APPLICATION OF NANOMATERIALS IN SENSORS

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **APPLICATION OF NANOMATERIALS IN SENSORS** in the partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE** and submitted in the **DISCIPLINE OF PHYSICS Indian Institute of Technology Indore**, is an authentic record of my own work carried out during the time period from July 2016 to June 2017 under the supervision of Dr. Sudeshna Chattopadhyay, Associate Professor, Discipline of Physics IIT INDORE. The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other institute.

JITHIN T S

This is to certify that the above statement made by the candidate is correct to the best of my/our knowledge.

Dr. Sudeshna Chattopadhyay

JITHIN T S has successfully given his/her M.Sc. Oral Examination held on 4th July 2017.

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Abstract

Metal nanoparticles are of strong interest for their unusual optical and electronic properties with potential for new and improved device applications. Specifically, the scattering enabled by Surface Plasmon Resonance on metal nanoparticles open up the potential for the implementation of very sensitive sensors. It has been demonstrated that Surface Plasmon resonance can be used for the detection of a single molecule. Gold (Au) nanoparticles (NPs) are free electron metals which shows strong resonance peak in the visible spectral region and their biocompatibility making them suitable for biosensors. Au NPs that are soluble in aqueous solution over a broad range of pH and ionic strength values and that are capable of selective uptake by folate receptor positive (FR+) cancer cells. The method of sensing relies on the change in the absorbance spectrum of a self-assembled monolayer of colloidal gold on glass, as a function of biomolecular binding to the surface of the immobilized colloids. In this work, Au NPs are conjugated with ligands DUPA and Pteroate, which have component functional groups compatible with disease cells (eg: prostate-specific membrane antigen, folate cells etc). The conjugated Au NPs are characterized using various spectroscopic techniques including UV-Visible, FTIR. The analysis of UV-Visible data is performed using classical electrodynamic theory (Mie theory). Theoretical calculation of various physical parameters such as dielectric function of the ligand and embedded medium, thickness of ligand layer over Au nanoparticle, size of the Au NPs, are calculated from the theoretical analysis and by fitting the experimental data with the theoretical model.

The dependence of the optical properties of spherical Au NPs on particle size and wavelength were analyzed theoretically using Mie scattering theory, where the complex refractive index of Au was corrected for the effect of a reduced mean free path of the conduction electrons in small particles. To compare these theoretical results to experimental data, gold nanoparticles of the size 5 nm and 100 nm were characterized with SEM and UV-Vis spectroscopy. Excellent agreement is found between the theoretical model and experiment.

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ACRONYMS

NPs:	Nanoparticles
Au:	Gold
NSM:	Nanostructured materials
0D:	Zero dimension
1D:	One dimension
3D:	Three dimensions
FTIR:	Fourier Transform Infrared Spectroscopy
SEM:	Scanning Electron Microscopy

Chapter 1

INTRODUCTION

Nanoscience primarily deals with synthesis, characterization, exploration, and exploitation of nanostructured materials. These materials are characterized by at least one dimension in the nanometer range. A nanometer (nm) is one billionth of a meter, or 10^{-9} m [1]. Nanomaterials have attracted lots of attention due to their tremendous potential in extensive fields, ranging from biology, physics, and chemistry to electronics, and also already proven their superior functionality to conventional materials [2–4]. The unique property of nanomaterials comes from their size, morphology, and structure, and frequently a ligand shell structure plays a key role in their distinct behavior and feature as it determines an interaction with environments [4–8]. They finds vast range of potential applications in the fields of sensors, nano-electronics, optoelectronics, energy conversion [4,9]. The development of nanotechnology in conjunction with biotechnology has significantly expanded the application domain of nanomaterials in various fields. For the last decades imperative utilization of nanomaterials has acquired paramount importance in the field of biosensor and this has led to the invention of precise and powerful analytical tools using biological sensing element as biosensor [10]. Noble metal nanoparticles, due to their prominent property of biocompatibility, are treated as a good candidate for the biosensor development. Gold nanoparticles (AuNPs) provide non-toxic carriers for drug and gene delivery applications [11]. With these systems, the Au core imparts stability to the assembly, while the monolayer allows tuning of surface properties such as charge and hydrophobicity. An additional attractive feature of Au NPs is their interaction with thiols, providing an effective and selective means of controlled intracellular release [12]. In another approach, optically responsive delivery systems can be designed by incorporation of Au nanospheres with small molecule ligands. In this chapter we emphasize on nanomaterials and their structural classification

[13], role of AuNPs in biosensors and scattering theories for spherical objects.

1.1 Nanomaterials

These are the materials characterized by at least one dimension in the nanometer range. A nanometer (nm) is one billionth of a meter, or 10^{-9} m. One nanometer is approximately the length equivalent to 10 Hydrogen or 5 Silicon atoms aligned in a line. On nanoscale, some physical and chemical material properties can differ significantly from those of the bulk structured materials of the same composition [3]; for example, the theoretical strength of nanomaterials can be reached or quantum effects may appear [14]; crystals in the nanometer scale have a low melting point (the difference can be as large as 1000°C) and reduced lattice constants, since the number of surface atoms or ions becomes a significant fraction of the total number of atoms or ions and the surface energy plays a significant role in the thermal stability. Therefore, many material properties must now be revisited in light of the fact that a considerable increase in surface-to-volume ratio is associated with the reduction in material size to the nanoscale, often having a prominent effect on material performance. Historically, fundamental material properties such as elastic modulus have been characterized in bulk specimens using macroscopic and more recently microscopic techniques. However, as nanofabrication advances continue, these bulk properties are no longer sufficient to predict performance when devices are fabricated with small critical dimensions. Promising applications of nanomaterial in medicine and/or biology have attracted a lot of attention and have become a fast-growing field. One of the attractive applications in nanomedicine is the creation of nanoscale devices for improved therapy and diagnostics [11]. Such nanoscale devices or nanorobots serve as vehicles for delivery of therapeutic agents, detectors or guardians against early disease and perhaps repair of metabolic or genetic defects. For applications in medicine, the major challenge is "miniaturization": new instruments to analyze tissues literally down to the molecular level, sensors smaller than a cell allowing to look at ongoing functions, and small machines that literally circulate within a human body pursuing pathogens and neutralizing chemical toxins.

Researchers expect to develop new commercial applications for nanotechnology for the next several years. They include: advanced drug – delivering systems, including implantable devices that automatically administer drugs and sense drug levels; medical diagnostic tools, such as cancer – tagger mechanisms and "lab-on-a-chip" diagnostics for physicians; cooling chips or wafer to replace compressors in cars, refrigerators, air conditioners and other devices using no chemicals or moving parts; sensors for airborne chemicals or other toxins; solar fuel cells and portable power to provide inexpensive, clean energy; etc.[11,15,16].

1.2 Classification of nanostructured materials (NSMs)

One of the basic results of the materials science is the insight that most properties of solids depend on the microstructure. A reduction in the spatial dimension, or confinement of particles or quasi-particles in a particular crystallographic direction within a structure generally leads to changes in physical properties of the system in that direction. Hence the classification of nanostructured materials and systems essentially depends on the number of dimensions which lie within the nanometer range [13]:

1.2.1 Two-dimensional nanostructures (2D): 2D nanostructures (or quantum well) [13,17] have two dimensions outside of the nanometric size range; e.g. filamentary structures where the length is substantially greater than the cross-sectional dimensions 2D nanostructures have been extensively studied by the semiconductor community because they can be conveniently prepared using techniques such as molecular beam epitaxy (MBE) [18].



Figure 1 (a): Schematic representation of two dimensional nano structure. Along z direction dimension of the structure is reduced in nanoscale.

1.2.2 One-dimensional nanostructures: Nanostructures – wires rods, tubes and belts – whose lateral dimensions fall anywhere in the range of 1-100 nm. It is generally accepted that one dimensional (1D) nanostructures provide a good system to investigate the dependence of electrical and thermal transport or mechanical properties on dimensionality and size reduction (or quantum confinement) [13,19]..



Figure 1 (b): Schematic representation of one dimensional nano structure. Along z and x direction dimension of the structure is reduced in nanoscale.

1.2.3 Zero-dimensional nanostructures : Zero dimensional (0D) structures are the systems confined in three dimensions, e.g. structures typically composed of consolidated equiaxed crystallites; The most common representation of zero-dimensional nanomaterial is nanoparticles or quantum dots and nanopores [20].



Figure 1 (c): Schematic representation of one dimensional nano structure. Along z, x and y direction dimension of the structure is reduced in nanoscale.

1.3 Sensors

In the broadest definition, a sensor is an object whose purpose is to detect events or changes in its environment, and then provide a corresponding output. Major part of a sensor is transducer whose purpose is to convert one form of signal to another easily accessible form. A simple sensor classification based on transducer action are: biosensor, physical sensor and chemical sensor [21,22]. A self-contained integrated device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is in direct spatial contact with a transducer element is called biosensor (e.g. glucose sensor) [9]. Physical sensor is a device detects physical stimulus (e.g.: light intensity, temperature etc.) and chemical sensor transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal (e.g.: moisture sensor) [21] .Designing a good sensor depends on many factors which includes, sensitivity, range, stability, repeatability, linearity, error time and frequency response.



Figure 2: Components of biosensor

1.3.1 Role of nanomaterials in biosensors

The nanostructured materials (NSMs) employed in sensor development include nanowires, semiconductor particles, various allotropes of carbon and imprinted polymeric spheres [23]. Nanoparticles, in general, exhibit physical properties that not only differ from the parent bulk material, but also from other nanoparticles that are of different dimensions. This uniqueness offers more opportunity to fine-tune a sensor in order to discriminately detect one component of a complex mixture. Nanomaterials have shown tremendous potentials to impact the broad field of biological sensing. One of the main challenge in bio-sensor development is the efficient signal capture of bio-recognition event[24]. Nanomaterials are promising candidates in order to increase sensitivities and lower detection limit down to even an individual molecule due to their ability to immobilize an enhanced quantity of bioreceptor units at reduced volume. Nanomaterials, with extremely small sizes and appropriate surface modifications, allow intimate interaction with target biomolecules. A variety of nanostructured materials with unique optical, electric, magnetic, electrochemical and thermal signatures have been used to detect molecular biomarkers with extreme high sensitivities [4,7,25].

The sensing mechanism is based on converting target binding events into physical signals that can be amplified and detected. Nanomaterials have been widely used for in vitro detection of molecular biomarkers of many diseases (such as cancer, neurodegenerative diseases, and infectious diseases, among others) released into patient's body fluids during disease progression. Biomarkers are molecules that increase in concentration during the onset of cancer, and they can be measured in serum and tissue for early cancer detection [11]. Accurate, sensitive measurement of proteins is of general importance to a broad range of applications in addition to cancer detection and monitoring, including systems biology, medical diagnostics for other diseases, proteomics, and drug development. Measurement of collections of protein cancer biomarkers is the most promising for reliable early cancer detection. For example, detecting 5 or more biomarkers for a given cancer by liquid chromatography-mass spectrometry (LC-MS) has provided nearly 100% reliable diagnostics [26]. In contrast, single biomarkers often have low positive predictive value.

1.4 Gold nanomaterials

Au based structures such as NPs have widely been used as building blocks for sensing devices in chemistry and biochemistry fields because of their unusual optical, electrical and mechanical properties [27]. Au NPs, with diameter between 1 nm and 100 nm, have been extensively used in chemical and biological sensors because of their excellent physical and chemical properties. When illuminated with electromagnetic waves, Au NPs exhibit a characteristic phenomenon which is known as surface plasmon resonance (SPR) and it depends greatly on particle size and shape [22]. The SPR peak is tunable by manipulating the size of Au NPs, and this property cannot be observed on bulk Au and Au NPs with a diameter smaller than 2 nm. The SPR peak is not only sensitive to size and the shape, but also many factors such as protective ligand, refractive index of solvent, inter particle distance and temperature [28]. Therefore, the redshifting and the broadening of the peak are observed when Au NPs are aggregated due to analyte binding. Aptamers are single-stranded DNA or RNA molecules that can bind target molecules with high affinity and specificity [29]. The conformation of an aptamer usually changes upon binding to its target analyte, and this property has been used in a wide variety of sensing applications, including detection based on fluorescence intensity, polarization, energy transfer, electrochemistry or color change. Researchers have described a protocol for the preparation of aptamer-linked Au NPs purple aggregates that undergo fast disassembly into red dispersed nanoparticles upon binding of target analytes. This method has proved to be generally applicable for colorimetric sensing of a broad range of analytes. Apart from absorption and scattering properties, Au NPs also display fluorescence properties which could be utilized in sensor fabrication. Photoluminescence can be generated from GNPs under certain conditions, such as laser and ultraviolet (UV) excitation [30]. Au NPs can enhance or quench the fluorescence of fluorophore depending on the distance between the fluorophore and the particle [31]. In addition to all these, Au NPs features include bio-compatibility, good optical and electronic properties, relatively simple production and modification [4,12].

1.5 Plasmons

The electronic and optical properties of NPs are determined by both their size and shape. Metal NPs have mainly been studied because of their unique optical properties. Nanoparticle colloidal solutions of noble metals, copper, silver, and Au show very intense color, which is absent in the bulk material as well as for the individual atoms. Their origin is attributed to the collective oscillation of the free conduction electrons induced by an interacting electromagnetic field [32]. These resonances are also denoted as surface plasmons. Mie was the first to describe them quantitatively by solving Maxwell's equations with the appropriate boundary conditions for spherical particles [33]. The total extinction cross section composed of absorption and scattering is given as a summation over all electric and magnetic multipole oscillations. The Mie theory has the advantage of being conceptually simple and has found wide applicability in explaining experimental results [33]. One of the most simple but nevertheless very utile models to describe the response of a metallic particle exposed to an

electromagnetic field was proposed by Paul Drude at the beginning of the 20th century and further extended by Hendrik Lorentz five years later [22]. Drude assumed a microscopic description of the electron dynamics in a metal in classical terms, and obtained the equation of motion of a damped oscillator where the electrons are moving between heavier, relatively immobile background ions:

Drude-Sommerfeild model of a free electron gas [34]

$$m_e \frac{\partial^2 r}{\partial t^2} + m_e \gamma_d \frac{\partial r}{\partial t} = e E_0 e^{-i\omega t}$$

where γ_d describes a phenomenological damping term, m_e the effective free electron mass, e the free electron charge and ω and E_0 are the frequency and amplitude of the applied electric field respectively. Solving above equation which directly leads to the dielectric function of Drude form

$$\in (\omega) = \in^{\infty} - \frac{\omega_p^2}{(\omega^2 + i\gamma_d \omega)}$$
 where $\omega_p^2 = \frac{4\pi N e^2}{m_0}$

Here ω_p is the volume or bulk plasma frequency (electron density, $n_e = 3/(4\pi r_s^3)$, r_s is the electron gas parameter and takes the value 0.16 nm for Au and silver and \in^{∞} describes the ionic background in the metal[4]. If we neglect γ_d and \in^{∞} for the moment, the Drude dielectric function simplifies to $\in (\omega) = 1 - \omega_p^2 / \omega^2$ and we can distinguish two frequency regions: If ω is larger than ω_p , $\in (\omega)$ is positive and the corresponding refractive index $n = \sqrt{\in} (\omega)$ is a real quantity. But if ω is smaller than ω_p , $\in (\omega)$ becomes negative and *n* is imaginary. An imaginary refractive index implies that an electromagnetic wave cannot propagate inside the medium.

1.6 Mie scattering and Rayleigh scattering

The Mie theory and Rayleigh approximation are two basic methods to study the electromagnetic (EM) scattering of uncharged spherical particle, and when the particle radius is much smaller than the incident wavelength, they are equivalent, but whether the Rayleigh approximation is still equivalent to Mie theory when we use them to calculate the EM scattering of small charged particle, there is still no any report published to discuss this problem [35].

Formal light scattering theory may be categorized in terms of two theoretical frameworks. One is the theory of Rayleigh scattering that is, strictly speaking as originally formulated, applicable to small, dielectric (non-absorbing), spherical particles [35,36]. Another theory is the Mie scattering [37] that encompasses the general spherical scattering solution (absorbing or non-absorbing) without a particular bound on particle size. Accordingly, Mie scattering theory has no size limitations and converges to the limit of geometric optics for large particles. Mie theory, therefore, may be used for describing most spherical particle scattering systems, including Rayleigh scattering. However, Rayleigh scattering theory is generally preferred if applicable, due to the complexity of the Mie scattering formulation. The criteria for Rayleigh scattering is that $x \ll 1$ and $|m| x \ll 1$, where x is the dimensionless size parameter given by the expression [35]

 $x = \frac{2 \pi r}{\lambda}$, where x is the spherical particle radius, and λ is the scattering wavelength and *m* is the complex refractive index of the scattering particle commonly represented as

m = n - ik. In this notation, *n* indicates the refraction of light (i.e. n equals the speed of light in vacuum divided by the speed of light in the material), while the complex term is related to absorption. The magnitude of the refractive index m, as needed for the Rayleigh criteria, is given by the expression $|m| = (n^2 + k^2)^{1/2}$

When the particle is charged, the values from Rayleigh approximation are much larger than the ones from Mie theory, and with the inclination angle increasing, the value from Rayleigh approximation keeps a constant [35,36], but for Mie theory it has a step change, for example, the scattering intensity in particle charged zone is much smaller than the one when particle uncharged, but for the zone where no charge distributed, the scattering intensity equal to the value when particle entirely uncharged. So, the Rayleigh approximation and the Mie theory are not still equivalent when used them to calculate the scattering field of charged spherical particle. That's because Rayleigh method is an electrostatic approximation, which just consider the modification of the dipole polar moment in particle interior, but ignored the effect of incident electromagnetic wave on the movement of net electron which distribute on the particle surface. However, the Mie method which based on Helmholtz equation hasn't this shortage, so the Rayleigh approximation and the Mie theory are not still equivalent in this condition.

Chapter 2

Theoretical background

2.1 Formulation of theoretical model using Mie theory for Homogeneous sphere

An electromagnetic field incident on an object will be scattered in all directions. Hence, incident and scattered fields have different properties, which depend on the physical (optical properties) and geometrical (size and shape) characteristics of the target and its surroundings [33,38]. Mie theory presents the solution for the electromagnetic scattering by a sphere of radius, R embedded in a homogeneous and isotropic medium illuminated by a plane wave.

Mie theory of light scattering by a homogeneous sphere is used for many problems of nanomaterials, atmospheric, optic and also in other fields in physics. The application of Mie theory still needs modern computers for numeric calculations of many functions and coefficients involved [31].

The two independent variables in Mie theory are the

- (1) size parameter $x = \frac{2 \pi r}{\lambda}$ where *r* is the radius of scattering sphere, λ wavelength of incident plane wave.
- (2) Complex refractive index of sphere relative to surrounding medium.

The key parameters for Mie calculations are the Mie Coefficients a_n and b_n to compute the amplitudes of the scattered field. The coefficients are determined by the boundary conditions of the fields at the sphere surface [33].

The Mie total extinction efficiency Q_{ext} for a homogeneous sphere is expressed as infinite series:

$$Q_{\text{ext}} = \frac{2\pi}{x^2} \sum (2n+1) \operatorname{Re}\left(a_n^2 + b_n^2\right)$$
(1)

Where a_n and b_n are expressed as

$$a_n \qquad \frac{\Psi_n(\alpha)\Psi_n^{\dagger}(x) - m\Psi_n(mx)\Psi_n^{\dagger}(x)}{\xi(x)\Psi_n^{\dagger}(mx) - m\Psi_n(mx)\xi_n^{\dagger}(x)} \tag{2}$$

$$b_n = \frac{m\Psi_n(x)\Psi_n^{\dagger}(x) - \Psi_n(mx)\Psi_n^{\dagger}(x)}{\xi(x)\Psi_n(mx) - m\Psi_n(mx)\xi_n(x)}$$
(3)

where *m* is the ratio of refractive index of the sphere *n* to that of the surrounding medium n_m , *x* is the size parameter given as $2\pi n_m R/\lambda$, Ψ_n and ξ are the Riccati-Bessel functions, and the prime represents first differentiation with respect to the argument in parentheses. The Index *n* runs from 1 to ∞ , but the infinite series occurring in Mie formulas can be truncated at a maximum, n_{max}

$$n_{max} = x + 4 x^{\frac{1}{3}} + 2 \tag{4}$$

Below characteristic sizes (2 R << λ) the electrodynamic effect of the local field enhancement, described by Mie theory, become independent of radius of homogeneous sphere. In this case retardation effects that lead radiative plasmon damping disappear and the extinction cross section is given by dipole absorption, only

$$Q_{ext} = \frac{9\epsilon_m^{\frac{3}{2}} V_0 \omega}{c} \frac{\epsilon_2}{[\epsilon_1 + 2\epsilon_m]^2 + \epsilon_2^2}$$
(5)

 V_0 is the spherical particle volume and \in_m and $\in (\omega) = \in_1 (\omega) + \in_2 (\omega)$ denote the dielectric functions of the surrounding medium and particle material respective. The cluster properties are thus attributed to the material properties of the bulk material. This cross section has a narrow resonance whose position λ_1 and the shape is governed merely by the dielectric function and the dipole resonance frequency is determined by the condition

$$\epsilon_1(\omega) = -2 \cdot \epsilon_m \quad (if \ \epsilon_2 \ is \ small) \tag{6}$$

2.2 Mie theory for Core – shell spherical nanoparticle particle

Efficiency equation (1) remains same for core-shell nanoparticles but their Mie coefficients a_n and b_n gets modified and are given by [39]

$$b_n = \frac{\Psi_n(y) \left[\Psi_n^{\dagger}(n_2 y) - A_n \chi_n^{\dagger}(n_2 y) \right] - n_2 \Psi_n^{\dagger}(y) \left[\Psi_n(n_2 y) - A_n \chi_n(n_2 y) \right]}{\xi_n(y) \left[\Psi_n^{\dagger}(n_2 y) - n_2 \chi_n^{\dagger}(n_2 y) \right] - \xi_n^{\dagger}(y) \left[\Psi_n(n_2 y) - A_n \chi_n(n_2 y) \right]}$$
(7)

$$b_n = \frac{n_2 \Psi_n(y) \left[\Psi_n^{\dagger}(n_2 y) - B_n \chi_n^{\dagger}(n_2 y) \right] - \Psi_n^{\dagger}(y) \left[\Psi_n(n_2 y) - B_n \chi_n(n_2 y) \right]}{n_2 \xi_n(y) \left[\Psi_n^{\dagger}(n_2 y) - A_n \chi_n^{\dagger}(n_2 y) \right] - \xi_n^{\dagger}(y) \left[\Psi_n(n_2 y) - B_n \chi_n(n_2 y) \right]}$$
(8)

Where A_n and B_n are given by

$$A_n = \frac{n_2 \Psi_n(n_2 x) \Psi_n^{\dagger}(n_1 x) - n_1 \Psi_n^{\dagger}(n_2 x) \Psi_n(n_1 x)}{n_2 \chi_n(n_2 x) \Psi_n^{\dagger}(n_1 x) - n_1 \chi_n^{\dagger}(n_2 x) \Psi_n(n_1 x)}$$
(9.1)

$$B_n = \frac{n_2 \Psi_n(n_1 x) \Psi_n^{\dagger}(n_2 x) - n_1 \Psi_n^{\dagger}(n_2 x) \Psi_n(n_1 x)}{n_2 \chi_n^{\dagger}(n_2 x) \Psi_n(n_1 x) - n_1 \chi_n(n_2 x) \Psi_n^{\dagger}(n_1 x)}$$
(9.2)

In these expressions, n_1 and n_2 are the refractive indices of the core and shell relative to the surrounding medium, $x = k r_1$ and y = k. r_2 are the size parameter of core and shell nanoparticle respectively. $r_1 r_2$ are the radius of core and shell, respectively and Ψ , ξ and Ψ^{\dagger} , ξ^{\dagger} are Riccati–Bessel functions and their derivatives [33].

The extinction cross section (Q_{ext}) of a small concentric sphere that is below characteristic size is given by,

$$Q_{ext} = 4 . x Im \left\{ \stackrel{(\in_{shell} - \epsilon_m)(\in_{core} - 2 \in_{shell}) + (1-g)(\in_{core} - \epsilon_{shell})(\in_m + 2 \in_{shell})}{(\epsilon_{shell} + 2 \epsilon_m)(\in_{core} + \epsilon_{shell}) + (1-g)(2 \in_{shell} - \epsilon_m)(\in_{core} - \epsilon_{shell})} \right\}$$
(10)

Where $\in_{core} (or \in_c)$ is the complex dielectric function of the core material, $\in_{shell} (or \in_s)$ is that of shell, \in_m is the dielectric function of surrounding medium, g is the volume fraction of the shell layer and x is the size parameter [33,40].

$$g = \frac{\left[\left(R_{core} + R_{shell}\right)^3 - R_{core}^3\right]}{\left(R_{core} + R_{shell}\right)^3} \tag{11}$$

 $x = \frac{2\pi R \epsilon_m^{1/2}}{\lambda}$. R, λ are radius of sphere and wavelength respectively.

Extinction coefficient (EC) of the metal particle is given by

EC = $\frac{Q_{ext} V_F}{(2.303R)}$ where V_F is the Fermi velocity. According to Drude freeelectron model, the complex dielectric function $\in (\omega)$ of core is,

 $\in_{core} (\omega) = \in^{\infty} - \frac{\omega_p^2}{\omega(\omega + \frac{i}{\tau})}, \ \omega_p$: bulk metal optical plasmon resonance frequency and $\omega_p^2 = \frac{4\pi N e^2}{m_0}, \ \in^{\infty}$: dielectric constant of bulk metal.

In the case of many metals, the region of absorption up to the bulk plasma frequency (in the UV) is dominated by the free electron behavior, and the dielectric response is well described by the simple Drude model. According to this theory, the real and imaginary parts of the dielectric function may be written as $\in_1 = \in^{\infty} - \frac{\lambda^2}{\lambda_p^2}$, $\in_2 = \frac{\lambda^3}{\lambda_p^2} \tau^*$, $\tau^* = 2\pi c \tau$, τ : relaxation time.

Therefore,

$$\epsilon_{core} (\omega) = \epsilon^{\infty} - \frac{\lambda^2}{\lambda_p^2} + i \frac{\lambda^3}{\lambda_p^2} \tau^*$$

$$= \epsilon_1 + i \epsilon_2$$
(12)

equation (12) in (11) gives

$$\therefore Q_{ext} = 4. x. Im\left(\frac{N_1 + N_2}{D_1 + D_2}\right)$$
(13)

Where,

$$N_{1} = (\epsilon_{s} - \epsilon_{m})(\epsilon_{1} + i \epsilon_{2} - 2 \epsilon_{s})$$

$$N_{2} = +(1 - g)(\epsilon_{1} + i \epsilon_{2} - \epsilon_{shell})(\epsilon_{m} + 2 \epsilon_{shell})$$

$$D_{1} = (\epsilon_{s} + 2 \epsilon_{m})(\epsilon_{1} + i \epsilon_{2} + \epsilon_{s})$$

$$D_{2} = (1 - g)(2 \epsilon_{s} - \epsilon_{m})(\epsilon_{1} + i \epsilon_{2} - \epsilon_{s})$$
Simplifying equation (13)

Simplifying equation (13),

$$\Rightarrow Q_{ext} = 4. x. Im \left(\frac{N_3 + N_4}{D_3 + D_4}\right)$$

Where,

$$N_{3} = (\epsilon_{s} - \epsilon_{m})(\epsilon_{1} + 2 \epsilon_{s}) + (1 - g)(\epsilon_{1} - \epsilon_{s})(\epsilon_{m} + 2 \epsilon_{s})$$

$$N_{4} = (\epsilon_{s} - \epsilon_{m}) \epsilon_{2} i + (1 - g)(\epsilon_{m} + 2 \epsilon_{s}) \epsilon_{2} i$$

$$D_{3} = (\epsilon_{s} + 2 \epsilon_{m})(\epsilon_{1} + 2 \epsilon_{s}) + (1 - g)(2 \epsilon_{s} - 2 \epsilon_{m})(\epsilon_{1} - \epsilon_{s})$$

$$D_{4} = \epsilon_{2} i[(\epsilon_{s} + 2 \epsilon_{m}) + (1 - g)(2 \epsilon_{s} - 2 \epsilon_{m})]$$

On rearranging,

$$\Rightarrow Q_{ext} = 4. x. Im\left(\frac{(N_5 + N_6)(N_7 - N_8)}{(D_5 + D_6)(D_7 - D_7)}\right)$$
(14)

Where,

$$\begin{split} N_{5} &= (\in_{s} - \in_{m})(\in_{1} + 2 \in_{s}) + (1 - g)(\in_{1} - \in_{s})(\in_{m} + 2 \in_{s}) \\ N_{6} &= (\in_{s} - \in_{m}) \in_{2} i + (1 - g)(\in_{m} + 2 \in_{s}) \in_{2} i \\ N_{7} &= (\in_{s} + 2 \in_{m})(\in_{1} + 2 \in_{s}) + (1 - g)(2 \in_{s} - 2 \in_{m})(\in_{1} - \in_{s}) \\ N_{8} &= \in_{2} i[(\in_{s} + 2 \in_{m}) + (1 - g)(2 \in_{s} - 2 \in_{m})] \\ D_{5} &= (\in_{s} + 2 \in_{m})(\in_{1} + 2 \in_{s}) + (1 - g)(2 \in_{s} - 2 \in_{m})(\in_{1} - \in_{s}) \\ D_{6} &= \in_{2} i[(\in_{s} + 2 \in_{m}) + (1 - g)(2 \in_{s} - 2 \in_{m})] \\ D_{7} &= (\in_{s} + 2 \in_{m})(\in_{1} + 2 \in_{s}) + (1 - g)(2 \in_{s} - 2 \in_{m})(\in_{1} - \in_{s}) \\ D_{8} &= \in_{2} i[(\in_{s} + 2 \in_{m}) + (1 - g)(2 \in_{s} - 2 \in_{m})] \\ Substituting for &\in_{1} = \in^{\infty} - \frac{\lambda^{2}}{\lambda_{p}^{2}} \text{ and } \in_{2} = \frac{\lambda^{3}}{\lambda_{p}^{2}} \tau^{*} \text{ in equation (14)} \end{split}$$

$$\Rightarrow Q_{ext} = 4x \frac{\lambda^3}{\lambda_p^2} \tau^* \left(\frac{V_1 - V_2}{V_3 + (\frac{\lambda^3}{\lambda_p^2} \tau^*)^2 V_4} \right)$$
(15)

Where,

$$V1 = [(\epsilon_{s} - \epsilon_{m}) + (1 - g)(\epsilon_{m} + 2 \epsilon_{s})] \left[(\epsilon_{s} + 2 \epsilon_{m}) \left(\epsilon^{\infty} - \frac{\lambda^{2}}{\lambda_{p}^{2}} \right) + (1 - g)(2 \epsilon_{s} - 2 \epsilon_{m}) \left(\epsilon^{\infty} - \frac{\lambda^{2}}{\lambda_{p}^{2}} - \epsilon_{s} \right) \right]$$

$$V2 = [(\epsilon_{s} + 2 \epsilon_{m}) + (1 - g)(2 \epsilon_{s} - 2 \epsilon_{m})] [(\epsilon_{s} + 2 \epsilon_{m})]$$

$$V3 = [(\epsilon_{s} + 2 \epsilon_{m})(\epsilon^{\infty} - \frac{\lambda^{2}}{\lambda_{p}^{2}} + 2\epsilon_{s}) + (1 - g)(2 \epsilon_{s} - 2 \epsilon_{m})(\epsilon^{\infty} - \frac{\lambda^{2}}{\lambda_{p}^{2}} - \epsilon_{s})]^{2}$$

$$V4 = [(\epsilon_{s} + 2 \epsilon_{m}) + (1 - g)(2 \epsilon_{s} - 2\epsilon_{m})]^{2}$$

 \therefore expression for extinction coefficient becomes

$$EC = \frac{V_F}{(2.303R)} \times 4 \frac{2\pi R \epsilon_m^{1/2}}{\lambda} \frac{\lambda^3}{\lambda_p^2} \tau^* \left(\frac{V_{1-V_2}}{V_{3+(\frac{\lambda^3}{\lambda_p^2} \tau^*)^2 V_4}} \right)$$

or
$$EC = 10.92 \frac{V_F \epsilon_m^{1/2}}{\lambda} \frac{\lambda^3}{\lambda_p^2} \tau^* \left(\frac{V_{1-V_2}}{V_{3+(\frac{\lambda^3}{\lambda_p^2} \tau^*)^2 V_4}} \right)$$
(16)

In order to include both the peaks (bare Au and thiolate), above equation is modified as,

EC= 10.92
$$\frac{\epsilon_m^{1/2}}{\lambda} \frac{\lambda^3}{\lambda_p^2} \tau^* \left(\frac{V_{1-V_2}}{V_3 + (\frac{\lambda^3}{\lambda_p^2} \tau^*)^2 V_4} \right) + A \frac{w}{((x-x_c)^2 + (\frac{w}{2})^2)}$$
(17)

The monolayer of the shell coating of the Au core modifies the condition for plasmon oscillations. Surface plasmon modes occur in the composite particle when the denominator in equation (16)

is zero, i.e., when

$$\epsilon_c = -2.\epsilon_s \frac{(\epsilon_s.g + \epsilon_m (3 - g))}{\epsilon_s (3 - 2.g) + 2.\epsilon_m.g)}$$

For monolayer shells that are thin relative to the core, $g \ll 1$, the condition for resonance becomes

$$\epsilon_c = -2. \epsilon_m - 2. g \frac{(\epsilon_s - \epsilon_m)}{3}$$

which reduces to the usual resonance condition for uncoated spheres as $g \rightarrow zero$:

$$\epsilon_1(\omega) = -2 \epsilon_m$$

2.3 Size dependence of optical material function

Mie theory for metal clusters shows cluster-size effects in the optical spectra. This leads to theoretical models for the size dependencies of the dielectric functions and the definition of a relevant parameter which can be compared to experiment. Within the Drude-Lorentz-Summerfield free-electron model [4,41]

$$\in (\omega) = \ \epsilon^{\infty} - \frac{\epsilon_p^2}{\omega^2 + i\omega\gamma}$$

Where the plasma frequency $\omega_p = \frac{n e^2}{\epsilon_0 m_{eff}}$ depends on the electron density n and on the proper electron effective mass m_{eff} . Using the expression for $\in (\omega)$ in equation (Q_{ext} for bare) and putting value of g = 0, and rewriting equation (17) in terms of a simple Lorentzian, the full width at half maxima being given by phenomenological damping constant γ .

$$Q_{ext} = C_0 \frac{\gamma}{(\omega - \omega 1)^2 + (\frac{\gamma}{2})^2}$$
(18)

In classical theory of free electron metals, the damping is due to the scattering of the electrons with phonons, electrons, lattice defects, or impurities. Mean free path (MFP) in Au is about 45 nm with an electron phonon collision time of 35 fs. For very small particles these interactions (collisions) of the conduction electrons with the particle surface dominate and this results in a reduced effective MFP. According to this model, the damping constant γ then depends on the particle radius *R*:

$$\gamma(R) = \gamma_{bulk} + \frac{AV_F}{R} \tag{19}$$

where **A** includes details of the scattering processes. The approximation can be viewed in another form,

$$k . a \ll 1 \tag{20}$$

 $k = 2 \pi / \lambda$ is the wave vector. For the Au NP, the equation (20) ideally holds good for particle size less than 25 nm [30]

Chapter 3

EXPERIMETAL TECHNIQUES

3.1 Fourier Transform IR Spectroscopy

Fourier-transform infrared spectroscopy (FTIR) is a vibrational spectroscopic technique [42], meaning it takes advantage of asymmetric molecular stretching, vibration, and rotation of chemical bonds as they are exposed to designated wavelengths of light. Fourier transform is to transform the signal from the time domain to its representation in the frequency domain. In Infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis [42,43]. FTIR is a method for measuring all of the infrared frequencies simultaneously, rather than individually, was needed. A solution was developed which employed a very simple optical device called an interferometer. The interferometer produces a unique type of signal which has all of the infrared frequencies "encoded" into it. The signal can be measured very quickly, usually on the order of one second or so. Thus, the time element per sample is reduced to a matter of a few seconds rather than several minutes. FTIR can provide the following information [42]:

- It can identify unknown materials
- It can determine the quality or consistency of a sample
- It can determine the number of components in a mixture



Figure 3: Schematic representation of FTIR measurement.

In this work, FTIR spectra of Au NPs conjugated with small ligands in the range of 500–4000 cm⁻¹ were recorded using Fourier Transform Infrared Spectrometer (FTIR), Tensor 27, BRUKER. FT-IR spectroscopy provides information about the components (functional group) of the conjugated system.

3.2 UV-Visible spectroscopy

Molecular spectroscopy that involves study of the interaction of Ultra violet (UV)-Visible radiation with molecules is called UV-Vis spectroscopy [45]. The visible and ultra violet spectra of ions and molecules are associated only with the transitions between the electronic energy levels of certain types or functional groups of molecules. UV range extends from 100-400 nm, of which 100-190 nm range is known as ultraviolet and 190-400 nm.

The absorption of radiation involves transfer of energy to the medium and this process is a specific phenomenon related to the characteristic molecular structure [45]. For a given excitation process a molecule absorbs a discrete quantity of energy corresponding to only one frequency which should give rise to an absorption line. However, since a group of same molecules exist in a number of vibrational and rotational energy state corresponding to each electronic level, a series of absorption peak or band is obtained. The UV– vis absorption spectra of bare Au NPs and small molecule conjugated Au NPs dispersed in buffer were monitored by UV-Vis spectrophotometer (Perkin Elmer lamba-35). Results are as given in Fig.6.

3.3 Scanning Electron microscopy (SEM)

The SEM is a microscope that uses electrons instead of light to form an image. The main components of a typical SEM are electron column, scanning system, detector(s), display, vacuum system and electronics controls [46]. The electron column of the SEM consists of an electron gun and two or more electromagnetic lenses operating in vacuum. The electron gun generates free electrons and accelerates these electrons to energies in the range 1-40 keV in the SEM. The purpose of the electron lenses is to create a small, focused electron probe on the specimen. Most SEMs can generate an electron beam at the specimen surface with spot size less than 10 nm in diameter while still carrying sufficient current to form acceptable image. Typically the electron beam is defined by probe diameter (d) in the range of 1 nm to 1 μ m, probe current (i_b) – pA to μ A; and probe convergence (α) – 10⁻⁴ to 10⁻² radians [46,47].

In order to produce images, the electron beam is focused into a fine probe, which is scanned across the surface of the specimen with the help of scanning coils. Each point on the specimen that is struck by the accelerated electrons emits signal in the form of electromagnetic radiation. Selected portions of this radiation, usually secondary (SE) and/or backscattered electrons (BSE), are collected by a detector and the resulting signal is amplified and displayed on a TV screen or computer monitor. The resulting image is generally straightforward to interpret, at least for topographic imaging of objects at low magnifications. The electron beam interacts with the specimen to depth approximately 1 μ m. Complex interactions of the beam electrons with the atoms of the specimen produce wide variety of radiation. The need of understanding of the process of image formation for reliable interpretation of images arises in special situations and mostly in the case of high-magnification imaging. In such case knowledge of electron optics, beam-specimen interactions, detection, and visualization processes

is necessary for successful utilization of the power of the SEM. In this study, we investigated Au NPs using Field Emission Scanning Electron Microscope (FE-SEM), Supra-55 Zeiss.

Chapter 4

RESULTS AND DISCUSSIONS

4.1 FTIR measurements and results

Optical measurements of ligands DUPA, Pteroate and bare silicon substrate were carried out using Bruker Tensor 27 FTIR spectrometer with wavenumber range 4500-500 cm⁻¹. Figure.4 shows the FTIR spectrum of small ligands DUPA, Pteroate and its conjugation on Au NP of size dimensions 5 and 100 nm.



Figure 4 (a): FTIR spectra of Pteroate



Figure 4 (c): FTIR Spectra of 5 nm Au NP conjugated with Pteroate



Figure 4 (d): FTIR Spectra of 5 nm Au NP conjugated with DUPA



Figure 4 (e): FT-IR Spectra of 100 nm Au NP conjugated with Pteroate.



Figure 4 (f): FT-IR Spectra of 100 nm Au NP conjugated with DUPA



Figure 5 (b): Structure of DUPA

Functional	Observed	peak position	Expected peak position	
group	(c <i>m</i> ⁻¹)		(c <i>m</i> ⁻¹)	
	Pteroate	DUPA		
	ligand	ligand		
N-H stretch –	3304	3301	3400-3250	
Amines,				
Amides				
O-Si-O	803.2	788.8	798	
			3217, 3210 (symmetric	
С-Н	3223.5,	3217.4,	stretches), 3185	
	3210.4,	2307.6,	(asymmetric stretch)	
	3183.5	3183.1		
C-0	1400.4	1402.7	1400 (carboxylic acid)	

Table 1: Results from FTIR measurement

It is well known that vibrational properties are a manner of structural characterization, and a great number of reports present the infrared (IR) and Raman spectra covering the mid- IR range, corresponding to modes with a fundamental frequency of $350-3500 \text{ cm}^{-1}$ [33]. These modes are localized in the organic tail-group (R-), allowing one to establish that it remains intact; the R-group conformation and ordering may also be deduced from the details of such spectra. Unfortunately, this region is notoriously insensitive to the structure and bonding in the headgroup region, that is, the Au–S bonding network, as well as to the Au–Au modes of the core.

From the FTIR spectrum presence of functional groups such as NH, CH₂, CH, CO in ligands are detected. Hence, we confirm about the conjugation of ligands onto the Au NPs while studying the spectra of Au NPs conjugated with ligands. The thiol group which is active in both IR and Raman mode but Raman spectroscopy can produce proper intense peak

compared to the IR spectrometer result. Unfortunately, thiol stretching frequency is not obtained from FTIR spectra of this study. In general, for thiol detection Raman spectroscopy is the preferred one.

4.2 UV-Vis measurements and results

Figure.6 shows the UV-Vis spectra of bare Au NP and its conjugation with small ligands DUPA and Pteroate. It is observed that a spectral blue shift that depends on the nanoparticle size. As the size of the nanoparticle increases, surface plasmon peak position is shifted towards the longer wavelength region. In addition to the peak position, shape and width of absorbance spectra also depends on the size of nanoparticle.



Figure 6 (a): UV-Vis spectra of 5 nm Au NP conjugated with DUPA.



Figure 6 (b): UV-Vis spectra of 5 nm Au NP conjugated with Pteroate.



Figure 6 (c): UV-Vis spectra of 100 nm Au NP conjugated with DUPA.



Figure 6 (e): UV-Vis spectra of 5 nm bare Au NP.



Figure 6 (f): UV-Vis spectra of 100 nm bare Au NP.

From the UV-Vis spectra (Fig.6) it is clear that, resonant wavelength redshifts strongly with increasing dimension of core (Au). Red shift observed in the resonant peak of coated NPs with respect to the bare NPs, indicates the successful conjugation ligand over the surface of Au NPs. For 5nm Au NP peak shift is 4.4 ± 0.3 nm, and for 100 nm it is 5.8 ± 0.3 nm. Also, Au NP conjugated with ligands shows larger full width at half maxima (FWHM) than bare Au NPs.



4.3 Fitting of the experimental data with Theoretical model from Mie theory

Figure 7 (a): Theoretical fitting of spectra of Au NP conjugated with DUPA using equation (17). Red dotted line represents calculated data. Black line represents experimental data.



Figure 7 (b): Theoretical fitting of spectra of Au NP conjugated with Pteroate using equation (17). Red dotted line represents calculated data. Black line represents experimental data.

Figure 7 (a) represents the calculated absorbance (dashed line) spectra of Au NP conjugated with Pteroate using equation (17). Solid line represents experimental spectra. For the calculation of extinction coefficient, the bulk Au dielectric constant (\in^{∞}) is taken as 12.2 and bulk Au plasmon wavelength (λ_p) is taken the value 131 nm [40]. In the absorbance versus wavelength spectrum, two peaks are observed in which one peak is due to the Au NPs and the other one is contributed by the small molecule conjugated on the surface of the Au nanoparticle. A good theoretical fit is obtained which matches with the experimental data. Fitting parameters includes dielectric constant of shell, dielectric constant of medium and volume fraction. Value obtained volume fraction from fitting data is used for the further calculations for finding thickness of the small molecules conjugated with the Au NPs.

Material	Dielectric	Dielectric	Volume fraction	
	constant of	constant of	(dimensionless)	
	shell	medium		
Au NP	2.13±0.03	2.11±0.02	0.50±0.002	
conjugated				
with DUPA				
Au NP	2.14 <u>±</u> 0.03	2.05±0.02	0.50 <u>±</u> 0.002	
conjugated				
with				
Pteroate				

 Table 2: Extracted parameters from theoretical fits



Figure 8: Fitting of calculated absorption spectra (dashed red line) of bare AuNP with experimental data (black line) using equation (18).

Figure.8 represents calculated absorption spectra of bare Au NP using equation (18). FWHM value from the fit is 104.06 nm for the bare Au NPs For the calculation part Fermi velocity of surface free electrons in bulk Au is taken to be $V_F = 1.9 \times 10^6 \text{ ms}^{-1}$ and the value for bulk Au damping constant is taken

 $\gamma_{bulk} = 1.1 \times 10^{14} Hz$ [48]. Core size of the Au NPs thus obtained from calculation is 5.3 nm.

4.4 Calculation of shell thickness

Volume fraction is a dimensionless quantity which is related to the radius of spherical Au NPs and thickness of the shell (small ligand molecules). The expression for volume fraction is

$$g = \frac{\left[(R_{core} + R_{shell})^3 - R_{core}^3)\right]}{(R_{core} + R_{shell})^3}$$

Radius of the spherical nanoparticle can be calculated by obtaining full width at half maxima (FWHM) from theoretical fit of the bare Au nanoparticle. FWHM (equivalent to the damping constant of oscillations of surface plasmons in Au NPs) thus obtained is substituted in equation (19) and hence radius of the spherical Au nanoparticle can be estimated.

Substituting value of R_{core} in equation (11) and expanding, we get

$R_{shell}^{3} + A R_{shell}^{2} + B R_{shell} + C = 0$

Where A, B and C are constants which depends on the radius of the sphere. Above equation is a cubic equation whose solution gives the shell thickness. It can be solved by some numerical methods like Bisection formula or Newton Raphson formula.

Material		Dielectric	Dielectric	Volume	Size of	Thickness
		constant	constant of	fraction	core	of shell
		of shell	medium	(dimensionless)	(nm)	(nm)
Au	NP	2.14±0.02	2.05 <u>+</u> 0.02	0.50 <u>+</u> 0.002	5.3 <u>±</u> 0.2	1.4 <u>±</u> 0.21
conjugate	ed					
with						
Pteroate						
Au	NP	2.11 <u>±0.02</u>	2.11 <u>±</u> 0.02	0.49±0.002	5.3 <u>±</u> 0.2	1.3 <u>+</u> 0.21
conjugate	ed					
with DUF	PA					

Table 3: Results from Mie Theory calculations

4.5 Final Model

4.5.1 Model 1



Figure 9 (a)

Figure 9 (b)

Figure 9 represents the schematic diagram of the Au NP-ligand structure. Calculated Au NP core size is 5.3 nm with an error bar ± 0.2 . Theoretically predicted thickness of small ligand DUPA around Au NP is 1.3 ± 0.21 and corresponding thickness of Pteroate around Au NP is 1.4 ± 0.28 nm. Dielectric constant for DUPA and Pteroate are 2.11 and 2.14 respectively with in error of 0.034.

4.5.2 Model 2

In this model the sample is assumed to be a mixture of Au NP and ligands. A combination of equation (18) (for core) and Lorentzian function (for ligand dye) has been used for the theoretical fit of UV-Vis data.



Figure 10 (a): UV-Visible spectra of AuNP conjugated with Pteroate. Dotted line and solid line are respectively theoretical fitting and experimental spectra (model 2).



Figure 10 (b): UV-Visible spectra of Au NP conjugated with DUPA. Dotted line and solid line are respectively theoretical fitting and experimental spectra (model 2).

It is found that model two result in poor fit compared to model 1. Goodness of fit is estimated by calculating reduced Chi square value of fit.

4.5.3 Comparison of model 1 and model 2

In model 1, the Reduced Chi-square value of the systems Au + DUPA and Au + Pteroate are 3.4×10^{-8} and 6.7×10^{-8} respectively. From the model 2, the corresponding values of Reduced Chi-square are 4.84×10^{-7} and 5.07×10^{-7} . This confirms the acceptability of model 1 than model 2.

4.6 Development of general theoretical expression to fit the experimental data for the UV-Vis absorption of the metal (Au) nanoparticles (corresponding to the plasmon resonance) for wide range of particle size (0.02 nm to 4 micron) from general form of Mie theory

According to Mie theory the expression for extinction coefficient for bare nanoparticle is expressed as [33]

$$Q_{\text{ext}} = \frac{2\pi}{x^2} \sum (2n+1) \operatorname{Re} \left(a_n^2 + b_n^2 \right)$$
(21)

The equation (21) is used for simulating extinction spectra of Au NPs ranging from 5 to 100 nm in diameter. The mean free path approximation did in Mie theory and thus obtained extinction formula (equation 17) is applicable only for small particles which satisfies the condition

 $k \cdot a \ll 1$, where k is the wave vector and a is the diameter of the nanoparticle. However, for larger particles which does not satisfies the condition $k \cdot a \ll 1$, then we adopt for general Mie theory formula.

Figure 11 shows the calculated spectra of the extinction coefficient Q_{ext} for Au nanospheres (5, 10, 50, 95,100 and 105 nm). It is observed that, blue shift in resonance peak position as the size of the Au NP decreases.

For the calculation part, the complex refractive index for Au at different wavelengths are obtained from reference work [34]. Refractive index values at intermediate wavelengths are obtained the interpolation of experimental data. Simulation of the extinction spectra has performed using Matlab software. The code for calculating Mie coefficients and extinction coefficient are taken from Christian Matzler [35] and we vectorized code for the simulation.



Figure.11 (a): Wavelength dependence of the imaginary part of complex refractive index for Au.



Figure 11 (b): wavelength dependence of the real part of complex refractive index for Au.



Figure 12: Schematic representation; steps for calculating Extinction coefficient from Lorentz-Mie theory



Figure 13: Calculated extinction efficiency (Q_{ext}) in dependence of λ for particle diameters of 5, 10, 50, 95,100,105 (from bottom to top).



Figure.14: Comparison of experimental absorption spectra (solid lines) of bare Au NP with predictions of the Mie theory (dotted lines). Diameter of Au NP calculated from theoretical fit is 100 ± 0.8 nm.



Figure.15: Comparison of experimental absorption spectra (solid lines) of bare Au NP with predictions of the Mie theory (dotted lines). Diameter of Au NP calculated from theoretical fitting is 5 ± 0.4 nm

Figure.13 shows calculated extinction efficiency (Q_{ext}) in dependence of λ for particle diameters of 5, 10, 50, 95,100,105 (from bottom to top).The spectrum indicates that as the size of the particle Figure 14 and 15 shows the comparison of calculated extinction coefficient with experimental data using general formula for Mie theory for bare Au NP of diameter 100 nm and Bare 5 nm respectively. Good agreement between the theoretical data with the experimental data shows that general formula for Mie theory holds well for nanoparticle diameter ranging from 5 to 100 nm.



Figure 16: SEM image of Au NP sample. Diameter is found to be 103 nm.

Table 4:	Results	from	Mie	Theory	calculations
----------	---------	------	-----	--------	--------------

Diameter of the Au NP	Surface Plasm		Plasmon	
(nm)	Resonance position			
	(nm)			
5	519	FWHM	: 69 nm	
10	521	FWHM	I: 52 nm	
15	522	FWHM	I: 48 nm	
45	544			
50	546			
55	549			
95	571			
100	574			
105	577			

Table.4 shows results obtained from general form of Mie theory. It is found that as particle size decreases the peak resonance peak position is shifted towards lower wavelength region. This phenomena of shifting of resonance position called blue shift is attributed from quantum confinement effect of free electrons present in the metal [14]. For the particle size below the mean free path length of metals (for Au = 45 nm) ; plasmon width increases with decrease in size (inversely proportional). These results are shown in table.4.

Chapter 5

Summary conclusion and scope for future work

5.1 Summary and conclusion

We have shown that Mie theory can provide an excellent method for studying the behavior of scattered light. Using strictly defined limitations basic Mie theory can be used to model scattering of an evanescent wave by a spherical particle. With Drude's approximation in complex dielectric function for the metal nanoparticle Mie theory get simplified and is applied to relatively small NPs [34]. From the theoretical fit, shell thickness, Dielectric constant for shell (DUPA and Pteroate) and medium are calculated. Using expanded version of Mie theory size of the core structure is calculated which agrees with the SEM results. Since Drude approximation is not valid for particles having larger size in general above 45 nm (mean free path at room temperature) we adapted general form of the Mie theory for the size calculation of Au NP. The general form of Mie theory can also be applied for particles with dimension below characteristic size of the metal NP.

Conjugation of small molecules DUPA and Pteroate on to the Au NP are confirmed by studying FTIR spectra of the conjugated system. Unfortunately, thiol stretching frequency is not found in the FTIR spectra of the sample.

MATLAB software was used for simulating extinction spectra of Au NPs. Simulated spectra shows that as the particle size decreases blue shift in the resonance peak position is observed. This blue shift is attributed by Quantum confinement phenomena.

5.2 Plan for future work

• We have Au NP conjugated with ligands DUPA and Pteroate with core size 100 nm for which thickness of the ligand has to be calculated. General form of Mie theory can be applied to this system for investigating thickness of ligand attached with the core.

- FTIR spectrum shows no characteristic frequency for thiol stretch. Study of sample using Raman spectra can give visible intense peak for thiol stretch.
- Mie theory can also be extended for studying band gap energy of metal NPs, cluster effects and determining the concentration of sample.
- Study of temperature and PH effects on the surface plasmon resonance of the Au-ligand core-shell structure is inevitable for the real life application of the samples.
- In our Mie theory calculations the only parameter which varies for different metals is their respective refractive index relative to the medium refractive index. Therefore with known refractive indices our calculation methodology can be applied to other free electron metals like Silver and Copper.

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