# Plasmonic Nanostructures for Pollutant Sensing

A Dissertation for
Course code and Course Title: PHY-651 Dissertation
Credits: 16
Submitted in partial fulfillment of Masters degree in Physics
By

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# DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, **”Plasmonic Nanostructures for Pollutant Sensing”** is based on the results of investigations carried out by me in the Masters of Physics at the School of Physical and Applied Science, Goa University, under the Supervision of UGC Assistant Prof. Dr Sudhir Cherukulappurath. I have not submitted the same elsewhere for a degree or diploma award. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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# COMPLETION CERTIFICATE

This is to certify that the dissertation report **"Plasmonic Nanostructures for Pollutant Sensing"** is a bonafide work carried out by Miss Alisha Joancia Dourado under my supervision in partial fulfilment of the requirements for the award of the degree of M.Sc in Physics at the School of Physical and Applied Sciences, Goa University.

Signature and Name of Supervisor: Dr.Sudhir Cherukulappurath

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# ACKNOWLEDGEMENT

I would like to express my profound gratitude to my project guide Dr.Sudhir Cherkulappurath, for his guidance and constant support to the completion of my project titled Plasmonic Nanostructures For Pollutant Sensing.

I would like to express my thanks to Mr.Shubhankar Bandekar for his time and efforts he has provided throughout. A special thanks to Ms Namita and Mr.Praveen for encouraging me and contributing their ideas and perspective which greatly enriched the project.

I would like to acknowledge the support received from the faculty for providing necessary resources and encouragement. Lastly, a heartfill gratitude to my parents and pals for constant motivation and support towards the completion of my project.

# ABSTRACT

Heavy-metal contamination is one of the most challenging problems of the twenty-first century. In particular, the mercury(II) ion is frequently considered to be among the most dangerous pollutants due to its detrimental effects on the environment used for production as well as human health. Therefore, developing a sensitive and focused mercury ion detection method is essential. In this project different nanostructures of Gold nanoparticle were synthesized using the chemical reduction approach for Gold nanopheres, Gold nanostar and seed mediated growth approach for Gold nanorods.These nanostructures were examined using field emission scanning electron microscopy (FE-SEM) and UV-Vis absorption techniques.Detection methods such as colorimetric, fluorometric and SERS were used for sensing of mercury ions using plasmonic nanostructures and Rhodamine-B (Rh-B) in aqueous environment. In general, the project advances knowledge of different geometries of Gold nanostructures and their possible uses in sensing applications, here specifically $Hg^{2+}$. Gold particles could sufficiently quench the fluroscence of Rh-B dye and the mixture of this fluorescent dye/nanoparticle mixture showed a possibility to detect mercury.

# TABLE OF CONTENTS

## Contents

# List of Figures

# 1 INTRODUCTION

$Hg^{2+}$ has been identified as one of the most dangerous pollutants and highly toxic metallic elements. It has the potential to enter the food chain and accumulate in human bodies, potentially leading to serious health problems such as renal failure, brain damage, and movement disorders. Due to its serious toxicity to human nervous and environmental systems, useful methods for detecting $Hg^{2+}$ are urgently needed. Traditional detection techniques for $Hg^{2+}$ include inductively coupled plasma (IDP) mass spectrometry, electrochemical method, and AAS/AES mass spectrometry. However , most of these methods require time-consuming sample preparation and complicated instrumentation, hence limiting their practical applications[1].

## 1.1 Mercury: Its toxicity and health effects

In terms of the most dangerous elements or compounds on Earth, mercury is ranked third by the US Government Agency for dangerous compounds and Disease Registry, after arsenic and lead, which are still being poured into our land and streams, poured into our atmosphere, and eaten in our food and drink. Mercury levels in the atmosphere have nearly tripled due to human activity, and the burden is rising by 1.5 percent year. Plants and livestock may absorb mercury-contaminated soil or contaminated water that has been redistributed, which could enter the food chain. Mercury can bioaccumulate once it enters the food chain and have a negative impact on people's health. It is still mostly unclear how precisely mercury enters the food chain, and it most likely differs among ecosystems[2].

Since the Minamata disaster, even the general public has become aware of the toxicity of mercury. A significant quantity of data about acute mercury exposure was provided by earlier studies, but information about mercury toxicity at low doses has just recently begun to surface. Nobody is safe when mercury taints soils, rain, and seawater. Mercury-tainted fish, meat, and vegetables are a result of contaminated water. Exposure is increased by the toxin's easy access to humans through a variety of channels, including food, drink, cosmetics, air, and even vaccinations. Children and fetuses are particularly vulnerable to mercury exposure. Breastfeeding mothers who eat a diet high in mercury expose their unborn children to the poison. Children exposed to levels of mercury thought to be safe have shown reduced performance in memory and motor function tests. Similarly, persons exposed to low amounts of mercury were also found to have disruptions in verbal memory, fine motor skills, and attention. It is a risk to their overall health. Mercury is known to cause a wide range of illnesses, including those related to the nervous system, kidneys, immune system, heart, muscles, reproduction, and even genetics.

Klinghardt's axiom says that ”Most, if not all, chronic infectious diseases are not caused by a failure of the immune system, but are a conscious adaptation of the immune system to an otherwise lethal heavy metal environment”. Mercury has long been known to negatively impact immune system function, most likely through its negative effects on polymorphonuclear leukocytes(PMN). Mercury inhibits the natural stimulation of PMN synthesis by suppressing the generation of adrenocorticosteroids. It also impacts PMN function by preventing PMNs from destroying foreign compounds. Mercury sensitivity is associated with increased susceptibility to allergies, asthma, and autoimmune-like symptoms, particularly rheumatoid-like symptoms. Mercury has the ability to change immune cell formation and function as well as trigger an immunological response in the central nervous system[3]. Lately, disorders like Alzheimer's, Parkinson's, autism, lupus, amyotrophic lateral sclerosis, etc. have been connected to heavy metal-mediated toxicity. In addition, it endangers the fauna. Therefore, it becomes essential to alert the public and experts alike about the dangers of mercury exposure. It is also crucial to have selective and sensitive detection methods to prevent its exposure to living beings.

## 1.2 Plasmonic Nanoparticles and their properties

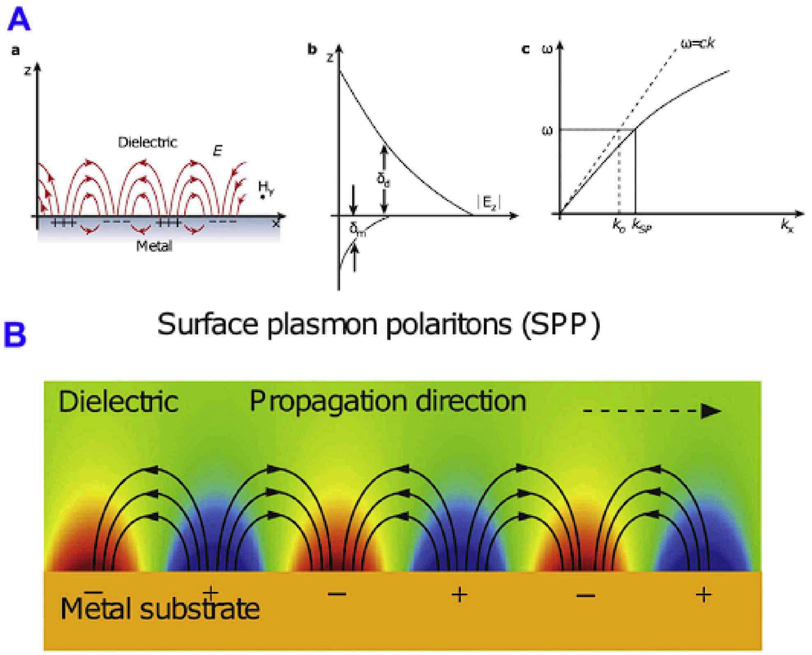


Figure 1: Surface Plasmon Polaritons

In the above picture (A) Surface Plasmon Polaritons (a) Propagation of surface plasmon polariton (SPP) at the metal dielectric interface. (b) SPPs are evanescent in the z-direction (toward the metal and medium) such that there is an exponential decay of energy. The decay length into the metal is determined by its skin depth. (c) Typical dispersion curve of SPP which is always lying below the light line. This means that it is not possible to directly excite SPPs. (B) Electromagnetic field distribution of surface plasmons at the interface[4].

Analytical chemistry has shown a considerable deal of interest in nanoparticles, which are distinguished by their small size, high surface area to volume ratios, and good adsorption ability. Because of their high sensitivity, broad linear range metal nanoparticle -based analytical techniques have gained significant traction in the fields of clinical, pharmaceutical, environmental, and food safety. Since metal nanostructures offer so many unique characteristics and functions compared to their bulk counterparts, they have attracted a lot of attention from a fundamental and technological standpoint. The optical characteristics of nanoparticles are among their most significant features. Many metals, including gold and silver, display high absorption in the visible spectrum at the nanoscale realm. The optical characteristics of nanoscale metal particles are determined by multiple factors, including the particles' size, form, metal composition, optical characteristics, and surrounding media. The last several years have seen a great deal of enthusiasm due to the amazing optical capabilities of metal nanostructures. Changes in shape, size distribution, surrounding medium,and high absorption caused these materials to become inorganic chromophores from the visible to near-infrared range, which accounts for the color fluctuations.

Nanoparticles are intricate multi-electron systems in which the confinement of electronic motion brought about by the size reduction produces intriguing effects that may be adjusted in accordance with the size and form of the particle. Furthermore, practically any shape can be created. These variously formed nanostructures display various colors or absorption spectra. The surface plasmon resonance (SPR) that gives metal nanoparticles their optical characteristics is responsible for the oscillation frequency at which conduction electrons respond to the electrical field of incident electromagnetic radiation. The collective oscillations with the free electrons in the metal cause the surface plasmons, which are basically electromagnetic waves trapped at the metal/dielectric contact. Mie theory, which is based on the Maxwell equation for scattering, provided an explanation for this occurrence. The SPR can be supported by materials with real and imaginary dielectric constants that are both positive and negative. This resonance is caused by surface conduction electrons that are stimulated by light's electromagnetic radiation and oscillate coherently. The study of these specific light interactions, known as plasmonics, has made a number of applications possible such as biological and chemical sensing, lithographic fabrication of the materials, and surface-enhanced spectroscopies .But only NPs made of gold, silver, and copper show plasmon resonance effects in the visual range, producing highly pigmented particles. This is because the size, shape, and surrounding medium of the particles affect the SPR's resonance frequency. Because of their surface accessibility for further functionalization and surface plasmon absorption, Gold and Silver nanoparticles are important in the visible area[5].

# 2 LITERATURE REVIEW

The literature study is an essential basis for understanding the present state of knowledge for a specific subject. This resource not only offers a thorough summary of current research, but also highlights areas where further exploration is needed, as well as inconsistencies and gaps in the existing literature. In this study, I thoroughly examined the many viewpoints and discoveries related to ”Plasmonic Nanostructures for Pollutant Sensing”.

1) A Rhodamine-Based Fluorescent and Colorimetric Chemodosimeter for the Rapid Detection of Hg2+ Ions in Aqueous Media. -Journal of the American Chemical Society, 2005

In this study, a new fluorescent and colorimetric chemodosimeter has been developed for the rapid detection of $Hg^{2+}$ ions in aqueous media. Through extensive research, a chemodosimeter has been created that is both highly selective and sensitive for detecting $Hg^{2+}$ ions in aqueous solutions. The system utilizes a reaction of a rhodamine derivative that is promoted by $Hg^{2+}$ ions. This system exhibits an exceptional level of selectivity for $Hg^{2+}$ compared to other metal ions, and its sensitivity in aqueous solutions is impressively low, measuring below 2 ppb. These findings indicate that this method has the potential to be a practical tool for quickly measuring $Hg^{2+}$ concentration[6].

2) L-cysteine functionalized gold nanoparticles for the colorimetric detection of $Hg^{2+}$ induced by ultraviolet light. -Nanotechnology, 2009

In this research, they developed a rapid and sensitive colorimetric sensor for the detection of $Hg^{2+}$, utilizing L-cysteine functionalized gold nanoparticles induced by ultraviolet radiation. This sensor is simple and cost-effective. The sensitivity and selectivity of detection were also examined. When exposed to $Hg^{2+}$ and ultraviolet radiation, the L-cysteine modified gold nanoparticles exhibit rapid aggregation. Gold nanoparticles can be used to detect the presence of $Hg^{2+}$ based on their colorimetric response. With the UV-vis spectra measurements, it is possible to detect $Hg^{2+}$ with a detection limit of 100 nM. Also, other divalent metal ions have been studied to determine the selectivity of this method. This colorimetric sensor claims to be highly effective and can be utilized for on-site and real-time detection of $Hg^{2+}$[7].

3) Recyclable Multifunctional Magnetic $Fe_3O4@SiO_2@Au$ Core/Shell Nanoparticles for SERS Detection of $Hg^{2+}$. -Chemosensors, 2023

In this study, a novel SERS sensor was created to detect Hg ions in water, making it easily detectable in the field. This new nano sensor demonstrates exceptional resistance to interference and adaptability to various environments. It also exhibits remarkable sensitivity and selectivity specifically towards Hg ions.The $Fe_3O_4$ core exhibits excellent magnetism. $SiO_2$ is employed to shield the $Fe_3O_4$ from any external disturbances. The dense gold shell offers a remarkable SERS enhancement effect, while 4-MPy is utilized for surface modification. 4-MPy has the ability to react with $Hg^{2+}$ and induce alterations in the spectrum. Furthermore, following the EDTA treatment, the sensor has the capability to be recycled and used again. It is worth noting that the Raman image of the macrophages treated with $Hg^{2+}$ ions provides solid evidence of the successful functioning of the MNPs sensors at the cellular level. With these advantages, the nano sensor offers a convenient and efficient solution for detecting Hg ions on-site. This SERS sensor exhibits excellent stability, accuracy, and anti-interference capabilities, making it a promising tool for detecting real samples. Additionally, it is magnetically separable and recyclable, further enhancing its practicality[8].

4) Mercaptopyridine-Functionalized Gold Nanoparticles for Fiber-Optic Surface Plasmon Resonance $Hg^{2+}$ Sensing. -ACS Sensors, 2019

This study presents the development of a highly responsive and specific fiber-optic surface plasmon resonance (SPR) sensor for the detection of Hgg that the Raman image of the macrophages treated with $Hg^{2+}$ ions. The sensor utilizes Au/4-MPY as signal amplification tags. The signal amplification tag was created through the self-assembly of 4-MPY onto the surface of Au nanoparticles. A particles-on-mirror structure was created during the sensing process, which is dependent on the concentration of Hg that the Raman image of the macrophages treated with $Hg^{2+}$. This structure exhibits a significant localized surface plasmon resonance (SPR) between gold nanoparticles (Au NPs), and the presence of an Au film leads to a noticeable shift in the wavelength of the SPR. This shift can be utilized for the detection and quantification of Hgg that the Raman image of the macrophages treated with $Hg^{2+}$. The Hg that the Raman image of the macrophages treated with $Hg^{2+}$ sensor was calibrated using a range of water solutions containing varying amounts of mercury. An extensive linear gradient was detected ranging from 8 to 100 nM. The minimum observed value of Hgg that the Raman image of the macrophages treated with $Hg^{2+}$ was determined to be 8 nM, which falls below the maximum allowable level for drinking water set by the World Health Organization (WHO). The findings demonstrate that this chemical sensor has exceptional selectivity towards Hgg that the Raman image of the macrophages treated with $Hg^{2+}$ in comparison to other prevalent metal ions present in water. In addition, we utilized the sensor to detect Hg that the Raman image of the macrophages treated with $Hg^{2+}$ in tap water samples that were intentionally contaminated, achieving outstanding recoveries ranging from 108 percent to 112 percent. The fiber-optic SPR sensor offers the benefits of simplicity, high selectivity, high sensitivity, and cost-effectiveness. This makes it highly suitable for on-site detection of Hgg that the Raman image of the macrophages treated with $Hg^{2+}$ in different environments, with significant practical potential[9].

# 3 METHODOLOGY

## 3.1 Materials

Silver Nitrate, Ascorbic acid, Cetyl trimethyl ammonium bromide, Gold Chloride, Trisodium Citrate, Sodium Borohydride, TX-100, Mercury Chloride, Rhodamine-B.

## 3.2 Procedure

**A) Gold Nanospheres**

Gold Nanopheres were synthesized by the reduction of $HAuCl_4.3H_2O$ with trisodium citrate. 50 mL $HAuCl_4.3H_2O$ solution of 1 mM was heated to boiling under vigorous stirring. Then 15 mL trisodium citrate solution was added dropwise till colour changes. Next, the reactant was heated for 15 minutes and then cooled to stop the reaction[10].

**B) Gold Nanorods**

Gold nanorods were synthesized through seed mediated growth process.

Seed Solution: 5 ml CTAB solution was mixed with 5 mL of $HAuCl_4.3H_2O$. To the stirred solution, ice-cold $NaBH_4$ was added, which resulted in the formation of a brownish yellow solution. After the solution was stirred, it was kept at 25°C.

Growth solution: 5 ml CTAB was added to 0.25 mL of $AgNO_3$ solution at 25°C. To this solution, 5 mL of $HAuCl_4.3H_2O$ was added, and after gentle mixing of the solution 70 microlitre of ascorbic acid was added. Ascorbic acid as a mild reducing agent changes the growth solution from dark yellow to colorless. The final step was the addition of 12 microlitre of the seed solution to the growth solution at 27-30°C. The color of the solution gradually changed within 10-20 min[11].

**C) Gold Nanostars**

In a beaker 3.959 ml of TX-100 , 0.457 ml of $HAuCl_4.3H_2O$ and 4.726 ml of distilled water was taken in 50 ml beaker under constant stirring and kept 40°C for an hour. Then, 1.5 ml of $AgNO_3$ was added to the solution followed by the addition of Ascorbic Acid till bright yellow colour of the solution changes to dark green[12].

# 4 CHARACTERISATION

The term ”characterization” in science and research refers to the broad and general process of analyzing and quantifying a material’s properties. It is necessary to determine a scientific understanding of the synthesized materials. In the realm of research and study, it is a crucial process.The several instrumental methods that have been used to characterize the prepared samples are described in depth in this chapter.

## 4.1 UV Visible Spectroscopy

Absorption spectroscopy is in portions of the ultraviolet and the entire, nearby visible parts of the electromagnetic spectrum is referred to as ultraviolet (UV) spectroscopy or ultraviolet-visible (UV–VIS) spectrscop. This methodology is frequently employed in a variety of practical and basic applications due to its ease of implementation and relative affordability. All that is needed for the sample to be a chromophore is for it to absorb in the UV-Vis range. Aside from the measurement wavelength, the parameters of interest are absorbance (A), transmittance, or reflectance, and how they vary over time. An analytical tool called a UV-vis spectrophotometer calculates how much ultraviolet (UV) and visible light a sample absorbs. It is a commonly used method for identifying and quantifying chemicals in a range of samples in the domains of chemistry, biochemistry, and other sciences[13]. A light beam is passed through the sample using a UV-vis spectrophotometer, which measures the amount of light absorbed at each wavelength. The concentration of the absorbing chemical in the sample determines how much light is absorbed. The visible and ultraviolet parts of the electromagnetic spectrum have wavelengths between 200 and 800 nm. Visible light is from 400 to 800 nm, while ultraviolet light is between 200 and 400 nm[14]. The energy of this radiation is high enough to cause the outer valence electrons to undergo an electronic transition. By absorbing radiation from the UV-visible region, electrons from bonding (outermost) orbitals in both organic and inorganic compounds are promoted to antibonding orbitals or higher energy levels. Coupled with these electronic transitions are vibrational and rotational transitions.

### 4.1.1 Principle

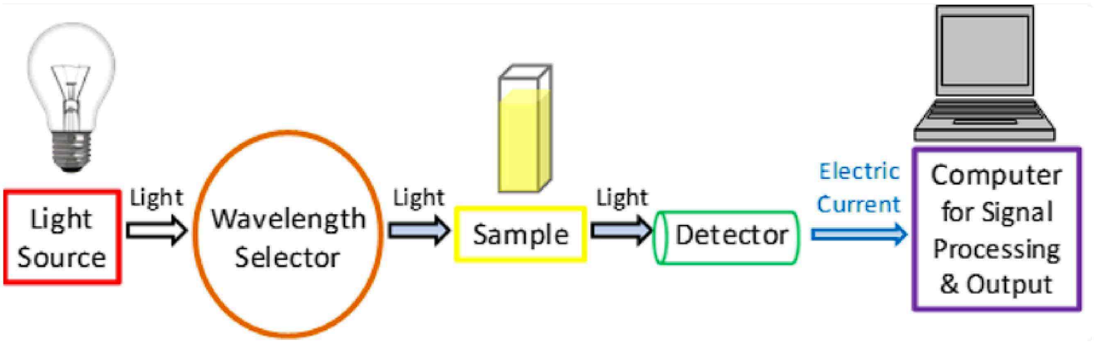


Figure 2: A simplified schematic of the main components in a UV-Vis spectrophotometer.

In the above picture a simplified schematic of the main components in a UV-Vis spectrophotometer is shown[15]. If a substance preferentially absorbs light from the visible spectrum, it appears coloured. The molecule is raised from its ground energy state to a higher excited energy state by the absorbed energy. Absorption in the UV or visible spectrum occurs due to the degree of electron bonding, which determines the energy difference E. Because the electrons in sigma bond compounds are tightly bound together, UV radiation will be absorbed. Because there is no absorption in the visible spectrum, the molecule appears colourless. The loosely bound electrons cause absorption in the visible spectrum, giving the combination a coloured appearance[15].
Molecules have three different kinds of orbitals in the ground state:

a) Molecular orbital a bonding sigma does not show absorption in the UV region because the electrons in this orbital are strongly bound and require high energy for excitation.

b) Pia bonding molecular orbital: Electrons in this orbital are typically mobile and present in many bonds. Because pia bonds are weak, UV energy can stimulate its electrons from lower energy levels to higher ones.

c) Non-bonding molecular orbital or n electron is the name for valence electrons in a molecule that does not contribute to the formation of a chemical bond. These are usually lone pairs of electrons that can be stimulated by UV light.

### 4.1.2 Applications

The analytical method known as UV-Vis spectroscopy counts the number of distinct UV or visible light wavelengths that a sample absorbs or transmits when compared to a reference or blank sample. It aids in the understanding of the optical characteristics, band gap determination, and electrical structure of materials in condensed matter physics. Researchers can study optical transitions, charge carrier dynamics, and excitonic effects in semiconductors, quantum dots, and nanomaterials by measuring the absorption or transmission of UV-visible light.

UV-visible spectroscopy allows for the accurate measurement of molecule structure, bond energies, and electronic configurations in atomic and molecular physics. It grasp processes like photoionization, photodissociation, and photoisomerization require a grasp of chemical kinetics, photochemical reactions, and molecular interactions[16]. Furthermore, UV-visible spectroscopy makes it easier to analyze the atmospheres, temperatures, and compositions of celestial bodies in astrophysics. Through analyzing absorption and emission lines found in stellar spectra, researchers can gain an understanding of cosmic processes, nucleosynthesis, and star development. Additionally, environmental physics uses UV-visible spectroscopy to identify contaminants, track the quality of the air and water, and analyze the composition of the atmosphere. It is also essential to biophysics, helping to understand protein folding, enzymatic processes, and biomolecular structures.

## 4.2 Field Emission Scanning Electron Microscopy(FE-SEM)

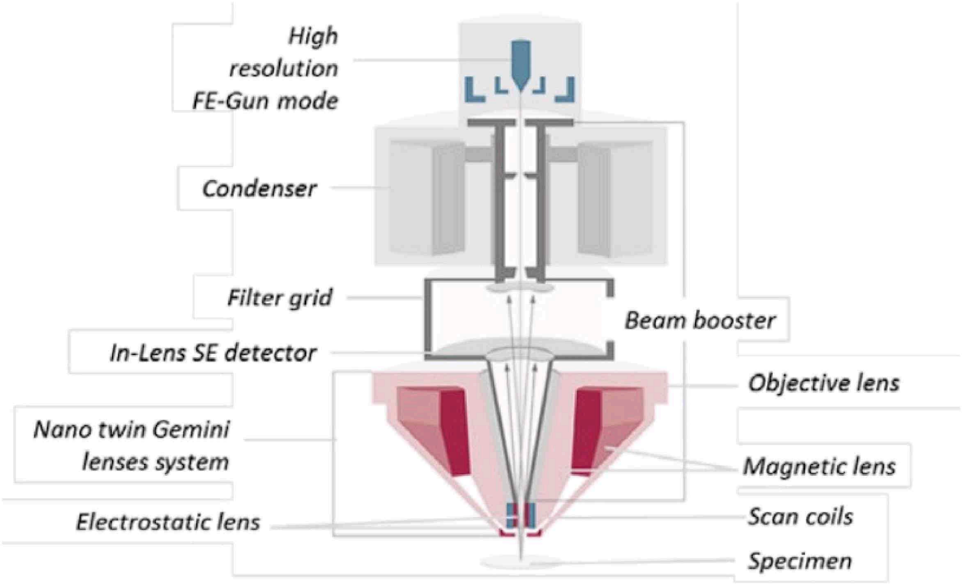


Figure 3: FE-SEM main parts

The above picture depicts the main parts of FE-SEM[17]. A microscope that employs negatively charged particles called electrons rather than light is known as a FE-SEM, or field emission scanning electron microscope. The Field allows for imaging at low accelerating voltages and small working distances. Several detectors are included into this system to distinguish between various signals, such as a secondary electron (SE). With image resolution as low as 0.6 nm at 15 KV and 1.2 nm at 1 KV, it offers a wide range of applications in chemistry, physics, electronics, mineralogy, ceramics, polymers, metallurgy, and life sciences, allowing for the analysis of nanopowders, nanofilms, and nanofiber's top surface. This system has several detectors to distinguish between various signals, such as a back-scattered electrons (BSE) detector for material composition contrast and a secondary electron (SE) detector for topographic information. With reduced electrostatic distortion, field emission scanning microscopy produces images with a spatial resolution down to 11/2 nanometers that is three to six times better. FESEM are still large, expensive pieces of equipment. They are still favored by due to their numerous applications and qualities, which include the ability to produce intricate, three-dimensional, high-resolution images.

### 4.2.1 Principle

A concentrated electron beam is employed by FE-SEM to examine or create a picture of the specimen. The cannon head, the column, and the specimen chamber must be evacuated in order to operate. The specimen chamber is evacuated by the turbo pump and pre-vacuum pump. A penning gauge is used to measure the vacuum in the specimen chamber. As long as the detected pressure is not ready for operation, the column chamber value stays closed. Nitrogen gas enters the specimen chamber through the vent value after the vent command is issued, closing the column chamber value. For applications that need the highest level of resolution, a field emission cathode in the electron gun of a scanning electron microscope produces narrower probing beams at both low and high electron energy, resulting in both better spatial resolution and minimal sample charging and damage.

### 4.2.2 Applications

Similar to the SEM in that it offers a wide range of information from the sample surface, the Field Emission Scanning Electron Microscope (FE-SEM) has a far larger energy range and higher resolution. Similar to a traditional SEM, it operates by scanning the sample surface with an electron beam while a monitor shows the data that is of interest based on the available detectors. The electron generation system is the primary distinction between a FE-SEM and a SEM. The FE-SEM's electron source is a field emission gun, which produces highly focused high and low energy electron beams. Applications of FE-SEM include, semiconductor device cross section analyses for gate widths, gate oxides, film thicknesses, and construction details, advanced coating thickness and structure uniformity determination.

## 4.3 Colorimetry

Noble metal nanoparticles have unique characteristics due to Surface Plasmon Resonance. Surface Plasmon Resonance is the collective oscillation of metal nanoparticle conduction electrons when a specific frequency of electromagnetic radiation interacts with the particles. Modifications to the nanoparticles' size, shape, composition, and sensitivity to the dielectric properties of their surroundings can adjust the Surface Plasmon Resonance Frequency. Metal nanoparticles, especially gold and silver nanoparticles, are unique due to these distinguishing characteristics. Numerous sensors have been developed based on this characteristic of metal nanoparticles.

### 4.3.1 Principle

Colorimetry is one of the methods of spectroanalytical methods. The variation of the colour of a solution with a change in concentration of some solute component in the solution forms the basis of colorimetry. The colour is either the inherent colour of the constituent itself or due to the formation of a coloured species by the addition of a suitable reagent. The intensity of the colour can then be compared with that obtained by treating a known amount of the chemical substance in the same manner. This technique provides a simple way to use colorimetry to see mercury ions in aqueous solution.

### 4.3.2 Applications

The widespread adoption of colorimetric sensors that are both sensitive and selective can be attributed to their ease of use, speed, accuracy, and accessibility of basic instruments for usage in both laboratory and field settings. This category includes applications that rely on basic visual detection by the unassisted eye without the need for costly technology. Recently, colorimetric sensing techniques are being developed to detect mercury in aqueous solutions. Gold nanoparticles have been useful in the development of visualizing sensors for chemical and biosensing because of their high absorption coefficient and the surface plasmon resonance (SPR) alterations they cause during binding-induced particle aggregation.

## 4.4 Fluorimetry

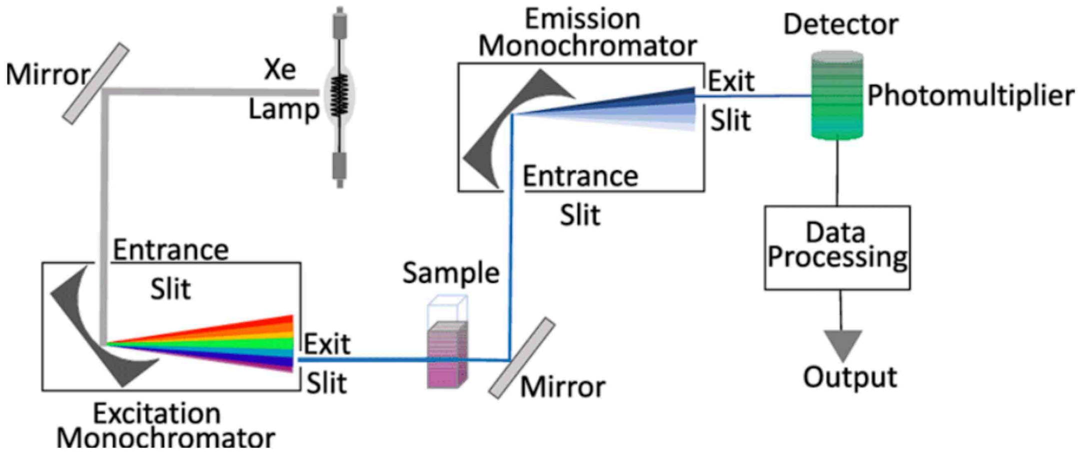


Figure 4: Major Components Of Fluorescence Spectrophotometry

The above figure shows the major components of Fluorescence Spectrophotometry[18]. To identify and quantify fluorescence in substances, this analytical method stimulates the compounds with ultraviolet light, causing them to produce visible light. The material releases more energy or light with a longer wavelength than it takes in. The technique of creating radiation with a longer wavelength than what is absorbed is known as luminescence.Fluorescence is one type of luminescence that happens when photons excite a molecule and bring it to an electronic excited state. This is an optical phenomenon in which molecules that absorb photon energy emit fluorescent photons with longer wavelengths.

### 4.4.1 Principle

In a fluorescence excitation spectrum, the emission wavelength remains constant while the excitation monochromator wavelength is varied. By analyzing the spectrum, valuable insights can be gained regarding the wavelengths at which a sample absorbs and emits light. This allows for precise observation of the chosen emission wavelength. It functions similarly to an absorbance spectrum, but offers greater sensitivity in terms of detecting limits and molecular specificity. Excitation spectra are distinct for a single emitting wavelength/species, unlike an absorbance spectrum that measures all absorbing species in a solution or sample. The emission and excitation spectra for a given fluorophore exhibit a symmetrical relationship. In most cases, the emission spectrum is observed at longer wavelengths (lower energy) compared to the excitation or absorbance spectrum. These two spectral types, emission and excitation, are utilized to observe the alterations in a sample. Changes in spectral intensity and peak wavelength can occur due to factors like temperature, concentration, or interactions with surrounding molecules. This involves the use of quencher molecules and other materials that are capable of energy transfer. Certain fluorophores can be influenced by factors like pH, polarity, and ion concentrations in the solvent environment[19].

### 4.4.2 Applications

In order to do fluorescence spectroscopy, a laser beam is used to excite the electrons in specific compounds' molecules, causing them to release light. The purpose of directing that light is to measure and identify the molecule or changes in the molecule by passing it through a filter and onto a detector.When molecules are excited by a constant source of light, they emit fluorescence. The emitted photons, or intensity, are then detected as a function of wavelength. Fluorescence emission spectra involve fixing the excitation wavelength and scanning the emission wavelength to obtain a plot of intensity versus emission wavelength.This technique can also be used for determination of Vitamin B (B1 thiamine and B2 riboflavin) in the food samples like meat, cereals, etc. It is employed to carry out both qualitative and quantitative analyses for various aromatic compounds present in cigarette smoke, air-pollutant, concentrates, and automobiles exhaust[19].

## 4.5 Surface Enhanced Raman Spectroscopy

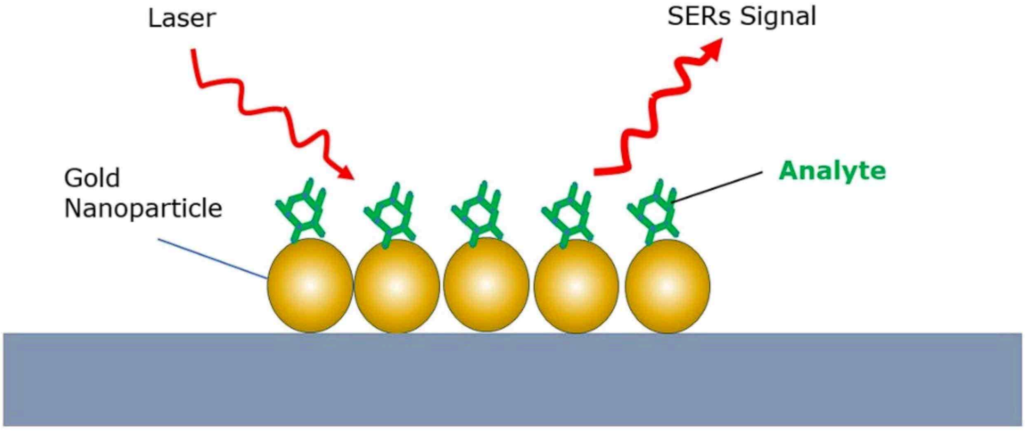


Figure 5: Surface Enhanced Raman Spectroscopy

Surface enhanced Raman spectroscopy is a method that involves molecules undergoing higher scattering efficiencies when adsorbed on metal colloidal nanoparticles and rough metal surfaces[20]. Surface enhanced Raman spectroscopy (SERS) is a potent vibrational spectroscopy method that amplifies electromagnetic fields produced by stimulating localized surface plasmons to enable highly sensitive structural identification of low concentration analytes. From model system investigations on roughened electrodes to extremely complex studies like single molecule spectroscopy, SERS has advanced. SERS is essentially a method of greatly boosting the signal from the structurally rich but weak Raman scattering technique. Although stimulated Raman processes and electronic resonance enhancement have been used by researchers to boost the effectiveness of Raman scattering, SERS provides the greatest Raman signal amplification.Fundamentally, Surface Enhanced Raman Scattering(SERS) is a method of greatly enhancing the signal from the structurally rich but weak Raman scattering technique. Although electronic resonance enhancement and stimulated Raman processes have been used by researchers to increase the efficiency of Raman scattering, SERS provides the most notable Raman signal amplification.

### 4.5.1 Principle

It is now widely acknowledged, following decades of controversy, that the electromagnetic enhancement mechanism is the primary factor in the majority of SERS processes. The light is amplified by the stimulation of localized surface plasmon resonances (LSPRs), which produces the enhancement. Phosphorite materials, which are essentially noble and coinage metals (such as silver, gold, and copper) with nanoscale characteristics, exhibit a preference for light concentration in their gaps, cracks, or sharp features. Chemical enhancement, which mostly involves charge transfer mechanisms where the excitation wavelength is resonant with the metal-molecule charge transfer electronic states, is the other mechanism involved in signal enhancement [21].Enhanced surface Raman scattering of molecules supported by nanostructured materials can be enhanced by the use of Raman spectroscopy, an incredibly sensitive technique. This implies the possibility of structural fingerprinting analytes at low concentrations. Chemical enhancement or electrical field amplification via plasmon mediated means is used to accomplish this technique.

Molecules can scatter light in two ways: inelastic (Rayleigh scattering) or elastic (Raman scattering). The primary distinction is that in elastic scattering, the scattering events do not alter the photon's energy or the state of the molecule. This means that information about molecular structures and states is not greatly revealed by light scattered using the Rayleigh approach.When monochromatic light frequencies interact with a molecule's modes and vibrational states, they alter in inelastic scattering. As laser sources have advanced, Raman spectroscopy has emerged as a very sensitive technique. However, the uses of conventional Raman spectroscopy have been restricted due to the low-scattering cross-sections and ensuing low sensitivity.Surface enhanced Raman spectroscopy is a great tool for tracking pollution levels and water quality. With its high sensitivity and versatility, SERS is a non-destructive testing method that provides information on both organic and inorganic contaminants found in water.

### 4.5.2 Applications

Surface-enhanced Raman spectroscopy (SERS) is a highly versatile technique that has found applications in various fields such as
*food safety
*environmental monitoring
*signaling pathway and neurotransmitted detection
*detection of pathogenic micro-organisms
*biomarkers of infectious diseases
*application in cellular and histological examination
*medical diagnosis and treatment
*drug analysis and monitoring
Its ability to provide rapid and accurate identification of molecular structures makes it a valuable tool in these areas[22].

# 5 RESULT AND ANALYSIS

## 5.1 UV-Vis Spectroscopy

The optical properties of the gold nanoparticles were characterized by UV–Vis spectroscopy.

A) Gold Nanospheres

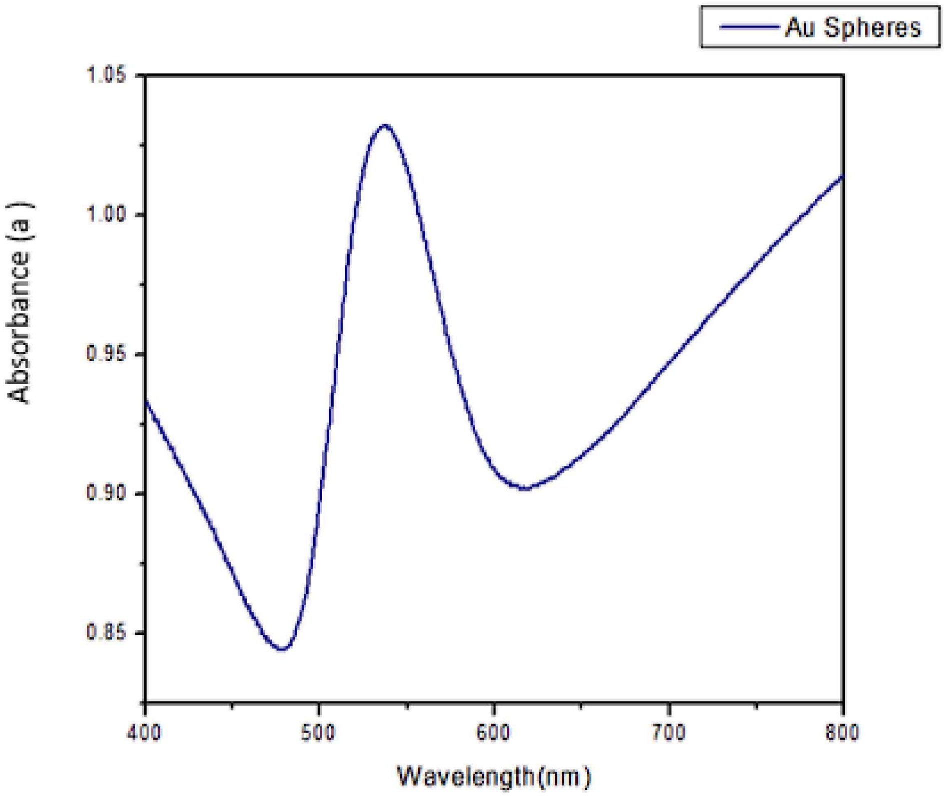


Figure 6: The absorption peak for Au Spheres

An absorption band around a wavelength of 536 nm was observed for Gold nanospheres and this peak position suggests the presence of small spherical particles of gold(figure 6).

B) Gold Nanorods

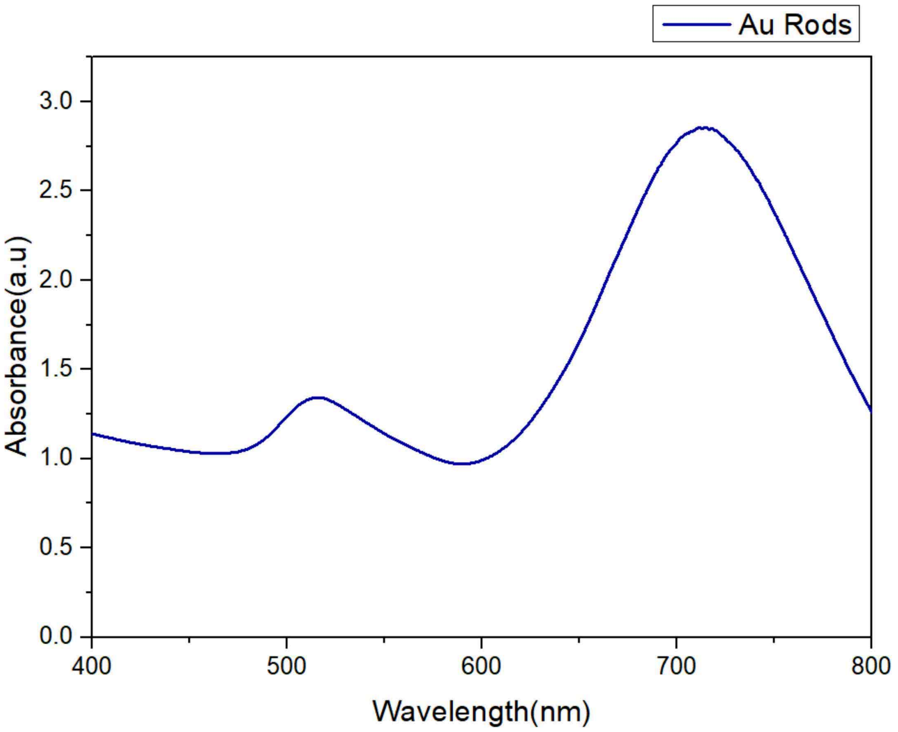


Figure 7: The absorption peak for Au Rods

UV-Vis absorption spectra of Gold nanorods produced using seed-mediated synthesis exhibited a characteristic longitudinal localized surface plasmon resonance (LSPR) at a wavelength of 714 nm and a transverse LSPR at a wavelength of 515 nm (Figure 7).

C) Gold Nanostars

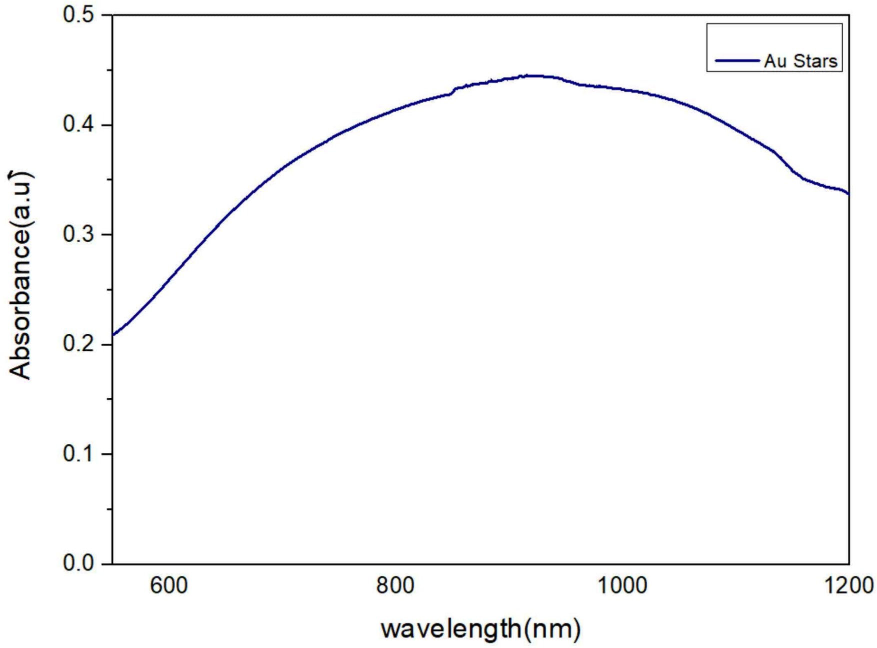


Figure 8: The absorption peak for Au Stars

A broad plasmon band mainly ranging from 600 nm to 1200 nm with maxima at 916 nm was observed for star nanoparticles (Figure 8). This broad absorption pattern corresponds to the blue colloidal solution of gold.

## 5.2 FE-SEM

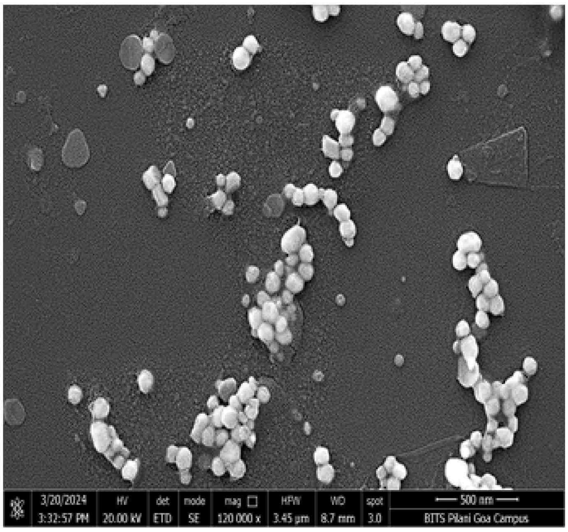


Figure 9: FE-SEM image of Gold Nanospheres

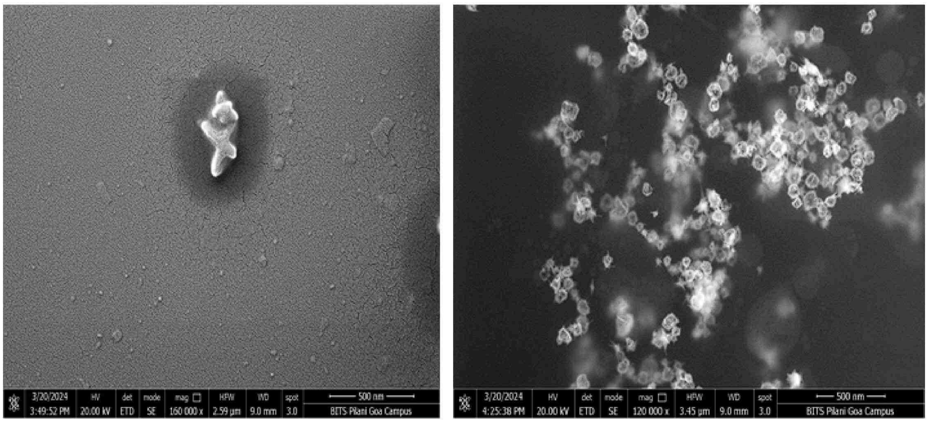


Figure 10: FE-SEM images of Gold Nanostars

The morphology of the synthesized Gold nanoparticles were evaluated by Field Emision Scanning Electron Microscopy(FE-SEM). Figure 9, shows the FE-SEM image of the Gold nanospheres. Figure 10, shows the FE-SEM image of Gold nanostars. Gold nanostars have a non-uniform structures with various numbers of tips of different lengths. FE-SEM analysis also demonstrates that the Gold nanostars have a branched structure with at least four tips.

## 5.3 Colorimetry

A) Gold Nanospheres

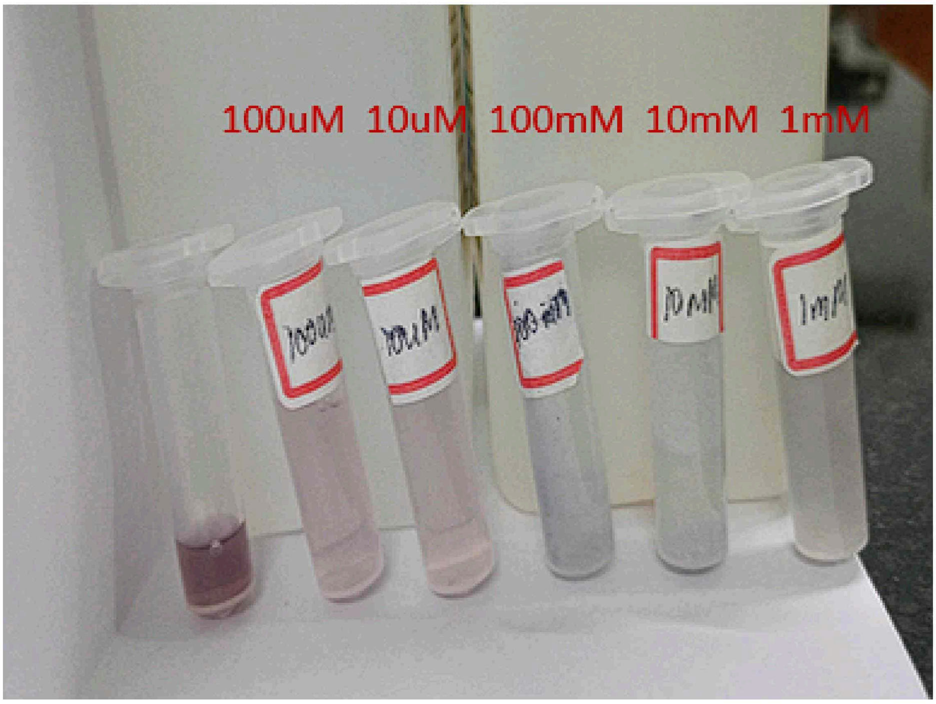


Figure 11: Colorimetric detection of mercury using Gold nanospheres.

The pristine Gold nanosphere solution was combined with different amounts of mercury solution until a change in color was seen. Upon the addition of a mercury solution, it was noted that the Gold nanosphere solution became nearly colourless at higher mercury concentrations and slightly lighter at lower mercury concentrations. The color change occurs because mercury forms an aggregation with gold particles.

B) Gold Nanorods

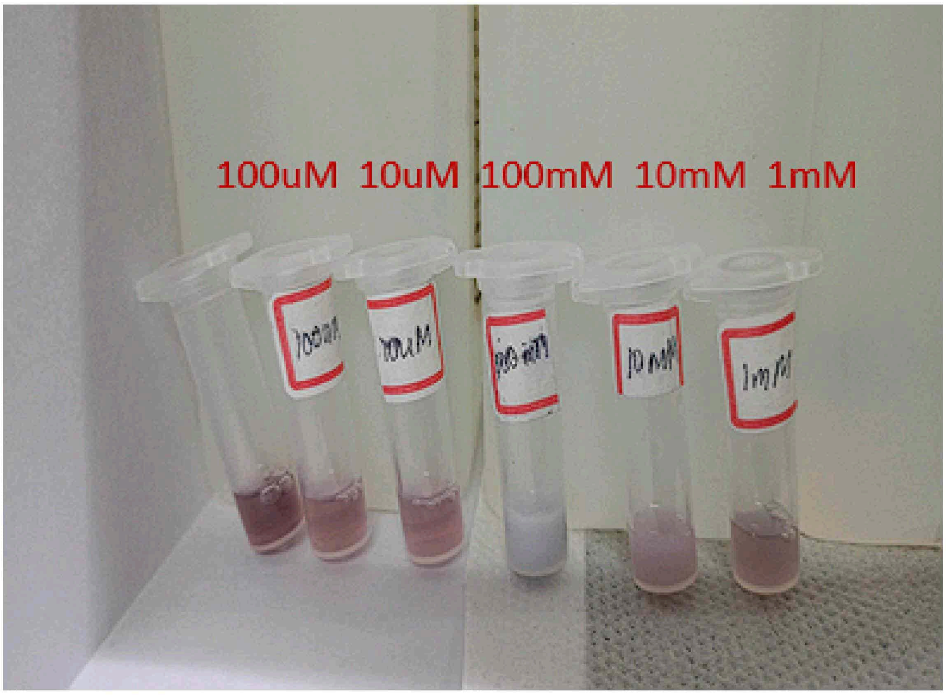


Figure 12: Colorimetric detection of mercury using Gold nanorods.

The pristine Gold nanorods solution was combined with different amounts of mercury solution until a change in color was seen. Upon the addition of a mercury solution, it was noted that the Gold nanorods solution underwent a slight lightening in color and at 100 mM a precipitate was formed. The color change occurs because mercury forms an aggregation with gold particles.

C) Gold Nanostars

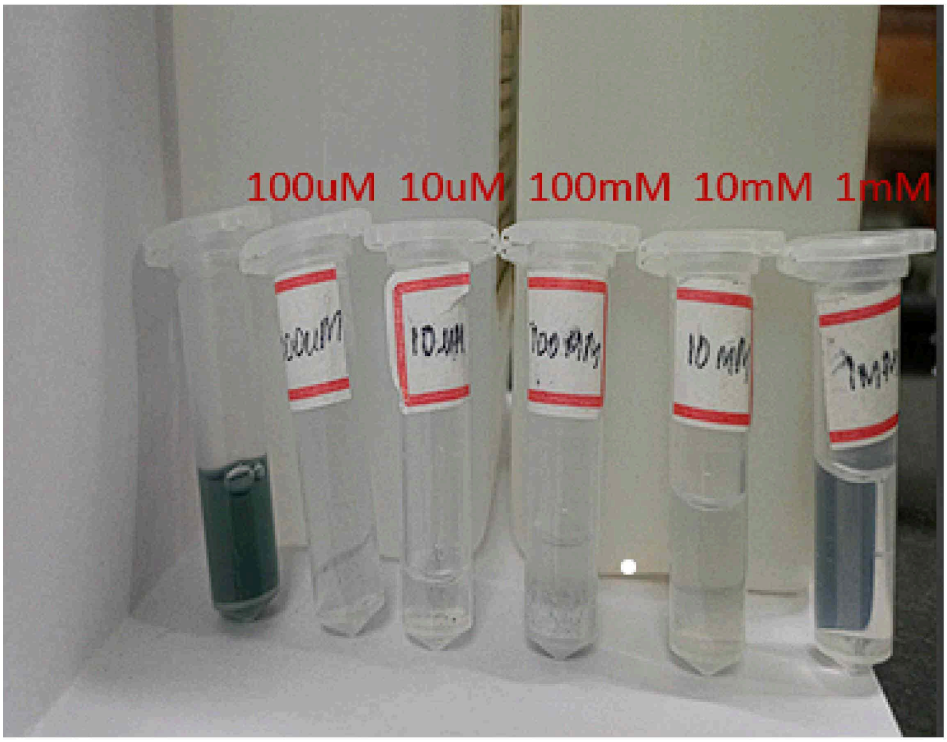


Figure 13: Colorimetric detection of mercury using Gold nanostars.

The pristine Gold nanostar solution was combined with different amounts of mercury solution until a change in color was seen. After adding a mercury solution, it was observed that the gold nanostar solution lost its color and turned colourless. The color change occurs because mercury forms an aggregation with gold particles.

## 5.4 Fluorimetry

A) Gold Nanospheres

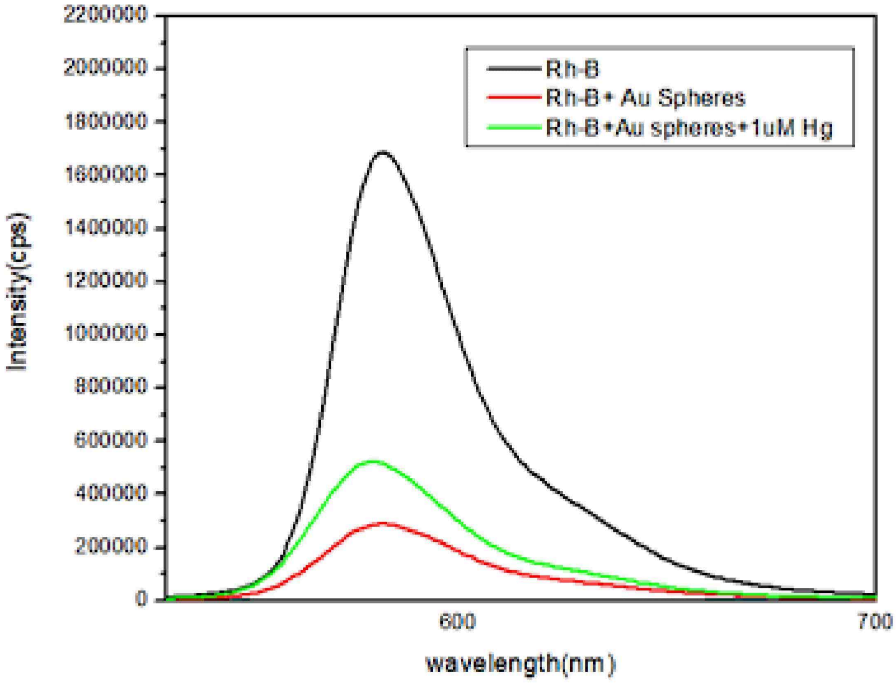


Figure 14: Fluorimetric detection for of 1uM Hg using Gold nanospheres and Rh-B dye

The Rh-B dye was employed as a fluorophore. The black curve represents the concentration of 1 uM Rh-B. Upon the addition of Gold nanosphere solution to the Rh-B solution, the fluorescence of the latter was suppressed, as seen by the red curve. This quenching phenomenon is a result of Fluorescence Resonance Energy Transfer (FRET). The green curve demonstrates that the introduction of 1 uM mercury which resulted in a partial restoration of fluorescence in the Rh-B dye. This is because gold nanoparticles exhibit a higher affinity for mercury in comparison to Rh-B dye.

A) Gold Nanospheres

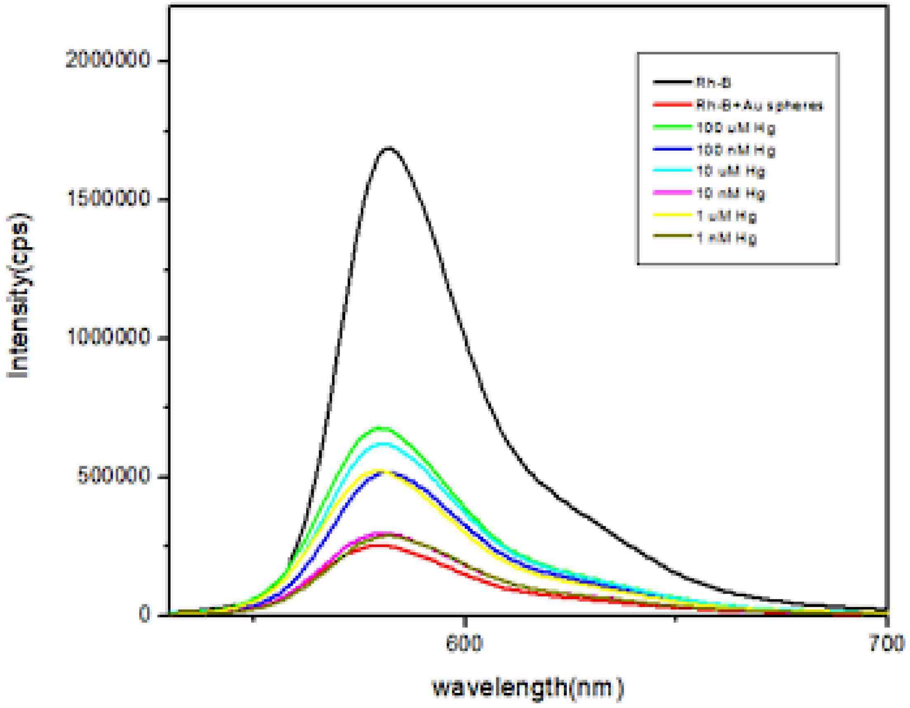


Figure 15: Fluorimetric detection for various concentration of Hg using Gold nanospheres and Rh-B dye

The graphic above illustrates the fluorimetric detection of different concentrations of Hg using Gold nanospheres and Rh-B dye. Fluorimetric detection was performed using concentrations of Hg at 100 uM, 10 uM, 1 uM, 100 nM, 10 nM, and 1 nM. It was observed that all the mercury concentrations were able to partially restore the fluorescence of the Rh-B dye.

B) Gold Nanorods

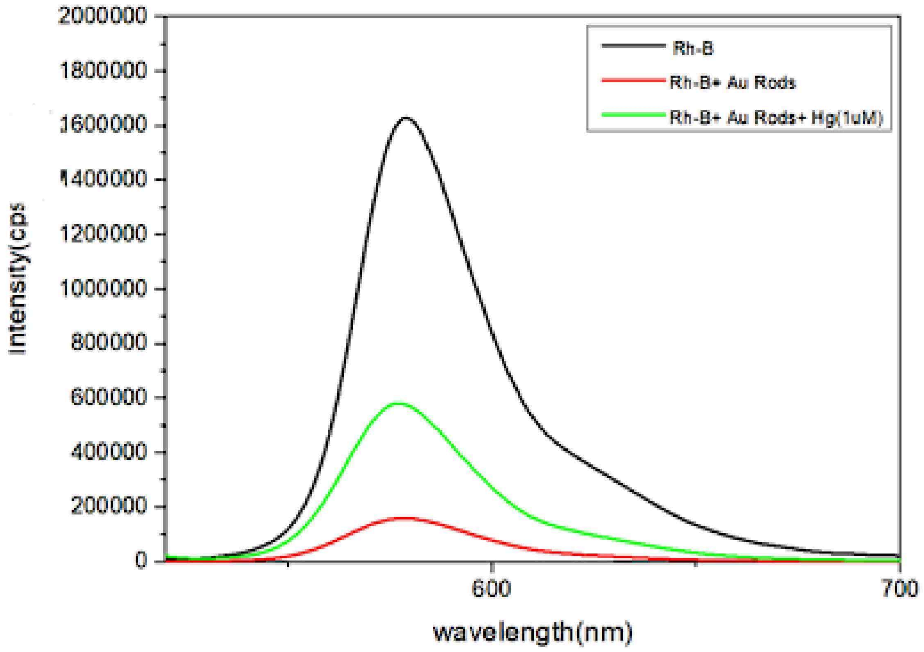


Figure 16: Fluorimetric detection for of 1uM Hg using Gold nanorods and Rh-B dye

The Rh-B dye was employed as a fluorophore. The black curve represents the concentration of 1 uM Rh-B. Upon the addition of Gold nanorods solution to the Rh-B solution, the fluorescence of the latter was suppressed, as seen by the red curve. This quenching phenomenon is a result of Fluorescence Resonance Energy Transfer (FRET). The green curve demonstrates that the introduction of 1 uM mercury which resulted in a partial restoration of fluorescence in the Rh-B dye. This is because gold nanoparticles exhibit a higher affinity for mercury in comparison to Rh-B dye.

B) Gold Nanorods

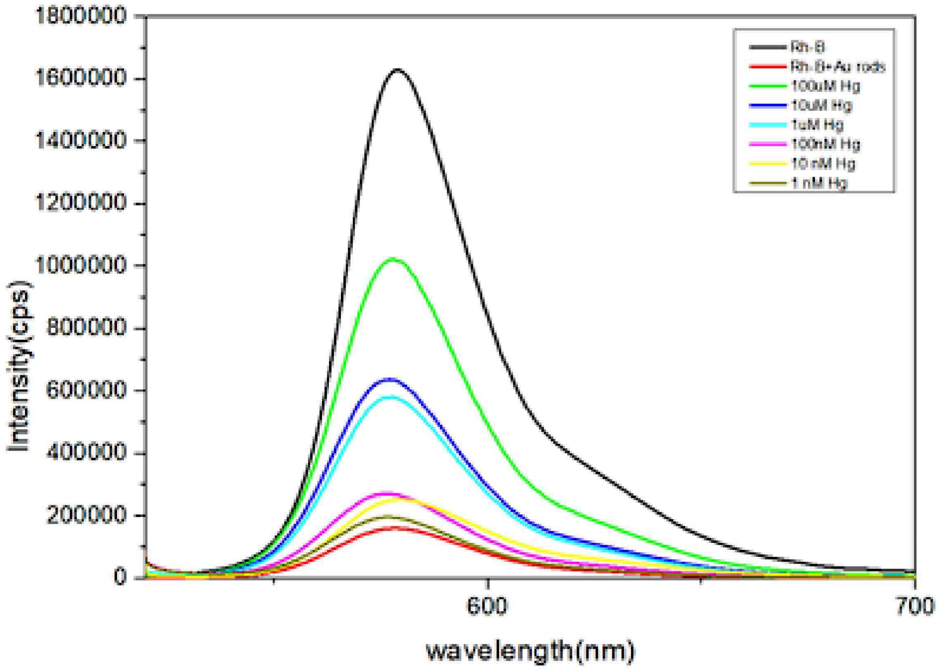


Figure 17: Fluorimetric detection for various concentration of Hg using Gold nanorods and Rh-B dye

The graphic above illustrates the fluorimetric detection of different concentrations of Hg using Gold nanorods and Rh-B dye. Fluorimetric detection was performed using concentrations of Hg at 100 uM, 10 uM, 1 uM, 100 nM, 10 nM, and 1 nM. It was observed that all the mercury concentrations were able to partially restore the fluorescence of the Rh-B dye.

C) Gold Nanostars

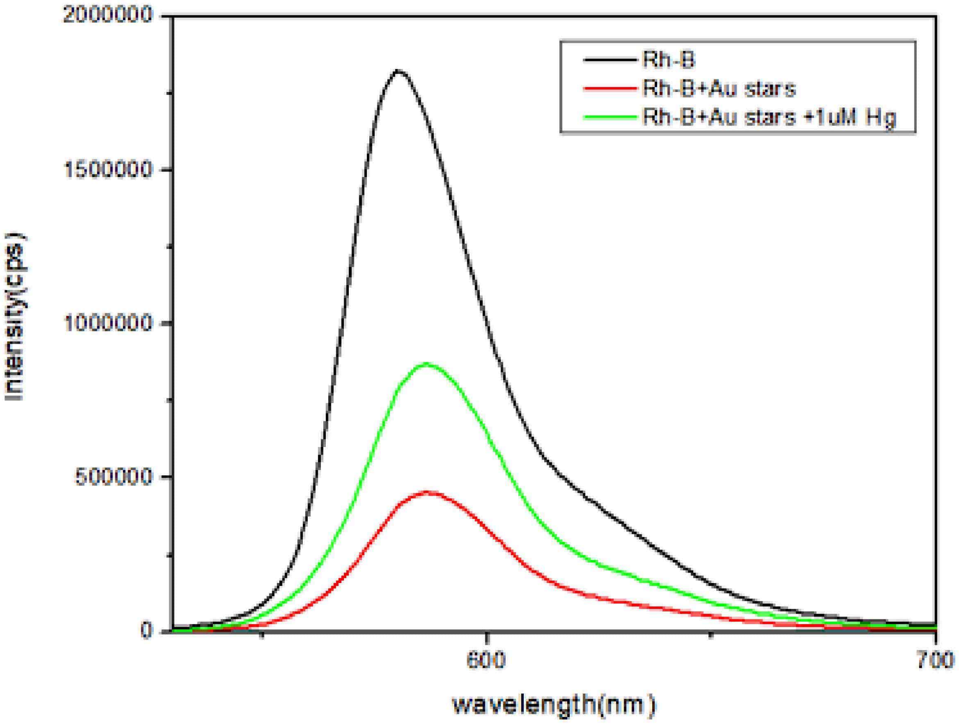


Figure 18: Fluorimetric detection for of 1uM Hg using Gold nanostars and Rh-B dye

The Rh-B dye was employed as a fluorophore. The black curve represents the concentration of 1 uM Rh-B. Upon the addition of Gold nanostars solution to the Rh-B solution, the fluorescence of the latter was suppressed, as seen by the red curve. This quenching phenomenon is a result of Fluorescence Resonance Energy Transfer (FRET). The green curve demonstrates that the introduction of 1 uM mercury which resulted in a partial restoration of fluorescence in the Rh-B dye. This is because gold nanoparticles exhibit a higher affinity for mercury in comparison to Rh-B dye.

C) Gold Nanostars

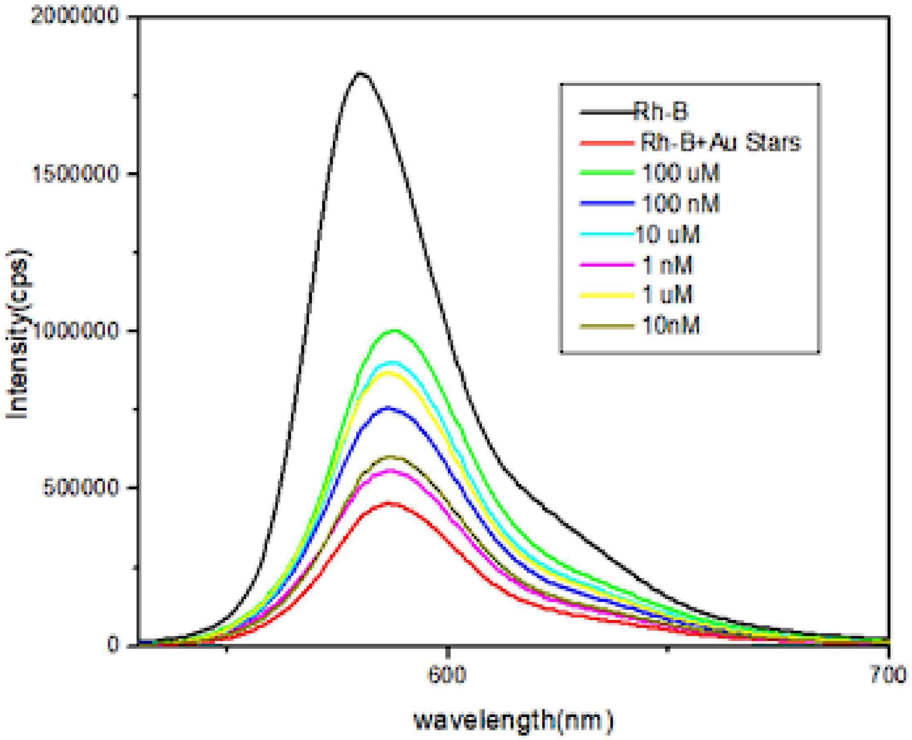
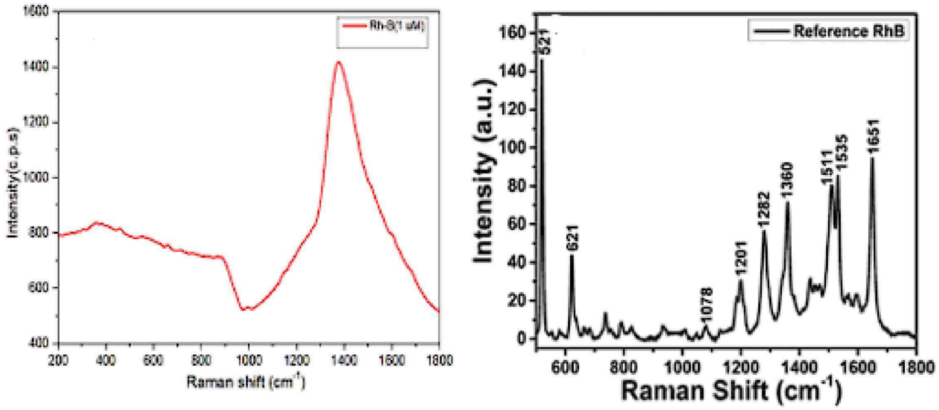


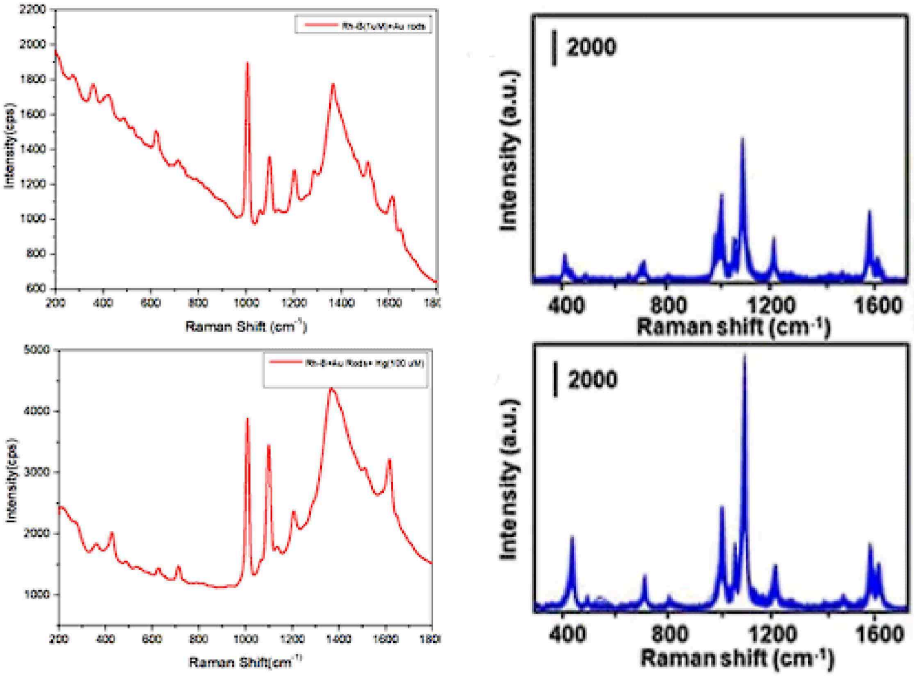
Figure 19: Fluorimetric detection for various concentration of Hg using Gold nanostars and Rh-B dye

The graph above shows the fluorimetric detection of various Hg concentrations using gold nanostars and Rh-B dye. Fluorimetric detection was carried out at concentrations of Hg of 100 uM, 10 uM, 1 uM, 100 nM, 10 nM, and 1 nM. It was discovered that all mercury concentrations could partially restore the fluorescence of the Rh-B dye. Gold nanostars were more effective in restoring the fluorescence of Rh-B dye than gold spheres and rods.

## 5.5 SERS



A Raman Spectrum of Rh-B
-ref20



$Fe_3O_4$@$SiO_2$@Au/4-MPy microspheres in the absence and presence of $Hg^{2+}$ ions, respectively
-Ref21

Figure 20: SERS data

The above figure shows observed data (left column) and expected data(right column). During the SERS experiment for mercury detection, I made an error by incorrectly following the technique. I synthesized a mixture of RH-B and Gold nanoparticles coated with 4-MPy for the purpose of detection.The investigation of mercury detection should be carried out independently, utilizing either Rhodamine dye or 4-MPy. When the Rh-B is used as a reporter molecule, adding gold nanoparticles is expected to boost the SERS signal from Rh-B. However, when mercury ions are added, the Rh-B will be removed from the surface of the gold nanoparticles, leading to a drop in the Rh-B signal[23].

4-MPy exhibits a great affinity for gold surfaces due to its mercapto group, and it can coordinate with mercuric species by using the nitrogen atom of the pyridine ring in water to produce a complex known as Hg(pyridine).When mercury ions are added to a solution of gold nanoparticles coated with 4-MPy, it is anticipated that there will be a rise in the signal peaks of 4-MPy[24].

# 6 SUMMARY

Different nanostructures of Gold nanoparticles were synthesized by the chemical reduction method for Gold nanospheres, Gold nanostars, and the seed mediated growth method for Gold nanorods. The morphology of these nanostructures was analyzed using field emission scanning electron microscopy (FE-SEM). Gold nanostars have a non-uniform structures with various numbers of tips of different lengths. FE-SEM analysis also demonstrates that the Gold nanostars have a branched structure with at least four tips. The UV–Vis spectroscopy was used to analyze the optical characteristics of the gold nanostructures. An absorption band around a wavelength of 536 nm was observed for Gold nanospheres and this peak position suggests the presence of small spherical particles of gold. UV-Vis absorption spectra of Gold nanorods produced using seed-mediated synthesis exhibited a characteristic longitudinal localized surface plasmon resonance (LSPR) at a wavelength of 714 nm and a transverse LSPR at a wavelength of 515 nm. A broad plasmon band mainly ranging from 600 nm to 1200 nm with maxima at 916 nm was observed for star nanoparticles. This broad absorption pattern corresponds to the blue colloidal solution of gold. The sensing of mercury ions in an aqueous environment was accomplished using plasmonic nanostructures and Rhodamine-B (Rh-B) through the utilization of detection methods such as colorimetric, fluorometric, and SERS. In colorimetric detection the pristine Gold nanostructure solution was combined with different amounts of mercury solution until a change in color was seen. The color change occurs because mercury forms an aggregation with gold particles. In fluorometric detection, Gold nanoparticles could effectively quench the fluorescence of Rh-B dye. The presence of $Hg^{2+}$ could be measured using a mixture of fluorescent dye and nanoparticles.One potential future approach for detecting mercury is to experiment with the use of silver nanoparticles. One may determine selectivity and sensitivity by employing different cations. The synthesized nanostructures can be enhanced with appropriate ligands to improve their stability and selectivity.

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