration of melamine present in the samples, confirming that the silver nanoparticles aggregate more strongly in the presence of higher concentrations of melamine. The absence of a detectable peak at 0.5 ppm indicates the limit of detection (LOD) for this method, beyond which the sensitivity is insufficient to produce measurable optical changes.

The UV-Vis spectroscopic results highlight the reliability of the developed silver nanoparticle-based detection method for melamine concentrations as low as 1 ppm. This concentration is below the safety limits set by the USA and EU (2.5 ppm) and comparable to that of China (1 ppm), making the method suitable for practical applications in food safety monitoring.

The spectral data could be represented graphically with absorbance on the y-axis and wavelength on the x-axis for all tested concentrations (100 ppm, 2.5 ppm, 1 ppm, and 0.5 ppm). This will help visualize the trend of decreasing peak intensity with lower melamine concentrations.

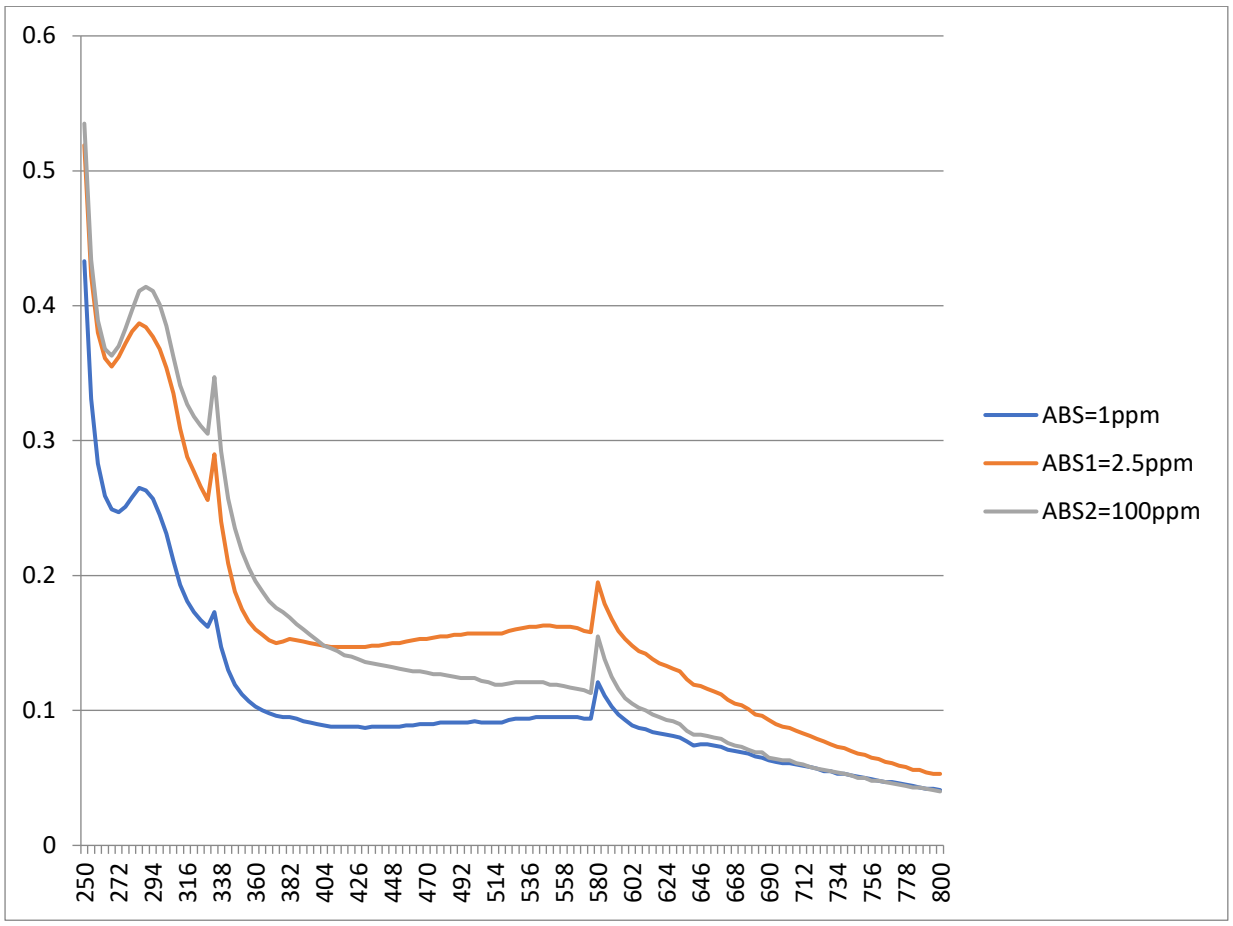

Fig 4.7: UV-Vis Spectroscopy Graph

The UV-Vis spectrometric analysis confirms that the silver nanoparticle-based method is effective for detecting melamine concentrations down to 1 ppm with observable and quantifiable peaks. This validates the method's potential for real-time monitoring of melamine in milk samples within regulatory limits.

CHAPTER 5

CONCLUSION

5.1 CONCLUSION

This project successfully developed a silver nanoparticle-based colorimetric detection method for melamine in milk samples. The method relies on the in-situ formation of silver nanoparticles through a modified Tollens process, which reacts with the nitrogen-rich triazine groups in melamine. The interaction results in significant changes in the optical properties of the nanoparticles, allowing for the visual and spectroscopic detection of melamine. The method is rapid, simple, and cost-effective, making it a promising alternative to conventional techniques.

The detection limit of the developed method was found to be 1 ppm using UV-Vis spectroscopy, with distinct absorbance peaks observed at higher melamine concentrations of 100 ppm and 2.5 ppm. Below 1 ppm, specifically at 0.5 ppm, no detectable peak was observed, indicating the sensitivity threshold of the method. The detection limit of 1 ppm aligns with the safety standards set by China and is well below the limits set by the USA and EU (2.5 ppm). This confirms the method's practical applicability for food safety monitoring and compliance with regulatory requirements.

The method's simplicity and reliability are evident in its ability to process samples in under 30 minutes, without requiring complex instrumentation or additional reagents. Pre-treatment of milk samples was optimized to remove fat and protein interferences, ensuring accurate detection. This highlights the robustness of the approach in dealing with real-world samples. Additionally, the distinct color change from yellow to brown provides a qualitative, visual indication of melamine presence, further enhancing its usability in low-resource settings or rapid on-site analysis.

Overall, this project demonstrates the effectiveness of using silver nanoparticles for the colorimetric detection of melamine in milk. While the method meets the current regulatory standards, future work could focus on further enhancing sensitivity to detect melamine at lower concentrations, such as 0.5 ppm or below. This could involve modifications to the synthesis process or leveraging alternative nanoparticles, such as gold, for improved visual contrast. The findings of this study provide a strong foundation for developing scalable and accessible tools for monitoring food safety.

5.2 FUTURE SCOPE OF WORK

This project establishes a foundation for detecting not only melamine but also other common adulterants in milk, such as urea and hydrogen peroxide. These substances, like melamine, are added to milk to alter its composition, often for economic gain, while posing significant health risks to consumers. Urea, for instance, is used to increase the apparent protein content of milk, while hydrogen peroxide can be used to extend its shelf life but harms the nutritional quality and safety. Therefore, it is essential to detect these adulterants alongside melamine for comprehensive milk quality monitoring.

Building upon the detection methods developed in this project, we envision creating a versatile, integrated device capable of simultaneously detecting multiple adulterants in milk with high sensitivity and accuracy. This device would combine the principles of colorimetric detection and nanoparticle-based sensing, which has already proven effective for melamine. The future iteration could incorporate additional reagents or sensors tailored to detect other adulterants like urea and hydrogen peroxide, thereby broadening the scope of adulterant detection in milk.

Such a device would offer multiple advantages, including portability, cost-effectiveness, and ease of use, making it accessible to consumers, dairy producers, and regulatory bodies alike. With real-time detection capabilities, this device could empower individuals and organizations to monitor milk quality on-site, reducing reliance on centralized laboratory testing, which is often time-consuming and expensive.

In the future, the integration of more advanced technologies such as wireless connectivity could allow for data collection and reporting, offering a more robust and dynamic approach to food safety. Furthermore, this technology could be expanded to detect adulterants in other food products, promoting broader applications for public health and safety. Ultimately, the goal is to provide a comprehensive solution that enhances food safety standards globally, reduces the risk of foodborne illnesses, and ensures consumers can trust the quality and safety of the products they consume.

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