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On

"Detection of Gluten by Surface Plasmon Resonance Spectroscopy"

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Submitted by

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July-2019

CERTIFICATE

I hereby certify that the work, which is being presented in the thesis, titled "***Detection of Gluten by Surface Plasmon Resonance Spectroscopy***" in partial fulfilment of the requirement for the award of the degree of **Master of Science in Physics** and submitted to Thapar Institute of Engineering and Technology, is an authentic record of my own work carried out during the period **Jan 2019** to **June 2019** under the supervision of **Dr. B.N. Chudasama.** I have also cited the reference about the text(s) from where they have been sourced.

The matter presented in this has not been submitted elsewhere for the award of any other degree or diploma from any Institute.

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

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ABSTRACT

Gluten is detected by Surface Plasmon Resonance Spectroscopy in this thesis. This detection is primarily based on the interaction of protein with silver and gold Nanospheres. Silver nanospheres are prepared by seedless method and gold nanospheres are prepared by seed mediated technique. Bovine Serum Albumin (BSA) is used as standard/test protein. Concentration dependent interaction of BSA and Gluten with silver nanospheres is exponential in nature while gold shows linear dependency for with both the proteins. Moreover, AgNSPs has nearly thousands times lower detection limit for both BSA and Gluten as compared to AuNSPs. Improved plasmonic sensitivity of silver nanospheres as compared to gold nanospheres make it better probe for bimolecular detection of proteins.

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Chapter 1 Introduction

1.1 Gluten

Gluten is a protein contained by wheat, barley and rye [1]. It is having major contribution towards providing unique properties to their dough like how much water it can absorb, its elasticity and viscosity, etc. In other words, we can say that it affects their baking properties [2]. Wheat gluten is obtained from wheat by removing extra components like globulins and albumin [3]. In other words we can say that wheat contains three kinds of proteins. Two of them are globulins and albumin which are soluble in salt or water. While other one is Gluten which is composed of gliadin and glutenin which is water insoluble component of wheat. Gliadin is soluble in alcohols while glutenin is soluble in alkali solvents or dilute acids [4]. Becarri, an Italian has firstly prepared gluten protein from wheat by washing its flour with water [5].

1.1.1 Gluten Intolerance

Wheat is world's most commonly used cereal. Due to presence of gluten in wheat some people have adverse effect on their health, which is named as gluten sensitivity or gluten intolerance. It may cause diarrhea, abdominal-pain, cramps, vomiting, weight-loss, etc. generally in children and younger adults [6]. In case of such patients, due to presence of gluten in wheat an auto-immune disease gets triggered which leads to destruction of internal structure of small intestine [7]. The internal structure of small intestine consists of small finger like projections named as villi (Figure 1.1).

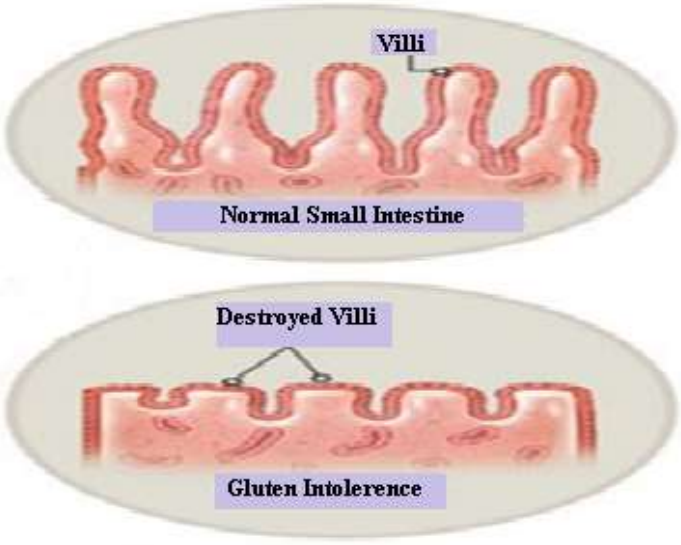

Figure 1.1 Villi structure before and after Gluten Intolerance [8]

Important nutrients of food like minerals, vitamins etc. gets absorbed with the help of these villi. With their destruction due to gluten, ability of small intestine to absorb nutrients gets reduced which leads to malabsorption and different kind of diseases [9].

1.1.2 Gluten Free Diet

The only effective solution for gluten intolerance till today which is proven scientifically is Gluten free diet. A person suffering from intolerance must have to stick lifetime to gluten free diet, which is only cure. All foods, which containing Gluten and derivative should be avoided because even a trace presence of gluten may have dangerous consequences to a person suffering from intolerance [10]. In gluten free products, flour of wheat is replaced with another type of cereals which are having flour that is gluten free [11].

Since, a person has to stick for lifetime to gluten free diet; therefore it is not easy to follow. There are lots of challenges regarding this. One of them is availability of gluten free products which are not generally available on everywhere [12]. Another major challenge is cost of gluten free products. Generally, gluten free products are much more expensive than their counter-parts. A person sticking for lifetime to gluten free diet must have to pay a huge amount of cost. Therefore, a family having multiple members, their life will also be affected. In other words large cost and affordability are major obstructions for the regulations of gluten free diet [13].

1.2 Nanoscience and Nanotechnology

It is related to sub-microscopic level objects [14]. In other words, this is related to world of objects with dimensions ranging from few nanometers to less than 100 nanometers [15]. Due to improvement in optical, electrical and magnetic properties at nano scale they are widely used in different fields as given in table 1.1[16].

Table 1.1 *Applications of Nanoscience and Nanotechnology in different fields*

Electronics [17]	Semiconductor nanowires, high voltage insulator & nano fluids based transformer, etc.
Agriculture [18]	Taking care of crop and soil, removal of pesticides contamination, dairy devices insulation, plant genetic engineering, etc.
Food Industry [19]	Detection of microorganism, food protection from dust and moisture, nutrients quick delivery, etc.
Medical [20]	Drug delivery, artificial implant of organs, biomedical imaging, etc.
Devices [21]	LEDs, AFM, QLEDs, MEMS etc.

1.3 Nanoparticles

Latin word 'Dwarf' from which 'nano' word is derived, is one thousands millionth part of a meter (1nm = 10^9). By reaching to nano scale the properties of materials like surface area, solubility, activity, etc. gets modified. Therefore, particle size plays very important role in deciding its properties [22]. As compared to bulk having infinite number of atoms combined, nanoparticles consist of few atoms only. Forces governing the nature of particles at nano scale are totally different from bulk because of increase in surface area, activity, defects, increase in band gap, etc. [23].

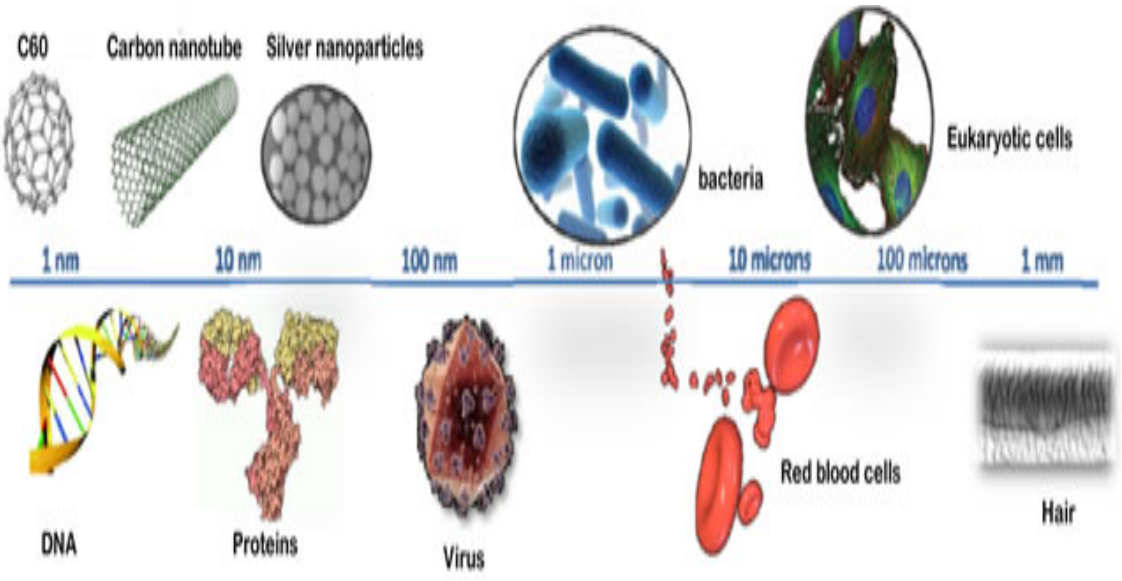

Figure 1.2 *Particle dimensions on relative size scale* [24]

1.3.1 Properties of Nanoparticles

1) Optical Properties:

Band gap increases by going from bulk to nano. Therefore, there is drastically change in the optical properties. For example nanoparticles absorption peak shifts toward lower wavelength while decease in size i.e. there is a blue shift in the optical absorption spectra [25].

2) Mechanical Properties:

There is increase in the mechanical strength while shifting from bulk to nano due to nano-fabrication or nano-manufacturing [25].

3) Electrical Properties:

In nanoparticles, there is enhancement in number of defects due to increase in surface area results in decease in electrical conductivity [25].

4) Magnetic Properties:

Some nanoparticles have magnetic properties and so, they are named as magnetic nanoparticles (MNP). According to studies, from last half century these MNP are widely used in storage devices and biomedical applications. They show supramagnetic behavior which is important for drug delivery [26].

5) Melting Point:

Nanoparticles have lower melting point than corresponding bulk material due to increase in surface to volume ratio which results in enhancement of broken bonds or surface defects. Melting point of a material is largely affected by bonding characteristics of that material [27].

1.3.2 Applications of Nanoparticles

Properties of nanoparticles are totally different from their bulks. Technologies related to nanoparticles have applications in every aspect of science. From past two decades, scientists and engineers are trying to use the complexity of nanoworld for wide range of applications and some of them are listed in table 1.2 as given below [28]:

Table 1.2 *Application of nanoparticles in different fields*

Drug delivery	Cancer treatment and other diseases can be efficiently being treated with precise manufactured nanostructures sometimes based on light and heat. Diseased cell and states can also be diagnosed.
Nano films	Nano films are being widely used because they are anti-reflecting, repellent to water, UV-IR resistant, etc. LEDs, camera and glasses also make use of nano films in various applications.
Nanoelectronics	Due to low power consumption, light weight and thickness they are widely used in display screens, memory chips, transistors and many other electronic devices.

Medical Nano-robotics	Nanorobots with highly equipped nanosensors are used by doctors in medical applications.
Nanotechnology and Space	Space flights are made more practical and light weight by nanotechnology. Decrease in weight during flights costs less fuel therefore reducing cost and efficiency.

1.3.3 Synthesis of Nanoparticles

All methods of synthesis of nanoparticles can have two categories named 'Top-down' and 'Bottom-up' named two categories. For an example to get a brick we can break a wall or can combine soil and water. The former one will be thus called top-down method while later one is known bottom-up method. In top down approach we start with bulk and reducing it nano size by any physical or chemical methods like ball milling, laser ablation or may be techniques related to lithography [29]. Instead of this, in bottom-up approach we start with a single atom or molecule and making their cluster to nano range by chemical reduction methods, sol-gel method, etc. [30].

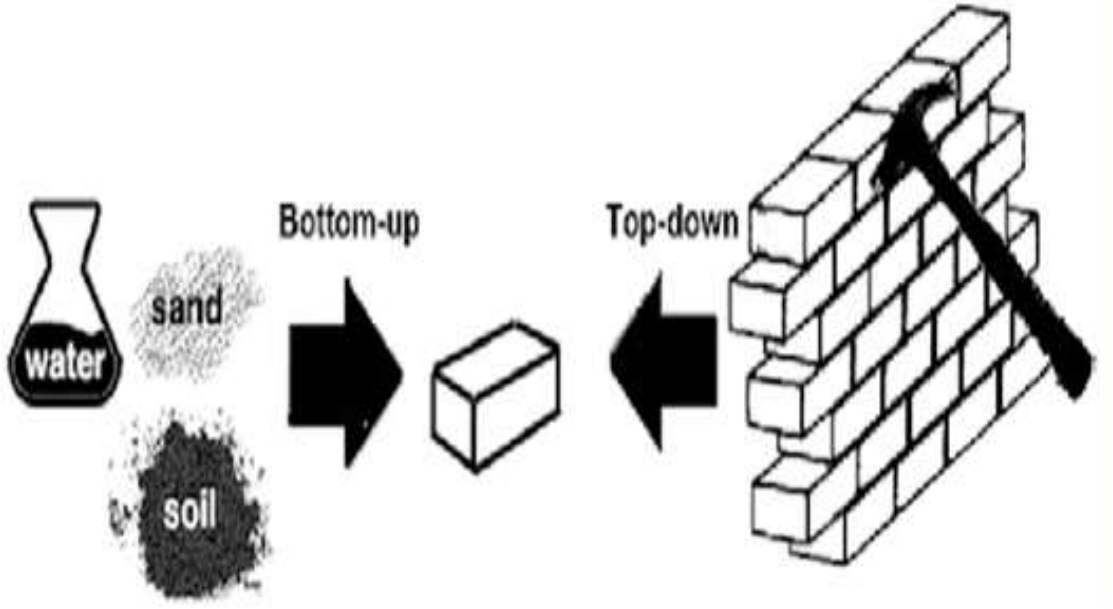

Figure 1.3 *Bottom-up and Top-down methods of nanoparticles synthesis* [29]

1.3.4 Surface Plasmon Resonance

When light hits with the metal surface, then electromagnetic radiation interacts with some electrons in the metal surface. Electrons oscillate at a particular frequency and when it matches with frequency of oscillations of electrons then light gets absorbed and gets amplified. A quantum of theses oscillations is named as "Plasmon" and this phenomenon is named as Surface Plasmon Resonance (SPR) and corresponding frequency is called plasmonic frequency [31].

Surface Plasmon Resonance is an optical phenomenon is shown by nanoparticles only. This depends on size and refractive index of environment near the surface. In a positively charged lattice, the displaced cloud of electrons having negative charge is named as Plasmon according to 'Fermi liquid model' because of its resemblance to the plasma [32]. The simplest diagram representing surface plasmon resonance is shown in Figure 1.3.

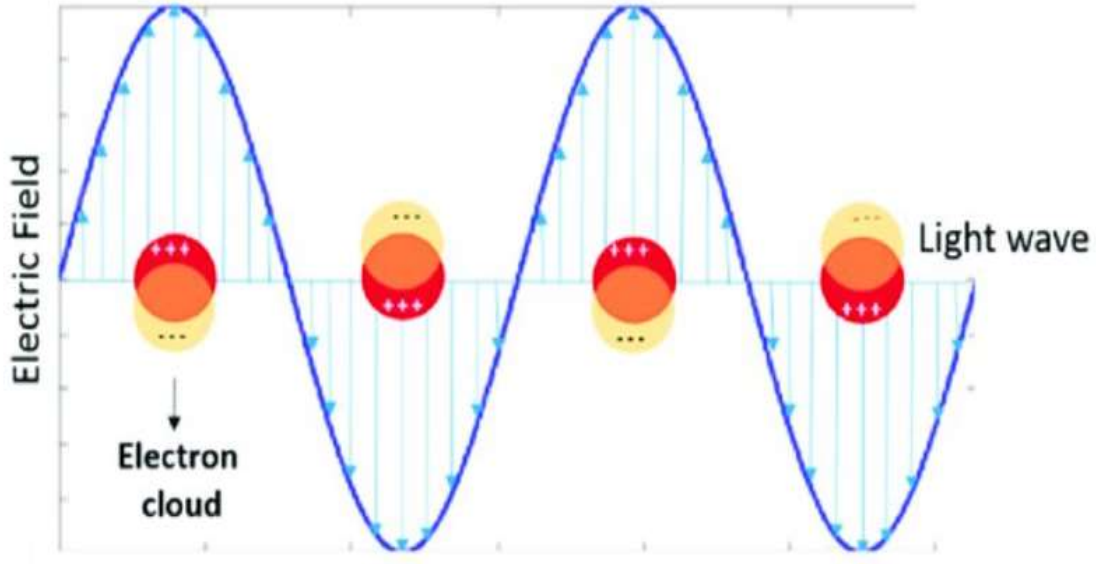

Figure 1.4 *Representation of Surface Plasmon Resonance* [33]

1.3.5 Nanoparticles and their Shapes

Due to surface plasmon resonance nanoparticles absorb and scatter electromagnetic radiations of a particular wavelength which is mainly related to the shape and size of the nanoparticles. Nanoparticles can have a number of shapes like spheres, cubes, truncated cubes, triangles, tetrahedral, octahedral, rods, nanorice, nanostars, etc. with varying sizes. Some of them are shown in Figure 1.4. [34].

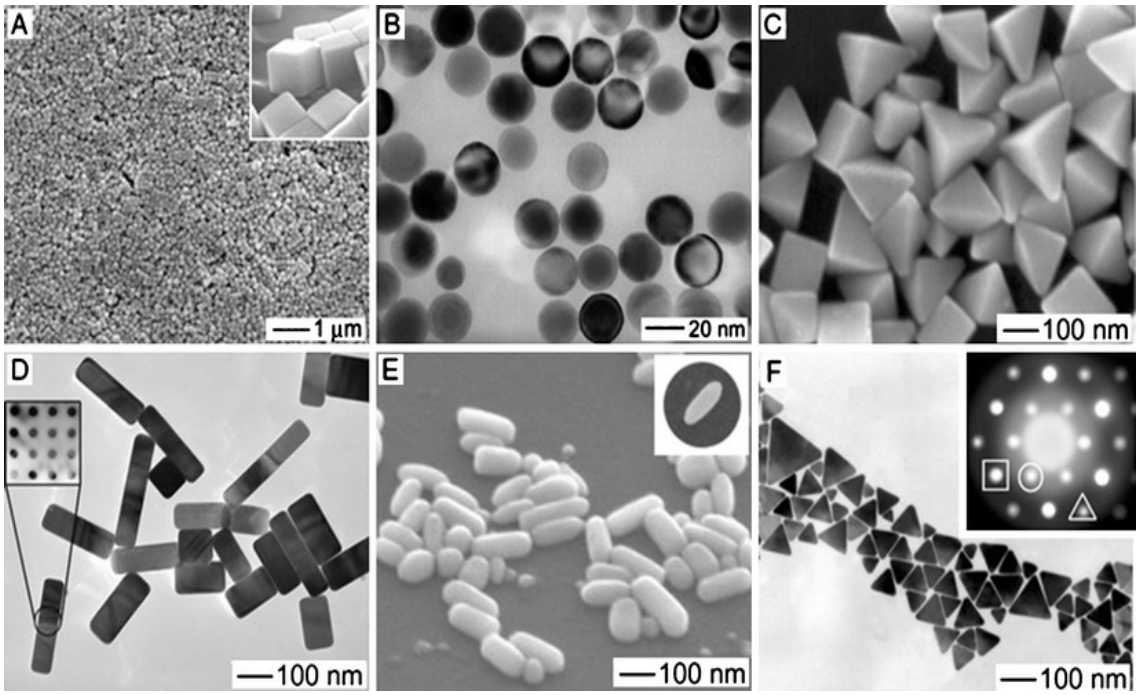

Figure 1.5 *Shapes of different types of Nanoparticles* [34]

Silver Nanoparticles

Strong localized surface plasmon resonance i.e. LSPR, which falls in visible region makes silver nanoparticles very entrancing and applicable for different kinds of applications. They are widely used in recent years for different kinds of application like surface Enhanced Raman Spectroscopy, different kinds of sensors, etc. [35]. Silver nanoparticles have number of shapes which can be tuned by precisely changing reducing agent, precursor salt, temperature, solvent, stabilizing agent and many other parameters during their synthesis. They have ability to enhance SERS signal to 10^{14}-10^{15} times [36].

Gold Nanoparticles

Gold nanoparticles are non-toxic which makes them bio-compatible as compared to all other types of nanoparticles. Therefore, they are better option for bio-sensing applications than others. In addition to bio-sensing they are effectively used for drug delivery, cancer hyperthermia, gene delivery, etc. [37]. Gold nanoparticles also have a number of shapes like spheres, rods, nanostars, nanocubes, etc. which are widely used in photography, catalytic applications and optoelectronic devices. Reducing agent, surfactant and reaction time also plays very important role in deciding their morphological properties [38].

1.4 Surfactants

Surfactants are the molecules having two parts; one is solvent loving part called hydrophilic part the other one which is solvent repellent named as hydrophobic part. In other words, surfactants are amphiphilic in nature. Hydrophobic part is also pronounced as head while the hydrophilic part having long chain is tail (figure 1.5) [39]. When they are dissolved in a solvent, then they form specific types of shapes like micelles, vesicles etc. [40].

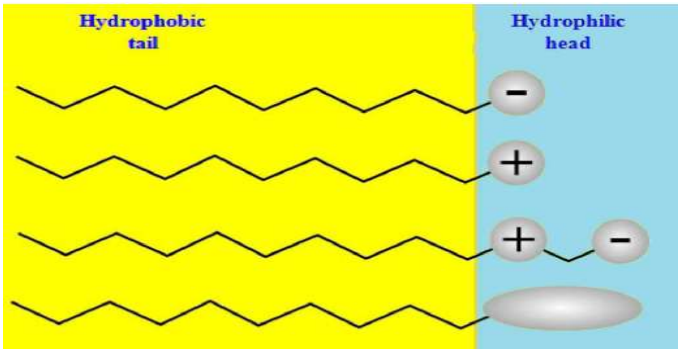

Figure 1.6 *Schematic diagram of amphiphilic surfactant* [41]

1.4.1 Types of Surfactant

Surfactant is activator which reduces tension by migrating into the surface. Therefore, they enhance the surface activity by reducing the surface tension. On the basis of their usage surfactant can be classified into following categories [42].

Anionic Surfactant

These are soluble in water having cationic and anionic parts. Cationic part mainly consists of alkaline (Li, Na, K, etc.) or quaternary ammonium (NH_4^+). Alkyl benzene-sulphonate, lauryl-sulphate etc. also comes in the category of anionic surfactant [42].

Cationic Surfactant

When they are dissolved in water they also form cationic and anionic parts but most of them are amines or halogen type having large alkyl chain. They are very costly as compared to the anionic surfactant because during their synthesis hydrogenation is carried out which takes place at very high atmospheric pressure [42].

Zwitterionic Surfactant

Surfactant has two parts named cation and anion. When, Surfactant molecule consisting of both parts in a single unit, then it is termed as Zwitter-ionic surfactant. Some synthetic

compounds, for example amino-acid, phospo-lipds, etc. comes under this category while natural one is betaines [42].

Non-ionic Surfactant

Approximately 45% of total production of the surfactants is non-ionic surfactants. Due to the presence of non-dissociable nature of their hydrophilic part, they don't give ions in the solutions consisting water i.e. which are aqueous in nature. They generally consists of groups like -NH_2, -OH, R-O-R etc. [42].

1.4.2 Role of Surfactant during synthesis of Nanoparticles

Well defined shape and stability are two important factors for the success of any synthesis method of nanoparticles. Therefore, surfactant plays very important role for deciding shape and enhancement of stability of the nanoparticles. Surfactants have the ability of capping a particular face while other grows. Hence, different shapes are generated by using different kinds of surfactants [43]. The commonly used surfactants are given as [44]

- Pollyvinynlpyrrolidone
- Cetyl trimethylammonium bromide
- Trisodium citrate
- Sodium citrate
- Oleylamine
- Gluconic acid

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Lots of methods are being used for the synthesis of silver and gold nanoparticles depending on their applications. Different methods for synthesis of silver and gold nanoparticles and their interaction with proteins are reviewed in this chapter.

2.1 Synthesis of Silver Nanoparticles

Author Name	Title	Important Findings
Cobley, Claire M., et al. Plasmonics 4.2 (2009): 171-179.	Shape-controlled synthesis of silver nanoparticles for plasmonic and sensing applications [1]	• Silver nanoparticles show LSPR which depends on their shape. • By controlling the shape we can tune the LSPR. • LSPR and SERS techniques are widely used as sensor for the detection of different kinds of chemicals or bio-molecules.
Liang, Hongyan, et al. The Journal of Physical Chemistry C 114.16 (2010): 7427-7431.	Controlled synthesis of uniform silver Nanospheres [2]	• Silver nanospheres having uniform diameter are prepared by this method. • Solvent and reducing agent is PEG, while capping agent is PVP. • Average diameter of silver nanosphere is 50nm.
Lah, Nurul Akmal Che, and Mohd Rafie Johan. Applied Surface Science 257.17 (2011): 7494-7500.	Facile shape control synthesis and optical properties of silver nanoparticles stabilized by Daxad 19 surfactant [3]	• Dexad 19 used as stabilizing agent. • Synthesized nanoparticles having uniform shape along with size and also they are well dispersed in nature. • The molar ratio of different kinds of chemicals and

		temperature of the reaction plays very important role.
Guzman, Maribel, Jean Dille, and Stéphane Godet. Nanomedicine: Nanotechnology, Biology and Medicine 8.1 (2012): 37-45.	Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria [4]	• At room temperature, silver nanospheres having average diameter 9, 14, 24 and 30 (in nm) are synthesized using this method. • Two chemicals are used as reducing agent which is hydrazine-hydrate and sodium-citrate. • 405 nm - 418 nm is the range in which LSPR peak of the silver nanospheres was observed.
Tran, Quang Huy, and Anh-Tuan Le. Advances in Natural Sciences: Nanoscience and Nanotechnology 4.3 (2013): 033001.	Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives [5]	• Due to fascinating properties of silver nanoparticles they are commercially used. • They are used for medical applications and also in electronics devices. • Silver nanospheres of different sizes and forms like powder or aqueous colloids are used for different types of microbial-strains.
Jose, M., and M. Sakthivel. Materials Letters 117 (2014): 78-81.	Synthesis and characterization of silver nanospheres in mixed surfactant solution [6]	• Having enhanced activity at nanoscale, silver nanospheres are widely used in catalytic and Surface Plasmon Resonance Spectroscopy applications. • In the combined environment of CTAB and ammonia silver nanospheres are synthesized. • As synthesized silver nanospheres shows surface plasmon peak at 422 nm wavelength

		confirmed by UV-Visible spectroscopy.
Khodashenas, Bahareh, and Hamid Reza Ghorbani. Arabian Journal of Chemistry (2015).	Synthesis of silver nanoparticles with different shapes [7]	• Due to unique shape-size dependent optical properties AgNPs are widely used. • AgNPs have a number of shapes like cube, rod, wire, triangle, prism, spheres etc. • 9 nm AgNPs are synthesized by using precursor salt $AgNO_3$ while $NaBH_4$ and PVP as reducing and surfacing agent.
Hu, Guansong, et al. Applied Physics A 122.10 (2016): 874.	Antibacterial activity of silver nanoparticles with different morphologies as well as their possible antibacterial mechanism [8]	• Silver nanoparticles are synthesized by seed mediated method. • Synthesized nanoparticles have a mixture of nanospheres, nanorods and nanoplates. • Silver nanospheres have surface plasmon peak at 420 nm. • Seed solution volume greatly affects the formation and size of the silver nanoparticles.

2.1 Synthesis of Gold Nanoparticles

Author Name	Title	Results
Long, Nguyen Ngoc, et al. Journal of Physics: Conference Series. Vol. 187. No. 1. IOP Publishing, 2009.	Synthesis and optical properties of colloidal gold nanoparticles [9]	• Reduction and photochemical techniques are used. • In reduction technique, reducing agent trisodium citrate is used, which controls the size. • In photochemical

		technique, X-ray irradiation time and volume of $HAuCl_4$ affects the size. • For gold nanospheres CTAB is used as stabilizing agent.
Sivaraman, Sankar K., Sanjeev Kumar, and Venugopal Santhanam. Gold Bulletin 43.4 (2010): 275-286.	Room-temperature synthesis of gold nanoparticles Size control by slow addition [10]	• Gold nanospheres are synthesized at room temperature. • Adsorption of stabilizing agent and pH controls the shape and nanoparticle formation. • Monodispersity can be controlled by speed of addition of tetrachloroaurate solution.
Joseph, Virginia, et al. Journal of Raman Spectroscopy 42.9 (2011): 1736-1742.	SERS enhancement of gold nanospheres of defined size [11]	• Monodisperse gold nanospheres are synthesized which are having diameter ranging from 15 nm to 40 nm. • Nanospheres are stabilized by sodium citrate and formation is confirmed by XRD and UV-Visible spectroscopy. • Each gold nanosphere produce enhancement of signal about 10^2 to 10^3 orders.
Fenger, R., et al. Physical Chemistry Chemical Physics 14.26 (2012): 9343-9349.	Size dependent catalysis with CTAB-stabilized gold nanoparticles [12]	• As compared to small nanospheres of size 3.5 nm or bigger like 28 nm, the intermediate ones having 13 nm size which are stabilized by the CTAB, they are most active. • Reaction rate of intermediate ones is 60 times and 3 times faster than the bigger

		and smaller ones respectively.
Leopold, Nicolae, et al. Colloids and Surfaces A: Physicochemical and Engineering Aspects 436 (2013): 133-138.	One step synthesis of SERS active colloidal gold nanoparticles by reduction with polyethylene glycol [13]	• Highly stable gold nanoparticles are prepared whose sized can be varied by ratio of gold salt and PEG stabilizer. • Diameter varies from 15 nm to 60 nm. • 15 nm nanoparticles are mainly spherical in nature while 60 nm are having polygonal shape. • Nanospheres have LSPR peak at 520 nm while polygonal having peak at 562 nm.
Zheng, Yiqun, et al. Particle & Particle Systems Characterization 31.2 (2014): 266-273.	Successive, Seed-Mediated Growth for the Synthesis of Single-Crystal Gold Nanospheres with Uniform Diameters Controlled in the Range of 5–150 nm [14]	• Seed mediated by which diameter can be varied from 5 nm to 150 nm which are having single crystal nature. • Firstly 5 nm to 16 nm nanospheres are prepared with the help of CTAB. • Then from above prepared 10nm nanospheres are used as seed to prepare 15 nm to 80 nm sized gold nanospheres. • In further step 46 nm are used for the synthesis of 75 nm tom 150 nm gold nanospheres.
Favi, Pelagie Marlene, et al. Journal of Biomedical Materials Research Part A 103.11 (2015): 3449-3462.	Shape and surface effects on the cytotoxicity of nanoparticles: Gold nanospheres versus gold nanostars [15]	• Gold nanospheres and nanostars are synthesized and their toxicity is compared. • Corresponding to toxicity with

		fibroblast-cells gold nanostar of diameter 39.69 nanometer have less toxicity than gold nanospheres of diameter 61.46 nanometer size.
Piella, Jordi, Neus G. Bastús, and Victor Puntes. Chemistry of Materials 28.4 (2016): 1066-1075.	Size-Controlled Synthesis of Sub-10-nanometer Citrate-Stabilized Gold Nanoparticles and Related Optical Properties [16]	• Gold nanospheres are synthesized by seed mediated technique. • Synthesized nanospheres have diameter of 10 nm which are stabilized by citrate. • They are well dispersing in nature which is biocompatible. • Seeds having very small size distribution of average diameter 3.5 nm.

2.2 Interaction of Proteins with Nanoparticles

Author Name	Title	Results
Chakraborty, Soumyananda, et al. Langmuir 27.12 (2011): 7722-7731.	Contrasting effect of gold nanoparticles and nanorods with different surface modifications on the structure and activity of bovine serum albumin [17]	• CTAB stabilized gold nanospheres and gold nanorods are synthesized and their interaction with BSA is compared. • Binding of BSA with gold nanorods and gold nanospheres is endothermic and exothermic respectively. • Gold nanospheres have 3.5 times greater coverage of the outer surface in comparison to the gold nanorods.

		• Gold nanorods have less biocompatibility due the presence of CTAB but they are widely used for cancer treatment.
Dominguez-Medina, Sergio, et al. Langmuir 28.24 (2012): 9131-9139.	In situ measurement of bovine serum albumin interaction with gold nanospheres [18]	• Gold nanospheres are prepared which are stabilized by citrate. • Scattering-correlation-spectroscopy is used to study adsorption of BSA on gold nanospheres surface by its Brownian movement. • There is blue shift after the BSA adsorption.
Dasgupta, Nandita, et al. Chemico-biological interactions 253 (2016): 100-111.	Bovine serum albumin interacts with silver nanoparticles with a "side-on" or "end on" conformation [19]	• There is increase in particle size which is resulted from conjunction of BSA with AgNPs i.e. BSA is attached to the silver particle surface. • UV-Visible spectrum has blue shift after the conjugation.
Wang, Gongke, et al. RSC advances 7.15 (2017): 9393-9401.	Probing the binding behavior and kinetics of silver nanoparticles with bovine serum albumin [20]	• Different kind of spectroscopic techniques are use to study the binding of silver nanospheres and BSA. • The process of conjugation of BSA with silver nanospheres is static quenching. • The kinetics followed by the adsorption the surface of silver nanospheres is second order kinetics. • The study of binding behavior is very

		important for medical applications.

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12. Fenger, R., et al. "Size dependent catalysis with CTAB-stabilized gold nanoparticles." Physical Chemistry Chemical Physics 14.26 (2012): 9343-9349.
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3.1 Introduction

Depending on the applications or use of nanoparticle a number of ways are adapted to synthesize nanoparticles which include physical or chemical methods. In the category of physical methods we have vapor-condensation, arc-discharge, laser-ablation etc. techniques that are widely used, while in chemical methods, chemical reduction methods is mostly preferred [1]. In chemical reduction method, first precursor salt is reduced to neutral atoms which coagulate themselves in the presence of suitable surfactant to form particular shape [2]. This is called seedless chemical reduction technique. While, in seed mediated technique nucleation takes place first which are used as seed for further growth in the presence of growth solution and surfactant to synthesize specific shape. Therefore, salt volume i.e. growth solution and seed on which salt has to grow efficiently affects the shape and stability of nanoparticles [3].

3.2 Material used and Instrumentation

3.2.1 Materials

Silver Nanospheres

Silver nanospheres are synthesized by seedless method during which the used chemicals along with their molecular formula, molecular weight and chemical company from which they are purchased are listed in Table 3.1.

Table 3.1 *List of chemicals used for the synthesis of Silver nanospheres*

Name	Molecular Formula	Molecular Weight (gm/mol)	Chemical Company
Silver nitrate	$AgNO_3$	169.87	Sigma-Aldrich
Polyvinylpyrrolidone (PVP)	$(C_6H_9NO)_n$	10,000	Sigma-Aldrich
Sodium chloride	NaCl	58.44	MilliporeSigma
Diethylamine (DEA)	$(CH_3CH_2)_2NH$	73.14	Loba Chemicals
Dimethylforamide (DMF)	$(CH_3)_2NC(O)H$	73.09	Loba Chemicals

Gold Nanospheres

Seed mediated technique is used for the synthesis of gold nanospheres and corresponding chemicals with their details are listed in Table 3.2.

Table 3.2 *List of Chemicals used for synthesis of gold nanospheres*

Name	Molecular Formula	Molecular Weight (gm/mol)	Chemical Company
Tetrachloroaurate	$HAuCl_4$	339.79	Sigma-Aldrich
Trisodium citrate	$C_6H_5Na_3O_7.2H_2O$	294.10	Sigma-Aldrich
Sodium borohydride	$NaBH_4$	37.83	Sigma-Aldrich
Silver nitrate	$AgNO_3$	169.87	Sigma-Aldrich
Hydrochloric acid	HCl	36.46	Loba Chemicals
Ascorbic acid	$C_6H_8O_6$	176.12	MilliporeSigma
Cetrimethylammonium bromide	$C_{19}H_{42}BrN$	364.45	SD Fine Chemicals Limited

Protein Solutions

All types of protein solutions are prepared in ultrapure water. Different kinds of proteins and chemicals used for their solution preparation with their details are listed in the table 3.3.

Table 3.3 *List of chemicals used for the preparation of Protein solutions*

Name	Molecular Formula	Molecular Weight (gm/mol)	Chemical Company
Bovine Serum Albumin	-	66430.3	Himedia
Gluten from Wheat	-	-	Sigma-Aldrich
Urea	NH_2CONH_2	60.06	Loba-Chemicals

3.2.1 Instrumentation

Different kinds of instruments used for synthesis and characterization of nanoparticle are:

- Labman digital Ultrasonic Cleaner
- DLS (Brookhaven 90 plus particle size analyzer)
- Mega17R Centrifuge Machine
- UV-Vis-NIR Spectrometer (Shimadzu, UV-2600)

3.3 Synthesis of Nanoparticles

3.3.1 Silver Nanospheres

Preparation of Silver Nanospheres

Synthesis of silver nanospheres is based on digestive ripening process. This is reverse case of Ostwald ripening in which bigger nanospheres grow on the expense of smaller one while in the case of the digestive ripening bigger one decays into smaller one to provide uniform size distribution. Diemethylforamide (DMF) is preferred as solvent for digestive ripening because it provide uniform distribution.

In this experiment, 4 mL of polyvinylpyrrolidone (PVP) solution, which is prepared by dissolving 5% weight of PVP in diemethylforamide (DMF), 0.8 mL diethyl amine, 2.5mL of 0.1 M silver nitrate ($AgNO_3$) and 0.1 mL of 1 M sodium chloride (NaCl) are added in sequence with vigorous stirring in 8.4 mL of diemethylforamide and keep stirring for 5 min till its color changes to milky white. Immediate after this start heating and keep at 50 °C for 15 hours under stirring. Silver nanospheres are thus prepared and can be used for further characterization/measurement.

3.3.2 Gold Nanospheres

Preparation of Seed Solution

Take 9.6 mL ultrapure water and add to it 0.125 mL of 0.01 M tetrachloroaurate and 0.25 mL of 0.01 M trisodium citrate in sequence. Now, add freshly prepared 0.15 mL of 0.01 M sodium borohydride with vigorous stirring for two minutes till color changes to light pink. Keep this undisturbed for two hours so that sodium borohydride added react completely. Seed solution is ready for use after two hours.

Preparation of Growth Solution

Prepare 40 mL of 0.1 M CTAB solution in ultrapure water. Now, add 0.4 mL of 0.01 M silver nitrate, 2 mL of 0.01 M tetrachloroaurate, 0.8 mL of 1 M hydrochloric acid and 0.32 mL of 0.1 M ascorbic acid in sequence with vigorous stirring. Growth solution is prepared and ready to be use.

Preparation of AgNSPs

For the synthesis of gold nanospheres, add 1 mL of seed solution into growth solution with gentle stirring for two minutes. Color changes to dark pink which shows formation of gold nanospheres.

3.4 Preparation of Protein Solutions

3.4.1 Bovine Serum Albumin (BSA) Solution

To prepare 5 mM solution, add 6.64 gm of BSA in 20 mL of water. Sonicate it for 10 minute and keep undisturbed for next 10 minute. Repeat this sonicate process for 4 times till clear dense yellow colored solution is obtained. Preserve this in refrigerator and was further used to prepare different concentrations of BSA.

3.4.2 Gluten Solution

Gluten is water insoluble protein i.e. it doesn't dissolve in water at all. Its solution can be obtained by dissolving it in urea. To prepare 2% gluten solution, take 100 mL of water add of it 40 gm of urea with stirring. A clear solution of urea is thus obtained. Heat it to 60°C and when it becomes sufficiently hot, add 2 gm of gluten with stirring. Heat it for 2 hours till all gluten gets dissolved and milky solution obtained. Now, filter this with the help of Buchner funnel using Whatman filter papers. Gluten solution is prepared and preserves this in refrigerator for future use. This was also used to prepare different concentration of Gluten by diluting it with water.

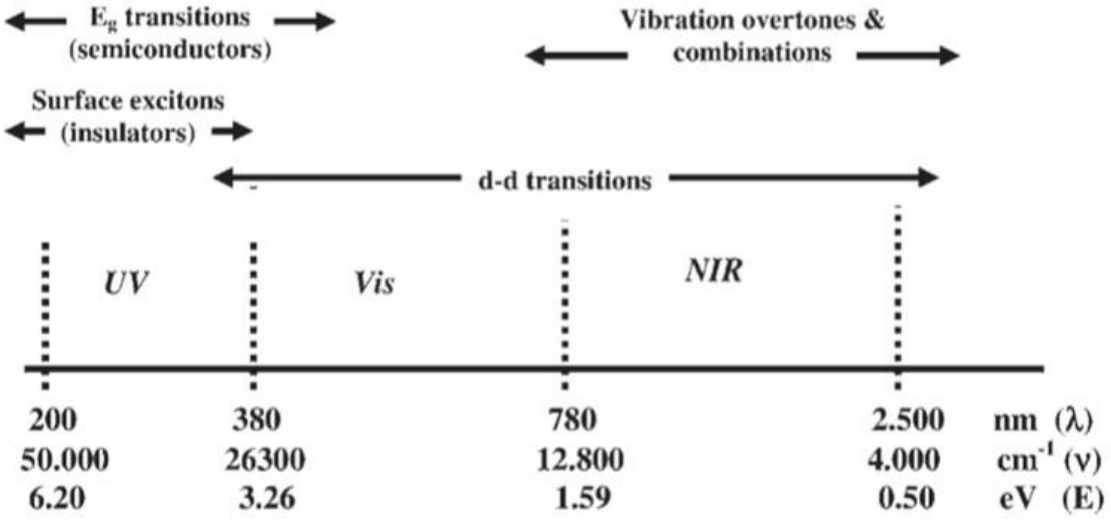
3.4 Characterization

The following two different types of techniques are used for the characterization of synthesized nanoparticles:

- UV-Visible-NIR Spectroscopy
- Dynamic Light Scattering (DLS)

3.4.1 UV-Visible-NIR Spectroscopy

Electromagnetic radiation interaction with matter is studied with the help of spectroscopic techniques. Atomic and molecular spectroscopic techniques are based on study of light interaction with matter and are analyzed with the help of UV-Visible-NIR spectrometer [4]. When electromagnetic radiations falls on the matter like UV, visible or infrared then they get absorbed by the matter and different types of transitions takes place between them like metal-centered, charge-transfer, between energy bands of electrons, between vibration levels etc. [5]. For UV-Visible-NIR spectrometer sample may be powder, liquid or solid and we can measure absorbance, reflectance and transmittance. In case of the, metal nanoparticles, we can easily get idea about the shapes of simple geometry like spheres or rods. For example, in case of nanospheres we have single peak while in case of rods we have two peaks named as transverse and longitudinal peaks [6].



Fiure 3.1 *Types of transition in UV-Visible-NIR regions* [5]

The apparatus used for the characterization for UV-Visible-NIR spectroscopy is Shimadzu UV-2600 which consist of two lamps made of tungsten-halogen and deuterium for production of Visible, NIR and UV radiations. Two different slots one for sample and another for reference sample are used. In case of liquid, reference sample may be water or DMF in which nanoparticles are prepared. In case of films glass slide is used as reference while in case of powder sample barium sulphide is used as reference sample.

3.4.2 Dynamic Light Scattering (DLS)

Nanoparticles have different shapes and within a shape they have different sizes and size distributions. A schematic diagram of DLS is shown in the figure 3.2.

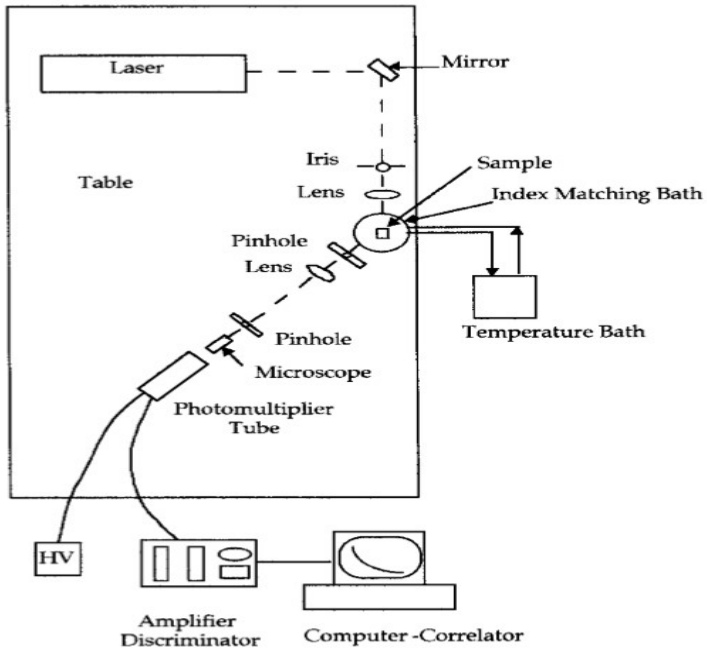


Figure 3.2 *Schematic diagram* of *Dynamic light scattering* [7]

All the parameters related to nanoparticle can be measured by a simple technique simultaneously in small time with the apparatus named DLS i.e. Dynamic Light Scattering. Photon Correlation Spectroscopy i.e. PCS name is used for dynamic light scattering [7]. When in liquid solution particle are in random motion i.e. they have Brownian movement then they scatter light when light falls on them. By measuring the scattered light diffusion coefficient D_T can be computed. With the movement intensity fluctuations takes place which are analyzed by autocorrelation function which gives important information about the dissolved particles. DLS is based on solid state laser system [8]. Brookhaven 90Plus is used for the measurement of particle size and their size distribution.

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4.1 Silver Nanospheres

4.1.1 UV-Visible and DLS of Silver Nanospheres

Silver nanoparticles are spherical in shape, which is confirmed with UV-Visible spectrometer and DLS. Single peak in UV-Visible spectrum is signature of spherical nanoparticles which is shown by synthesized nanoparticles. It is centered around 420 nm. The hydrodynamic size of the nanoparticles is determined by the dynamic light scattering. The silver nanoparticles have hydrodynamic size of 29.32 nm which is very good for surface plasmonic applications. UV-Visible and dynamic light scattering (DLS) of nanoparticles are shown in figure 4.1.

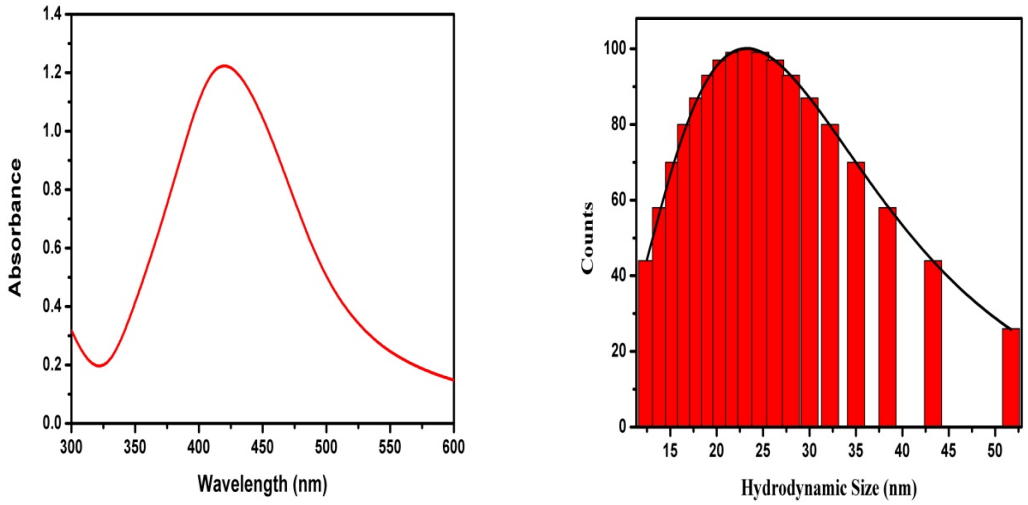

Figure 4.1 *UV-Visible Spectrum & Dynamic Light Scattering of Silver Nanoparticles*

4.1.2 Effect of Time on growth of Silver Nanospheres

Many factors have role in the synthesis. For example precursor salt, reducing agent, stabling agent, solvent and reaction time have very important effect on the synthesis on nanoparticles. During the synthesis at constant temperature of 50 °C, it is observed that with time, there is increase in the absorption. This increase in absorption signifies that number of spherical or size nanoparticles are increasing with time i.e. there is growth of silver nanoparticles. UV-Visible spectra of silver nanoparticles are recorded after every hour, till 15 hours. There is continuous increase in the absorption. There is slight peak shift till 7 hours but after 8 hours there is no change in the peak i.e. peak becomes constant at 420 nm. The UV-Visible spectrum showing effect of temperature on the synthesis of silver nanospheres is shown in figure 4.2.

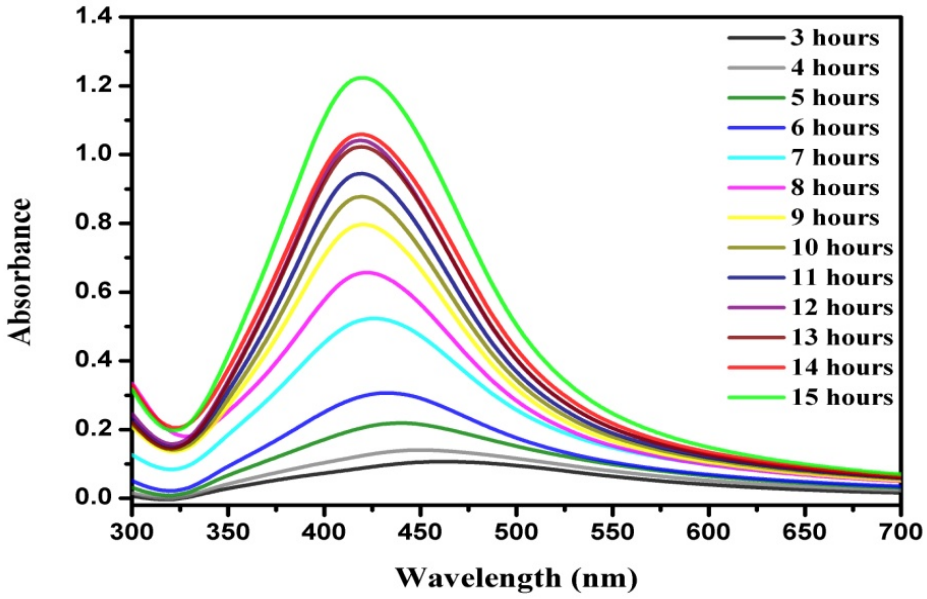

Figure 4.2 *Effect of temperature on synthesis of AgNSPs*

4.1.3 Interaction of BSA with Silver Nanospheres

The interaction of different volumes (5 µL, 10 µL and 30 µL) of silver nanosphere with Bovine Serum Albumin (BSA) is observed. There is blue shift in the UV-Visible spectra due to interaction of BSA with silver nanospheres [1,2]. The volume range in which silver is active for with BSA is 0-25 µM and readings are taken in the steps of 2.5 µM.

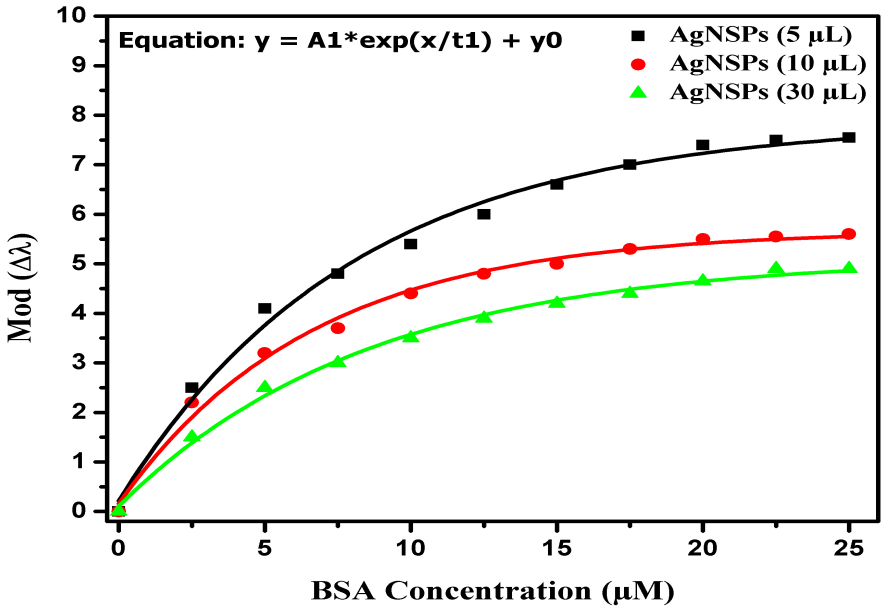

Figure 4.3 *Interaction of BSA (0-25 µM) with AgNSPs (5, 10 & 30 µL)*

Table 4.1 *Modulus of peak shift (|Δλ|) of silver nanospheres with BSA*

BSA Concentartion (mM)	|Δλ| of AgNSPs (5 µL)	|Δλ| of AgNSPs (10 µL)	|Δλ| of AgNSPs (30 µL)
0.0	0	0	0
2.5	2.5	2.2	1.5
5.0	4.1	3.2	2.5
7.5	4.8	3.7	3
10.0	5.4	4.4	3.5
12.5	6	4.8	3.9
15.0	6.6	5	4.2
17.5	7	5.3	4.4
20.0	7.4	5.5	4.65
22.5	7.5	5.55	4.9
25.0	7.55	5.6	4.9

Exponential nature is shown by |Δλ| (w.r.t. concentration of BSA) of bouvine serum albumin interaction with silver nanoparticles. Among all volumes, 5 µL of AgNSPs showing effectivly highest activty followed by 10 µL and 30 µL as showm in figure 4.4.

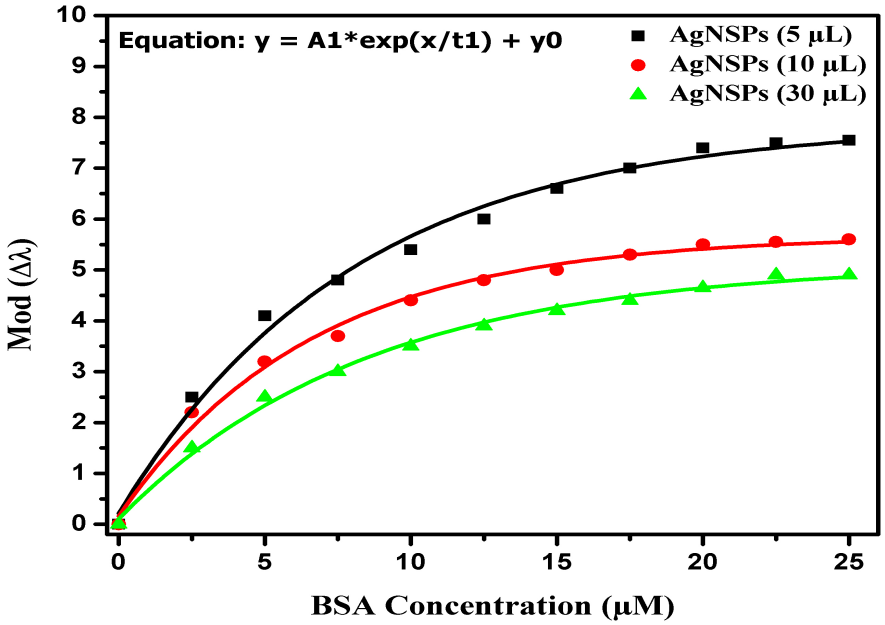

Figure 4.4 *Effect of BSA on |Δλ| with different volumes of AgNSPs (5, 10 & 30 µL)*

Every exponential has a particular decay constant. The decay constant of exponential growing graphs of different volumes of silver nanospheres are listed in table 4.2. By using them any unknown concentration of BSA can be determined. AgNSPs with 5 µL volume as probes gives highest sensitivity of detection.

Table 4.2 *Decay constant of exponential lines of BSA interaction with AgNSPs*

AgNSPs Volume	Decay Constant
5µL	-8.02558
10µL	-6.59246
30µL	-8.41206

4.1.4 Interaction of Gluten with Silver Nanospheres

Gluten prepared by dissolving in urea is used to study the interaction with silver nanospheres. To remove un-dissolved part if any left is filtered by Buchner filter because of its concentrations makes it difficult to filter by simple filter paper. The concentration in which silver has its characteristics is 0% - 0.008%. The UV-Visible spectrum of different volumes of AgNSPs with gluten is shown in figure 4.5.

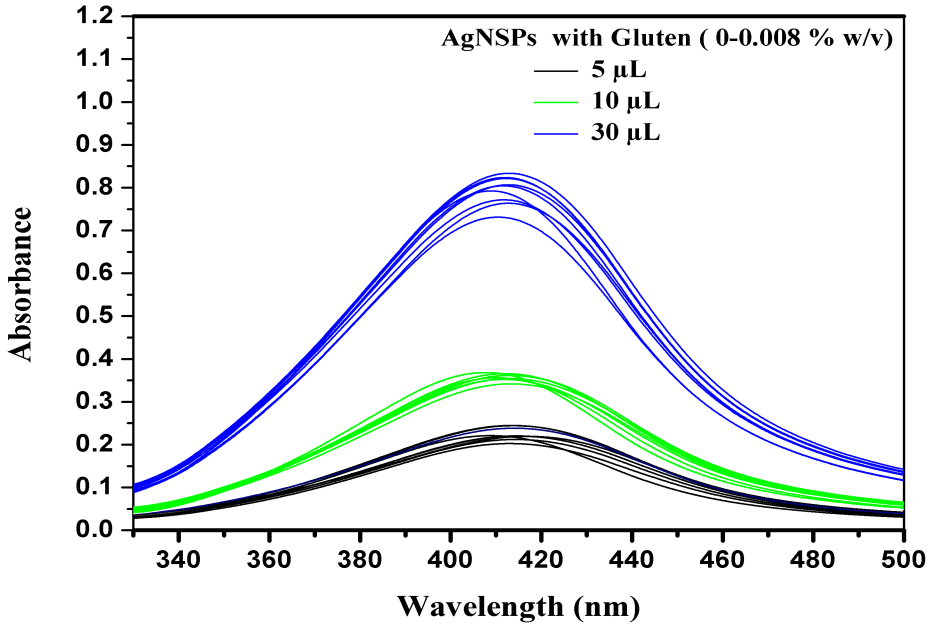

Figure 4.5 *Interaction of Gluten with AgNSPs (5, 10 & 30 µL)*

The UV-Visible spectrum shows that there is red shift in the SPR peak due to interaction of gluten with silver nanospheres.

Table 4.3 *Modulus of peak shift (|Δλ|) of silver nanospheres with Gluten*

Gluten Concentration (% w/v)	|Δλ| of AgNSPs (5µL)	|Δλ| of AgNSPs (10µL)	|Δλ| of AgNSPs (30µL)
0.000	0	0	0
0.001	2.35	1.45	1.1
0.002	3.95	2.7	2.1
0.003	5.1	3.2	2.5
0.004	5.4	3.8	2.9
0.005	5.5	4.1	3.3
0.006	5.55	4.2	3.6
0.007	5.6	4.2	3.7
0.008	5.5	4.2	3.8

When gluten interacts with AgNSPs, |Δλ| (w.r.t. concentration of Gluten) also show exponential behavior as shown by the BSA. Among all volumes, 5µL of AgNSPs showing effectivly highest activty followed by 10µL and 30µL (as shown in Figure 4.6 and Table 4.4). Again highest deflection senstivity is observed with lowest volume of AgNSPs (5 µL).

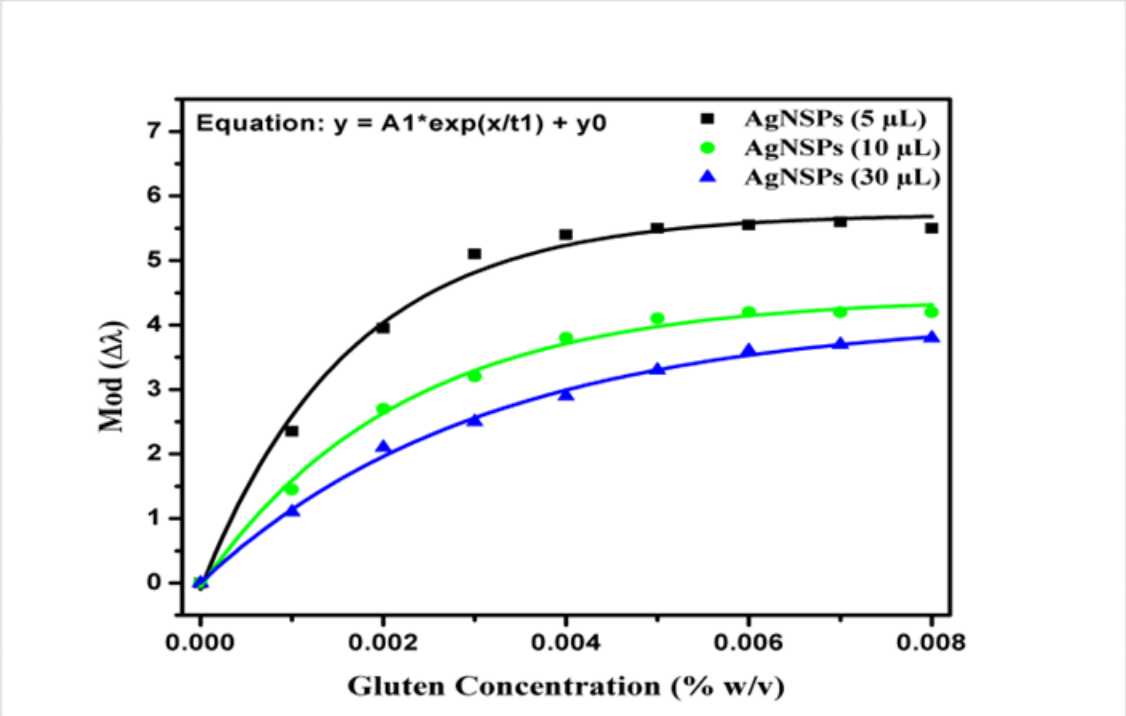

Figure 4.6 *Effect of Gluten on |Δλ| with different volumes of AgNSPs (5, 10 & 30 µL)*

Table 4.4 *Decay constant of exponential lines of Gluten interaction with AgNSPs*

AgNSPs Volume (µL)	Decay Constant
5	-0.00162
10	-0.00219
30	-0.00319

4.2 Gold Nanospheres

4.2.1 UV spectra of Gold Nanospheres

Synthesized gold nanoparticles by seed mediated method have spherical shape which is confirmed with UV-Visible spectra and DLS. Gold nanospheres have a single peak at 531.8 nm which confirms its spherical shape. Hydrodynamic size is given by DLS which is 22.32 nm which includes coating of the surfactant. UV-Visible spectra and DLS of synthesized nanospheres are shown in Figure 4.7.

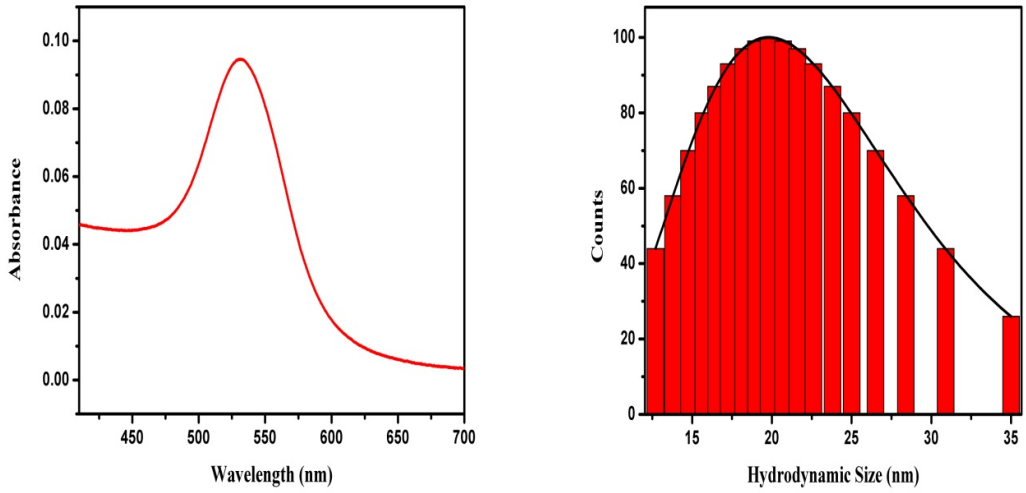

Figure 4.7 *UV-Visible Spectra & Dynamic Light Scattering (DLS) Gold Nanoparticles*

4.2.2 Interaction of BSA with Gold nanospheres

BSA interaction with AuNSps of different volumes (5 µL, 10 µL and 30 µL) is studied. Due to interaction there is blue shift in the UV-visible spectrum with BSA [3,4]. 0-5 mM range of concentration of BSA is studied for interaction with gold nanospheres. The step size of BSA

concentration for study is 0.5 mM. The UV-Visible spectrum of interaction of BSA with different volumes i.e. 50 , 100 and 200 µL of gold are shown in Figure 4.8, 4.9 and 4.10 respectively.

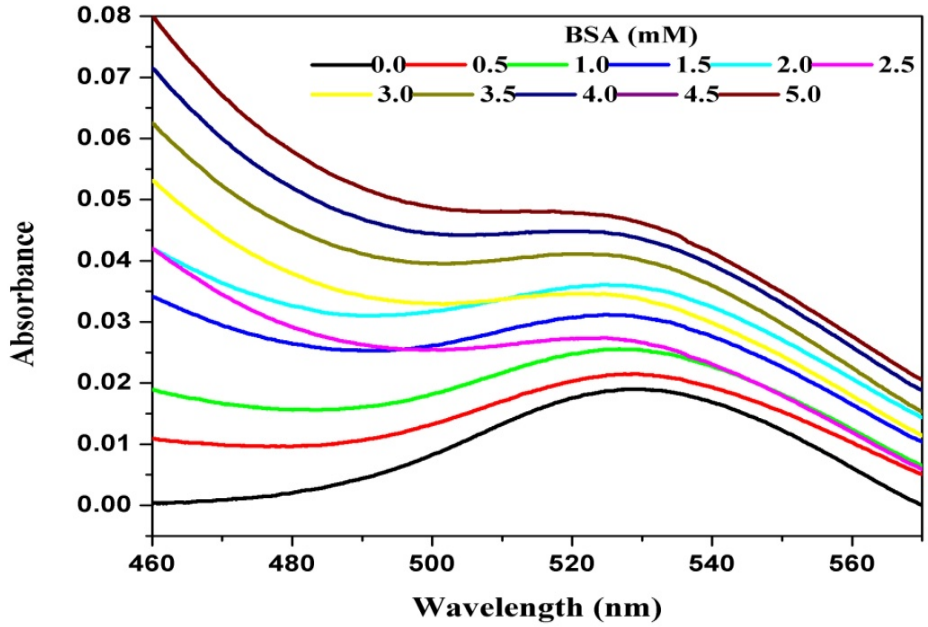

Figure 4.8 *UV-Visible spectrum of interaction of BSA with Gold (50 µL)*

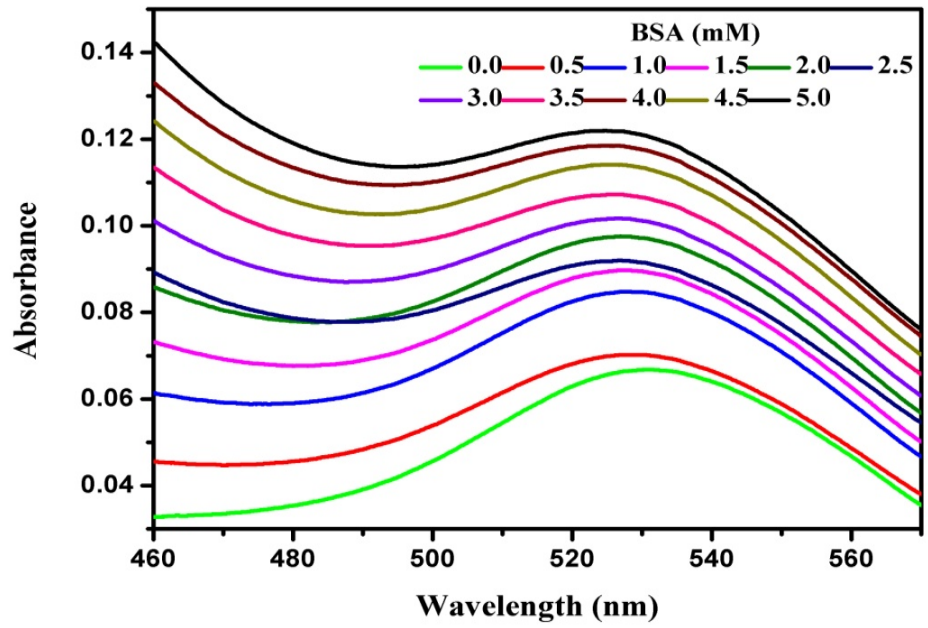

Figure 4.9 *UV-Visible spectrum of interaction of BSA with Gold (100 µL)*

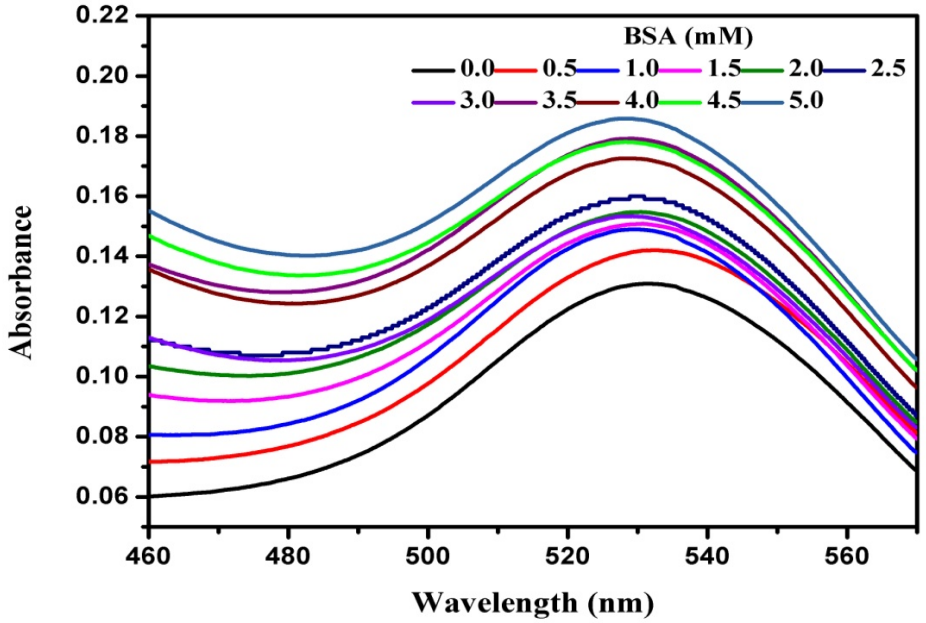

Figure 4.10 *UV-Visible spectrum of interaction of BSA with Gold (200 µL)*

It is clear from the above UV-Visible spectrum that there is continuously increase in the absorption with increasing concentration of BSA. The corresponding peak shift w.r.t. original peak is noted and is listed in Table 4.5 as given below.

Table 4.5 *Modulus of peak shift (|Δλ|) of BSA interaction with gold nanospheres*

BSA Concentartion (mM)	|Δλ| of AgNSPs (5µL)	|Δλ| of AgNSPs (10µL)	|Δλ| of AgNSPs (30µL)
0	3	0	0.05
0.5	5.6	1.5	0.4
1.0	7.3	3.4	1
1.5	8.65	4.1	1.5
2.0	9.95	4.4	2
2.5	12.15	4.7	2.15
3.0	13.45	4.9	2.25
3.5	14.3	5.4	2.65
4.0	15.75	6	3
4.5	-	5.9	3.3
5.0	-	6.8	3.25

Peak shift (Δλ) show linear relationship with BSA concentration at all volumes of gold nanospheres i.e. 50 µL, 100 µL and 200 µL. BSA has highest activity towards 50 µL volume

of gold nanospheres followed by 100 µL and 200 µL. The effect of BSA on peak shift is shown in Figure 4.11.

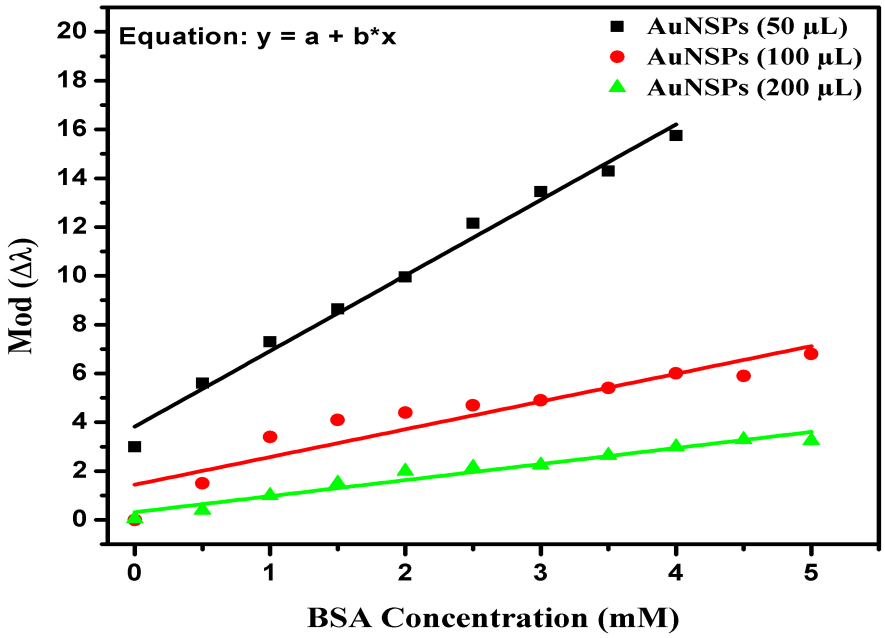

Figure 4.11 *Effect of BSA on |Δλ| with different volumes of AuNSPs (5, 10 & 30 µL)*

The activity of BSA towards gold nanosphere volumes can be estimated from slope of linear relationship between Δλ and BSA concentration which maximum for 50 µL is followed by 100 µL and 200 µL as listed in table 4.6 as given below. Thus 50 µL of AuNSPs have highest sensitivity for BSA detection.

Table 4.6 *Slope of straight lines of BSA interaction with AuNSPs*

AuNSPs Volume (µL)	Slope of Line
50	3.09667
100	1.13636
200	0.65270

4.2.3 Interaction of Gluten with Gold nanospheres

Gluten of concentration 0-2 % w/v is used here for the interaction study with gold nanospheres having different volumes 50, 100 and 200 µL. The UV-Visible spectrum of

interaction of gluten with gold nanospheres is shown in figure 4.12, 4.13 and 4.14, which shows that there is continuous increase in absorption with change in gluten concentration from 0-2 % w/v. The step size of gluten for interaction study is 0.2 % w/v and there is blue shift due to interaction of gluten with gold nanospheres.

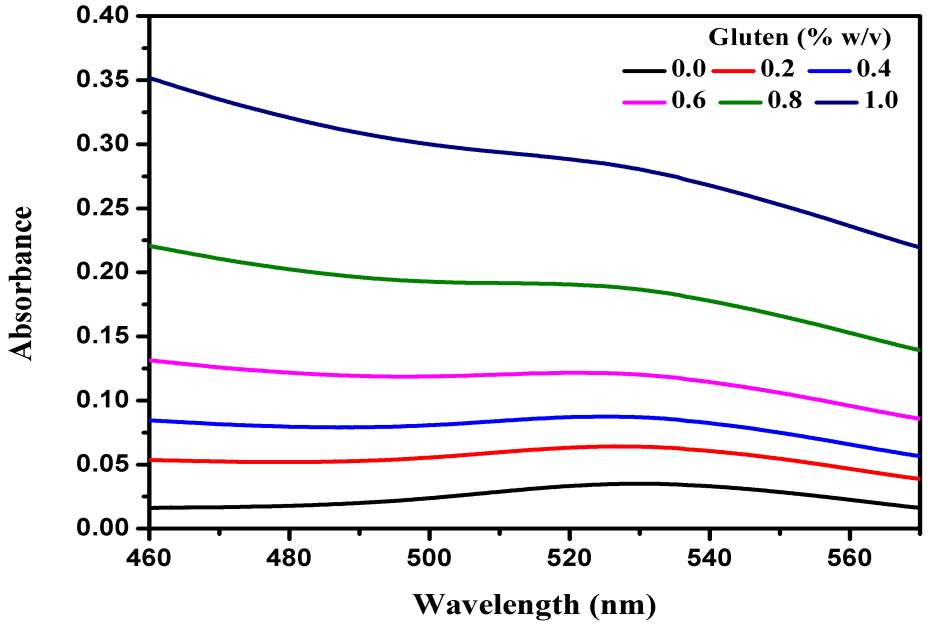

Figure 4.12 *UV-Visible spectra of Interaction of Gluten with AuNSPs (50 µL)*

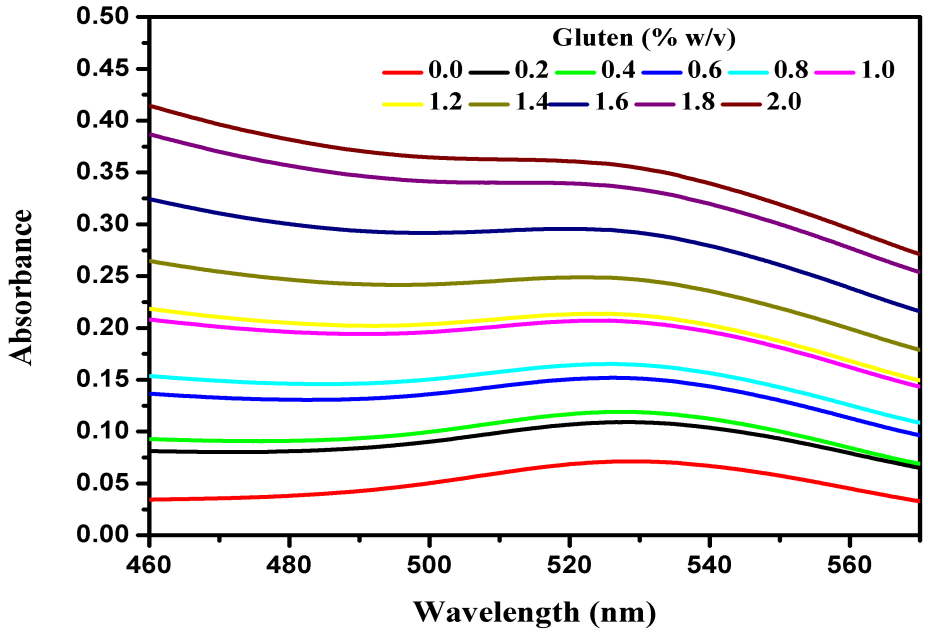

Figure 4.13 *UV-Visible spectra of Interaction of Gluten with AuNSPs (100 µL)*

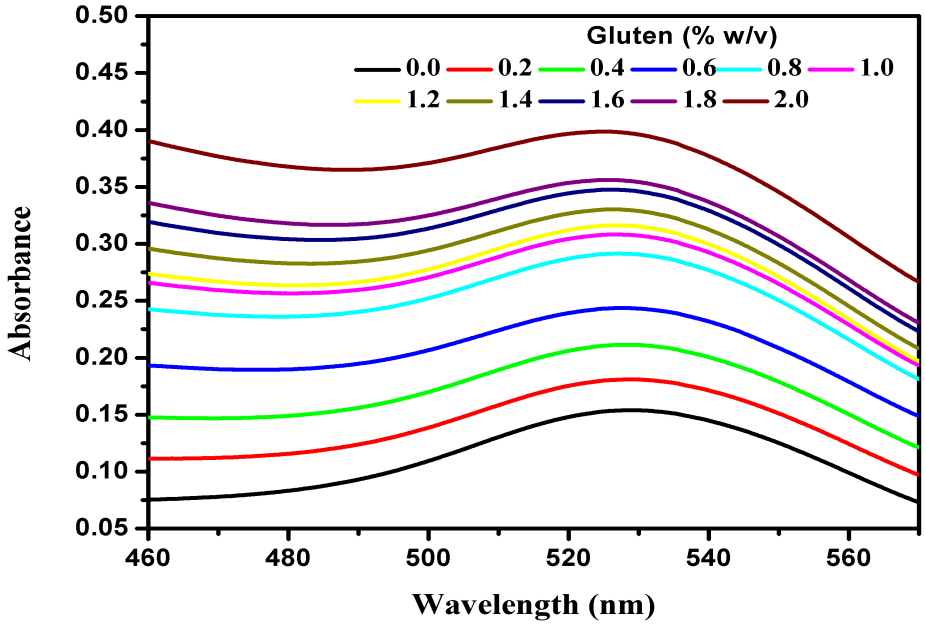

Figure 4.14 *UV-Visible spectra of Interaction of Gluten with AuNSPs (200 µL)*

Gluten concentration at all volumes of gold nanospheres i.e. 50 µL, 100 µL and 200 µL shows linear relationship with peak shift. Peak shift corresponding to different volumes of AuNSPs is shown in Table 4.7.

Table 4.7 *Modulus of peak shift ($|\Delta\lambda|$) of Gluten interaction with gold nanospheres*

Gluten Concentartion (% w/v)	$\|\Delta\lambda\|$ of AuNSPs (50 µL)	$\|\Delta\lambda\|$ of AuNSPs (100 µL)	$\|\Delta\lambda\|$ of AuNSPs (200 µL)
0	0	0	0
0.2	2.75	0.8	0.05
0.4	4.75	1.1	0
0.6	8.3	1.5	0.05
0.8	--	2	0.1
1.0	--	2.35	0.3
1.2	--	2.65	0.3
1.4	--	3.05	0.3
1.6	--	3.35	0.6
1.8	--	3.6	0.8
2.0	--	4.2	0.8

The activity of Gluten towards gold nanosphere volumes (50, 100 and 200 µL) can be estimated from the graph and slope of linear relationship between Δλ and Gluten concentration which shows that activity is higher towards 50 µL followed by 100 µL and 200 µL as shown in figure 4.15 and listed in table 4.8. Highest sensitivity of deflection is observed with 50 µL of AuNSPs.

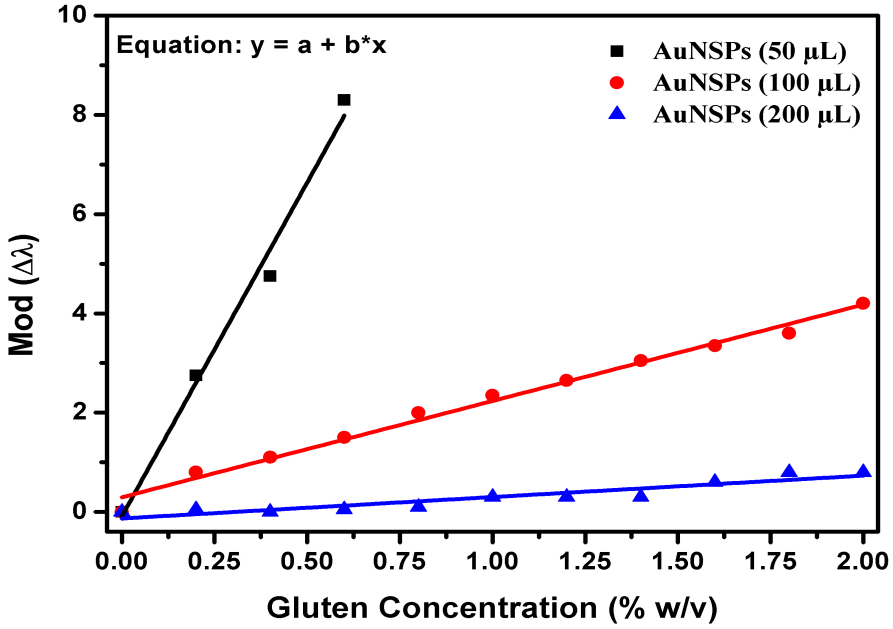

Figure 4.15 *Effect of Gluten (0-2% w/v) on peak shift (Δλ) of gold nanospheres*

Table 4.8 *Slope of straight lines of Gluten interaction with AuNSPs*

AuNSPs Volume (µL)	Slope of Line
50	13.45
100	1.94
200	0.43

References

1. Dasgupta, Nandita, et al. "Bovine serum albumin interacts with silver nanoparticles with a “side-on” or “end on” conformation." Chemico-biological interactions 253 (2016): 100-111.
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3. Chakraborty, Soumyananda, et al. "Contrasting effect of gold nanoparticles and nanorods with different surface modifications on the structure and activity of bovine serum albumin." Langmuir 27.12 (2011): 7722-7731.
4. Dominguez-Medina, Sergio, et al. "In situ measurement of bovine serum albumin interaction with gold nanospheres." Langmuir 28.24 (2012): 9131-9139.

Conclusions

Silver nanospheres are synthesized by seedless method and gold nanospheres are synthesized by seed mediated method. Synthesized silver and gold nanoparticles are highly stable and spherical in shape which is confirmed by UV-Visible spectroscopy and DLS. Interaction of BSA and Gluten is studied with different volumes of silver and gold nanospheres and it is concludes that with same concentration of proteins smaller volume of nanoparticles has better detection sensitivity for BSA and Gluten. The lowest concentration of BSA that could be detected with AgNSPs and AuNSPs is 2.5 μM and 0.5 mM, respectively. The lowest detection limit of Gluten with AgNSPs and AuNSPs is 0.001 % w/v and 0.2 % w/v, respectively.