EVALUATION OF NATURAL GUMS AS POTENTIAL PRECURSORS FOR SYNTHESIS OF GOLD AND SILVER NANOPARTICLES: THEIR CHARACTERIZATION AND APPLICATIONS

Ph.D Thesis

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(JANUARY, 2017)
ABSTRACT

Herbal medicines are recognized as multi-component remedies for the treatment of complicated diseases because of their better therapeutic value and fewer side effects compared to modern medicine. The work presented in this thesis consists of “Evaluation of natural gums as potential precursors for synthesis of gold and silver nanoparticles: their characterization and applications” which includes the synthesis, optimization, characterization, applications and bioactivity evaluation of gold and silver nanoparticles stabilized with different gums.

Four different gums of plants origin and one gum of microbial origin were selected for the synthesis of gold and silver nanoparticles. Gums of plants origin were collected from three different plant families i.e. family Fabaceae (genus: Acacia), family Rosaceae (genus: Prunus), family Bixaceae (genus: Cochlospermum), while the microbial “Xanthan gum” was obtained from Xanthomonas campestris.

In this study gold and silver nanoparticles (Au- and Ag-NPs) were biosynthesized by using the aqueous extracts of different gums. Synthesized nanoparticles were characterized with different spectroscopic and microscopic techniques such as UV–Vis spectroscopy, Fourier Transform Infrared (FTIR), Scanning electron microscopy (SEM), energy dispersive X-ray (EDX), X-ray diffraction (XRD) and atomic absorption (AA). The effects of gum, metal ions concentration, reaction temperature and time on the synthetic stability of nanoparticles was studied along with their post synthetic stability against varying pH and salt concentrations, long term storage and extreme of temperature. Formation of Au- and Ag-NPs was confirmed from the surface Plasmon resonance in the range of 500-560 and
400-450 nm respectively and was further corroborated from the results of FTIR, EDX, XRD and Atomic absorption. SEM analysis showed that Au- and Ag-NPs stabilized with different gums were mostly spherical and in the size range of 5–50 nm. FTIR analysis revealed that the abundance of hydroxyl, carboxylate, amide and acetyl groups of the gum biopolymers are involved in the reduction and stabilization of metal cations. The EDX analysis confirmed the presence of Au and Ag in the respective gum stabilized nanoparticles. No peaks were observed in the XRD spectrum of pure gums, thus indicating their non-crystalline nature; while the XRD pattern of gum stabilized Au- and Ag-NPs have characteristic peaks, and so are crystalline in nature. Atomic absorption analysis showed almost complete reduction of the metal ions into the respective nanoparticles. The TGA spectrum further confirmed their thermal stability, where three successive weight losses were observed in the temperature range of 50–800 °C. It was observed that reaction temperature of 80°C and reaction time of 4 h was most suitable for the efficient synthesis of Au- and Ag-NPs. The results of post-synthetic stability revealed that Au- and Ag-NPs were stable in different concentrations of NaCl (1–3 M), neutral to acidic pH (7–2) and without any long term storage (eight months) or thermal (100 °C) induced degradable changes.

Gold and silver nanoparticles stabilized with different gums were evaluated for their prospective anticancer, antibacterial, urease inhibition, anti-inflammatory and analgesic properties. Gold and silver nanoparticles possessed mild to moderate antibacterial activity and can observed from their zone of inhibition against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Gold nanoparticles significantly alleviated the acetic acid induced writhes at much lower doses of 40 mg/kg (P < 0.01) and 80 mg/kg (P < 0.001)
compared to that of gum extract at 200 and 400 mg/kg (P < 0.001). At similar doses, Au-NPs also significantly inhibited the carrageenan induced paw edema during the 1st h (P < 0.05) and 2–5 h (P < 0.001) of the study duration. Different gums stabilized Au- and Ag-NPs were tested to evaluate their cytotoxic potential against human cervical cancer cells (HeLa). Potential anticancer effect was showed by the respective gold and silver nanoparticles. Biosynthesized Au- and Ag-NPs were also able to inhibit urease with better affinity.
CHAPTER: 1

INTRODUCTION TO GUMS
1.1 Introduction to gums

Natural gums are thought to be pathological products produced by the plants due to adverse conditions, such as drought or injury [1]. Chemical analysis shows that natural gums are promising biodegradable polymeric materials. Plant gums are adhesive substances having a variable chemical composition, and are usually produced as exudates from the bark of shrubs or trees. Some natural gums of plants origin, such as gum Arabic, are water soluble and produce clear solution when dissolved in water, whereas some natural gums produce mucilages due to absorption of large quantities of water.

From centuries plant gums produced in several countries of the world are considered to be the most significant item in international trade of pharmaceutical, textile, paper, food and other industries. Gums of plant origin are generally classified as 'non-food' and 'food' or 'technological grade' gums depending upon their major uses. The previous class has major uses in non-food industries, such as textile, paper and includes 'gum talha' 'gum ghatti', and many other gums. The later can be used as food additives in different kinds of beverages, foods, confectioneries, such as gum tragacanth, gum arable, gum carob and karaya.

1.2 Classification of gums

A wide variety of plants, seaweeds, animals, microbial sources and fungi produces gums in high quantity. They perform a lot of metabolic and structural functions inside their bodies. Huge quantities of gums are obtained from plant sources. The various available gums can be classified as follows.

1.3 Classification of gums on the basis of charge
1.3.1 Non-ionic seed gums: These gums are less common as compared to gums obtained from other sources. As these gums are produced in seeds so are called seed gums. Locust (carob) bean gum, guar, tamarind, starch, amylose, arabinans, galactomannans are some common examples of seed gums. These gums are present in the center endosperm of a seed and thus are analogous to wheat. Locust bean gum does not come from insects regardless of its name, but relatively from the seed of the carob pod. Carob is known as the usual health food store substitute for chocolate. Inside of the seed becomes locust bean gum and the external covering is converted into carob powder. Non ionic seed gums, unlike flour or corn starch did not need heat for thickening, which is a big advantage in processing some foods.

![Structure of non ionic seed gum](image)

**Figure 1.1:** Structure of non ionic seed gum

1.3.2 Anionic gums: Acacia gum (gum Arabic), agar, gellan, tragacant, algin, pectic acid, karaya, carrageenans etc are examples of anionic gums.

1.4 Classification of gums on the basis of source
On the basis of source/origin gums can be classified as follows.

1.4.1 **Plant origin:** Plants are the major contributors in the production of natural gums. About 80% of the gums used in industries and pharmaceuticals are obtained from plants. All plants do not produce gums and mucilages, whereas some plant families are very famous for gums and mucilages production in large quantities. Family Fabaceae (genus accasia), family Rosaceae (genus prunus) and family Bixaceae (genus regeligosum) are the major contributors in the production of gums worldwide.

1.4.2 **Tree/ shrubs exudates:** Exudates is an alternative name for sap, similar to maple syrup. Chemically there is no distinction between gums and maple syrup, as the gums like gum karaya, gum tragacanth, gum ghatti and gum arabic are formed from these saps after solidification as they seep from the tree. The tough sap is collected from the tree in the form of gum and ground down to fine powder. Gums obtained from different plants have different chemical composition, and every gum hold exclusive properties and applications. Gum arabic is extensively used as flavoring agent and emulsifier.
Albizia, tragacanth, arabic, karaya and ghatti are the commonly known natural gums of plant origin.

1.4.3 Animal origin: These gums are obtained from animal sources. Chitosan, chitin, hyaluronic acid and chondroitin sulfate are some famous gums of animal origin.

1.4.5 Marine origin/ (seaweed) gums: These gums are produced by the marine plants or seaweeds. A variety of algae and seaweeds comprise of marine gums as component of cell walls and membranes and are also present in the intracellular regions where they actually serve as reserve food material. Carrageenan, alginate, and agar-agar are commonly known marine gums. Some of the marine gums like agar form gels and are used in science laboratories for the growth of molds. Agar, carrageenans, alginic acid, laminarin can be
obtained from the respective marine plants by boiling the plant materials after drying and grinding.

![Figure 1.3: Pictures of marine plants producing gums](image)

1.4.5 **Microbial origin:** These gums are synthesized by using microorganisms. They are essentially foods modified by microbes. Dextran, curdian, xanthan, pullulan, emulsan, zanflo, schizophyllan, Baker’s yeast, glycan, krestin, scleroglucan, lentinan etc are some common examples of microbial gums. Xanthan gum is the most famous microbial gum and is produced by the microorganisms “*Xanthomonas campestris*” using a natural (microbial) fermentation process which converts corn syrup to Xanthan gum. The gum is produced as a protective coating on green leaves, such as cabbage due to the action of bacteria. The gum has many industrial and pharmaceutical applications. In food industry it is extensively used in salad dressings, sauces, ice cream and also gluten free foods.
1.5 **Semi-synthetic gums**: Semi synthetic gums include decomposed starch and its derivatives. Following are the two major groups of semi synthetic gums.

1.5.1 **Starch derivatives**: Starch acetate, hetastarch, starch phosphates.

1.5.2 **Cellulose derivatives**: Hydroxyethyl cellulose, methylcellulose (MC), microcrystalline cellulose (MCC), carboxymethyl cellulose (CMC), hydroxypropyl methylcellulose (HPMC).

1.6 **Classification of gums according to shape**

1.6.1 **Linear**: These gums are linear in arrangement. Algins, amylose, cellulose, pectins are the common examples.

1.6.2 **Branched**: These gums are branched in shape and are further of two types

(1) Short branches: Xylan, galactomanan, Xanthan etc
(2) Branch-on-branch: Arabic, tragacanth, amylopectin etc.

1.7 **Classification according to manomeric units in chemical structure**

1.7.1 **Homoglycans**: Cellulose, arabinanas, amylose etc.

1.7.2 **Diheteroglycans**: Galactomannans, carragennans, algins etc.
1.7.3 **Tri-heteroglycans:** Arabinoxylans, gellan, Xanthan etc.

1.7.4 **Tetra-heteroglycans:** Seed gum, psyllium penta-heteroglycans, gum arabic, tragacanth, ghatti gum etc.

1.8 **Applications of gums**

Many natural and synthetic gums are considered as safe for human consumption and therefore are used in the food industry. Gums obtained from various origins and their derivatives constitute a group of polymers commonly used in pharmaceutical dosage forms. However, there is rising apprehension regarding the safety of pharmaceutical excipients resulting from natural sources. Screening of gums has become a vital pharmaceutical area due to extensive uses of various gums in textiles and cosmetics. Now a day’s plant exudates and gums are screened for their application as pharmaceutical adjuvants. Gums of various sources are also applied in usual dosage forms of different drugs for their thickening, humidifying, stabilizing and binding properties in medicine. However, a variety of gums used as pharmaceutical adjuvants have severe stipulations, which hardly any ordinary agents can perform. Natural gums have the following significant industrial and pharmaceutical applications.

1.8.1 **Applications in the food industry**

Natural gums have several uses in food manufacturing [2]. Natural availability, low cost, biocompatibility and bioavailability of gums make them as suitable candidates for their use in foodstuff production. Various gums have special applications, such as water preservation, stabilization (locust bean and guar gum) instant pudding, (carrageenanas) dairy, meat products, and confectionary (agar) beverages, sauces and backed products (pectins, xanthan gum, tragacanth, gum arabic, alginates).
1.8.2 Pharmaceutical applications

Natural gums have enormous applications in pharmaceutical industries. Owing to their demulcent properties, they are used in medicines for cough suppression. They can be used as mass laxatives and as a component of teeth and several other important adhesive materials. These hydrophilic polymers are useful as gelling agents, emulsifiers, suspending agents, tablet binders, disintegrants and film making agents in periodontal and transdermal films, thickening agents, stabilizing agents, sustaining agents in matrix tablets as well as buccal tablets and coating agents in microcapsules as well as those applied for protein delivery.

1.9 Benefits of natural gums in pharmaceutical sciences

Natural plant based materials have the following advantages.

1.9.1 Non-toxic nature and bio-compatibility: Almost each of these plant products are chemically carbohydrates and proteins and are composed of repeating units of sugar (monosaccharides) and amino acids. As they are biopolymers therefore they are non-toxic and biocompatible.

1.9.2 Biodegradability: Biopolymers produced by all living organisms are biodegradable. They are readily decomposed by the microbes present in the environment and thus signify really renewable resource and have no bad impact on environmental or humans health (e.g. eye + skin irritation).

1.9.3 Low cost: People in many developing countries are poor and cannot afford expensive products. Use of natural sources is always cheaper because the production cost of synthetic material is much high compared with that of natural one.
1.9.4 Environmental-friendly processing: Owing to the simple manufacture procedures involved, gums from various sources can be readily collected in various times in huge amounts.

1.9.5 Local availability: Natural gums are mostly of plant origin and are therefore locally available. Due to excessive use in different industries governments in advance countries encourage the production of plants, like tragacanth and guar gum.

1.9.6 Public acceptance as well as better patient tolerance: Synthetic materials have more side effects as compared to that of natural ones and therefore are not acceptable to the public. Gums are mostly of natural origin therefore are publically accepted e.g. PMMA, povidone.

1.9.7 Edible sources: The majority of gums are obtained from edible sources.

1.10 Industrial uses

Natural gums are used in textiles (cellulose, dextrin, tamarind gum, starch and pectins), cosmetics (tragacanth, gum arabic and gum karaya), lithography (gum Arabic, locust bean gum, tragacanth, adhesives (acacia gum, and tragacanth), paper production (cellulose, tamarind) and paints (resins, pectins, hemicellulose).
2.1 Introduction to Nanotechnology

Nanotechnology, nanomaterials, nanoscience or nanostructures are some latest nano containing terminologies that occurs mostly in newspapers and in scientific reports. Since the last few years the engineering and scientific communities have been making remarkable development in the field of nanotechnology and nanoscience. Nanotechnology is the study of tiny materials and structures in the range of a few nanometers to less than 100 nanometers in size. Because of their sizes and shapes nano-materials show novel physical and chemical properties. Nanomaterials are typically classified on the basis of sizes and shapes, like nanotubes, nanowires and nano-layers etc. Nanotechnology is the synthesis, characterization, and application of materials which has at least one dimension between 1-100 nm scales [3].

For synthesizing nanoscale materials there are two principal ways, bottom – up nano synthesis starts with individual molecules or atoms and make up them upto nano level, while Top – down nano synthesis initiates with huge materials and proceeds to build it smaller through consecutive cuttings. Due to variation in their morphology, size, shape and surface to volume ratio, nano-materials display novel and improved physical, chemical and biological properties [4]. In literature many examples are available regarding variation in physical properties, like Cu is transparent at nano scale and is opaque in bulk [5]. Platinum is inert in bulk, while at nanoscale it shows excellent catalytic properties [6]. Aluminum is stable in bulk, while it becomes inflammable at nanoscale [7]. Similarly silicon is insulator in bulk and shows better conductive properties at nano level [8].

2.2 Background of Nanotechnology
The history of nanomaterials is quite old, however during the last two decades major development has occurred in the field of nanoscience and nanotechnology. Michel Faraday (1791-1867), determined systematically the optical behavior of delicate film of gold by using solution of gold chloride salt. He employed phosphorus as a reducing agent and synthesized small size Au-NPs in aqueous system [9]. Rechard Feynman in 1957 acknowledged the second clue of nanotechnology. He worked as physicist at institute of technology California and published one of his article in 1960 entitled, “There is plenty of room at the bottom” Growing interest of investigating controlling matter at the nanoscale, Feynman envisaged that whole encyclopedia of Britannica can be transcribed on the head of a pin by restricting material at a degree of individual atoms and molecules. He introduced novel logical terminologies, like nanotechnology, nanomaterials, nano engineering and nanoscale, in modern research. He also used terms, such as nanoscale objects, small materials and miniaturization in his speech [10]. The potential of nano-engineering substances were described by ‘Norio Taniguchi; a researcher at the University of Tokyo in 1874. In 1980s, AFM and STM invented by Henrich Rohrer and Gerd Binnig, performed significant role in scheming substances at nano-level and copying images of atom or compounds in nanotechnology. Fullerene C60, was discovered by Kroto’s and Smalley’s et al in 1985 and after that nanotechnology got development and popularity through speeches, books and articles. In 1986 Eric Drexler wrote a book entitled, (Engines of creation, the coming era of nanotechnology) for promoting nanotechnology and nanoscience. Saumio Iijima in 1991 discovered carbon nanotubes for the first time. Since 2000, National Nanotechnology Initiative program (NNI) was started by united state government for promoting nanoscience in collaboration with different 16 US autonomous
states. Richard Zsigmondy used ultra-microscope to observe the size of nanoparticles for the first time. All of the above stated discoveries lined strong way for growth and development in the era of nanotechnology.

2.4 Different Types of Nanostructures (NSs)

In nanotechnology there are different classifications for nanostructures; NSs are generally classified by their size, shape and morphology. All bulk materials including metals, polymers, semiconductors, glass, and ceramic etc can be converted into nanomaterials by using different analytical techniques. Nanostructures typically consist of nanocrystallites, nanobelts, nanofibers, nanoneedles, nanoflowers, nanoflakes, nanocages, nanocomposites, nanopinfilms, nanofoams, nanoparticles, nanofabrics, nanomeshes, nanorings, nanopillars, nanorods, nanoshells, nanowires, nanotubes, quantum dots, nanoclusters, nanopowders, sculptured thin films and quantum hetero etc [11].

2.5 Forms of Nanomaterials (NMs)

Following are a few significant characteristics and features of nanomaterials

2.5.1 Nanoparticles (NPs)

In modern material science a metal nanoparticle fabrication is one of the most gorgeous and exciting research areas and is rising quickly. Researchers have succeeded in developing new materials with lower cost, more functionality and improved physical properties than existing ones in the field of nanotechnology. In order to enhance performance of nanomaterials, numerous wonderful methods have been developed displaying enhanced characteristics with intend to boost excellent command over particle morphology, distribution and size [12]. Nanoparticles exhibit electrical [13] and magnetic [14] properties completely different from their bulk materials, surfaces and individual atoms.
They have a broad variety of uses, in nearly all fields of science, including, biotechnology [15], bio labeling [16], biosensors [17] chemical sensor [18], catalysis [19], drug delivery [20] and electrochemical sensors [21].

For better practical application of Metal nanoparticles (M-NPs), some vital properties, like matching shapes, uniform size distribution and well dispersion is necessary. Controlling size of M-NPs at nanoscale level is also essential.

2.5.2 Fullerenes

Smalley’s and Kroto’s discovered Fullerene C60 in 1985, by evaporating graphite through laser [22]. Besides diamond and graphite these are thought as the third allotropic form of carbon and consist of 60, 70 or more carbon atoms; eventually forming 5 and 6 member structures. Structurally they are different from diamond and graphite. Generally fullerenes are classified on the bases of their shapes. Those having cylindrical shapes are called nanotubes or "Bucky tubes" where as spherical shaped fullerenes are known as "Bucky balls". Fullerenes derivatives show many important properties, and therefore have many potential applications in electronic devices such as, transistors, batteries and sensors [23].

Due to their exclusive structures and attractive properties nowadays engineers, physicists, chemists, and biologists, are paying much attention towards Fullerene. These materials are applied in fabricating chemical sensors. Fullerenes commonly termed as C60 possess 60 pi- electrons and have remarkable capacity to absorb non-polar and partially polar organic compounds and therefore can be used as a superior adsorbent, however they are not capable of absorbing polar organic species, metal cations and anions. In medical science they are used for HIV inhibition [24], in electro sensors [25] and as gas adsorbent [26].

2.5.3 Nanotubes (NTs)
Nanotubes show a close resemblance in structure to fullerenes. The cluster is made up of nanowires, nano belts, nanotubes and nanorods. Nanotubes have more applications due to their better characteristics over utmost available nano structures, such as carbon fibers and metal nanoparticles. Iijima in 1951 introduced multi walled carbon nanotubes (MWCNTs) for the first time, by vaporizing carbon graphite in inert environment with the use of an electric arc applying chemical vapors deposition (CVD) method [27]. Fabrication of MWCNTs or SWCNTs entirely depends upon synthetic environment, SWCNTs are made up of single cylinders of graphite sheet and MWCNTs consist of several packed cylinders of graphite sheets. Network of carbon nanotubes consist of strong C-C covalent bond [28]. Incredible mechanical properties of carbon nanotubes are due to sp2 hybridization of carbon atoms that increase the strength of C-C bond [29]. Properties of carbon nanotubes depend upon its structure and some of them behave like metallic or semiconductors, while others show extra ordinary electronic properties. Owing to its electronic properties its breaking or tensile is 100 times more than steel. Carbon nanotubes are used to construct electronic devices at nanoscale [30]. In the field of nano science carbon nanotubes have broad range applications, such as in sensing devices and scanning probe microscopes [31].
2.5.4 Carbon Nanofibers (CNFs)

Carbon nanofibers are formed by breakdown of CO or natural gases by using some metal nanoparticles such as Fe, Co, Ni as catalyst at high temperature. Hollow cylinders of CNFs having a size of 200 nm reshuffles and form various structures such as cups, cones or plates [32]. Electrical and mechanical properties of CNFs and CNTs are almost similar, but CNFs are superior over CNTs due to their extraordinary cost difference. CNFs can build physical bonding with other materials in a better way due to having sticky graphene edge planes. CNFs are 100 times greater than SWCNTs or MWCNTs in size; furthermore naturally SWCNTs or MWCNTs are more crystalline [33]. CNFs materials are extensively used in the production of high surface area electrodes for
useful applications, like power storing [34], in electro analysis [35] or as support for catalysts [36], biotechnology [37] and biosensor [38].

2.5.5 Nanocomposites

Nanocomposites are those substances that consist of more than one phase. This material interacts internally; therefore nanocomposites demonstrate better properties to each those usual composites or pure one phase [39]. For a variety of uses, a lot of concentration has been paid to produce nanocomposites materials with well-controlled structures. Nanocomposites with elevated surface area can provide a considerable and useful scheme for catalysis [40], sensing [41], in field separation [42], sorption [43], in fuel cells [44], and e/m transport mechanisms [45]. Numerous synthetic methods of well controlled nanostructures composites materials are illustrated in literature and among them simple and highly effective method for introducing nanoparticles or nanotubes into polymer matrices [46].

2.5.6 Microporous and Mesoporous Materials

Porous solids with distinct size between 1-100 nm are called microporous and mesoporous materials [47]. Porous substances can further be divided into three groups on the basis of pore size (1) Microporous solids have pore size less than 2 nm (2) Mesoporous solids have pore size between 2 -50 nm (3) Macro porous solids have pore size greater than 50 nm.

In the field of adsorption and catalysis microporous and mesoporous materials display extraordinary performance. When combined with other materials, these solid porous materials illustrate enhanced and outstanding properties with extra functionality. Zeolite is the most suitable commercially available microporous material [48].

2.6 Metal Nanoparticles
CHAPTER 2
INTRODUCTION TO NANOTECHNOLOGY

The field of metal nanoparticles has a wonderful growth during the last two decades. For the first time Fe-NPs were synthesized from Fe (CO)\textsubscript{5} using organic solvents; similar method was used for the synthesis of CO-NPs. At low temperature the carbonyl was decomposed sonochemically and amorphous Fe-NPs were synthesized [49]. Nanoparticles can be synthesized with different physical, chemical and biological methods, and have enormous applications in the field of nanotechnology.

2.7 Different techniques for synthesis of gold and silver nanoparticles

Due to broad range applications of metal nanoparticles, a lot of methods have been developed for their preparation. The following methods are generally applied for the preparation of gold and silver nanoparticles.

2.7.1 Physical Synthesis

Numerous physical techniques have been applied for the production of Au- and Ag-NPs in various solvent systems, such as laser ablation [50], photolysis [51], sonochemical [52], radiolysis [53], low temperature vapors co-condensation [54], microwave assisted [55], supercritical fluids [56] etc. Some of the chemical techniques leading to the synthesis of nanoparticles can provisionally be thought as physical ones owing to the action of a physical feature on the system under study. For example, radiolysis, sonication or photolysis may produce free radicals or solvated electrons that act as possible initiators for subsequent transformations.

2.7.2 Biosynthesis
In biosynthetic method plant extract, natural gums, microorganism’s cells or enzymes are employed for the preparation of M-NPs. For M-NPs fabrication only those metals can be used which are situated below hydrogen in the electrochemical series. Different high and low molecular weight bioorganic molecules can be used as reducing agents or surface stabilizers for the biosynthesis of Au- and Ag-NPs [57]. ‘Brassica juncea’ plant extract has been used as surface stabilizer for the biosynthesis of Ag-NPs [58]. Bacterium, *Brevibacterium casei* has been used as a reducing and stabilizing agent for production of spherical shaped gold and silver nanoparticles [59]. Nanoparticles were also biosynthesized using natural plant gums such as “gum khundakugo” as reducing and stabilizing agent [60].

2.7.3 Electrolytic Methods

Morphology, shape and size of nano-structured materials can be controlled by using this method. Electrochemically Au- and Ag-NPs can be prepared by passing electric current among electrodes in an appropriate medium. In literature there are several interesting information regarding electrochemical production of Ag, Au, Pd, Cu and Pt-NPs. On the surface of electrode various metallic salts make stable M-NPs, whereas others are stabilized by surfactants. The entire electrolytic methods follow the same mechanism, where the cations are liberated on cathode and are consequently stabilized and displaced by the surfactants in solution [61].

2.7.4 Chemical Methods

The chemical techniques for the synthesis of Au- and Ag-NPs are superficial and efficient than physical one, as they offer easiness, quickness and enhanced influence over shape, morphology and functionalization. A wide range of nanomaterials, such as one dimensional
nanotubes and nanowires, two-dimensional nanowalls and nanofilms as well as zero-dimensional nanocrystals, have been synthesized by using the chemical methods. Different organic reducing and stabilizing agents along with unusual strategies (aqueous and organic media, homogeneous and heterogeneous) have been used in broad range of reaction condition. Chemical vapor deposition (CVD), impregnation, deposition–precipitation, co-precipitation, chemical vapor impregnation, reverse micelle emulsions and micro emulsions are some chemical methods used for the production of noble nanoparticles [62].

2.8 Reducing Agents

Generally for the synthesis of gold and silver nanoparticles reducing agents react with metallic salts (chloride and nitrates) according to following chemical equation.

\[ mM^+ + n \text{Red} \rightarrow mM^0 + n^{ox} \]

A number of reducing agents have been applied for the fabrication of Au- and Ag-NPs.

2.9 Capping agents

In nanoparticles synthesis capping agents are becoming increasingly predominant, and are mainly applied for stabilization of incredibly reactive nanoparticles surfaces to dissolution and aggregation. Excess surface energy of the nanoparticles is decreased when the stabilizers interact with the surface atoms of the nanoparticles. Capping agents increase stability of the product through steric stabilization, electrostatic repulsion, or a combination of both mechanisms. Sulfur-containing organic compounds (disulfides thiols), organic compounds containing polar functional group and surfactants are mostly used as stabilizer. Capping agents are generally classified into the following three categories: neutral (2, 2, 2 [mercaptoethoxy(ethoxy)]ethanol and polyvinylpyrrolidone), anionic (tannic acid and citrate) and Cationic (mercaptopentyl (trimethylammonium) etc. Several organic
compounds have been applied as reducing and capping agents for the production of gold and silver nanoparticles. Conversely the catalytic activity of M-NPs is affected by capping agents or surfactants. The surfactants pack around the surface of the catalyst and thus affect the convenience of reacting species to the M-NPs and can even alter the mechanism of catalytic reactions.
2.10 Characterization Techniques

After synthesis, nanoparticles are generally characterized with some spectroscopic and microscopic techniques to corroborate their shape, size and morphology. The following characterization techniques are mostly used for characterization of gold and silver nanoparticles.

2.10.1 X-Ray Diffraction

To determine the geometry, lattice constants, orientation of single crystals, recognition of unidentified substances and crystal structure of solids, X-ray diffraction technique is usually applied. The average size of the nanoparticles can be anticipated from the XRD studies. The X-ray diffraction spectrum is obtained by measuring the angles at which an X-ray beam is diffracted by the crystalline phases in the specimen. Degree of crystallinity, unit cell parameters of the nano-crystalline substances, phase purity and the uniqueness of nanocrystal structure can be fined out from the diffraction spectra. XRD method is broadly applied in material analysis, since it is undisruptive and does not need complex sample preparation. For determination of the crystal size of nanoscale materials X-ray diffraction expansion study has been commonly used. By using the Debye-Scherrer equation: $D = k\lambda/\beta \cos \theta$

where $k$ is a constant, $D$ is the thickness of the nanocrystal, $\beta$ is the full width at half maxima of (111) reflection at Bragg's angle $2\theta$ and $\lambda$ is the wavelength of X-rays [63].

2.10.2 Scanning Electron Microscopy (SEM)

For characterization of nonmaterial’s and nanostructures one of the most widely used techniques is the scanning electron microscopy. Like optical microscopes this practice simply not only gives topographical information, but also information of chemical composition of the sample. A beam of electrons is generated by the instrument and the
solid sample is scanned back and forth over it. Different types of signals are generated due to contact between the beam and the solid sample and provide detailed information regarding the morphology and surface structure of the sample.

2.10.3 Transmission Electron Microscopy (TEM)

For compositional and nanostructural analysis of a solid sample, Transmission electron microscopy is typically used. Transmission electron microscopy involves: (i) irradiation of an enormously slim sample through a rich electron ray that is scattered by the lattices of the semi-crystalline or crystalline substances and propagated along various sides, (ii) angular distribution analysis and imaging of the forward-diffracted rays and (iii) energy study of the secreted radiations. Structural identification and characterization of different phases of nonmaterials, namely lamellar, cubic or hexagonal can be determined from the information obtained through TEM. Selected area diffraction (SAD) can be used to determine the crystal structure of individual nanocrystals, nanomaterials, and nanorods. In SAD, parallel illumination by defocusing the condenser lens and the selected-area slit is applied to bind the diffracting level. Lattice parameters and Bravais lattices of crystalline substances are often determined by using “SAD” through a similar method as applied in XRD [64]. TEM has so many other uses in nanotechnology, beside its potential of chemical and structural analysis. For example melting points of nanocrystals can be determined through TEM, where an electron beam is applied for heating the crystals and the melting points are find out in the absence of electron scattering [65]. Measurement of the electrical and mechanical properties of individual nanotubes and nanowires is another example.

2.10.4 UV-Vis Spectroscopy
UV-Vis Spectroscopy is frequently applied as a primary tool in nanoparticles fabrication. Electronic transitions among bands of atoms, orbitals, molecules or ions in solid, liquid or gaseous state are studied in the UV-Vis Spectroscopy. The metallic nanoparticles are known to display different distinctive colors. Metallic nanoparticles absorb electromagnetic radiations due to consistent alternation of the outer shell electrons owing to a strong interaction with the applied electromagnetic field. These resonances are identified as SPR, and do not happen in the case of bulk metallic particle. Consequently usual optical properties of nanoparticles can be studied by using UV-Visible spectroscopy [66].

2.10.5 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR is used specifically to know the functional groups involved in the reduction of metal cations during nanoparticles synthesis. The vibrations of chemical bonds in the compounds at different frequencies are studied by using this technique and depend on the atoms and nature of intramolecular attractive forces. The vibration frequency of a bond increases after absorbing electromagnetic radiation, causing an excitation among the low and higher energy states. These absorption frequencies enhance vibrational frequencies of the chemical bonds and therefore are definite to the group of atoms and the type of bond involved in the vibration. The FTIR spectroscopic method can be applied to observe the presence of protein compounds in the medium as the FTIR spectra in the 1400~1700 cm⁻¹ area give information regarding the occurrence of-NH- and -CO- groups. The energy equivalent to these frequencies match to the IR (4000-400 cm⁻¹) of the electromagnetic spectrum [67].

2.10.6 Atomic Absorption Spectroscopy
Atomic absorption is based on the energy absorption through transitions among electronic energy levels of an atom. Once a definite magnitude of energy is given to an atom in the stable state by a source, such as a glow, the outer electrons are excited to higher energy state. The absorbed energy as a result of this excitation among energy levels can be applied for quantitative analysis of metalloids and metals. For determination of elemental concentration comparative intensity values are compared with reference standards [68].

2.10.7 Energy dispersive X-ray analysis (EDX)

Chemical analysis of a sample is conducted by using EDX analysis. It is based on a source of X-ray excitation and interface of a sample. The analysis capacity of this instrument is based on the basic rule that every atom has an exclusive atomic structure and on X-ray emission spectrum gives a unique set of peaks. In order to excite the emission of specific X-rays from sample high energy rays of charged particles, like a beam of X-rays, electrons or protons is concentrated into the sample. Inside the sample the atoms in the rest possesses ground state electrons in distinct energy levels and holes are created in the inner shells due to excitation when the incident beam falls on it. The hole created in this way is packed via an electron from an external, higher-energy level and the distinction in energy among the lower and higher-energy level is liberated in the form of an X-ray.

2.11 Properties
Many important properties are exhibited by metal nanoparticles, which may be applied in different fields of analytical chemistry, medicines, catalysis and optical activity etc. A few properties are discussed below.

2.11.1 Optical properties

Nanoparticles provide light based sensors for diagnosing cancer, and might also be applied for covering anti-reflection goods, consequently fabricate a refractive index for different surfaces. Due to their optical properties, Ag-NPs can be used as a valuable element in different sensors.

Silver nanoparticles robustly interact with light, resulting in extremely strong dispersion and absorption properties, as the diffusion of electrons on the metal crust experience a collective fluctuation formerly animated at a specific frequency of light identified as SPR. When strike with white light, 60 nm Ag-NPs elucidate as brilliant blue points under a dark field microscope (Figure 2.2).
Figure 2.2: Dark field microscopy image of 60 nm silver nanoparticles

Sphere-shaped Ag-NPs might too show illuminations at 400 nm to 530 nm depending on their particle size. Rod or plate shaped Ag-NPs show illumination even in the infrared region. Consequently the color of the nanoparticles depends on shape and size of the particles.

Figure 2.3: Color of nanoparticles depends on the size of particles

2.11.2 Magnetic properties

Nanostuctured materials show distinctly different magnetic properties from that of bulk structures. As soon as the particle size is decreased under a definite size, ferromagnetic particles become unstable as the raise in surface energy gives adequate energy for domains to instinctively change polarization sites and become paramagnetic. Different methods have been developed for the synthesis of magnetic nanoparticles and their special characteristics can be used for various purposes, for instance, if the M-NPs are magnetized, they can enhance the contrast and detail of MRI images.

2.11.3 Thermal properties
Semiconductor and metal nanoparticles have a considerably poor phase transition temperature or melting point compared to their bulk counterparts. Lowering of the phase transition temperature is normally seen as the size of particles decreases than 100 nm and is ascribed to raise in surface energy with a decrease of particles dimensions. The conduction of heat may be increased by the particularly modified particles from gatherers of solar energy to the storing containers.

2.11.4 Energy properties

Owing to its long lasting nature and more energy density, nanoparticles can be used in energy storage batteries. Metal nanoparticles might be used for storing hydrogen gas and tremendously proficient energy cells might be generated by applying the property of electro-catalysts for these instruments. The engines turn into more profitable and proficient, when metal nanoparticles are used as catalysts in ignition engines.

2.11.5 Electronic properties

For the synthesis of more efficient delicate electronics small sizes of nanoparticles is very suitable as nanoparticles carry constituents with better conductivity. Nowadays nanoparticles electronics are used for the production of digital displays, which are less expensive and brighter in color.

2.11.6 Biomedical properties

Several organic compounds can identify and attach to other compounds with tremendously high specificity and selectivity. One of the significant biological uses of colloidal nanoparticles is molecular detection. Antibodies and oligo-nucleotides are widely used as receptors for molecular recognition applications. Antibacterial covered nanoparticles might be used for wound directly. Similarly antibiotics stabilized nanoparticles can boost
their biological activity against microbes and thus provide novel types of drugs. “Quantum dots” may be used for drug loading and its transport to a specific affected area, like tumor and cancer cells, and also to recognize and diagnose certain diseases. Although the majority of quantum dots are noxious, consequently supplementary harmless fluorescent nanoparticles may be used for the similar purposes.

2.12 Applications

Nanotechnology has a wide variety of potential uses from biological systems, optical communications and electronics to present elegant substances. The broad variety uses of nanomaterials are owing to (i) the bulky surface area, like Au-NPs supported on metal oxides are applied as short temperature catalyst and for different sensors, (ii) the remarkable physical characteristics, e.g. Au-NPs can be used as an inorganic colorant for coloration of certain glasses, and (iii) the small size. New nanomaterials with novel characteristics have been fabricated for many applications. A few of the applications of nanostructures and nanoparticles are discussed here.

In the fields of analytical chemistry nanoparticles have a wide of range applications, such as biological and chemical sensing or optoelectronics. Numerous M-NPs are extremely appropriate for catalyzing the re-doxy reaction of certain compounds that can be monitored with electro analytical techniques. Different properties, such as chemical, optical, mechanical, magnetic and catalytic exhibited by metal nanoparticles in comparison to bulk materials are due to their huge surface area [69]. Nanoparticles play a significant role in the fields of antibiotics where experts have explained a complete mechanism of action for it [70]. Similarly, Au-NPs play a significant task in different fields, such as sensing [71]
and chemical separations [72]. They are also applied in the medical science [73], such as the analysis and cure of certain tumors/cancers [74].

Silver nanoparticles could simply interact with microorganisms, like virus and bacteria due to their minute size. Nanoparticles are adequately adjustable as they can precisely be the particles of any material, and can be used in numerous technical applications i.e. from innovative biomedical procedures to modern electronics.

The nanoparticles boast numerous therapeutic uses within the human body as their minute size is very appropriate to brawl against a range of unnecessary attackers, and can work greatly in a similar manner as immune system’s cells accomplish in the body.

12.13 Brief Background of gold and silver Nanoparticles

12.13.1 Revive on Gold Nanoparticles

Liu et al. used axial amine 5 poly amido-amine cascade molecules as stabilizing agent and sodium borohydride as reducing agent for the synthesis of Au-Ag alloy nanoparticles. Different molar ratios of dendrites, silver and gold ions were used in these synthetic reactions. The axial amines of the dendrimer were acylated. Newly prepared nanoparticles were analyzed by using different spectroscopic methods. Synthesized Au–Ag alloy nanoparticles were relatively stable at a temperature range of 5-40 °C and pH 3-9. The effect of different parameters on nanoparticles synthesis was studied, and it was noted, that the optical properties and size of nanoparticles were reliant upon metal concentration i.e. decrease in nanoparticles size was observed by increasing the metal ions concentration. X-ray absorption co-efficient measurements reveal that acetylation and increasing the gold ratio in alloy nanoparticles, the X-ray attenuation intensity of nanoparticles increases. An increase was also observed in the cyto compatibility of the nanoparticles by acetylation.
Owing to these characteristics the Au-Ag alloy nanoparticles could be effectively used in several therapeutic applications having CT imaging [75].

Misra et al reported the synthesis of Au-NPs by Gamma radiolysis of H\textsubscript{Au}Cl\textsubscript{4}·3H\textsubscript{2}O precursor in aqueous solution of PVP in the presence of 2-propanol, acetone and small amount of silver. The effect of molecular weight of PVP and reactants concentration on nanoparticles synthesis was studied. Size, shape and morphology of nanoparticles were studied using a transmission electron microscope. Spherical shaped Au-NPs were observed by using 361,000 molecular weight of PVP. Nanoparticles synthesized in this way were used for the determination of H\textsubscript{2}O\textsubscript{2}, as they react with the reaction product of O-PDA and H\textsubscript{2}O\textsubscript{2} and enhance the intensity of absorption peaks in the chemical reaction. The intensity of absorption peak was dependent on H\textsubscript{2}O\textsubscript{2} concentration [76].

Nalawadi et al reported the synthesis of multi spiked Au-NPs by the reduction of (Au III) ions in ethylene glycol using sodium hydroxide as a catalyst in the chemical reaction. Various spectroscopic techniques, such UV-Vis spectroscopy, XRD, SEM and TEM were used for nanoparticles characterization. Average diameter of synthesize nanoparticles was 63 ± 23 nm. Nanoparticles prepared by using PVP as stabilizing agent were used as a catalyst in the reduction of o-nitro aniline to benzenediamine [77].

Nguyen et al reported the use of functionalized Au-NPs on the basis of controllable size and shapes in cell imaging, small organic compounds, metal ions, protein, DNA and RNA. Owing to their luminous characteristics and the capability to generate immobilization of bio-molecules without affecting their bioactivities, these nanoparticles can also be used as
biosensors. It was also studied that size, shape, functional groups and aggregation affect the optical properties of these nanoparticles [78].

Nurozi et al synthesized Au-NPs by using aqueous extract of rose petals as a reducing and stabilizing agent. The characteristics of biosynthesized Au-NPs were studied by using different analytical techniques, such as FTIR, EDX, XRD, TEM, DLS and UV-Vis spectroscopy. Purity and Crystalline nature of the nanoparticles was determined by XRD studies. Different shapes of nanoparticles were determined by TEM, and FTIR was used to find out functionalization with different biomolecules having –OH, –NH₂ and other stabilizing functional groups. DLS was used to determine particle size. Average particles size was about 10 nm in this study [79].

Peng et al synthesized dendrimer entrapped Au-NPs by modification with Polyethylene glycol and thus enhanced their biocompatibility of CT imaging. By adjusting the molar ratio of reactants, the size of nanoparticles was restricted in the range of 2–4 nm. Formed nanoparticles were stable at a temperature of 0–50°C and pH range of 5–8 in aqueous solution. Synthesized nanoparticles were extremely successful for CT blood pool imaging mostly in cancer analysis due to their lengthy half life time [80].

Akhavana et al reported the synthesis of Au-NPs by using γ -irradiation. Bovine serum albumin (BSA) protein was used as stabilizer. Different spectroscopic and microscopic techniques were used for characterization of biosynthesized nanoparticles. The sizes, shapes and morphology of the respective Au-NPs were finding out by using AFM, XRD and UV-Vis spectroscopy. UV-Vis spectroscopy was used as a primary tool for nanoparticles synthesis, and absorption peak at 525 nm was observed as surface Plasmon
resonance band. The $\gamma$-irradiation doses affect the size of nanoparticles. The conjugation of BSA to nanoparticles was indicated by Dynamic light scattering and FTIR studies. The effect of irradiation on BSA structure was demonstrated by SDS-PAGE analysis. The non-cytotoxic nature of Au-NPs to mammalian cells was indicated by MTT assay. After nanoparticles synthesis in the experiment a partial protection was observed for BSA from aggregation and degradation [81].

Zeng et al synthesized regular hexagonal lead oxide nano-sheets by anisotropic enlargement, using Au-NPs at different temperatures. Nano-sheets were produced in the presence of gold nanoparticles. For seeding the growth of nano-crystals, nanoparticles provided nucleation sites, which then aggregates in the shape of nano-sheets. By using this technique highly symmetrical and high class lead oxide nano-sheets with controlled edge length were synthesized [82].

Larguino et al applied nanoscience in medical diagnostics and disease treatment. Various nanoscale instruments have been introduced for analysis of DNA, RNA and proteins. Through the application of nanoscience a lot of improvement has been occurred in biomedical diagnostics [83].

Seol et al reported the microwave fabrication of Au-NPs with homogeneous sizes of 11.14 ± 1.25nm. Nanoparticles with improved quality and uniformity were produced by adjusting different reaction parameters, like pH and temperature. By using high power microwave temperature the ramping rate was changed during the synthesis reaction and within a few min of time the reaction was completed [84].
Paclawski et al reported flow micro-reactor system for controlled fabrication of Au-NPs. For the reduction of AuCl$_3$ ions glucose was used as reductant, while varying amounts of PVP were used as stabilizer. Concentration of various components and flow rate in the micro-reactor were optimized by Batch reactor. UV-Vis spectroscopy and DLS techniques were used to determine effects of temperature and reducing agent concentrations on the rate constants of nanoparticles escalation and nucleation. By using these method nanoparticles with narrow well-defined spherical shapes and size distribution could be prepared [85].

Stresziwski et al reported the mechanisms and kinetics of Au-NPs synthesis from AuCl$_4$ and hydrazine sulfate. Dynamic light scattering (DLS) and UV-Vis spectroscopic methods were used to find out the effect of reactants concentration on nanoparticles synthesis. The reaction mechanism consists of several steps. The bimolecular reduction of Au (III) complex ions followed by autocatalytic reduction of Au (III) ions, and synthesis of Au-NPs by nucleation and growth are some major steps of this mechanism. By using the kinetic data and modified Finke Watzky model, values of rate constants were determined. TEM and DLS studies confirmed the autocatalytic reduction mechanism [86].

Arshi et al presented the synthesis of Au-NPs by using single step microwave irradiation technique. Cetyl-trimethyl ammonium bromide and citric acid were applied as stabilizing and reducing agents respectively. By using two different irradiation times in the reaction i.e. 50 and 80 seconds, two different types of Au-NPs were produced. Nanoparticles were analyzed by using UV-Vis spectroscopy and TEM. From UV-Vis analysis maximum absorptions were observed at 580 nm for 40s and 555 nm for 70s Au-NPs. E. coli was used to study the effect of Au-NPs concentration on the antibacterial activity. Approximately 22
mm zone of inhibition was observed for these two kind nanoparticles. Almost similar antibacterial activity was observed for both kinds, but for Au-NPs synthesized at 70s irradiation time slightly better antibacterial property was observed [87].

Rudolf et al reported the synthesis of Fe complexes. The disulfide group of metallo carbonylsuccinamidene complex was involved in covalent bonding with noble metals. This Fe-complex was absorbed on the surface of Au-NPs of unusual sizes. Properties of Au-NP-metallo carbonyl conjugate were found out through DLS, TEM, IR, ICP-MS and UV-Vis spectroscopy. A surface enhancement IR absorption effect of Au-NP-metallo carbonyl conjugates was observed from IR analysis [88].

Mandal et al reported the synthesis of multi-functionalized Au-NPs coated with fluorescent dye, folic acid and multi layers of boron phenylalanine and were applied for release of boron to tumor cells. In vitro confocal fluorescence microscopy was used to determine the absorption of nanoparticles in cancer cell and its tracking up to cellular level [89].

Fayaz et al reported the biosynthesis of Au- and Ag-NPs by reacting metal salts in solution with the cell free extract of Geobacillus stearothermophilus. Properties of the biosynthesized nanoparticles were measured by applying different spectroscopic and microscopic methods, such as TEM, FTIR, XRD and UV-Vis spectroscopy. Maximum absorption for Au- and Ag-NPs was observed at 522 and 423 nm from UV-Vis spectroscopic analysis. Silver nanoparticles were poly dispersed, while gold nanoparticles were observed to be mono dispersed as shown by TEM. Bacteria secreted certain proteins in the reaction mixture and were demonstrated by FTIR, PAGE and SDS in the reaction mixture. These proteins increase stability of nanoparticles in the reaction mixture [90].
Berzina et al. fabricated and characterize the electrical properties and structure of Au-NPs poly aniline complex materials. Nanoparticles synthesized in this way were of various sizes in the range of 10–15 nm and were dependent on the use of different stabilizing agents. For polymerization of anilinium salt a mixture of Au-NPs stabilized by dodecylbenzenesulfonic acid and 2-mercapto-ethanesulfonic acid were used. The obtained composite material showed excellent results. Schottky barriers were noted among the nanoparticles and polyaniline and were confirmed by electrical properties [91].

Chu et al. used luminescent polymers and modified the surface of Au-NPs. Photoluminescence quenching and the morphological actions of the respective nanoparticles were studied by AFM. Owing to difference in the electron donating capacity of MeO and Me substituent’s present on the side chains of polymers (PBT1–PBT3) and (PBOT1–PBOT3) the extent of hydrogen bonding and dipole-dipole interaction was different. Highest KSV (Stern–Volmer quenching constant) was observed for the nano-composite obtained from PBOT1 and Au-SCOOH [92].

Gupta et al. synthesized 16-Mercaptohexadecanoic acid stabilized Au-NPs by single phase synthesis. The lack of un-reacted thiol in Au-NPs was determined by XPS and FTIR analysis. The presence of nanoparticles on silicon surface was confirmed by XPS analysis and HR-TEM showed the addition of particles to silicon surface. These studies might be applied for advance nano-fabrications [93].

Ara et al fabricated stable Au-NPs by using soluble starch as reducing as well as stabilizing agent for 4 h at 50°C. Formed Au-NPs were analyzed by using spectroscopic techniques, such as UV-Vis, TEM and Z-scan. Nanoparticles morphology and sizes were determined
through TEM, and were found in the range of 7–30 nm. The non-linear optical properties were studied using continuous wave He–Ne single laser beam technique. The non-linear refractive indices of nanoparticles were determined by close holes-scan studies, and were in the order of $10^{-7}$ cm$^2$/W. The non-linear absorption of Au-NPs in the order of $10^{-1}$ cm/W was determined by Z-scan studies [94].

Kumar et al reported the biosynthesis of Au-NPs via green technique. The extract of Terminalia chebula was used as a reducing and stabilizing agent. The SPR peak at 535 nm confirmed the synthesis of Au-NPs from UV-VIS spectroscopy. The sizes of anisotropic Au-NPs were in range of 5 to 45 nm from TEM analysis. Water soluble tannins were the chief constituents liable for reduction and stabilizing of gold nanoparticles in the aqueous solution of T. chebula. Antimicrobial potential of nanoparticles were checked by using the standard well diffusion method [95].

Ali et al. used the aqueous extract of Mentha and biosynthesized gold and silver nanoparticles by reduction of HAuCl$_4$ and AgNO$_3$ solutions. Both metallic salts were mixed with extract and incubated. UV-Vis spectroscopy, SEM, FTIR and EDS were used to study the biosynthesized gold and silver nanoparticles. The sizes of spherical Au- and Ag-NPs were 130 and 80 nm respectively. Very high antibacterial activities were observed for staphylococcus aureus and Escherichia coli [96].

Kumar et al. used extract of Cassia auriculata leaves for the biosynthesis of Au-NPs. Gold nanoparticles were synthesized at room temperature within 10 min the by reduction of gold salt. Biosynthesized Au-NPs were characterized via different spectroscopic techniques, such as SEM, EDX, XRD, FTIR, TEM and UV-Vis spectroscopy. Cassia auriculata
stabilized Au-NPs, ranged from 10-22 nm with spherical and triangular crystalline shapes. Stability of the resultant Au-NPs was checked at different PH values. The resulting nanoparticles could facilitate in promotion of anti-hyperglycemic action upon testing, as the plant used for reduction and stabilization of nanoparticles was an anti-diabetic plant [97].

Philip et al used the leaf extracts of Murraya Koenigii for the biosynthesis of Au- and Ag-NPs. Chemical reaction was conducted at 374 K for Au-NPs, and at ambient conditions for Ag-NPs. Biosynthesized Au- and Ag-NPs were elucidated by applying UV-Vis spectroscopy, XRD, FTIR and TEM. At room temperature the synthesis of Ag-NPs was completed within 5 min with SPR peak at 411 nm, and size of resultant nanoparticles was about 10 nm. By mixing plant extract to the boiling solutions of HAuCl₄, spherical Au-NPs were synthesized. Synthesized Au-NPs were uniformly distributed with a size of about 15 nm and SPR at 530 nm. Crystalline morphology of Au-NPs was confirmed by TEM, XRD and SAED studies. FTIR analysis showed that capping molecules in Au-NPs are different from that of silver nanoparticles [98].

Chili et al synthesized multi twinned Ag-NPs by using ultraviolet irradiation technique. Gold salt was reduced by polyvinyl pyrrolidone [99].

Gupta et al synthesized Au-NPs capped with short chain thiol (4-aminothiophenol). The effect of dispersion medium and reaction conditions was studied on the particles morphology. Anhydride was used as capping agent for preventing aggregation due to amine-amine hydrogen bonding. Nanoparticles were stabilized through covalent bonding with silicon dioxide. XPS analysis confirmed the lack of un-reacted thiol on silicon
surfaces and attachment of particles on Si surface. The particle shapes and sizes were measured by using TEM and UV-Vis spectroscopy. Cross-sectional HR-TEM images also confirmed the binding of particles to Si surfaces [100].

Bahadur et al reported the synthesis of silica coated Au-NPs and functionalized their surfaces with different groups. Au-NPs having mean size of 16 nm were produced by applying the citrate reduction method. Silica was coated on the surface of nanoparticles within 5 min, by microwave irradiation of the tetra-ethoxysilane and ammonia with synthesized gold solution. The mono-dispersion and size consistency of the nanoparticles were confirmed by TEM and DLS analysis. Different functional groups including alkyl, carboxylate and amino groups were used for surface functionalization of the synthesized silica coated Au-NPs, which improved their use in numerous applications. The functional groups on the surface of nanoparticles were identified by XPS studies and Zeta potential measurements [101].

Aroma et al used aqueous extract of Macrotyloma uniflorum for the synthesis of Au-NPs. The effect of changing pH, temperature, time and quantity of extract was studied on the preparation of gold nanoparticles. Different characterization techniques, such as TEM, FTIR, XRD and UV-Vis spectroscopy were used to know the particles morphology. SAED patterns, XRD and HRTEM images confirmed highly faced centered cubic structure, and highly crystalline nature of the nanoparticles. Different functional groups of the aqueous extract implicated in the reduction and stabilization of metal cations were confirmed from FTIR analysis [102]

2.13.2 Revive on silver nanoparticles
Guidelli et al synthesized Ag-NPs by heating aqueous solution of Ag with dl-alanine as stabilizing and reducing agent. Prepared Au-NPs were mostly spherical in shape with a fine size distribution. Average particle size was around 8 nm. The amine group of dl-alanine reduced the Ag+ ions on the surface of particles and consequently resulted in the stability of colloid. Lower energetic dependence and higher sensitivity was observed for the bio-hybrid composite containing nanoparticles as compared to pure dl-alanine [103].

Ghaseminezhad et al. synthesized Ag-NPs by adding the fungal supernatant to the mixture of starch and AgNO₃ solution. Properties of Ag-NPs synthesized by this green technique were compared to those synthesized by microbial and adapted polysaccharide methods. Sizes of the nanoparticles synthesized by microbial and modified polysaccharide methods were 15 and 75 nm while Ag-NPs synthesized by the reported novel method was found to be 15 nm in size. DLS technique was used to confirm sizes of the prepared Ag-NPs and XRD spectrum confirmed their crystalline nature. Nanoparticles synthesized by this method were found quite stable under different conditions and for longer period of time. FTIR showed the presence of different functional groups involved in the reduction and stabilization of the particles [104].

Al-Deyab and Abdel Halim synthesized Ag-NPs using hydroxypropyl cellulose samples by a green method. Hydroxypropyl cellulose samples with different molar ratios were synthesized by alkalization of cellulose followed by etherification with propylene oxide. Effect of reaction temperature, time, alkali concentration and propylene oxide concentration, on the synthesis of hydroxypropyl cellulose was studied. In the synthesis of Ag-NPs the prepared hydroxypropyl cellulose samples were used to reduce AgNO₃
solution. From UV-Vis spectra it was observed, that complete reduction of Ag\(^+\) to Ag-NPs occurred at pH 12.5 [105].

Kouvaris et al fabricated Ag-NPs by a green method, using leaf broth of Arbutus unedo as reducing and stabilizing agent. By reacting AgNO\(_3\) solution with leaf broth surface functionalized Ag-NPs were prepared. Different characterization techniques revealed single crystalline nature with narrow size distribution. The resultant discrete nanoparticles were stable over broad range of pH, temperature and time and the stability might be owing to covering of organic extract on particles surface causing development of tiny aggregates. Ag-NPs prepared in this way were found to be most suitable for coating procedures and have many important uses in biotechnology [106].

Vasileva et al developed another green synthetic method for the preparation of Ag-NPs with a mean diameter of 22.4 ± 4.4 nm. For the fabrication of nanoparticles aqueous solution of starch was applied as a reductant and stabilizer. Ultrasound method was applied for the reduction of silver nitrate solution with d-glucose. Various spectroscopic and microscopic techniques such as differential scanning calorimetry, UV-Vis spectroscopy, differential thermal analysis and XRD were used to study the nature of biosynthesized silver nanoparticles. Formed Ag-NPs showed excellent catalytic activity in the reduction of H\(_2\)O\(_2\). Observed absorbance strength of localized surface Plasmon resonance band was changed due to degradation of Ag-NPs in the said reduction and the change was dependent upon the concentration of H\(_2\)O\(_2\) [107].

Abdel- Mohsen et al applied an eco-friendly chemical procedure for the synthesis of hyaluronan fibers having Ag-NPs. Wet spinning technique was used for the fabrication of
hyaluronan fibers from a transparent solution of NaOH containing dissolved hyaluronic acid. The same technique was applied for the synthesis of Ag-NPs having fibers. Effect of different factors such as hyaluronan, AgNO₃ concentration, reaction temperature, time and pH of the mixture was studied upon the synthesis of fibers containing Ag-NPs. Different spectroscopic and microscopic techniques including TEM, SEM, XRD, ICP, OES and 2D SWAXS were applied to confirm the presence of Ag-NPs in fibers. Mechanical properties of the fibers having nanoparticles were measured [108].

Shukla et al. used agar extracted from the red alga “Gracilaria dura” for the synthesis of Ag-NPs and nanocomposite materials. Biosynthesized nanoparticles were characterized using XRD, SEM, TEM, SAED, EDX, UV-VIS and atomic absorption spectroscopic analysis. XRD and DSC studies confirmed crystalline index (CIDSC 0.73) of nanoparticles [109].

Quang et al synthesized Ag-NPs by using silica beads having size in the range of 0.5 mm -1 mm. Silver cations present inside the holes of silica beads were reduced by sodium borohydride. The effect of porous structure and pore size on Ag-NPs fabrication was fined out by applying silica beads having different pore sizes in the range of 4.8-18 nm. The amount of silver contained inside the pores was observed to be increased by increasing pore size of silica beads. It was also noted that the porous structure and pore size affect the particles sizes. Silica beads having different pore sizes were used in this study [110].

Shivaji et al used cell-free culture supernatants of psychrophilic bacteria i.e. arthrobacter gangotriensis and P. Antarctica for the synthesis of Ag-NPs. Size, shape and morphology of these biosynthesized nanoparticles were determined using AFM, TEM UV-Visible
spectroscopy. Stability of the biosynthesized Ag-NPs was studied, and it was found that they are stable in dark for about six months. The effect of different factors, such as bacterial species, pH and reaction time was evaluated on the stability and synthesis of nanoparticles. For different bacterial species the effect of cell-free culture supernatants was different. The synthesized Ag-NPs were found to be bactericidal. In this study genus Arthrobacter and culture supernatants of psychrophilic bacteria were used for the first time for the preparation of silver nanoparticles [111].

Bhatte et al. prepared Ag-NPs in powder form by using an environmental friendly method. Aqueous solution of AgNO₃ was reduced using hydrogen as a reducing agent and PVA as stabilizer. The adopted method is more eco-friendly owing to full biodegradable nature and very low cyto-toxicity value. Nanoparticles were studied by using TEM, AFM, EDX, XRD, DLS and UV-Vis spectroscopic techniques. Activities of prepared nanoparticles as eco-friendly catalyst were studied in the synthesis of various enaminones [112].

Tuan et al. used silver plate as basis of Ag⁺ ions for the synthesis of Ag-NPs colloid by using sono-electro deposition technique. Synthesized nanoparticles were dispersed in non-toxic solution and were from 4 to 30 nm in size. Activated carbon was produced from coconut husk and Ag-NPs were loaded on it. The most excellent activated carbon has a surface area of 780m²/g. The occurrence of Ag-NPs does not affect the methylene blue absorption ability of the activated carbon and also does not alter its morphology. The synthesized materials were very active in prevention of microbial infections and were found to have many applications in treatment of environmental contaminations [113].
Gunawidjaja et al. combined Europium-doped lutetium-oxide (Eu: Lu2O3) by a core-shell approach with Ag-NPs. Nanoparticles were the metal core component, while Eu: Lu2O3 acted as the phosphor shell component. An optically transparent SiO2 layer separates metallic nanoparticles core from the phosphor shell. By changing the size of the SiO2 sheet frequently in the series of nanometers, the interaction between shell and core was changed. To fabricate nano-composite, phosphor core-shell nanoparticles were entrenched into a translucent polymeric matrix [114].

Arunachalam and Rastogi used garlic aqueous extract for the synthesis of extremely stable Ag-NPs. Aqueous extract of garlic was mixed with 0.1 M [Ag(NH3)2]+ and was exposed to intense daylight for 20 min. The sunlight act as catalyst and the extract served as reducing and stabilizing agent in the formation of nanoparticles. FTIR, GA-XRD, TEM and UV-Visible spectroscopy were applied for the analysis. Synthesized nanoparticles were found to be 8.4 ± 5.5 nm in size and were mostly spherical in shape. FTIR studies confirmed the presence of protein as capping agents. Silver nanoparticles were formed in an excellent yield of approximately 80% by dry mass and 85% ICP-AES technique. Well diffusion assay was used to determine antibacterial activities of nanoparticles, and were active against gram negative and gram positive bacteria. Nanoparticles synthesized in this way were stable in colloidal solutions for a very long period of time and typically for more than a year retained their bactericidal property [115].

Johan and Lah synthesized anisotropic mono-dispersed Ag-NPs by using an easy chemical reduction method, using Daxad 19 surfactant as a reducing and stabilizing agent. The morphology of nanoparticles was affected by the mass ratios of the reactants and temperature. The conversion of Ag+ ions to Ag-NPs increased by rising mass ratios of
AgNO₃ and Daxad 19 under controlled temperature. Size of the resultant nanoparticles could be controlled easily and were uniform in size, morphology and shape [116].

Li et al. synthesized colloidal Ag-NPs by a process analogous to silver mirror reaction without the addition of Tollen’s reagent. Ammonia was applied as a substitute to C₁₉H₄₂BrN for reaction with Ag⁺ ions. Different spectroscopic techniques, such as XRD, FTIR, TEM and UV-Vis spectroscopy were used for characterization of the synthesized nanoparticles. A high concentration of Ag (0.05 mol/L) was observed in colloidal Ag-NPs and was found to be stable for more than 45 days. The formation of C₁₉H₄₂NBr-Ag⁺ complex was due to the presence of bromine in C₁₉H₄₂BrN, which acted as surfactant counter ion. Glucose was used as reducing agent for the reduction of the complex to nanoparticles. The effect of molar ratios of C₁₉H₄₂NBr to silver ion on the dispersion and size of nanoparticles were also studied [117].

De Matos et al. synthesized Ag-NPs by irradiating a solution of Euphorbia mili and silver nitrate. No additive like surfactants, solvents or reducing agents were used in this method. The production of nanoparticles was determined by UV-visible spectroscopy. Xenon lamp irradiation was applied to prepare Ag-NPs and laser pulses were used to decrease the size of Ag-NPs upto 8 nm. The effect of various factors, such as AgNO₃, leaf extract concentration, energy of laser irradiation and time were studied on the size and formation of nanoparticles [118].

Guzman et al. reported the synthesis of Ag-NPs by the reduction of aqueous solution of silver nitrate. Sodium citrate and hydrazine hydrate were used as reducing agent and sodium dodecyl sulfate as a stabilizing agent. The antibacterial activities of the obtained
Ag-NPs were determined using Kirby-Bauer method, and were found active against E. coli, S. aureus, P. aeruginosa [119].

Darroudi et al. used aqueous gelatin solution as an organic stabilizing agent for the synthesis of colloidal Ag-NPs by a sono-chemical method. The effect of various reaction conditions, such as reducing agent concentration, silver ions concentration, ultrasonic time and amplitude was studied on the synthesis and morphology of nanoparticles. An increase in particles size was observed with decrease in amplitude and an increase in ultrasonic time. Formed nanoparticles were well-dispersed with spherical shapes and 3.5 nm in size. The technique was recommended to be used for other noble metals, such as Pt, Pd and Au in numerous medicinal, technological and industrial processes [118].

**Aims and objectives**

Aims and objectives of the research work were

- To develop new drugs with multi-functions
- To develop nanomaterial based drugs
- To develop Metallic sensors
- To develop chemo sensors
CHAPTER: 3

METAL NANOPARTICLES STABILIZED WITH PRUNUS ARMENIACA (APRICOT) GUM
3.1 Introduction

Prunus armeniaca (family; Rosaceae) is widely consumed in abundant amounts either as fresh fruit or dried fruit during the summer season or processed into apricot juice, jam and nectar. It is a traditional drug that is used in oriental medicine to treat different diseases, such as nausea, bronchitis, emphysema, asthma, leprosy, leucoderma and constipation [121]. Apricot has been studied for its hepatoprotective [122], antinociceptive [121], anti-inflammatory [123], antioxidant and antiradical [124], gastroprotective [125], nephroprotective [126], anti-mutagenic [127], anti-carcinogenic [128], antibacterial, antifungal [129], cardioprotective [130], testicular protective [131] and tyrosinase [132] as well as trypsin [133] inhibition activities. The purported medicinal properties of apricot have been credited to the occurrence of rich phytochemical compounds including phytosterols, flavonoids, polyphenols and vitamins. P. armeniaca fruit has been used as a reducing and capping agent for the efficient synthesis of Au- and Ag-NPs, and showed considerable potential for free radical scavenging activity [134].

Apricot (Prunus armeniaca), produces a transparent gum known as apricot gum. The gum seems to be a lot like gum Arabic, and oozes out in the spring on apricot tree bark. Gums collected from apricot trees growing in different parts of the world are considered as exudates of ecological significance. It can entirely replace the more expensive gum Arabic, as well as its synthetic derivatives in the food and pharmaceutical industries. Apricot gum has been mentioned in ancient formulas for use in paint and varnishes additives. Furthermore, it is superior to gum Arabic in so many properties like emulsifying ability, durability and viscosity. It is also used for the preparation of oil
emulsions and sometimes as a coating and binding agent. Powdered (white or yellow) gum is used in medicine as an alternate for imported Acacia gum.

In the majority of plant mediated synthesis of nanoparticles, generally the leaves, roots or fruit parts of plants are used, whereas the gum part is usually ignored. Natural plant based gums offer a number of advantages, as they are non-toxic, low cost, biodegradable, biocompatible and are widely available [1]. We therefore presented primarily the biosynthesis of gold and silver nanoparticles by using the gum solution of P. armeniaca, which proves to be much cheaper as compared to the other reported methods. P. armeniaca gum has previously undergone phytochemical evaluation, and showed the presence of D-xylose, L-arabinose, D-galactose, D-glucuronic acid, and 4–0-methyl-D-glucuronic acid in a molar ratio of 0.6:1:0.3:3.2:3.2, and traces of D-mannose [135]. Different spectroscopic and microscopic techniques were used for characterization of these biosynthesized Au- and Ag-NPs. We elucidated their stability, and also studied the effect of gum, tetrachloroaauric acid trihydrate (HAuCl₄. 3H₂O) and silver nitrate concentration, reaction time and temperature on the synthesis of nanoparticles. We subjected the biosynthesized nanoparticles to varying NaCl concentration, pH, long term storage conditions, and high temperature. The apricot gum functionalized Au- and Ag-NPs were also tested for the first time against various pathogenic bacterial strains. Moreover, the gold nanoparticles (Au-NPs) were assessed for antinociceptive and anti-inflammatory activities, but at much lower doses compared to that of gum alone. Biosynthesized Au- and Ag-NPs were also studied for their prospective anticancer and urease inhibition properties.

3.2 Experimental set up
3.2.1 Materials and method

Apricot (Prunus armeniaca) gum, Tetrachloroauric acid trihydrate (HAuCl₄·3H₂O) and silver nitrate (AgNO₃) salts were used for the synthesis of Au- and Ag-NPs. Tetrachloroauric acid trihydrate and silver nitrate both of analytical grade were purchased from E. Merck (Germany). Fresh gum was purchased from the local market in April 2012. The gum was officially identified (RU-120) preceding to its use by Prof. Dr. Muhammad Ibrar of Department of Botany, University of Peshawar, Peshawar, Pakistan. Tetrachloroauric acid trihydrate and silver nitrate were used as a source of Au (II), and Ag ions for the biosynthesis of Au- and Ag-NPs. Doubly distilled water was used throughout experiments, and for supplementary analysis.

For the synthesis of gold and silver nanoparticles, 1mM stock solutions of HAuCl₄·3H₂O and AgNO₃ metallic salts were prepared by dissolving a calculated amount of these metallic salts in distilled water. Similarly 0.5% w/v stock solution of apricot gum was prepared by dissolving 5 mg of powder gum in 100 ml distilled water. The mixture was heated for complete dissolution, as the P. armeniaca gum powder does not dissolve readily at room temperature. For removal of impurities, centrifugation was carried out at 6000 rpm for 15 min and the apparent supernatant was used for all experiments. Nanoparticles were synthesized by reducing the aqueous solution of Au (III) and Ag⁺ ions in the presence of gum solution.

3.2.2 Synthesis of gold and silver nanoparticles functionalized with apricot gum

For the preparation of Au- and Ag-NPs, aqueous solutions of HAuCl₄ and AgNO₃ (From their 1mM stock solutions) were taken in two separate vials and a particular amount of gum solution (From 0.5% w/v aqueous gum solution) was added to it. The reaction mixtures
were stirred for 3-5 h and change in color was observed, which gave a primary indication of nanoparticles synthesis, as pure gold, silver nitrate and gum solutions are colorless (Figure 3.1).

Figure 3.1: Shows (A) color of gum solution, gold solution and gold nanoparticles (B) gum solution, silver nitrate solution and the silver nanoparticles.

Absorption spectra of the resultant nanoparticles were obtained on spectrophotometer, and a particular absorption Plasmon band was noted for nanoparticles in the UV-Vis region, whereas pure gum, gold and silver nitrate solutions did not show any type of absorption in this region.

Graphical representation PAG stabilized gold and silver nanoparticles
Figure 3.2: Shows graphical representation for the synthesis of (A) gold and (B) silver nanoparticles

3.2.3 Optimization of the bio-synthesized gold and silver nanoparticles

For complete optimization a number of reactions with different metal to gum ratios were carried out by changing concentration of one (metal or gum), while keeping the other one constant. For instance metal to ligand (gum) ratios of 1:1, 1:2……1:10, and also 2:1, 3:1……10:1 were reacted to obtained excellent results. Better results were observed by increasing concentration of gum in these reactions. Among the observed peaks the sharpest peak was selected for further studies. From UV-Visible absorption results 1:6 and 1:7 were the optimized ratio with sharpest peak for Au- and Ag-NPs respectively, (Figure 3.3). By increasing concentration of gum afar this ratio i.e. 1:6 and 1:7 the peak become broad, and thus indicates an increase in particle size and their irregular morphology.

Gold and silver nanoparticles prepared at different ratios were not similar in sharpness of their absorption peaks and had dissimilar colors (Figure 3.4). After completion of reaction, the nanoparticles were separated by freeze drying and were used for further analysis, like characterization and bioassays etc.
Figure 3.3: UV-Visible spectra of optimized (A) gold and (B) silver nanoparticles
CHAPTER 3  METAL NANOPARTICLES STABILIZED WITH P. ARMENIACA GUM

Figure 3.4: Color of (A) gold nanoparticles synthesized at different gold and gum ratio (B) silver nanoparticles synthesized at different silver and gum ratio.

3.3 Nanoparticles synthesis using different protocols/conditions

3.3.1 Effect of gum concentration on nanoparticles synthesis

UV-visible spectra revealed an increase in the absorbance at 555 and 450 nm with raise in gum concentration, therefore showing an increase in the concentration of nanoparticles (Figure 3.5). However, the characteristic absorption peak at 555 and 450 nm showed a significant red shift, which might be due to an increase in the particle size and is in accordance with earlier studies [136]. Moreover, the UV-Vis spectra were broader at lower and higher gum concentrations. It can be argued that the broad peaks at lower gum concentration was due to insufficient protection of the nanoparticles, while at high gum concentration, the increased intermolecular forces of gum molecules possibly hinder the dispersion of nanoparticles [137]. Furthermore, with an increasing gum concentration (0.1-0.5%), the colors of the nanoparticle colloidal solutions were also intensified from ruby red to dark red and light yellow to dark yellow, respectively, showing an increase in the concentration of nanoparticle (Figure 3.6).
Figure 3.5: UV-Vis spectra showing the effect of gum concentration on the synthesis of (A) gold (B) silver nanoparticles.
3.3.2 Effect of gold or silver ions concentration on nanoparticles synthesis

As shown in figure 3.7, with an increased concentration of Au or Ag ions, the intensities of SPR peaks at 555 and 450 nm increases with a slight red shift, and is in agreement with the earlier reports [138]. The increase in the intensities of absorption peaks might be owing to an increase in the number of metal nuclei, which resulted in the formation of larger amount of nanoparticles. On the other hand, the shift in the position of SPR peaks might be due to enhanced particle sizes as a result of high collision frequency among the metal nuclei or atoms. Relatively larger particle size and size distributions were noted, when metal ion concentrations was 5 mM. This might be due to the less protective effects of the gum and account for the longitudinal Plasmon resonance at approximately 558 and 455 nm for Au- and Ag-NPs respectively (Figure 3.7).
Figure 3.7: UV-Vis spectra showing the effect of metal ions concentration on the synthesis of (A) gold (B) silver nanoparticles.

3.3.3 Effect of reaction temperature on nanoparticles synthesis

Reaction temperature is also a significant factor that can affect nanoparticles synthesis [139]. The effect of different temperatures (20, 40, 60, 80°C) on the synthesis of
nanoparticles was studied and it was observed that absorption intensity of the UV-Vis spectra increased linearly without any shift in the position of SPR peaks, (Figure 3.8), indicating an enhancement in nanoparticles concentration of similar sizes. The highest absorption intensity of the characteristic absorption bands was noted at 80°C, and was indicative of the most optimum temperature for the synthesis of maximum concentration of nanoparticles in the solution.
Figure 3.8: UV-Vis spectra showing the effect of reaction temperature on the synthesis of (A) gold (B) silver nanoparticles.
3.3.4 Effect of reaction time on nanoparticles synthesis

The effect of reaction time on the biosynthesis of nanoparticles was evaluated, and an increase in the reduction capacity of the gum was observed with reaction time. The SPR occurred at 555 and 450 nm, and the intensity of the absorption peaks increased linearly, without any shift in the peaks position with time for Au- and Ag-NPs, respectively. This might be due to the persistent reduction of metal cations, resulting in an increase in the number of nanoparticles. No variation in absorption intensity of peaks was observed after 5 h for Au-NPs and 4 h for Ag-NPs, indicating the completion of the chemical reactions (Figure 3.9).
Figure 3.9: UV-Vis spectra showing the effect of reaction time on the synthesis of (A) gold (B) silver nanoparticles
3.4 Results and discussion

3.5 Characterization of gold and silver nanoparticles

The synthesis of Au- and Ag-NPs was assessed on a double beam UV-Vis spectrophotometer (Lambda 25, Perkin Elmer), in the spectral range of 250-800 nm. The nanoparticles were further characterized with a SEM (JSM-5910, England), EDX spectrometer (INCA-200, England), X-ray diffractometer (RX-III, Shimadzu, Japan) and FTIR spectrophotometer (Prestege-21 Shimadzu, Japan). Thermo gravimetric analysis was carried out by using Diamond TG/DTA Perkin Elmer, USA.

3.5.1 UV-Vis Spectroscopy

The synthesis of nanoparticles in aqueous colloidal solution was confirmed from the UV–Vis spectra, as well as from the appearance of ruby red and brown yellow color after 1 h of heating that became intensified as the reaction proceeded with the passage of time. As shown in figure 3.10, in comparison to the UV-Vis spectra of gold, silver nitrate and gum solution, a typical SPR band at 550 and 450 nm appeared for Au- and Ag-NPs respectively, after 4 h of heating, thus indicating the successful synthesis of respective nanoparticles in the solutions. The formation of nanoparticles can be further confirmed from the color of the corresponding solutions where a ruby red and brown yellow color indicates Au- and Ag-NPs in the colloidal solutions (Figure 3.10), whereas pure gold, silver nitrate and gum solutions appeared colorless. Variation in morphologies, shapes and sizes of the nanoparticles resulted in alteration of the observed colors. Color of the respective nanoparticles is actually due to the collective oscillation of the electrons in the conduction band; gold nanoparticles show characteristic red color, whereas silver particles are brown
yellow in color. For gold and silver nanoparticles the oscillation frequency is typically in the visible range, and thus gives rise to strong surface plasmon resonance (SPR) absorption [140].

Figure 3.10: UV-Vis spectra of (A) gold (B) silver nanoparticles. The inset photos show the corresponding color of the biosynthesized nanoparticles solutions.
3.5.2 Fourier Transforms Infrared Spectroscopy (FTIR)

The FTIR spectra of gum extract and the biosynthesized Au- and Ag-NPs is shown in figure 3.11. The apricot gum has mainly polysaccharide compounds, which along with glucuronic acid and its 4-O-methyl ether having O-H, -COOH, hemiacetal and ether groups, showed a broad O-H stretching band at 3300 cm\(^{-1}\). A mini peak at 2840 cm\(^{-1}\) merged with a broad carboxylic acid peak, was due to C-H stretching. A strong peak at 1717 cm\(^{-1}\) was owing to C=O stretching of -COOH. Similarly, a strong peak at 1600 cm\(^{-1}\) might be attributed to the conjugated C=C bond stretching which might be due to the presence of flavonoids and/or carotenoids. Bending vibration of C-H bond was observed at 1417 cm\(^{-1}\) and O-H bending at 1373 cm\(^{-1}\). A strong peak at 1037 cm\(^{-1}\) was due to C-O stretching vibrations.

In case of Au-NPs, the bonded O-H stretching shifted from 3300 cm\(^{-1}\) to higher frequency of 3311 cm\(^{-1}\), and showed the involvement of OH groups in the reduction and capping processes. A sharp peak of carbonyl carbon at 1717 cm\(^{-1}\) disappeared completely, thus representing the participation of carboxylic acid group in the reduction and stabilization process. The bending vibrations at 1417, 1373 cm\(^{-1}\) also disappeared due to attraction by the gold nanometal. A shift in C-O acyclic bond stretching from 1037 to 1022 cm\(^{-1}\) can be attributed to attraction by the gold nanometal during the capping process. Almost similar FTIR spectrum was observed for Ag-NPs (Figure 3.11). Moreover, the small merged peak at 2840 cm\(^{-1}\) can be seen as a separate entity in the FTIR spectra of biosynthesized gold and silver nanoparticles and was attributed to the interaction of carboxylic acid with Au- and Ag-NPs. These results confirmed that O-H, carbonyl and C-O groups not only reduced the Au and Ag metals, but also act as stabilizing agents.
Figure 3.11: FTIR spectra of (A) P. armeniaca gum, (B) gold and (C) silver nanoparticles

3.5.3 Characterization through Scanning Electron Microscope (SEM)
The morphology, size and shapes of nanoparticles were determined by scanning electron microscope (Figure 3.12). It was observed from the SEM images that the biosynthesized Au- and Ag-NPs were in the range of 10-40 nm and 5-30 nm respectively. Nanoparticles were mostly spherical in shape with different sizes, but a small number of anisotropic nanostructures such as nanorods, nanotriangles and a few polygonal and hexagonal nanoprisms were also observed. The uniform size distribution of nanoparticles in this study indicated the efficient stabilization of nanoparticles, whereas the large sizes and anisotropic shapes of some nanoparticles might be due to the aggregation of smaller ones.
3.5.4 EDX and XRD

The EDX study showed the presence of Au and Ag in the samples (Figure 3.13). Strong signals were observed from Au atoms in Au-NPs at approximately 0.6 and 2.6 keV, while weak signals were observed at 9.7 and 11.6 keV. In the case of Ag-NPs, strong signals
were observed at 0.6 and 2.6 keV, while a weak signal was observed at 3.7 keV. The appearance of Si signal might be owing to the use of silicon grid in the EDX analysis, while the signal for N indicated the presence of nitrogen containing organic compounds in the gum. Moreover, other strong signals for C and O were also due to the presence of bio-organic molecules that were involved in capping the nanoparticles. The presence of chlorine atom in the EDX spectra of gold nanoparticles was due to the presence of chlorine in tetrachloroauric acid molecule. The signals for K and Mg were owing to X-rays emission from various bio-molecules of the gum. The appearance of elemental Au and Ag in the EDX analysis supported the XRD results, thus showing the reduction of metal cations to elemental form.
Figure 3.13: EDX spectra of (A) gold (B) silver nanoparticles.

The nature of Au- and Ag-NPs synthesized was evaluated with XRD analysis. Figure 3.14 shows the XRD profile of the synthesized gum stabilized nanoparticles. The XRD peak positions are consistent with the metallic silver and gold. The peaks at 35 degree, or double peak at 45 degree also depicts the presence of silver oxide in addition to silver [141], and this might be due to air oxidation of silver ions during Ag-NPs synthesis as the reactions were carried out in open atmosphere. The XRD pattern of Ag-NPs exhibited typical peaks
at dispersion angles (2 Ø) of 38.431, 44.623, 64.531, 77.781 that can be indexed to the (111), (200), (220), (311) Bragg’s reflections of face centered cubic (FCC) structure of metallic silver, similar to the Joint Committee on Powder Diffraction Standards (JCPDS) file no: ICDD-PDF2, showing that the biosynthesized Ag-NPs were of pure crystalline silver. In the case of gold containing sample, the typical diffraction peaks of FCC metallic gold phase (4-0784) at 38.21°, 44.39°, 64.62°, and 77.59° were observed. No absorption peaks similar to other crystalline phases were observed indicating high purity of the products. The diffraction peak at 38° was only highly intense peak among the observed peaks for both Au- and Ag-NPs. In addition, the ratio between the (2 2 0) and (1 1 1) peaks was quite smaller than the normal value (0.1 versus 0.4). The mean particle diameters of Au- and Ag-NPs was calculated from the XRD data, which can be derived from the Debye Scherrer equation \( D = \frac{k \lambda}{\beta \cos \theta} \). This equation exploits the mentioned peak width at angle \( \theta \), where \( K \) is the shape factor, \( \beta \) is the width of the XRD peak at half height and \( \lambda \) is the X-ray wavelength. Average particle size was around 22 and 20 nm for Au- and Ag-NPs, respectively.
Figure 3.14: XRD patterns of (A) gold (B) silver nanoparticles
3.5.5 Thermogravimetric analysis (TGA)

For a comparative study of thermal stability, equal amounts (7.5 mg) of gum and nanoparticles were heated in alumina crucibles, and the TGA profiles were recorded from 50-800°C at a scan rate of 10°C/min in nitrogen atmosphere. Within the same temperature range different weight loss was observed for gum and biosynthesized nanoparticles. Three successive weight losses were observed for gum and nanoparticles in the temperature range of 50–800°C (Figure 3.15). The first decrease in weight of the sample was ascribed to the loss of entrapped water molecules from the polymer matrix. The subsequent weight loss might be owing to the thermal decomposition of the polymer and polymer stabilizing the nanoparticles [142], whereas, the third weight loss might be owing to the conversion of residual polymer to carbon residue. The observed thermal decomposition scheme for the gum is in best agreement with the earlier reports [143]. These findings showed that the gum stabilized Au- and Ag-NPs were thermally more stable than the gum alone.
3.6 Assessment of stability

3.6.1 Effect of sodium chloride on the biosynthesized gold and silver nanoparticles

Stability of the resultant P. armeniaca gum stabilized Au- and Ag-NPs were checked against 1M NaCl solution. Accurately measured 3 ml colloidal solutions of Au- and Ag-
NPs were taken in two separate vials and UV-visible spectra were recorded after adding 20, 40 and 60 µl of 1M NaCl solution. In the UV-Vis absorption spectra no changes were observed in the position and absorption intensity of peaks with varying concentrations of the salt solutions. The observed decrease in intensity of absorption peaks is actually due to water in the salt solution (dilution effect) and was further confirmed by the addition of 60 µl of distilled water to 3 ml of gold and silver nanoparticles solution (Figure 3.16). UV-visible spectra of the resultant gold and silver nanoparticles were also recorded after adding 20, 40 and 60 ul of distilled water (Figure 3.17).

Stability of gold and silver nanoparticles mainly depends on the surface properties of nanoparticles, such as ligand structure and surface charge. Normally, a raise in steric hindrance and electrostatic repulsion of nanoparticles surfaces can extensively increase the stability of nanoparticles in solution. In this case a slight decrease in the surface charge of particles was observed with an enhanced electrolyte concentration (NaCl), which led to aggregation of particles and this might be owing to a decrease in the electrostatic repulsion of particles. The biological activity of nanoparticles depends on many physico-chemical factors and is regulated by their stability, with decrease in stability results in aggregation and consequently leads to total or partial loss of nanoscale properties [144].
Figure 3.16: UV-Vis spectra showing the effect of NaCl on the biosynthesized (A) gold (B) silver nanoparticles.
Figure 3.17: UV-Vis spectra showing the effect of distilled water on the biosynthesized (A) gold (B) silver nanoparticles.
3.6.2 Effect of pH on the biosynthesized gold and silver nanoparticles

The effect of changing pH on the stability of biosynthesized nanoparticles was also studied. Optimized samples of nanoparticles were slightly acidic, having a pH value of 6-7, and showed maximum absorption in their UV-Vis spectra (Figure 3.18). It was noted that Au- and Ag-NPs were stable in acidic medium as there was a less significant change in their UV-Vis absorption spectra. However, in basic medium, the nanoparticles were unstable as significant peak broadening was observed in their UV-Vis spectra. The absorption intensity of nanoparticles was significantly dependent on their sizes. Earlier studied showed that aggregation of nanoparticles led to peak broadening, and a remarkable red shift in the absorption intensity, which depends on the distance between nanoparticles, the density of the assembly and the size of the particles [145]. Thus, at basic pH, the Au- and Ag-NPs exhibited aggregation, which increased their particle size and therefore peak broadening was noted in their respective UV-Vis absorption spectra. The extreme alkaline pH induced nanoparticles aggregation was further confirmed from the SEM images (Figure 3.19). pH plays vital role not only in the formation of stable nanoparticles, but in the stabilization of re-dispersed nanoparticles as well [146]. Solution pH affects nanoparticles interfacial free energy, the protonation of the ionic groups released by nanoparticles dissolution, and the dissociation equilibrium of a complexing agent [147].
Figure 3.18: Effect of different pH on the stability of biosynthesized (A) gold (B) silver nanoparticles
Figure 3.19: SEM images showing the effect of acidic (2-3), neutral (6-7) and basic (12-13) pH on the biosynthesized gold and silver nanoparticles.

3.6.3 Effect of storage on the biosynthesized gold and silver nanoparticles
The effect of long term storage on the biosynthesized Au- and Ag-NPs was studied by keeping the samples at room temperature for eight months. No change in the color and visual aggregation was observed during the entire storage duration. Moreover, no major change was noted in the position of SPR peak of the UV-Vis spectra (Figure 3.20). Thus the biosynthesized Au- and Ag-NPs were highly stable and well dispersed for prolonged period, without undergoing any significant changes. In order for the nanoparticles to be useful in biomedicine, they must satisfy certain criteria which include among other factors, their long term stability under physiological conditions [148]. The stability of nanoparticles carries out a significant role in finding mobility, bioavailability and toxicity [149].
Figure 3.20: UV-Vis spectra showing the effect of long term storage on the biosynthesized (A) gold (B) silver nanoparticles

3.6.4 Effect of high temperature on the biosynthesized gold and silver nanoparticles

The biosynthesized nanoparticles were subjected to extreme temperature by heating at 100°C for 30 min, and it was observed that they were quite stable as no significant changes
were noted in the position of absorption peaks (Figure 3.21). This high stability of nanoparticles could be ascribed to the protective effect of gum on the surface of nanoparticles. Temperature is one of the essential environmental factors that influence the stability, activity and chemical characteristics of materials [144]. Nano-coating of reactive substances is successful in shielding the active therapeutic moiety from environmental degradation, hydrolytic and oxidative processes and thus increases its shelf-life [150].
Figure 3.21: UV-Vis spectra showing the effect of high temperature (100°C) on the biosynthesized (A) gold and (B) silver nanoparticles.
3.7 Biological activities

3.7.1 Antibacterial activity

To assess antibacterial activity of P. armeniaca gum stabilized Au- and Ag-NPs disc diffusion against method was used. Growth suppression was noted in plates loaded with Au- and Ag-NPs, while the negative control plates with autoclaved gum were devoid of any zone of inhibition. The positive control plates loaded with the standard streptomycin antibiotic discs produced greater inhibition zones. The zones of inhibition for Au-NPs measuring 10 ± 0.3 mm, 9 ± 0.5 mm and 7.9 ± 0.3 mm were recorded, while that for the Ag-NPs were 18 ± 0.5 mm, 10.2 ± 0.8 mm, 11.2 ± 0.3 mm against the bacterial strains of S. aureus, E. coli and P. aeruginosa, respectively (Table 3.1). As no antibacterial activity was noted for the gum extract, it can be argued that the bactericidal activity might be due to the synergistic activity of gum stabilized nanoparticles and unreduced Au (III) or Ag + ions. A greater zone of inhibition was observed against gram positive bacteria; S. aureus as compared to gram negative strains, P. aeruginosa and E. coli, and is in accordance with the earlier studies [151]. Nanoparticles have emerged as new antimicrobial agents, and numerous types of antimicrobial nanoparticles and nanosized carriers for antibiotics deliverance have confirmed their efficacy for treating communicable diseases. Nanoparticles interrupt the reliability of the bacterial covering and triggered the initiation of oxidative pressure by free radical development [152]. Gold nanoparticles are important in the growth of antibacterial agents, as they are biocompatible than the other nanometals and can be engineered to possess chemical or photo thermal functionality [153]. Silver nanoparticles are used as antimicrobial agents and are typically conventional inorganic nanoparticles. Silver nanoparticles illustrate elevated antimicrobial action analogous to its
ionic state, and have demonstrated potential antibacterial effect against drug resistant bacteria [154]. Development of low cost and simple inorganic antimicrobial agents, such as metal oxide and metal nanoparticles as a substitute of conventional antibiotics may be encouraged for future of pharmaceuticals and medicine [153].

**Table 3.1:** Antibacterial activity of the biosynthesized gold and silver nanoparticles (zone of inhibition in millimeter)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prunus armeniaca gum</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Au-NPs</td>
<td>10 ± 0.3</td>
<td>9 ± 0.5</td>
<td>7.9 ± 0.3</td>
</tr>
<tr>
<td>Ag-NPs</td>
<td>18 ± 0.5</td>
<td>10.2 ± 0.8</td>
<td>11.2 ± 0.3</td>
</tr>
<tr>
<td>DMSO</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>22.5 ± 0.5</td>
<td>20.2 ± 0.3</td>
<td>15 ± 0.5</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of three separate experiments. NA = Not active

**3.7.2 Antinociceptive activity**

As revealed in figure 3.22, considerable antinociceptive effect \( (P < 0.001) \) was produced by the gum extract at doses of 200 and 400 mg/kg. Similarly, the Au-NPs significantly relieved the acetic acid induced nociception at doses of 40 mg/kg \( (P < 0.01) \) and 80 mg/kg \( (P < 0.001) \). The antinoceptive effect of both the extract and its Au-NPs was similar to that of typical diclofenac sodium, which significantly decreased \( (P < 0.001) \) the acetic acid induced writhes at 50 mg/kg. The nociceptive response in the acetic acid induced writhing test results from the action of cyclooxygenase-1 (COX-1) and COX-2 enzymes that create prostaglandins, which stimulate the sensory pathways in the mouse peritoneum and incite
a viscero-somatic reflex manifested as powerful abdominal constrictions [155]. The acetic acid induced writhing method is susceptible to analgesics [156] and sensory afferents in the peritoneum that transmit various receptors on their terminals [157] are activated by suitable agonists and therefore discourage the production of pain impulses [158]. The antinociceptive activity of prunus armeniaca has been shown to be attributed to the presence of amygdalin, which considerably decrease the formalin-induced tonic pain equally in untimely (the first 10 min following formalin injection) and tardy phases (10-30 min next to first formalin injection). Moreover, it subdued c-Fos appearance in the spinal cord and the gene appearance of IL-1b and TNF-α in the skin of the back foot induced by formalin injection [121]. In this study, a dose dependent antinociceptive action was noted for the gum extract and its Au-NPs; however, the antinociceptive doses employed for the Au-NPs (40 and 80 mg/kg) were much lower than that of the gum extract (200 and 400 mg/kg). The lower doses of Au-NPs significantly reduced the tonic chemical induced nociception, and the effect was similar to that of the standard, diclofenac sodium. Chronic and acute pain conditions affect a major part of the world. Individuals suffering from chronic pain experience several pain-related problems, such as increased psychological co-morbidities, sleep disturbances, as well as reduced functionality and an overall decrease in their distinction of living. Nanomedicine offers the prospective for growth of new drugs, technologies and drug release technologies to concentrate on many of the inclusive unmet requirements in pain administration [159]. Phyto-therapeutics needs a scientific approach in the form of nano drug delivery systems, which not only decrease their frequent management, but also assist to enhance their therapeutic worth by increasing the bioavailability and decreasing toxicity [160].
Figure 3.22: Antinociceptive effect of P.armenica loaded gold nanoparticles. One way ANOVA followed by Dunnett’s post hoc test. *$P < 0.05$, **$P < 0.01$ compared to saline treated group. $n = 6$ mice per group.

3.7.3 Anti-inflammatory activity

As revealed in table 3.2, only carrageenan treated animals showed considerable increase ($P < 0.001$) of paw volume at each hour of the study period. The gum extract at the tested doses (200 and 400 mg/kg) appreciably decreased the carrageenan induced paw edema. The Au-NPs at doses of 40 and 80 mg/kg created non considerable ($P < 0.01$) anti-inflammatory effect at the 1st hour of study; though, a high important ($P < 0.001$) protective effect was noted for the next 2-5 hours. The standard diclofenac sodium was found to have a robust anti-inflammatory activity ($P < 0.001$) at 50 mg/kg for the whole five h study duration. Edema development owing to carrageenan operation in mouse foot is a two phase event. The primary stage continuing about 1–5 hours is typically characterized by a non-phagocytic edema and has been ascribed to the feat of different intermediaries such as serotonin, bradykinin and histamine and on vascular permeability [161]. The preliminary
stage is followed by a succeeding stage having time of 2–5 hours and results from too much production of prostaglandins [162]. The succeeding stage of edema is susceptible to drugs akin to indomethacin, phenylbutazone and hydrocortisone. Prunus armeniaca exhibit anti-inflammatory properties, and has been tested against lipopolysaccharide (LPS)-induced inflammation model on mouse BV2 microglial cells, where the extract suppress the nitric oxide production and synthesis of prostaglandin E2 by inhibiting the inducible nitric oxide synthase mRNA appearance, and LPS-stimulated development of cyclooxygenase-2 [163]. The presence of amygdalin in Prunus armeniaca is efficient in relieving inflammatory pain, and could be applied as an analgesic through anti-inflammatory and antinociceptive properties [121].
Table 3.2: Anti-inflammatory activity of the biosynthesized gold and silver nanoparticles against carrageenan induced paw edema in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; h</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; h</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; h</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; h</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.240 ± 0.022</td>
<td>0.244 ± 0.020</td>
<td>0.258 ± 0.017</td>
<td>0.242 ± 0.013</td>
<td>0.254 ± 0.011</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Carrageenan</td>
<td>0.344 ± 0.023###</td>
<td>0.368 ± 0.027###</td>
<td>0.376 ± 0.027###</td>
<td>0.400 ± 0.025###</td>
<td>0.432 ± 0.037###</td>
</tr>
<tr>
<td><strong>Group 3</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Diclofenac (50 mg/kg)</td>
<td>0.252 ± 0.031***</td>
<td>0.254 ± 0.020***</td>
<td>0.268 ± 0.028***</td>
<td>0.292 ± 0.027***</td>
<td>0.302 ± 0.017***</td>
</tr>
<tr>
<td><strong>Group 4</strong></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(PAG 200 mg/kg)</td>
<td>0.248 ± 0.025***</td>
<td>0.266 ± 0.020***</td>
<td>0.276 ± 0.011***</td>
<td>0.304 ± 0.023***</td>
<td>0.312 ± 0.022***</td>
</tr>
<tr>
<td><strong>Group 5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(PAG 400 mg/kg)</td>
<td>0.254 ± 0.028***</td>
<td>0.258 ± 0.031***</td>
<td>0.270 ± 0.029***</td>
<td>0.300 ± 0.033***</td>
<td>0.306 ± 0.023***</td>
</tr>
<tr>
<td><strong>Group 6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Au-NPs 40 mg/kg)</td>
<td>0.276 ± 0.029**</td>
<td>0.288 ± 0.025***</td>
<td>0.306 ± 0.011***</td>
<td>0.302 ± 0.017***</td>
<td>0.330 ± 0.034***</td>
</tr>
<tr>
<td><strong>Group 7</strong></td>
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</tr>
<tr>
<td>(Au-NPs 80 mg/kg)</td>
<td>0.268 ± 0.028**</td>
<td>0.282 ± 0.025***</td>
<td>0.294 ± 0.011***</td>
<td>0.284 ± 0.026***</td>
<td>0.314 ± 0.028***</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD. One way ANOVA followed by Tukey’s post hoc test. ###$p < 0.001$ compared to group 1. **$p < 0.01$, ***$p < 0.001$ compared to group 2. n = 6.
3.7.4 Cytotoxicity assay of P. armeniaca gum stabilized gold and silver nanoparticles

P. armeniaca gum stabilized Au- and Ag-NPs, were tested to evaluate their cytotoxic potential against human cervical cancer cells (HeLa). Potential anticancer effect was demonstrated by P. armeniaca capped Ag-NPs having an IC50 value of 2.45 ± 0.25, followed by P. armeniaca loaded Au-NPs 3.71 ± 0.14 and P. armeniaca gum 6.57 ± 0.16. The standard anticancer drug cisplatin had the lowest IC50 value (1.89 ± 0.12) and therefore exhibited a robust inhibitory effect on HeLa cancer cells.

**Table 3.3:** Anticancer activity of P. armeniaca loaded gold and silver nanoparticles

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. armeniaca gum</td>
<td>6.57 ± 0.16</td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>3.71 ± 0.14</td>
</tr>
<tr>
<td>Silver nanoparticles</td>
<td>2.45 ± 0.25</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>1.89 ± 0.12</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of three separate experiments

3.7.5 Urease inhibition assay

P. armeniaca capped Au- and Ag-NPs were tested in vitro for their potential effect as inhibitor of urease. The percent inhibition values revealed that both the gum and its nanoparticles were able to inhibit urease with better affinity. Increase inhibitory effect was demonstrated by the P. armenica loaded silver (35.1 ± 0.44) followed by P. armeniaca gum (19.3 ± 0.95) and gold (16.1 ± 0.83) nanoparticles. The positive standard inhibitor thiourea, showed a robust efficacy by producing a potent inhibition (78.3 ± 2.33%) of urease (**Table 3.4**).

**Table 3.4:** Urease inhibition assay of P. armeniaca loaded gold and silver nanoparticles
<table>
<thead>
<tr>
<th>Sample</th>
<th>Percent inhibition (1 mg/mL)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>P. armeniaca gum</td>
<td>19.3 ± 0.95</td>
<td></td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>16.1 ± 0.83</td>
<td></td>
</tr>
<tr>
<td>Silver nanoparticles</td>
<td>35.1 ± 0.44</td>
<td></td>
</tr>
<tr>
<td>Thiourea (1 mM)</td>
<td>78.3 ± 2.33</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of three separate experiments.
CHAPTER: 4

METAL NANOPARTICLES STABILIZED

WITH PRUNUS DOMESTICA (PLUM) GUM
4.1 Introduction

Prunus domestica L. (family Rosaceae) is a deciduous, shrubby, tiny tree cultivated at high altitude. The fruit of P. domestica is used medicinally for the reduction of disturbed menses, debility and leukorrhea subsequent miscarriage. Moreover, high consumption of the fruit has been revealed to decrease low-density lipoprotein cholesterol in human plasma \[164\] as well as liver and plasma lipids in rats \[165\], prevent and improve ovariectomy-induced hypercholesterolemia \[166\] and bone mineral density loss \[167\], possess antiemetic activity against apomorphine induced emesis in dogs \[168\], and has analgesic \[169\] and antibacterial activities \[170\]. P. domestica dried fruit contains huge quantity of antioxidant compounds, such as cryptochlorogenic acid [(4-O-caffeoylquinic acid), (+)-abscisic acid, (6S,9R)-roseoside, (+)-β-D-glucopyranosyl abscisate, chlorogenic acid (5-O-caffeoylquinic acid), neochlorogenic acid (3-O-caffeoylquinic acid), and two lignan glucosides 3-(β-D-glucopyranosyloxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-(2R,3S)-dihydrobenzofuran and ((+)-pinoresinol mono-β-D-glucopyranoside) \[171\]. In addition the fruit contains flavonols [(myricetin, quercetin and kaempferol), carbohydrates (fructose, sucrose, glucocse, sorbitol), organic acids (citric acid, malic acid), vitamins (α-tocopherol, γ-tocopherol, β-carotene) and minerals (sodium, potassium, magnesium, calcium, iron, zinc)] \[172\].

Plum, P. domestica produces a reddish brown gum known as plum gum. The gum oozes out in the spring on plum tree bark and seems to be alike gum Arabic. Gums collected from plum trees growing in different parts of the world are considered as exudates of ecological significance. It can entirely replace the more expensive gum Arabic as well as its synthetic derivatives in the pharmaceutical and food production.
CHAPTER 4  METAL NANOPARTICLES STABILIZED WITH P. DOMESTICA GUM

P. domestica gum has been used as a reducing and stabilizing agent for the preparation of Au-NPs and showed a dose-dependent catalytic activity [173]. Keeping in view the nano-inspired multi-targeted phytotherapeutic approach and the multifactorial nature of diseases in this study we have evaluated the P. domestica gum stabilized Au- and Ag-NPs for their prospective anticancer, antibacterial, urease inhibition properties. In addition Au-NPs have also been tested for their respective anti-inflammatory and analgesic activities.

4.2 Experimental

4.2.1 Materials and method

Tetrachloroauric acid trihydrate and silver nitrate salts of analytical grade were purchased from Merck, Germany. P. domestica fresh gum was purchased from the local market in June 2013 and was formally identified (RA-85) prior to its use by Prof. Dr. Samen Jan of Department of Botany, Islamia College University Peshawar, Pakistan. Doubly distilled water was used throughout the experiments and for all types of analysis. Water was purified through a Milli-Q-SP ultra pure water purification system Institute of Chemical Sciences University of Peshawar.

4.2.2 Synthesis of gold and silver nanoparticles

The P. domestica mediated biosynthesis of nanoparticles was carried out by utilizing the stock solutions of tetrachloroauric acid trihydrate/silver nitrate and P. domestica gum at concentrations of 1 mM and 0.5% w/v, respectively. The stock solutions were centrifuged at 6000 rpm for 20 minutes to exterminate bulk impurities. The aqueous solutions of tetrachloroauric acid and silver nitrate were reduced by mixing with 0.5% P. domestica gum solution in differing ratios and stirred gently at temperatures of 20, 40, 60 and 80°C. The optimized product having SPR at 555 nm for Au-NPs was obtained by mixing 8 ml of
tetrachloroauric acid solution (1 mM) and 5 ml of 0.5% w/v P. domestica gum solution at a temperature of 80°C and a reaction time of 5 h. Similarly, the optimized Ag-NPs having SPR at 450 nm were obtained by use of 20 ml of silver nitrate solution and 8 ml of 0.5% w/v P. domestica gum solution at a temperature of 80°C.

4.3 In vitro biological assays

4.3.1 Cytotoxicity assay

The HeLa cervical cancer cell line (HeLa cells) was cultured in RPMI-1640, having 100 U/mL penicillin pyruvate (1 mM), 100 μg/mL streptomycin and heat-inactivated fetal bovine serum (10%), glutamine (2 mM) in T-75 cm2 sterile tissue culture flasks in a 5% carbon dioxide incubator at 37°C. For growing HeLa cells 96-well plates were used in the experiments by inoculating 5 × 104 cells per 100 μL per well, and plates were incubated at 37°C for 24 h in a humidified environment having 5% carbon dioxide. A homogeneous monolayer was produced, within 24 h that was used for experiments. To perform the cytotoxicity assay, a previously described method [174] was adopted with small modifications [175]. Temporarily the cells were cultivated in various 96 well plates for 24 h. Initially, 1 mg/mL of P. domestica gum solution and the nanoparticles were inoculated in test wells. Further, these solutions screened at different concentrations (1 mg/mL – 1 ng/mL) were inoculated in test wells. Cisplatin was used as a positive control. The well possessing culture media with cells having no drug or compound was taken as blank. After that all plates were incubated for 48 h. Following that, cells were fixed with 50 μL of 50% ice cold trichloroacetic acid solution and plates were incubated at 5°C for 1 h. Later on plates were rinsed, 5 times with phosphate-buffered saline and air dried. Fixed cells were more treated with 0.4% w/v sulforhodamine B dye and left at room temperature for 30 min.
Later on the plates were rinsed with 1% acetic acid solution and were dried. The dried plates were treated with 10 mM Tris base solution for 10 min at room temperature in order to solubilize the dye. The absorbance was calculated at 490 nm subtracting the background (blank) measurement at 630 nm [176]. All experiments were conducted in triplicate.

4.3.2 Urease inhibition assay

The indophenol method was used to determine the Urease inhibition assay of the synthesized compounds [177] with small modifications [178]. Reaction mixtures comprised of 40 μL of buffer (0.01 mol/L K₂HPO₄, 100 mmol/L urea, 1 mmol based on the synthesis of ammonia. The phenol reagent (0.005 % w/v sodium nitroprusside, 40 μL, 1 % w/v phenol) and alkali reagent (0.1 % active chloride NaOCl, 40 μL, 0.5 %, w/v NaOH) were put in every well, and, the absorption was calculated at 630 nm applying a microplate reader (Bio-TekELx 800TM, Instruments, Inc. Winooski, VT, USA) consequent to 10 min of incubation at 37°C. Thiourea was applied as the standard inhibitor. All experiments were performed in triplicate. The following equation was used to calculate the percent inhibition,

Percent inhibition = 100 – [absorption of nanoparticles/ absorption of control] × 100

4.3.3 Antibacterial assay

The P. domestica loaded Au- and Ag-NPs was evaluated against Gram positive and Gram negative strains of E. coli, S. aureus and P. aeruginosa, respectively, by the disc well diffusion method. For each bacterial strain three independent experiments were conducted using streptomycin as the standard. 5 μg of dried Au- and Ag-NPs were dissolved in DMSO and incubated at 30°C for 24 h.

4.4 In vivo biological assays
4.4.1 Animals

BALB/c mice of both sexes weighing 25-30 g were purchased from the National Institute of Health (NIH), Islamabad, for the in vivo experiments. The animals were acclimatized at 22 ± 2°C for one week before experiments. Throughout this period the animals boast open access to food and water. Before experiments, the animals were fasted for 2 and 4 h, respectively, for antinociceptive and anti-inflammatory assay. Every experimental procedure on animals was carried out in agreement with the NIH procedure for the care and use of laboratory animals.

4.4.1.1 Antinociceptive assay

The acetic acid induced abdominal constriction assay was used to determine the analgesic efficiency of P. domestica loaded Au-NPs against tonic visceral chemical induced nociception [179]. All drugs were dissolved in normal saline and were administered through an oral gavage tube, except diclofenac sodium, which was given as an intraperitoneal injection. Animals were arbitrarily alienated into six groups. Group I received normal saline and act as control. Group II was treated with the standard diclofenac sodium (50 mg/kg, i.p). Group III and IV received P. domestica gum at 200 and 400 mg/kg, while group V and VI were treated with P. domestica loaded Au-NPs at doses of 40 and 80 mg/kg, respectively. After 30 min of drugs treatment, the animals were injected with 1% acetic acid (10 ml/kg, i.p) and the abdominal writhes were counted for 20 min.

4.4.1.2 Anti-inflammatory assay

The inhibitory effect produced by P. domestica loaded Au-NPs against a phlogistic agent mediated paw swelling was evaluated by the carrageenan induced paw edema assay [180]. The animals were treated with P. domestica gum and its Au-NPs by an oral gavage tube.
Diclofenac sodium was used as standard and was injected i.p at a dose of 50 mg/kg. After 30 min of treatment, the entire animals were challenged with 50 μL of 1% solution of carrageenan injected into the plantar surface of the left hind paw. The anti-inflammatory effect was evaluated by measuring the paw size of each animal with a digital plethysmometer after each hour of the 5 h study duration.

4.6 Results and discussion

4.6.1 Characterization of P. domestica stabilized gold and silver nanoparticles

4.6.1.1 UV-Vis spectroscopy

Absorption spectra of P. domestica loaded Au- and Ag-NPs were recorded against P. domestica gum and pure metal salt solutions. The typical SPR peaks for Au- and Ag-NPs were obtained at 555 and 450 nm, respectively, however, no absorption peaks were observed for the pure chloroauric acid, silver nitrate, and gum solutions. The synthesis of nanoparticles were more inveterate from the appearance of wine red and light yellow color, respectively, while the pure gum, chloroauric acid and silver nitrate solutions appeared colorless (Figure 4.1).
Figure 4.1: UV-Vis spectra of P. domestica loaded (A) gold and (B) silver nanoparticles. The inset photos show the corresponding color of the biosynthesized nanoparticles solutions.

4.6.1.2 Characterization through Scanning Electron Microscope
P. domestica loaded Au- and Ag-NPs were mostly in the size range of 7-40 and 5-35 nm respectively, with most spherical shapes, but a small number of irregular nanoparticles, such as nanorods, nanotriangles, polygonal and hexagonal nanoprisms were also observed (Figure 4.2). The uniform distribution of Au- and Ag-NPs signify the complete stabilization of nanoparticles by capping agents, while the large sizes and anisotropic shapes of some particles might be due to the aggregation of the small size nanocrystals.
**Figure 4.2:** Scanning electron microscopic images of *P. domestica* loaded (A) gold and (B) silver nanoparticles.
4.6.1.3 EDX and XRD analysis

The presence of metallic Au and Ag in P. domestica loaded Au- and Ag-NPs was confirmed from the EDX studies. Strong signals were observed from Au- atoms in Au-NPs at approximately 0.6 and 2.6 keV, while a weak signal was observed at 9.7 keV. In the case of Ag-NPs, strong signals were observed at 0.6 and 2.6 keV, while a weak signal was observed at 3.7 keV. The appearance of the Si signal corresponded to the use of a silicon lattice in the EDX study, while the signal for N show the presence of nitrogen containing organic compounds in the biopolymer. Moreover, the other strong signals for C and O were due to the presence of different organic molecules capping the Au- and Ag-NPs. The appearance of the Cl signal in the EDX spectra of gold nanoparticles was due to the presence of chlorine in the tetrachloroauric acid trihydrate molecule. The signals for K and Mg were owing to the X-ray emission from various bio-molecules of the gum (Figure 4.3). The appearance of elemental Au and Ag in the EDX analysis supports the XRD results, which provides confirmation of the reduction of metal cations to elemental form.
Figure 4.3: EDX spectra of P. domestica loaded (A) gold and (B) silver nanoparticles.

In the XRD spectrum, no peaks were observed for the P. domestica gum, therefore indicating their non crystalline (amorphous) nature, while the XRD spectrum of gum loaded nanoparticles had characteristic peaks, thus indicating their crystalline nature. The XRD pattern of the Au-NPs (Figure 4.4) exhibited typical peaks at scattering angles (2 $\Theta$) of 38.431, 44.623, 64.531, 77.781 that may be indexed to the (111), (200), (220), (311)
Bragg’s reflections of the face centered cubic (FCC) structure of metallic gold analogous to the Joint Committee on Powder Diffraction Standards (JCPDS) file no: ICDD-PDF2, showing that the synthesized Au-NPs are of pure crystalline gold respectively. For the Ag-NPs, the typical diffraction peaks of FCC metallic silver phase (4-0784) were observed at 38.21, 44.39, 64.62 and 77.59. No peaks similar to supplementary crystalline phases were noted, indicating high purity of the resultant products. Among the observed peaks the diffraction peak at 38° was the only strong peak for both Au- and Ag-NPs. In addition, the ratio between the (1 1 1) and (2 2 0) peaks was less than the standard value (0.1 versus 0.4). From the XRD data, the mean particle sizes of nanoparticles were calculated which can be derived from the Debye Scherrer equation \( D = \frac{k \lambda}{\beta \cos \theta} \). This equation exploits the reference peak thickness at an angle \( \theta \), where \( k \) is the shape factor; \( \beta \) is the width of the XRD peak at half height and \( \lambda \) is the X-ray wavelength. Average particle size was around 20 and 17 nm for Au- and Ag-NPs, respectively.
Figure 4.4: XRD patterns of (A) P. domestica gum, (B) gold and (C) silver nanoparticles.

4.6.1.4 Thermogravimetric analysis
The thermogram obtained from the thermogravimetric analysis showed three successive weight losses for gum and nanoparticles within the temperature range of 50-800°C (Figure 4.5). The first observed weight loss was endorsed to the removal of entrapped water molecules from the gum and nanoparticles. The succeeding weight loss might be owing to the thermal decomposition of the gum polymer and the polymer surrounding the nanoparticles, whereas the third weight loss might be owing to the transformation of residual polymer to carbon scum. After nanoparticles synthesis a low percent weight loss was noted for the gum.
Figure 4.5: TGA spectra of P. domestica loaded (A) gold and (B) silver nanoparticles.

4.6.1.5 Characterization through FTIR

Significant changes were observed in the FTIR spectrum of P. domestica gum after nanoparticles synthesis. The major absorption bands were observed in the spectrum of P. domestica gum at 3323 [(O-H stretching), 2931 (C-H stretching), 1606, 1409, 1325, 1255 (Asymmetrical and symmetrical stretches of carboxylate group) and 1020 (C-O stretch of
carboxylic acids]) (Figure 4.6). Similarly for Au-NPs the absorption peaks were observed at 3317, 2931, 1602, 1402, 1352, 1022 cm\(^{-1}\), while for silver absorption occurred at 3306, 2931, 1595, 1114, 1024 cm\(^{-1}\). For gold particles, the bonded O-H stretching shifted from 3323 cm\(^{-1}\) to lower frequency of 3317 cm\(^{-1}\), and thus showed the involvement of OH groups during the reduction and capping process. The weak peak at 2931 cm\(^{-1}\) due to C-H stretching remained unchanged for the gold and silver nanoparticles. A sharp peak of carbonyl carbon at 1606 cm\(^{-1}\) was shifted to 1602 for the gold and 1595 cm\(^{-1}\) for the silver nanoparticles, representing the participation of carboxylic acid groups in nanoparticles formation as reducing and stabilizing agent. Significant changes were observed in the bending vibrations at 1409, 1325, 1255, and 1220 cm\(^{-1}\) in the spectrum of gum due to possible attraction by the gold and silver metals.
Figure 4.6: FTIR spectra of (A) P. domestica gum (B) P. domestica loaded gold and (C) Silver nanoparticles.
4.7 Assessment of stability

The effect of gum concentration on the synthesis of nanoparticles was studied by heating several concentrations (0.1-0.5%) of gum solutions with 1 mM of tetrachloroauric acid and silver nitrate solutions, respectively, for 1 h. The effects of gold or silver ions were evaluated by changing their concentration from 1 to 5 mM and then heating at 80°C for 3 hours. The thermal stability was studied by keeping the nanoparticles solution at 20, 40, 60 and 80°C each for 3 hours. The effect of varying reaction time (1-5 hours) was assessed with 0.5% gum at 1 mM gold or silver salt solution. The salt stability was checked by adding different volumes of sodium chloride solutions (20-60 ul) to 3 ml nanoparticles solution under continuous mixing for 3 hours. One molar solution of hydrochloric acid or sodium hydroxide was added drop wise to the colloidal solutions of nanoparticles and the resistance to varying pH conditions was measured at different pH (2-3, 4-5, 6-7, 8-9, 10-11, 12-13) values. The long term stability was estimated by keeping the nanoparticles at room temperature for eight months. The extreme thermal stability was evaluated by heating the nanoparticles at 100°C for 30 min.

4.7.1 Effect of salt

Stability of the resultant P. domestica gum stabilized Au- and Ag-NPs was checked against 1M NaCl solution. Accurately measured 3 ml colloidal solutions of Au- and Ag-NPs were taken in two separate vials and UV-visible spectra were recorded after adding 20, 40 and 60 µl of 1M NaCl solution. In the UV-Vis absorption spectra, no changes were observed in the position and absorption intensity of SPR peak with varying concentrations of the salt solution. The observed decrease in intensity of absorption peaks was actually due to water in the salt solution (dilution effect) and was further confirmed by the addition of 60 µl of
distilled water to 3 ml colloidal solutions of Au- and Ag-NPs (Figure 4.7). Moreover no obvious color change was observed when different volumes of 1M NaCl solutions were added to colloidal solutions of Au- and Ag-NPs.
Figure 4.7: UV-Vis spectra showing the effect of different concentrations of sodium chloride (NaCl) on P. domestica loaded (A) gold and (B) silver nanoparticles.
4.7.2 Effect of pH

Optimized samples of both Au- and Ag-NPs were slightly acidic, having a pH value of 6-7, and showed maximum absorption in the UV-Vis spectra (Figure 4.8). It was observed that formed nanoparticles were stable in acidic medium as there were less significant shifts in the UV-Vis absorption spectra. However, in basic media, the nanoparticles were unstable, as peak broadening and a red shift were observed in their UV-Vis spectra.
Figure 4.8: UV-Vis spectra showing the effect of different pH on P. domestica loaded (A) gold and (B) silver nanoparticles

4.7.3 Storage and thermal stability
P. domestica loaded Au- and Ag-NPs colloidal solutions were stored at room temperature for about six months. There was no change in color and visual aggregation was observed during the entire storage duration. From the UV-Vis spectra, a slight red shift was observed in the position of the SPR peak and a lesser absorption value, indicating an enhancement in the size of particles due to aggregation. Synthesized Au- and Ag-NPs were also studied for their thermal stability. Nanoparticles were heated at different temperatures and the UV-Vis spectra were recorded. It was observed that up to 80°C the nanoparticles were quite stable as no change was observed in the position of the SPR peak and absorption values; however when they were heated at 100°C for 30 min, a slight red shift was observed in the position of absorption peak in the UV-Vis spectra, indicating an increase in particle size.

4.8 Prunus domestica loaded nanoparticles inhibit cancer cells

P. domestica loaded Au- and Ag-NPs were tested to evaluate their cytotoxic potential against human cervical cancer cells (HeLa) (Table 4.1). A potential anticancer effect was showed by P. domestica loaded Au-NPs, having an IC50 value of 2.14 ± 0.15, followed by P. domestica loaded Ag-NPs (3.45 ± 0.23) and P. domestica gum (4.92 ± 0.31). The standard anticancer drug cisplatin had the lowest IC50 value (1.89 ± 0.12), and therefore exhibited a robust inhibitory effect on the HeLa cancer cells.

Table 4.1: Anticancer activity of P. domestica loaded gold and silver nanoparticles

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 (μg/mL)</th>
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<tbody>
<tr>
<td>Sample</td>
<td>IC50 (μg/mL)</td>
</tr>
<tr>
<td>P. domestica loaded Au-NPs</td>
<td>2.14 ± 0.15</td>
</tr>
<tr>
<td>P. domestica loaded Ag-NPs</td>
<td>3.45 ± 0.23</td>
</tr>
<tr>
<td>P. domestica gum</td>
<td>4.92 ± 0.31</td>
</tr>
</tbody>
</table>
Cancer remains a high unmet medical requirement. Usual chemotherapy kills cancer cells successfully; but in addition to cancer and tumor cells these cytotoxic drugs also kill healthy cells, leading to severe unpleasant side effects that limit their clinical efficacy. Recently, single-target chemotherapy is fading in favor of a multi-target approach [181]. The most excellent way to treat various cancers may be applying a substance that strike any specific position in numerous independent positions in the disease-causing medium or coupling multiple targeted-agents [182]. In this study, the *Prunus domestica* loaded Au- and Ag-NPs showed potential inhibitory susceptibility towards human cervical cancer cells (HeLa), and the anticancer result was similar to that of the standard cisplatin. Nanotechnology can play a significant role in realizing the objective of detecting transforming cell populations before time and will also permit the suitable combination of agents that act as “smart nanogrenades” by selectively targeting the early cancer lesions and therefore contain or eliminate them exclusive of collateral effects on well tissue [183]. Integration of nanocarriers as a novel drug delivery system in the traditional system of medicine can be proved beneficial to conflict chronic notorious diseases like cancer [160].

### 4.9 Prunus domestica loaded nanoparticles suppressed pathogenic bacteria

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<table>
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<tbody>
<tr>
<td><strong>Prunus domestica gum</strong></td>
<td>4.92 ± 0.31</td>
</tr>
<tr>
<td><strong>Gold nanoparticles</strong></td>
<td>2.14 ± 0.15</td>
</tr>
<tr>
<td><strong>Silver nanoparticles</strong></td>
<td>3.45 ± 0.23</td>
</tr>
<tr>
<td><strong>Cisplatin</strong></td>
<td>1.89 ± 0.12</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of three separate experiments.
The disk diffusion technique was applied to assess the potential antibacterial assay of P. domestica loaded Au- and Ag-NPs against the pathological strains of bacteria (Table 4.2). Prominent growth inhibition was noted in plates laden with nanoparticles, while the negative control plates and the autoclaved gum did not produce any visible zone of inhibition. The potential antibacterial effect was observed with P. domestica loaded silver nanoparticles against Gram positive strains of S. aureus (19.7 ± 0.4) and Gram negative strain of E. coli (14.4 ± 0.7) and P. aeruginosa (13.1 ± 0.2). Similarly, these bacterial strains were also inhibited by P. domestica loaded Au-NPs but with low affinity (10.5 ± 0.6, 10 ± 0.4 and 8.2 ± 0.3). The positive standard streptomycin also possessed an antibacterial effect of higher magnitude as compared to P. domestica loaded Au- and Ag-NPs against the tested pathological stains (23.6 ± 0.8, 21.8 ± 0.2 and 18.6 ± 0.3).

Table 4.2: Antibacterial activity of P. domestica loaded Au- and Ag-NPs (zone of inhibition in millimeter)
Infectious diseases remain among the leading cause of death and produce an extremely significant impact on global health and economies [184]. Infections caused by bacteria signify a chief community health burden, not only in provisos of mortality and morbidity, but also in terms of heavy costs on implementation of infection control measures and patient management. For bacterial infections conventional drugs typically give effective antibiotic treatment, however antibiotic resistance is an increasing problem and a continuing need for new solutions. Immediate actions should be taken to counter the antibiotic resistance and reduce the development and spread of life-threatening infections [185]. Several herbal preparations with antibacterial activity have shown efficacy in different clinical trials against various pathogenic species of bacteria [186]. Our study shows that the *Prunus domestica* loaded Au- and Ag-NPs possessed antibacterial properties while the *P. domestica* gum itself was unable to produce any zone of inhibition. Preferential inhibition was showed by the *P. domestica* loaded silver nanoparticles against both the Gram positive (*S. aureus*) and Gram negative (*E. coli, P. aeruginosa*) pathogenic strains, and the effect was comparable to that of standard antibiotic, streptomycin. Nanotechnology can provide important tools for designing and fabricating a new generation of substrates
with specific antimicrobial properties [187]. By applying green chemistry principles nanoparticles with smaller environmental impact, higher antimicrobial activity and biodegradable cores can be fabricated [188]. Herbal drugs incorporated into nano-based drug delivery systems have potential benefit for use in the treatment of wound healing or dermal bacterial infections [189].

4.10 Prunus domestica loaded nanoparticles modify the enzymatic activity of urease

P. domestica loaded Au- and Ag-NPs were tested in vitro for their potential effect as an inhibitor of urease. The percent inhibition values revealed that both the gum and its nanoparticles were able to inhibit urease with better affinity (Table 4.3). An increased inhibitory effect was showed by the P. domestica gum (26.7 ± 0.49%) followed by P. domestica loaded silver (21.5 ± 1.17%) and gold (19.2 ± 0.86%) nanoparticles. The positive standard inhibitor, thiourea showed a robust efficacy by producing a potent inhibition (78.3 ± 2.33%) of urease.

Table 4.3: Urease inhibition assay of P. domestica loaded gold and silver nanoparticles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percent inhibition (1 mg/mL)</th>
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<tbody>
<tr>
<td>Prunus domestica gum</td>
<td>26.7 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>Value</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>Gold nanoparticles</strong></td>
<td>19.2 ± 0.86</td>
</tr>
<tr>
<td><strong>Silver nanoparticles</strong></td>
<td>21.5 ± 1.17</td>
</tr>
<tr>
<td><strong>Thiourea (1 mM)</strong></td>
<td>78.3 ± 2.33</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of three separate experiments.

Ureas are metalloenzymes that hydrolyze urea into carbon dioxide and ammonia. Urease is necessary for the host organism in the maintenance of bacterial cells in tissues and is a virulence factor found in a variety of pathogenic bacteria [190]. The production of urease by *Helicobacter pylori*, a Gram-negative bacterium, performs a significant role in protecting this bacterium from the critical acidic condition of stomach. The colonization of *H. pylori* in the human stomach leads to gastric ulcer or even gastric carcinoma, if left untreated [191]. There is an urgent need of novel urease inhibitors for counteracting the catastrophe of *H. pylori* infection on the eve of rising antibiotic resistance. Our study showed that both the *P. domestica* gum and its Au-NPs possessed promising urease inhibitory potency. There is a great potential of plant secondary metabolites of different classes to negatively affect the activity of ureases, the knowledge of which can contribute to the design of novel, safe and, and less costly urease inhibitors with the aim to improve human life by fighting urease-related diseases [192]. For inhibiting urease, plant extract fabricated nanoparticles use may be beneficial for the growth of novel drugs for treatment of multidrug-resistant *H. pylori* by means of techniques such as creating synergistic mixture through typical drugs or their encapsulation with drugs [193].

4.11 *Prunus domestica* loaded gold nanoparticles ameliorate inflammation
As shown in table 4.4, the sub-plantar injection of carrageenan significantly elevated (P < 0.001) the normal paw volume during the entire study duration. After 1 h, considerable decrease in the carrageenan induced paw edema was observed through P. domestica gum at doses of 200 mg/kg (P < 0.05) and 400 mg/kg (P < 0.001) as well as with the P. domestica loaded Au-NPs at 40 mg/kg (P < 0.05) and 80 mg/kg (P < 0.01). Moreover, there was a significant antagonism (P < 0.001) of the phlogistic agent (carrageenan) induced increase in paw volume was confirmed by every tested doses of P. domestica gum (200 and 400 mg/kg) and P. domestica loaded Au-NPs (40 and 80 mg/kg) during the succeeding 2-5 h of experiment. A similar anti inflammatory profile was shown by the standard drug, diclofenac sodium, at a dose of 50 mg/kg during the 1st (P < 0.05) and 2-5 h (P < 0.001) of the study period.

Inflammation is a complex set of relations between the cells and soluble factors that can occur in every tissue in reaction to post-ischemic, infectious, traumatic and toxic, or autoimmune injury. The progression usually leads to revival from infection and to healing; but, if targeted obliteration and assisted repair are not correctly phased, inflammation can cause constant tissue destruction by lymphocytes, leukocytes, or collagen. Inflammation per se relics one of the chief healing targets in different disorders through a confounding combined impact [194]. In relation to the treatment and prevention of inflammatory circumstances natural products play an important role in human health [195]. Inflammation produced by carrageenan is nonimmune, and acute and generate the fundamental symbols of inflammation, i.e. erythema, hyperalgesia and edema, which increase instantly subsequent subcutaneous injection, ensuing from the action of pro inflammatory agents, such as histamine, tachykinins, bradykinin, complement, and reactive nitrogen and oxygen
species. Such agents could be produced in situ by infiltrating cells, or at the position of insult. The neutrophils eagerly move to inflammation sites and can produce pro-inflammatory reactive species such as oxygen etc. The inflammatory response which is maximal around 5 h post-carrageenan injection and is typically quantified by an enhancement in paw size (edema), and is modulated by inhibitors of particular molecules inside the inflammatory cascade [180]. In this study, P. domestica gum (200 and 400 mg/kg) succintly inhibited the carrageenan induced biphasic paw edema response. Similarly, the P. domestica loaded Au-NPs also exhibited a similar anti-inflammatory profile; however the beneficial effect was noted at much lower doses (40 and 80 mg/kg) compared to P. domestica gum, and the effect was comparable to that of a standard anti-inflammatory drug, diclofenac sodium. By the application of eco-friendly nanoplatforms present administration of inflammatory procedure can be enhanced which specially deliver anti-inflammatory compounds to swollen parts [196]. Nanobiomaterials based on Au-NPs conjugated with biomolecules possessed unique anti-inflammatory properties by in vivo decreasing the leukocyte-endothelium interaction and leukocyte influx to adjacent tissues after leukotriene B4 stimulation as well as create obvious reduction of oxidative burst activation and chemotaxis in vitro [197].
### Table 4.4: Anti-inflammatory activity of P. domestica gum stabilized gold nanoparticles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; h</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; h</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; h</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; h</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; h</th>
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<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
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<tr>
<td>Saline</td>
<td>0.247 ± 0.026</td>
<td>0.250 ± 0.022</td>
<td>0.256 ± 0.013</td>
<td>0.250 ± 0.014</td>
<td>0.250 ± 0.016</td>
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<tr>
<td><strong>Group 2</strong></td>
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<tr>
<td>Carrageenan</td>
<td>0.355 ± 0.013###</td>
<td>0.370 ± 0.026###</td>
<td>0.386 ± 0.021###</td>
<td>0.397 ± 0.022###</td>
<td>0.405 ± 0.013###</td>
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<tr>
<td><strong>Group 3</strong></td>
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<tr>
<td>Diclofenac (50 mg/kg)</td>
<td>0.287 ± 0.029***</td>
<td>0.265 ± 0.019***</td>
<td>0.265 ± 0.031***</td>
<td>0.287 ± 0.025***</td>
<td>0.305 ± 0.012***</td>
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<tr>
<td><strong>Group 4</strong></td>
<td></td>
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<tr>
<td>(Gum 200 mg/kg)</td>
<td>0.302 ± 0.009***</td>
<td>0.290 ± 0.008***</td>
<td>0.295 ± 0.013***</td>
<td>0.305 ± 0.013***</td>
<td>0.315 ± 0.019***</td>
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<td><strong>Group 5</strong></td>
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<tr>
<td>(Gum 400 mg/kg)</td>
<td>0.282 ± 0.017***</td>
<td>0.292 ± 0.012***</td>
<td>0.287 ± 0.012***</td>
<td>0.280 ± 0.022***</td>
<td>0.290 ± 0.018***</td>
</tr>
<tr>
<td><strong>Group 6</strong></td>
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<tr>
<td>(Au-NPs 40 mg/kg)</td>
<td>0.305 ± 0.021**</td>
<td>0.307 ± 0.012***</td>
<td>0.305 ± 0.015***</td>
<td>0.302 ± 0.017***</td>
<td>0.317 ± 0.026***</td>
</tr>
<tr>
<td><strong>Group 7</strong></td>
<td></td>
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<td></td>
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<tr>
<td>(Au-NPs 80 mg/kg)</td>
<td>0.295 ± 0.013**</td>
<td>0.287 ± 0.017***</td>
<td>0.295 ± 0.013***</td>
<td>0.287 ± 0.022***</td>
<td>0.300 ± 0.018***</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD. One way ANOVA followed by Tukey’s post hoc test n = 6 mice per group. ###P<0.001 compared to group 1 *P<0.05, **P<0.01, ***P<0.001 compared to group 2.
4.12 Prunus domestica loaded gold nanoparticles de-escalate nociception

A potent antinocicpactive effect was demonstrated by the P. domestica gum and its Au-NPs (Figure 4.9). Substantial attenuation of chemically induced nociception was noted by P. domestica gum at 200 (P < 0.05) and 400 mg/kg (P < 0.01). Similarly, the tested doses (40 and 80 mg/kg) of P. domestica loaded Au-NPs significantly abolished (P < 0.01) the acetic acid induced writhes. The single dose of diclofenac sodium (50 mg/kg), the standard drug also produced an efficient peripheral analgesic effect by significantly alleviating (P < 0.01) the chemical induced nociceptive pain.

![Figure 4.9: Antinociceptive effect of P. domestica loaded gold nanoparticles. One way ANOVA followed by Dunnett’s post hoc test. *P < 0.05, **P < 0.01 compared to saline treated group. n = 6 mice per group.](image)

Pain is an emotional and terrible sensory incident allied with potential or actual tissue damage or described in expressions of such damage [198]. Pain is typically elicited by the activation of particular nociceptors (nociceptive pain). It might also result from injury or damage to the
CNS itself or sensory fibers (neuropathic pain) [199]. Writhing, that is an obvious reaction to the extreme pain induced by acetic acid via nociceptors, is characterized by stretching of the hind limbs and episodes of renunciation of the abdomen. The signals carried to the central nervous system in reaction to pain, originate liberation of mediators, just like prostaglandins, which contribute to the enhanced sensitivity of nociceptors [200]. The acetic acid induced nociceptive test is susceptible to analgesics [156], and sensory afferents in the peritoneum that bring various receptors on their terminals [157] are activated by suitable agonists and consequently discourage the production of pain impulses [158]. P. domestica gum has potential peripheral antinociceptive properties since a marked reduction in chemically induced nociceptive response was noted at doses of 200 and 400 mg/kg. The extent to which P. domestica gum abolished the tonic visceral chemically-induced nociception was also exhibited by P. domestica loaded gold nanoparticles. However, the effect was observed at much smaller doses (40 and 80 mg/kg) of Au-NPs and was similar to diclofenac sodium used as a standard analgesic. Chronic pain, resulting from disease or injury, constitutes an enormous burden for the individual and society. The effectiveness of current pain therapies is inadequate through the degree of pain release provided and the incidence of considerable side effects. Nanotechnology has the potential to address multiple, major, unmet problems in the diagnosis, treatment, and symptom management of a large variety of diseases and conditions, like cancer, which are accompanied by pain [201]. Gold nanoparticles have shown prominence to be useful for enhancing the analgesic effects of medicinal plant extracts with potential antinociceptive properties [202]. Nanomedicine offers extraordinary opportunities in the progress of new pain relieving therapies to alter the frowning face of pain to a smile of relief [159].
CHAPTER: 5

METAL NANOPARTICLES STABILIZED WITH ACACIA GUM (GUM ARABIC)
5.1 Introduction

Acacia gum also known as meska, char goond, chaar gund or gum Arabic is a natural gum made up of the gloomy sap of many species of the genus acacia. It is historically cultivated in west Asia and Arabia, but the producers also harvest it commercially from wild trees, mostly in Sudan (80%) and all over the Sahel, from Somalia to Senegal.

Chemically this gum is a multifaceted mixture of glycoproteins and polysaccharides [203]. It is the extraordinary source of ribose and arabinose sugars and both of them were isolated for the first time from it. Arabingalactan is a biopolymer, made up of galactose and arabinose monosaccharides and is a main constituent of several plant gums, including gum Arabic. 8-5 Non cyclic diferulic acid had been known as covalently bonded to carbohydrate moieties of the arabinogalactan-protein part. Advance chemical analysis showed that gum Arabic is made up of largely three distinctive parts, that are referred as arabinogalactan-protein (AGP, 10.4% of the total) with a high protein content of 9.18% w/w, glycoprotein (GP, about 1% of the total) with a protein content of 50% w/w and arabinogalactan (AG, 88.4% of the total) with a little protein content (0.44% w/ w) [204].

In the food production, GA is mainly used as an emulsifier and stabilizer [205]. Its commercial E number is E414 and is edible. In medicine, it is used as anti-spasmodic, anti-hypertensive, anti-diabetic and anti-plasmodial. The gum is liver tonic, emollient, potent stringent, anti-asthmatic and antipyretic [206]. It is also a major constituent in conventional lithography and is used in paint, cosmetics, printing materials and glue preparation. It has a variety of industrial applications, such as for viscosity grip in inks, and is also used in cloth commerce. Although it is a low cost material, contend through it for several of these tasks. Furthermore, GA polymer can be applied for solid–liquid dispersion
applications [207]. This eco-friendly gum is broadly applied in soft drink and confectioneries dispensation as it have a natural capability to stabilize and emulsify flavor oil spread in an aqueous medium [208].

Herein, we studied the potential of gum Arabic for the fabrication of Au- and Ag-NPs. Au (III) and Ag ions were reduced and stabilized by the water soluble compounds present in the gum, without using any external reducing and stabilizing agents. Using gum acacia as stabilizing agent and dispersant can prevent the Au- and Ag-NPs from oxidation and aggregation, and also provide these particles with long term stability. This natural biopolymer is not only cheap and profusely available, but also has surface active and outstanding emulsifying assets, that are favorable for the synthesis of different types of nanoparticles [209]. Our tailored method is important due to use of natural biopolymer as an alternative of conventionally used chemicals such as Cetyltrimethylammonium bromide (CTAB) or poly vinyl pyrrolidone (PVP) as the stabilizing agents for the production nanoparticles. The effect of gum and metal ions concentration, reaction time and temperature on the synthetic stability of nanoparticles was studied along with their post synthetic stability against varying pH and salt concentrations, long term storage and extreme of temperature. Biosynthesized Au- and Ag-NPs were also studied for their prospective antimicrobial, anticancer and urease inhibition properties.
5.2 Experimental

5.2.1 Materials and method

Acacia gum (Gum Arabic), Tetrachloroauric acid trihydrate and silver nitrate were employed for the production of Au- and Ag-NPs. Gold and silver salts of analytical grade were purchased from E. Merck (Germany). Fresh acacia gum was collected from Acacia tree from District Kohat, Khyber Pakhtunkhwa, Pakistan in February 2013. The gum properly identified (RZ-85) before use by Prof. Dr. Zahir Shah of Department of Botany, Islamia College University, Peshawar, Pakistan. Chloroauric acid and silver nitrate were taken as precursors for the production of nanoparticles. Throughout the experiments doubly distilled water was utilize for the synthesis of nanoparticles and for additional characterizations. The same solvent (water) was used for recording the UV-Vis absorption spectra.

For Au- and Ag-NPs biosynthesis, 1mM stock solutions of chloroauric acid and silver nitrate salts were prepared by dissolving a calculated amount of these metallic salts in distilled water. Similarly the gum solution was prepared by dissolving 5 mg of powder gum in 100 ml cold distilled water. Within 20 min the gum powder readily dissolved forming viscous solution. For removal of unwanted aggregates, gum solution was centrifuged at 6000 rpm for 20 min, and the apparent supernatant was used for the preparation of nanoparticles. Nanoparticles were prepared by reducing the aqueous solution of HAuCl₄ and AgNO₃ solutions in the presence of gum solution. The effect of different factors such as gum, metal ions concentration, reaction temperature and time on nanoparticles synthesis was studied.
Graphical representation for the fabrication of nanoparticles

**Figure 5.1:** Graphical representation for the synthesis of (A) gold and (B) silver nanoparticles

### 5.2.2 Nanoparticles synthesis and Optimization

The conventional Turkevich Method was applied for the preparation of nanoparticles. For Au- and Ag-NPs biosynthesis different amounts of chloroauric acid and silver nitrate solutions (From their respective 1mM stock solutions) were taken in two different vials, and a desired amount of gum (From 0.5% stock solution of acacia gum) was added to it. Reaction mixtures were stirred gently at the desired temperatures of (20, 40, 60, and 80°C). After stirring for 1h synthesis of nanoparticles were confirmed by the emergence of ruby and golden colors in the reaction mixtures. The resulting mixtures were stirred for 4-5 h and the absorption spectra were recorded. For complete optimization of Au- and Ag-NPs, a lot of reactions were carried out by changing concentration of metal and gum in different ratios. The sharpest absorption peak was selected for further studies among the observed
peaks. The distinctive product having SPR at 555 nm for Au-NPs was obtained by mixing 1 ml of chloroauric acid solution ($10^{-3}$ M) and 3 ml of 0.5% w/v gum solution at a temperature of 80°C and a reaction time of 5 h. In case of Ag-NPs, the typical product having SPR at 450 nm was obtained by mixing 1 ml of silver nitrate solution ($10^{-3}$ M) and 4 ml of 0.5% w/v gum solution at a temperature of 80°C and reaction time of 4 h (Figure 5.2). The optimized ratios (1:3 and 1:4) were applied for the preparation of nanoparticles in bulk amount. The synthesized nanoparticles were isolated by freeze drying, and were used for supplementary analysis and bioassays. Nanoparticles synthesis was observed at all temperatures, but optimum results were obtained at a temperature of 80°C.
Figure 5.2: UV-Visible spectra showing optimization of (A) gold and (B) silver nanoparticles.
5.3 Proposed mechanism for synthesis of nanoparticles

Figure 5.4 illustrates a graphical representation of the synthesis of nanoparticles. After mixing metallic salt solutions with the gum polymer an ion exchange process take place initially, and the glycoprotein carboxylate group (−COOH) of GA polymer is changed into −COOAg. Laterally, these Au (III) and Ag ions bonded to COOH groups are transformed into the respective Au- and Ag-NPs in an in situ manner and are accordingly stabilized by the GA polymer chains.

![Figure 5.4: Proposed mechanism for the synthesis of gold and silver nanoparticles](image)

**Figure 5.4:** Proposed mechanism for the synthesis of gold and silver nanoparticles
CHAPTER 5 METAL NANOPARTICLES STABILIZED WITH GUM ARABIC

5.4 Results and discussion

5.4.1 Characterization

5.4.1.1 UV-visible spectroscopy

The synthesis of nanoparticles can be determined from the position of Plasmon band (extinction spectrum) on a conventional UV-Vis spectrophotometer [210]. The occurrence of Plasmon resonance band at 540 and 425 nm corresponding to that of Au- and Ag-NPs corroborate the biosynthesis of nanoparticles (Figure 5.5). The absorption spectra of the biosynthesized nanoparticles were recorded against the gum aqueous extract and pure gold and silver nitrate solution. It is evident from the UV-Visible spectra that pure gold, silver nitrate and gum aqueous extract have no absorption in the UV-visible region, but the resultant nanoparticles show absorbance at 540 and 425 nm indicating the synthesis of Au- and Ag-NPs in the reaction mixture. In addition, the appearance of ruby red and brownish yellow color after heating the reaction mixture further confirms the successful synthesis of nanoparticles in the presence of gum.
Figure 5.5: UV-Vis spectra of (A) gold and (B) silver nanoparticles. The inset photos show the corresponding color of the biosynthesized gold and silver nanoparticles.
5.4.1.2 X-ray diffraction analysis (XRD)

The XRD analysis is generally bring about to evaluate the crystalline property of nanoparticles. Crystalline nanoparticles are called nanocrystals. XRD analysis was carried out for acacia gum and their respective bio-synthesized Au- and Ag-NPs. No peaks were observed in the XRD spectrum of gum indicating their non crystalline (amorphous) nature, while the XRD spectrum of Au- and Ag-NPs presented characteristic peaks, indicating their crystalline nature. Figure 5.6 shows the XRD profile of the gum and their respective Au- and Ag-NPs. XRD pattern of Au-NPs exhibited distinctive peaks at scattering angles (2θ) of 38.431, 44.623, 64.531, 77.781 that can be indexed to the (111), (200), (220), (311) Bragg’s reflections of face centered cubic (FCC) structure of metallic gold equivalent to the Joint Committee on Powder Diffraction Standards (JCPDS) file no: ICDD-PDF2, illuminating that the synthesized Au-NPs are of pure crystalline gold respectively. For Ag-NPs the typical diffraction peaks of FCC metallic silver phase (4-0784) at 38.21, 44.39, 64.62 and 77.59 were recorded. No peaks similar to other crystalline phases were noted indicating high purity of the products. The diffraction peak at 38° was merely the strong peak among the observed peaks for both Au- and Ag-NPs (Figure 5.6). The mean particle diameters of nanoparticles were calculated from the XRD data which could be derived from the Debye Scherrer equation \( D = \frac{k \lambda}{\beta \cos \theta} \). This equation exploits the reference peak width at angle \( \theta \), where \( K \) is the shape factor, \( \beta \) \( \frac{1}{2} \) is the width of the XRD peak at half height and \( \lambda \) is the X-ray wavelength (1.5418). For Au- and Ag-NPs average particle size was approximately 17 and 20 nm respectively.
Figure 5.6: XRD patterns of (A) Acacia gum (B) gold and (C) silver nanoparticles
5.4.1.3 Characterization through SEM and EDX

Scanning electron microscope was used to determine morphology, size and shapes of gum stabilized nanoparticles. The SEM analysis confirmed that the biosynthesized nanoparticles were in the range of 5-25 and 10-35 nm. Biosynthesized Au- and Ag-NPs were mostly spherical in shape and uniformly distributed (Figure 5.7 (A, B). A uniform distribution of nanoparticles indicates complete stabilization of nanoparticles by the gum. From SEM analysis, it was also observed that increased gum concentration resulted in the production of large size nanoparticles (Figure 5.7 (C, D).

![SEM images of gold and silver nanoparticles at low (A, B) and high (C, D) gum concentrations.](image)

**Figure 5.7**: SEM images of gold and silver nanoparticles at low (A, B) and high (C, D) gum concentrations.
The EDX profile showed strong signals for Au atoms and weak signals for carbon, oxygen, nitrogen, magnesium, aluminum and potassium (Figure 5.8). The appearance of Al signal corresponds to the application of Al grid in the EDX analysis, while the signals for N, C and O corresponded to the presence of bio-organic molecules capping the nanoparticles. The signals for K and Mg were owing to the emission of X-ray from various biomolecules of the gum. The appearance of elemental Au and Ag in the EDX analysis supported the XRD results indicating the reduction of metal ions to elemental form.

![EDX spectra of (A) gold and (B) silver nanoparticles.](image)

**Figure 5.8:** EDX spectra of (A) gold and (B) silver nanoparticles.

5.4.1.4 Thermo-gravimetric analysis (TGA)
TGA study was performed to examine the thermal stability of gum and prepared Au- and Ag-NPs. Equal amounts (7.5 mg) of gum and nanoparticles were heated in alumina crucibles and the thermograms were recorded at 50-800°C (Figure 5.9). In this temperature range, three successive weight losses were observed for both the samples. The first weight loss might be owing to removal of entrapped water molecules from gum polymer. The subsequent weight loss might be due to thermal decomposition of the polymer and polymer stabilizing the nanoparticles. Whereas, the third weight loss might be owing to the thermal degradation of residual gum to carbon remains. The observed thermal decomposition scheme for the polymer was in good accordance with the previous reports [143]. The findings shows that gum stabilized Au- and Ag-NPs are thermally more stable than the gum alone and are in accordance with previous study [211].
Figure 5.9: TGA spectra of (A) gold and (B) silver nanoparticles.

5.4.1.5 Fourier Transforms Infrared Spectroscopy (FTIR)

FT-IR studies were brought about to know the various functional groups on the gum biopolymer which are particularly implicated in the biosynthesis of nanoparticles. Figure
5.10 illustrate the FTIR spectrum of acacia gum polymer and their biosynthesized Au- and Ag-NPs. The main absorption bands observed in the spectrum of gum polymer were at 3309, 2963, 2917, 1716, 1597, 1417, 1373, 1253, 1247, 1028 cm$^{-1}$, respectively. The broad band noted at 3309 cm$^{-1}$ might be attributed to particular stretching vibrations of O-H and different carbonyl groups of the gum. The bands at 2917 cm$^{-1}$ matched to symmetric and asymmetric stretching vibrations of methylene groups. The bands found at 1716 and 1597 and 1417 cm$^{-1}$ could be ascribed to distinctive symmetrical and asymmetrical stretches of amide and carboxylate groups. The peaks at 1373, 1253, 1247 and 1028 cm$^{-1}$ owing to C-O stretch of ether, alcoholic and carboxylic acids groups. After nanoparticles synthesis a great change in the absorption peak with reduced intensities were noted from 3309 cm$^{-1}$ to (3244 gold, 3296 cm$^{-1}$ silver) showing the attachment of gold and silver ions with carboxylate and hydroxyl groups of the polymer. The absorbance peak at 1716 cm$^{-1}$ completely disappeared for Au- and Ag-NPs. Similarly the absorbance peaks at 1597, 1417 cm$^{-1}$ also disappeared for gold and silver nanometals indicating the involvement of amide groups in the reduction process. From these changes it can be urged that carbonyl, hydroxyl and amide groups of the gum biopolymer are implicated in the reduction of Au (III) and Ag ions.
5.4.1.6 Atomic absorption spectroscopy

Atomic absorption study was carried out to confirm the amount of Au and Ag in pure HAuCl₄ and AgNO₃ solution and the resultant acacia gum stabilized nanoparticles. In 1M HAuCl₄ and AgNO₃ solution, the concentration of Au and Ag was 197 mg/ml and 107 mg/ml respectively while in the resultant Au- and Ag-NPs it was 172 and 101 mg/ml. No significant changes occurred in gold and silver concentration after nanoparticles synthesis, indicating almost complete reduction of Au (III) and Ag⁺ ions.
5.5 Stability studies of acacia gum stabilized gold and silver nanoparticles

5.5.1 Storage stability: The storage stability of Acacia gum stabilized nanoparticles was studied. Both colloidal solutions were stored at room temperature for about eight months, and no change in color and visual aggregation was observed during the entire storage time. From UV-Vis spectra a slight red shift was observed in the position of SPR peak and absorption values, indicating an increase in particles size after storage due to particles aggregation (Figure 5.11). The particles aggregation after storage was confirmed form SEM images (Figure 5.12).
Figure 5.11: UV-Vis spectra showing the effect of long term storage on the biosynthesized (A) gold and (B) silver nanoparticles.
5.5.2 Thermal stability: Synthesized Au- and Ag-NPs were also studied for their thermal stability. Colloidal solutions of Au- and Ag-NPs were heated at different temperatures for 30 minutes. Up to 80°C, no considerable variation was noted in the position of SPR peak and absorption values indicating that Au- and Ag-NPs are stable up to this temperature,
however at 100°C microscopic precipitates were observed in the colloidal solution of Au- and Ag-NPs, indicating particles aggregation at elevated temperatures. A slight red shift and broadening of peaks was observed in the UV-Vis spectra after heating (Figure 5.13). The change in shape and morphology after heating at 100°C was also confirmed from their SEM images (Figure 5.14). It was also observed that pure gum, gold and silver nitrate solutions did not show any type of absorption after heating at 100°C.
Figure 5.13: UV-Vis spectra showing the effect of high temperature (100°C) on the biosynthesized (A) gold and (B) silver nanoparticles.
Figure 5.14: SEM images of gold and silver nanoparticles before and after heating at 100°C.

5.5.3 Effect of sodium chloride

Colloidal solutions of acacia gum stabilized Au- and Ag-NPs were stable in 1M sodium chloride solution for about one week as no changes were observed in the position and
absorption values of SPR peak. After storing for one month in 1M salt solution microscopic precipitates were observed in the bottom of vials showing particles aggregation.

5.5.4 pH effect on the stability of gold and silver nanoparticles

The effect of varying pH on the stability of biosynthesized nanoparticles was studied. Optimized samples of both gold and silver nanoparticles were slightly acidic having a pH value between 6-7 and show maximum absorption in the UV-Vis spectra (Figure 5.15). It was noted that both Au- and Ag-NPs were stable in acidic medium, as there were less significant shifts in the UV-Vis absorption spectra. However, in basic medium, the nanoparticles were unstable as peak broadening were observed in their UV-Vis spectra. The UV-Vis absorption spectra of nanoparticles were significantly dependent on their sizes. It was reported that the aggregation of nanoparticles led to peak broadening in the absorption spectra which depends on the distance between nanoparticles, the density of the assembly and the size of the particles [145]. Similarly, in acidic and neutral pH, the colloidal solutions of Au- and Ag-NPs retained their characteristic ruby red and yellow color, however a deviation from this color was observed as the pH became alkaline (Figure 5.16). The extreme alkaline pH induced nanoparticles aggregation was confirmed from the SEM images (Figure 5.17). Thus it can be argued that at basic pH, nanoparticles exhibited aggregation, which might increased their particle size and therefore broadening of peaks was observed in their UV-Vis absorption spectra. pH plays a vital role not only in the synthesis of stable particles, but also in the stabilization of re-dispersed nanoparticles [146]. Solution pH affects the nanoparticles interfacial free energy, the hydroxylation or protonation of the ionic groups liberated by nanoparticles disbanding and dissociation equilibrium of a complexing agent [147].
Figure 5.15: UV-Vis spectra showing the effect of different pH on the biosynthesized (A) gold and (B) silver nanoparticles
Figure 6.16: Showing effect of different pH on the color of gum stabilized (A) gold (B) silver nanoparticles
Figure 5.17: SEM images showing the effect of acidic (2-3), neutral (6-7) and basic (12-13) pH on the biosynthesized gold and silver nanoparticles.

5.6 In vitro biological assays

5.6.1 Anticancer property of acacia stabilized gold and silver nanoparticles
Acacia gum stabilized nanoparticles were tested to evaluate their cytotoxic potential against human cervical cancer cells (HeLa). As revealed from Table 5.1, potential anticancer effect was afforded by acacia gum stabilized Ag-NPs having an IC50 value of (2.99± 0.21), followed by Au-NPs (4.72± 0.27) and acacia gum (9.05± 0.18). The standard anticancer drug cisplatin had the lowest IC50 value (1.89 ± 0.12) and therefore exhibited a strong inhibitory effect on HeLa cancer cells.

Table 5.1: Anticancer activity of acacia gum stabilized gold and silver nanoparticles

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia gum</td>
<td>9.05± 0.18</td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>4.72± 0.27</td>
</tr>
<tr>
<td>Silver nanoparticles</td>
<td>2.99± 0.21</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>1.89 ± 0.12</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of three separate experiments

5.6.2 Antimicrobial property of acacia stabilized gold and silver nanoparticles

The disk diffusion method was applied to assess the potential antibacterial assay of acacia gum stabilized Au- and Ag-NPs against the pathological stains of Gram positive and Gram negative bacteria. Prominent growth inhibition was noted in plates loaded with nanoparticles, while the negative control plates as well as the autoclaved gum were unable to produce any visible zone of inhibition. Potential antibacterial effect was observed with acacia loaded silver nanoparticles against Gram positive strains of S. aureus (18.3 ± 0.4) and Gram negative strain of E. coli (13.6 ± 0.4) and P. aeruginosa (12.1 ± 0.4). Similarly, these bacterial strains were also inhibited by acacia stabilized Au-NPs (10.3 ± 0.4, 9 ± 0.4
and 7.6 ± 0.6). The positive standard streptomycin also obsessed an antibacterial effect of higher magnitude as compared to acacia loaded Au- and Ag-NPs against the tested pathological stains (23.6 ± 0.8, 21.8 ± 0.2 and 18.6 ± 0.3) as shown in Table 5.2. A greater antimicrobial effect was observed in this study for Ag-NPs compared to that of Au-NPs.

**Table 5.2:** Antibacterial activity of acacia gum stabilized gold and silver nanoparticles (zone of inhibition in millimeter).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia gum</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>10.3 ± 0.4</td>
<td>9 ± 0.4</td>
<td>7.6 ± 0.6</td>
</tr>
<tr>
<td>Silver nanoparticles</td>
<td>18.3 ± 0.4</td>
<td>13.6 ± 0.4</td>
<td>12.1 ± 0.4</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>23.6 ± 0.8</td>
<td>21.8 ± 0.2</td>
<td>18.6 ± 0.3</td>
</tr>
<tr>
<td>DMSO</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of three separate experiments. NA = Not active

5.6.3 Urease inhibition study of Acacia gum stabilized gold and silver nanoparticles

Acacia gum stabilized Au- and Ag-NPs were tested in vitro for their potential effect as inhibitor of urease. The percent inhibition values revealed that both the gum and its nanoparticles were able to inhibit urease with better affinity. Increase inhibitory effect was
showed by the acacia gum (31.6 ± 1.31%) followed by acacia stabilized silver (29.1 ± 1.09%) and gold (12.5 ± 0.65%) nanoparticles. The positive standard inhibitor, thiourea showed a robust efficacy by producing a potent inhibition (78.3 ± 2.33%) of urease as shown in Table 5.3.

Table 5.3: Urease inhibition assay of acacia gum stabilized gold and silver nanoparticles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percent inhibition (1 mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia gum</td>
<td>31.6 ± 1.31</td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>12.5 ± 0.65</td>
</tr>
<tr>
<td>Silver nanoparticles</td>
<td>29.1 ± 1.09</td>
</tr>
<tr>
<td>Thiourea (1 mM)</td>
<td>78.3 ± 2.33</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of three separate experiments.
CHAPTER: 6

METAL NANOPARTICLES STABILIZED WITH KATIRA GUM (GOND KATIRA)
6.1 Introduction

Cochlospermum religiosum, is a flowering plant belongs to the family Bixaceae and is widely distributed in tropical regions of Indian Subcontinent, Pakistan and South East Asia. In Indian Subcontinent, it is frequently found in Maharashtra, Madhya Pradesh, Andhra Pradesh, Bihar, while in Pakistan it is found in south Punjab. Generally the tree is found in dry deciduous forests growing to a height of about 8 m. Other species of this genus are distributed throughout the tropics typically deciduous; xerophytes and most of them are ornamental, medicinal and produce timber, gum, floss and fiber. Seed cakes are used as cattle feed and manure. The dehydrated flowers and leaves are used as sedative, stimulants, antipyretic and laxative [212]. Powdered root is mixed with water and is used for reducing face wrinkles [213]. Bark Powder along with water is used for the treatment of jaundice [214].

Cochlospermum exudates a white yellowish transparent gum from its bark known as gum katira (gond katira). Chemically the gum consists of equimolecular proportion of D-galacturonic acid, L-rhamnose D-galactose and together with traces of a ketohexose. It has been reported that [1→2]-4-linked galacturonic acid is present in the linear chain of this polysaccharide with similar deposit of neutral sugars [215]. Low price, less toxic nature, bio-compatibility and stability as compared to tragacanth and acacia has led to so many novel applications for it in pharmaceuticals and industries. This gum has been exported in current years from India in rising quantity. Depending upon locality, the ideal season for collecting the gum is from October to June. In most of the American countries Katira gum (KG) is mainly used as an alternate of Tragacanth and gum Arabic. There are so many other applications of KG, such as in calico-printing, leather dressing and polishing paper.
Furthermore, it is applied for polishing tusser silk. In Latin America, it is imported for use in Ice cream and cigar industries. It is used as a laxative and is regarded to be better than other commonly used gums. It is an excellent alternate for tragacanth as an emulsifying agent. The gum is sweet, thermogenic and is applied in medicine for the treatment of diarrhea, dysentery, gonorrhoea, syphilis, pharyngitis, trachoma and cough [1]. The gum has been used externally in folk medicine for dressing burns, and it is now thought that it might have anti-tumor properties, and can excite the immune system.

Mustica is sold in Pakistan in crystal form for use in cooling drinks. It is also helpful in the treatment of constipation. It is used in makeup and book binding; the floss is used for stuffing pillows, life jackets, cushions and mattresses. About 20 g of the oral gum powder mixed with ghee works as an aphrodisiac (Savithramma).

Herein, we explicate the prospective of this gum for the biosynthesis of nanoparticles. The massive industrial and pharmaceutical applications of this gum motivated us to use this gum for the synthesis of nanoparticles and to study its applications at nano-scale. The
natural materials are better than synthetic ones as they are nontoxic, biodegradable, chemically inert, widely available and less expensive. They can compete with the available synthetic polymers as they can be modified through various methods for acquiring custom-made materials. The importance of biodegradable and biocompatible hydrophilic polymers has extensive uses in several fields such as chemical engineering, pharmaceuticals, polymer engineering, agriculture and food because of their propensity to combine with other materials [216].

Synthesized nanoparticles were analyzed with UV-Visible, IR, SEM, XRD, TGA and atomic absorption spectroscopy. The effects of different factors such as reaction temperature, time, gum and metal ions concentration was studied on the synthesis of gum capped Au- and Ag-NPs. Nanoparticles were then tested for anticancer (HeLa cervical cancer cells), antibacterial (Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa), urease inhibition (jack-bean urease) activities.

6.2 Experimental

6.2.1 Materials and method

Katira gum (KG), Tetrachloroauric acid trihydrate, silver nitrate and sodium hydroxide were used for the preparation of gold and silver nanoparticles. All chemicals of analytical grade were purchased from E. Merck (Germany). Fresh gum was purchased from the local market in March 2014. Metallic salts of tetrachloroauric acid trihydrate and silver nitrate were used as a source of Au (III) and Ag ions for the synthesis reactions. Adding of a calculated amount of sodium hydroxide open the glucose ring by abstracting the α-proton of the sugar ring oxygen and subsequently the metal ions oxidize glucose to gluconic acid
during the chemical reaction. In the synthesis reactions doubly distilled water was used as a medium in all chemical reactions and for further analysis.

For Au-NPs biosynthesis, 1mM stock solution of gold was prepared by dissolving 1g of Tetrachloroauric acid trihydrate (HAuCl$_4$·3H$_2$O) in 10 ml distilled water and then by further diluting 1 ml of it in 250 ml distilled water. Similarly, 1mM stock solution of silver nitrate was prepared by dissolving 17 mg of AgNO$_3$ in 100 ml of double distilled water. 0.5 % stock solution of KG was made by dissolving 5 mg of powder gum in approximately 100 ml distilled water. As the gum powder does not dissolve readily at room temperature, therefore the mixture was heated for complete dissolution. For removal of impurities, centrifugation was carried out at 6000 rpm for 20 minutes and the clear supernatant was used for all experiments. Nanoparticles were prepared by reducing the aqueous solution of Au (III) and Ag ions in the presence of gum solution. The effect of various parameters such as gum, metal ions concentration, reaction temperature and time on nanoparticles synthesis was studied.

### 6.2.2 Synthesis of KG stabilized gold and silver nanoparticles

Nanoparticles were prepared by using a classical method known as Turkevich Method. For nanoparticles biosynthesis, different volumes of gold and silver nitrate solutions (From their 1mM stock solutions) were mixed with desired volumes of gum solution (From 0.5% w/v stock solution) and stirred gently at different temperatures of (20, 40, 60, and 80°C). After stirring for 1h appearance of light red and light yellow colors in the reaction mixture showed the successful synthesis of Au- and Ag-NPs.
6.2.3 Optimization of synthesis reactions

For complete optimization of the chemical reaction different ratios of metallic salts and gum solutions were reacted by changing concentration of one (metal or gum), while keeping the other one constant and vice versa. First nanoparticles synthesis was observed by varying gum concentration, i.e. 1Au:1gum, 1Au:2gum, 1Au:3gum….1Au:10gum and then by changing metal Au (III) and Ag ions concentration i.e. 1gum:1Au, 1gum:2Au, 1gum:3Au….1gum:10Au. The mixtures were stirred for 4-5 h at different temperatures and UV-Vis spectra were monitored. The typical product having SPR at 555 nm for Au-NPs was obtained by stirring 1ml of gold solution (1mM) and 5 ml of (0.5% w/v) gum solution (1:5) at a temperature of 80°C and a reaction time of 4h, while in case of Ag-NPs typical product having SPR at 450 nm was obtained by stirring 1ml of gold solution (1 mM) and 4 ml of (0.5%) gum solution (1: 4) at a temperature of 80°C and a reaction time of 4h. Several colors were observed for the nanoparticles solutions and were due to the difference in sizes of the nanoparticles. The ratio of gum and metallic salt which gave excellent result in worth of possessing the highest absorption peak was chosen for additional analysis. Gold and silver nanoparticles were then prepared in bulk by using the above optimized ratio. Nanoparticles prepared in different ratios were different in sharpness of their peaks, and had different colors of the solutions. The nanoparticles were separated by freeze drying after completion of reaction and were used for further studies.
6.3 Proposed mechanism of reduction

For nanoparticles synthesis a bio-reduction mechanism has been proposed. Due to complex nature of these carbohydrates, it is believed that more than one mechanism is involved in the reduction and subsequent complexation of gold and silver ions by gum. We believe that the nanoparticles are produced on the surface of the polymer and not in solution; primarily the metal cations are trapped on the surface of polymer complex probably through electrostatic attractions among the negatively charged carboxylate and hydroxyl groups of the biopolymer and metal cations. During reaction the gum polymer expands and the functional groups on the biopolymer becomes more accessible to interact with Au (III) and Ag ions in the solution.

Metal nuclei are produced on hydrolysis of metal ions which grow subsequently and aggregate in the form of nanoparticles inside the gum matrix. Former chemical analyses show the abundance of hydroxyl, carboxylic and carbonyl functional groups in this polymer, and thus are involved in the complexation of metal cations during bio-reduction. Hydroxyl groups are first oxidized to carbonyl groups which subsequently reduce the gold and silver ions to elemental form. In addition to this intrinsic oxidation, the air present in the reaction mixture also oxidizes the available hydroxyl to carbonyl groups such as carboxylates and aldehydes. These strong reducing aldehyde groups beside the other available carbonyl groups in turn reduce excess of gold and silver ions to elemental form. Furthermore, the proteins along with polysaccharides present in the gum probably cape and stabilize these gold and silver nanoparticles.
6.4 Results and discussion

6.4.1 Characterization of synthesized gold and silver nanoparticles

6.4.1.1 UV–visible (UV–Vis) spectroscopy

The syntheses of the metal nanoparticles by reduction of metal ions in aqueous solutions were examined by using UV–Vis spectroscopy. Different types of nanoparticles are generally characterized by using light wavelengths in the 300–800 nm range [217]. From UV-visible absorption spectroscopy SPR spectra for Au- and Ag-NPs was obtained at 555 and 450 nm with win-red and light yellow color, respectively. The peak position was an indicative of the particles size and shape. UV–visible absorption spectra of the synthesized colloidal gold and silver were recorded against gum aqueous extract and pure gold solution. Figure 6.1 illustrates the absorption spectra of pure gold solution, silver nitrate solution and synthesized Au- and Ag-NPs colloidal solution. Pure gold and gum solutions had no absorption in UV-Vis region, but the resultant colloidal nanoparticles solution showed absorbance at 555 and 450 nm, indicating the synthesis of nanoparticles (Figure 6.1).
**Figure 6.1:** UV-Vis spectra of (A) gold and (B) silver nanoparticles. The inset photos show the corresponding color of the biosynthesized nanoparticles solutions.
6.4.1.2 Characterization through scanning electron microscope (SEM)

Scanning electron microscope was used to find out size and shapes of gum stabilized gold and silver nanoparticles. SEM images show that formed nanoparticles have different sizes and shapes. The irregular shapes of some particles might be due to aggregation (Figure 6.2). Formed nanoparticles were in the range of 5-30 and 7-35 nm from SEM analysis.

**Figure 6.2:** (A) SEM images of (A) gold and (B) silver nanoparticles.
The EDX study showed the presence of Au and Ag in the sample (Figure 6.3). Strong signals were observed from Au atoms in Au-NPs at approximately 0.6 and 2.6 keV, while a weak signal was observed at 9.7 keV. In the case of Ag-NPs, strong signals were observed at 0.6 and 2.6 keV, while a weak signal was observed at 3.7 keV. The appearance of Si signal was due to the use of silicon grid in the apparatus while the signal for N indicated the presence of nitrogen containing organic compounds in the gum. Moreover, the other strong signals for C and O were also due to the presence of bio-organic molecules capping the nanoparticles. The presence of chlorine atom in the EDX spectrum of gold nanoparticles was due to the presence of chlorine in tetrachloroauric acid trihydrate molecule. The signals for K and Mg were owing to emission of X-ray from various bio-molecules of the gum. The appearance of elemental Au and Ag in the EDX analysis supports the XRD results, which shows the reduction of metal cations to elemental form.
Figure 6.3: EDX spectra of (A) gold and (B) silver nanoparticles.

6.4.1.3 X-ray diffraction analysis

To identify the crystalline nature of the biosynthesized nanoparticles, XRD analysis was carried out. XRD analysis was conducted for KG and the respective nanoparticles. No absorption peaks were observed in the XRD spectrum of gum indicative of their non-crystalline nature. The XRD pattern of Au-NPs showed typical peaks at different angles (2
Ø) of 38.431, 44.623, 64.531, 77.781 that can be indexed to the (111), (200), (220), (311) Bragg’s reflections of face centered cubic (FCC) structure of metallic gold approximating to Joint Committee on Powder Diffraction Standards (JCPDS) file no: ICDD-PDF2, revealing that synthesized Au-NPs were of pure crystalline gold respectively. In case of silver containing sample, the typical diffraction peaks of FCC metallic silver phase (4-0784) at 38.21°, 44.39°, 64.62°, and 77.59° were noted. No absorption peaks similar to other crystalline phases were observed indicating high purity of the products without any impurity. The diffraction peak at 38° was the purely strong peak among the observed peaks for nanoparticles. The mean particle diameters of gold and silver nanoparticles was calculated from the XRD data which can be derived by Debye Scherrer equation \( D = \frac{k \lambda}{\beta \frac{1}{2} \cos \theta} \). This equation exploits the reference peak width at angle \( \theta \), where \( K \) is the shape factor, \( \beta \frac{1}{2} \) is the width of the XRD peak at half height and \( \lambda \) is the X-ray wavelength. Average particle size was around 18 and 22 nm for Au- and Ag-NPs, respectively.
Figure 6.4: XRD patterns of (A) gold and (B) silver nanoparticles

6.4.1.4 Thermogravimetric analysis
The thermal stability of the respective gum stabilized Au- and Ag-NPs and gum polymer was examined by thermo gravimetric analysis. For a comparative study, equal amounts of gum and their respective nanoparticles were heated in alumina crucibles and the profiles were recorded from 50–800°C, at a scan rate of 10°C/min, in inert nitrogen atmosphere. Figure 6.5 shows the thermogram of gum and the respective nanoparticles. Different weight losses were observed for gum and nanoparticles within the same temperature range. In the temperature range of 50–800°C, three subsequent weight losses were observed for gum and nanoparticles. The first observed weight loss was ascribed to removal of entrapped water from the gum biopolymer. The subsequent loss might due to the thermal degradation of the polymer and polymer capping the nanoparticles [142], whereas, the third weight loss might be owing to the alteration of residual gum to carbon scum. Thermal decomposition scheme for the biopolymer was in best agreement with previous reports [143]. After nanoparticle synthesis a little weight loss was noted for the gum as compared to that of before synthesis. These findings reveal that thermal stability of polymer increases after nanoparticles synthesis and is consistent with previous studies [211].
Figure 6.5: TGA spectrum of (A) gold and (B) silver nanoparticles.

6.4.1.1.5 Fourier transforms infrared spectroscopy (FTIR)
To recognize the capping agent on the surface of nanoparticles, the FT-IR spectra of pure gum and the respective nanoparticles stabilized with gum were compared (Figure 6.6). In the presence of nanoparticles, the major peaks shifted from 3352 cm\(^{-1}\) (O–H stretching), 2947 cm\(^{-1}\) (C–H stretching), 1734, 1633 cm\(^{-1}\) (C=O stretching), 1417, 1373, 1331 cm\(^{-1}\) (O–H deformation), and 1313–1018 cm\(^{-1}\) (C–OH stretching) to 3317, 2833, 1606, 1421, 1022 cm\(^{-1}\) for gold and 3306, 2931, 1595, 1114, 1040 cm\(^{-1}\) for silver nanoparticles respectively. All these changes indicate that –COOH and –OH groups are specifically associated with the Au- and Ag-NPs clusters.
Figure 6.6: FTIR spectra of (A) KG (B) gold (C) silver nanoparticles
6.5 Stability studies of KG stabilized gold and silver nanoparticles

6.5.1 Effect of pH on the stability of gold and silver nanoparticles

It was observed that KG stabilized Au- and Ag-NPs were stable over a broad range of pH. Formed Au-NPs were stable in neutral and acidic medium for a long time and could maintain the absorption capacity for several weeks. Although at high pH (basic medium) the absorption steadily declined and microscopic ppt were noted at pH 12-13. At high pH stability decreased and could also be seen from broadening of peaks in the UV-Vis spectra (Figure 6.7).
6.5.2 Effect of sodium chloride (NaCl)

Stability of the resultant Au- and Ag-NPs was studied by using different concentrations of sodium chloride solution, and it was observed that resultant gum stabilized gold and silver nanoparticles remained stable in salt solution for a long time.
6.5.3 Storage and thermal stability

The storage stability of gum stabilized nanoparticles was studied. Synthesized nanoparticles were stored at room temperature for about six months, and no change in color and microscopic ppt was observed during the intact storage time, signifying that the gum furnished an outstanding defending environment for the metal hydrosols throughout their development process. From UV-Vis spectra, no important alteration was noted in the position of SPR peak and absorption values. A little particles aggregation was observed after storing for one year and was confirmed from red shift and decreased in absorption values in the UV-Visible spectra. Synthesized nanoparticles were also studied for their thermal stability. Formed nanoparticles were stable up to boiling temperature. Colloidal solutions of Au- and Ag-NPs were heated at various temperatures (40, 60, 80 and 100°C) for 30 min and the stability was tested by noting the growth of ppt as seen from naked eye and from the absorption spectra. No major shift was noted in the position of SPR peak and absorption values up to 80°C. Above 80°C the Ag-NPs started coagulation and formed precipitates which showed instability of Ag-NPs at higher temperature.

6.6 In vitro biological assays

6.6.1 Cytotoxicity assay of KG stabilized gold and silver nanoparticles

KG stabilized nanoparticles were tested to evaluate their cytotoxic potential against human cervical cancer cells (HeLa). Potential anticancer effect was afforded by KG capped Ag-NPs having an IC50 value of 2.73± 0.09, followed by KG loaded Au-NPs 3.13± 0.14, and KG 8.93± 0.19. The standard anticancer drug cisplatin had the lowest IC50 value (1.89 ± 0.12) and therefore demonstrated a strong inhibitory effect on HeLa cancer cells.

**Table 6.1:** Anticancer activity of KG loaded gold and silver nanoparticles
### Values are expressed as mean ± SEM of three separate experiments

#### 6.6.2 Urease inhibition assay

KG capped Au- and Ag-NPs were tested in vitro for their potential effect as inhibitor of urease. The percent inhibition values revealed that both the gum and its nanoparticles were able to inhibit urease with enhanced affinity. Increased inhibitory effect was demonstrated by the KG (22.6 ± 0.82) followed by KG loaded silver (23.6 ± 0.87) and gold (7.29 ± 0.47) nanoparticles. The positive standard inhibitor, thiourea showed a strong efficiency by producing a potent inhibition (78.3 ± 2.33\%) of urease (Table 6.2).

#### Table 6.2: Urease inhibition assay of KG loaded gold and silver nanoparticles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percent inhibition (1 mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KG</td>
<td>22.6 ± 0.82</td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>7.29 ± 0.47</td>
</tr>
<tr>
<td>Silver nanoparticles</td>
<td>23.6 ± 0.87</td>
</tr>
<tr>
<td>Thiourea (1 mM)</td>
<td>78.3 ± 2.33</td>
</tr>
</tbody>
</table>

#### 6.6.3 Antibacterial study of KG stabilized gold and silver nanoparticles

The disk diffusion method was used to assess the potential antibacterial assay of KG loaded nanoparticles against the pathological stains of Gram positive and Gram negative
bacteria. Prominent growth inhibition was noted in plates loaded with nanoparticles, whereas the negative control plates as well as the autoclaved gum were unable to produce any visible zone of inhibition. Potential antibacterial effect was observed with KG loaded Au-NPs against Gram positive strains of S. aureus (19.7 ± 0.4), and Gram negative strain of E. coli (14.4 ± 0.7) and P. aeruginosa (13.1 ± 0.2). Similarly, these bacterial strains were also inhibited by KG loaded Au-NPs, but with low affinity (10.5 ± 0.6, 10 ± 0.4 and 8.2 ± 0.3). The positive standard streptomycin also possessed an antibacterial effect of higher magnitude as compared to KG loaded Au- and Ag-NPs against the tested pathological stains (23.6 ± 0.8, 21.8 ± 0.2 and 18.6 ± 0.3). **Table 6.3**: Antibacterial activity of KG stabilized gold and silver nanoparticles (zone of inhibition in millimeter)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>KG</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>10.5 ± 0.6</td>
<td>10 ± 0.4</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>Silver nanoparticles</td>
<td>19.7 ± 0.4</td>
<td>14.4 ± 0.7</td>
<td>13.1 ± 0.2</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>23.6 ± 0.8</td>
<td>21.8 ± 0.2</td>
<td>18.6 ± 0.3</td>
</tr>
<tr>
<td>DMSO</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of three separate experiments. NA = Not active
CHAPTER: 7

METAL NANOPARTICLES STABILIZED WITH XANTHAN GUM
7.1 Introduction

Xanthan gum (XG) was discovered by USDA scientists for the first time in 1950. The gum is obtained from the fermentation of corn sugar with a bacterium ‘Xanthomonas campestris. It’s the same bacteria that create black spots on broccoli and cauliflower. Like yeast and vinegar it is also a very important natural material, and is one of the most important discoveries in food science after yeast.

Chemically XG is high molecular weight, exo-polysaccharide anionic gum, produced by aerobic fermentation of sugars. The primary chain is made up of a $\beta$-(1 $\rightarrow$ 4) linked glucose backbone with the substitution of a charged tri-saccharide side-chains of $[\beta$-(1 $\rightarrow$ 3)-mannose-$\alpha$-(1 $\rightarrow$ 2)-glucuronic acid-$\beta$-(1 $\rightarrow$ 4)-mannose] on alternate glucose residue [218]. It is cheap, eco-friendly, biodegradable and hydrophilic gum, and is frequently used in pharmaceutical, ornamental and food manufacturing. The gum is soluble in both cold and hot water [219]. The gum has been used for formulation of both liquid and solid dosage forms. In liquid formulations, it is applied as suspending agent, emulsion stabilizer and thickening agent [220], whereas in solid form it is applied as controlled release agent [221]. The gum hydrates quickly and at very low concentration produces high viscosity. At very low concentrations, it can thicken liquids as small as 0.1% by weight, and 0.5% by weight can produce a thick paste. Xanthan gum is additionally one of the major valuable food additive, and is used in a wide range of viscosities, pH and temperature levels. It has no taste, can be easily used and usually works very well.

In the present study we used this gum for the biosynthesis of environment friendly gold and silver nanoparticles. The importance of the current work is due to the application of a
non-toxic biopolymer instead of using toxic and non bio-degradable materials that produces hostile effects on the environment. The effect of gum and metal ions concentration, reaction temperature and time on the synthetic stability of nanoparticles was studied along with their post-synthetic stability against varying pH and salt concentrations, long term storage and extreme of temperature. UV–Vis spectroscopy, XRD, SEM, FTIR, atomic absorption and EDX were used for complete structural analysis of nanoparticles. Biosynthesized Au- and Ag-NPs were also evaluated for their antibacterial activity.

7.2 Experimental

7.2.1 Materials and method

Xanthan gum, Tetrachloroauric acid trihydrate and silver nitrate were used for the biosynthesis of gold and silver nanoparticles. Tetrachloroauric acid trihydrate (HAuCl₄.3H₂O) and silver nitrate (AgNO₃) of analytical grade were purchased from E. Merck (Germany). Xanthan gum was purchased from Zenta pharmaceutical industry Hayatabad, Peshawar, Pakistan. Tetrachloroauric acid trihydrate and silver nitrate were used as precursors for the preparation of nanoparticles. Doubly distilled water was used throughout the experiments for the synthesis and analysis of nanoparticles.

For synthesizing Au-NPs, 1mM stock solution of gold, 1g of gold salt was dissolved in 10 ml distilled water and by further diluting 1 ml of it in 250 ml distilled water. Similarly for 1mM stock solution of silver nitrate, 17 mg of silver salt was dissolved in 100 ml of distilled water. 0.5% w/v stock solution of XG was prepared by dissolving 5 mg of powder gum in approximately 100 ml distilled water. The gum powder was heated for complete dissolution, as it does not dissolve readily at room temperature. For removal of impurities the gum solution was first filtered and then centrifuged for 30 min and the clear supernatant
was used for all experiments. Nanoparticles were prepared by reducing the aqueous solution of metal cations in the presence of gum solution. The effect of different factors such as pH, reaction temperature, time, gum and metal ions concentration on nanoparticles synthesis and morphology was studied.

7.2.2 Synthesis of gold and silver nanoparticles stabilized with Xanthan gum

The previously described method (Turkevich method) was employ for the synthesis of XG stabilized nanoparticles. For the synthesis of Au- and Ag-NPs different volumes of gold and silver nitrate solutions (From their 1mM stock solutions) were mixed with desired volumes of gum solution, (From 0.5% w/v stock solution) and stirred gently at different temperatures of (20, 40, 60, and 80°C). After stirring for 1h appearance of light red and light yellow color in the reaction mixture indicates the successful synthesis of nanoparticles in the reaction mixture.

Graphical representation of XG stabilized gold and silver nanoparticles
7.2.3 Optimization of synthesis reactions

For optimization a lot of reactions were carried out by varying concentration of one specie, (metal or gum) while keeping the other one constant and vice versa. First nanoparticles synthesis was observed by varying ligand (XG) concentration i.e 1Au:1gum, 1Au:2gum, 1Au:3gum….1Au: 10gum and then by changing metal Au (III) and Ag ions concentration i.e. 1gum:1Au, 1gum:2Au, 1gum:3Au…. 1gum:10Au. UV-Visible absorption spectra were recorded after stirring the mixture for 4 h at different temperatures. Nanoparticles synthesis was observed at all ratios, but the typical product having SPR at 555 nm for Au-NPs was obtained by stirring 1ml of gold solution (1mM) and 3 ml of (0.5% w/v) gum solution (1:3) at a temperature of 80°C and a reaction time of 5 h. While in case of Ag-NPs typical product
having SPR at 450 nm was obtained by stirring 1 ml (1 mM) of gold solution and 6 ml of (0.5%) gum solution (1: 6) at a temperature of 80°C and a reaction time of 5 h. Due to difference in the size of resultant nanoparticles different colors were observed. The gum and metallic salt ratio that furnished excellent result in reverence of having the sharpest peak was chosen for further studies. Nanoparticles were then synthesized in bulk using the above optimized ratio. Nanoparticles prepared in different ratios were different in sharpness of their peaks and had different colors of their solutions. After completion of the chemical reactions, the nanoparticles were separated by freeze drying which were used for further studies.

7.3 Proposed mechanism of reduction

We proposed a tentative representation to demonstrate the binding of the Au- and Ag-NPs to helical and dispersion in XG solution on the basis of above mentioned results. Although there continued to exist some disagreements, the ordered structure is recognized as a 5-fold helix and exhibited an ordered rigid structure during the cooling state, whereas subsist in a chaotic coil shape when denaturation occurs at the temperature above 60°C. After heating, the formation of helica gum is frequently detached and the molecular chain is exposed. The outer hydroxyl groups present on the gum polymer exert a reduction effect during nanoparticles synthesis. In the first step the hydrogen of the hydroxyl group is replaced by the metal cations by an ion exchange process and then these Au (III) and Ag ions bonded to COOH groups are converted into Au- and Ag-NPs in an in situ manner and are consequently stabilized by the gum polymer chains. After denaturation, the outer oxhydrolys of gum provide reduction capability for nanoparticles synthesis. Synthesized Au- and Ag-NPs were prohibited from aggregation and showed better dispersion due to attachment to
XG chain through the high electronegativity. However the distance between Au- and Ag-NPs decreases after storage during the gum renaturation owing to inter and intra molecular hydrogen bonding. The helical structure of XG was found difficult to reform as previous one due to the Au- and Ag-NPs steric hindrance (Figure 7.2). Au- and Ag-NPs were found to bind to the chain surface and entrap in the hydrophobic cavity of the folded helix. As previously reported, the Au- and Ag-NPs did not cluster in the dense arrangement and the size almost remain stable [222]. The fascinating event suggests that the outer OH groups are involved in reduction and stabilization of these biosynthesized nanoparticles.

Figure 7.2: Showing the reduction and stabilization of gold and silver nanoparticles.

7.4 Results and discussion

7.4.1 Characterization

7.4.1.1 UV–visible (UV–Vis) spectroscopy

From UV-visible absorption spectroscopy surface Plasmon resonance spectra for Au- and Ag-NPs were observed at approximately 550 and 445 nm with win-red and light yellow color, respectively. The size and shape of nanoparticles could be guess from the peak position of the UV-Vis absorption spectra (Figure 7.3).
Figure 7.3: UV-Vis spectra of XG stabilized (A) gold and (B) silver nanoparticles. The inset photos show the corresponding color of the biosynthesized nanoparticles solutions.

7.4.1.2 Characterization through Scanning Electron Microscope (SEM)
Scanning electron microscopy is generally used for structural characterization at the nanometer to micrometer scale [223]. The size, morphology and shapes of nanoparticles in
aqueous solutions were observed with scanning electron microscope. SEM images showed that formed Au- and Ag-NPs had different sizes and shapes depending upon concentration of gum and metal ions. Nanoparticles were mostly spherical in shapes, but some irregular shaped nanoparticles were also present which might be owing to the accretion of the smaller ones. Formed nanoparticles were in the range of 5-30 and 7-35 nm from SEM analysis (Figure 7.4).

Figure 7.4: SEM images of XG stabilized (A) gold and (B) silver nanoparticles

7.4.1.3 EDX analysis of XG stabilized nanoparticles
The EDX profile showed strong signals for Au and Ag atoms and weak signals for nitrogen, oxygen, carbon, magnesium, silicon and potassium. The appearance of silicon signal corresponds to the use of silicon grid in the EDX analysis, while the signals for N, C and O correspond to the presence of bio-organic molecules capping the Au- and Ag-NPs (Figure 7.5). The signals for K and Mg were owing to the emission of X-ray from different bio-molecules of the gum. The appearance of elemental gold and silver in the EDX analysis supports the XRD results, which showed the reduction of gold and silver ions to elemental form.
Figure 7.5: EDX spectra of XG stabilized (A) gold and (B) silver nanoparticles.

7.4.1.4 X-ray diffraction analysis of XG stabilized nanoparticles

The nature of nanoparticles formed in this approach was evaluated with XRD analysis. Figure 7.6 shows the XRD profile of the synthesized gum stabilized Au- and Ag-NPs. The XRD peak positions are consistent with the metallic silver. The peaks at 35 degree or double peak at 45 degree also depicts the presence of silver oxide in addition to silver and this might be due to air oxidation of silver ions during Ag-NPs synthesis as the reactions were carried out in open atmosphere. The XRD pattern of Ag-NPs exhibited characteristic peaks at scattering angles (2 Ø) of 38.431, 44.623, 64.531, 77.781 that can be indexed to the (111), (200), (220), (311) Bragg’s reflections of face centered cubic (FCC) structure of metallic silver similar to the Joint Committee on Powder Diffraction Standards (JCPDS) file no: ICDD-PDF2, revealing that the biosynthesized Ag-NPs were of pure crystalline silver. In the case of gold containing sample, the diffraction peaks of FCC metallic gold phase (4-0784) at 38.21°, 44.39°, 64.62°, and 77.59° were noted. No peaks similar to other crystalline phases were observed indicating high purity of the products. The mean particle diameters of Au- and Ag-NPs was find out from the XRD data, which can be derived from the Debye Scherrer equation (D =k λ/ β½ cosθ). This equation exploits the reference peak width at angle θ, where K is the shape factor, β½ is the width of the XRD peak at half height and λ is the X-ray wavelength. Average particle size was around 22 and 20 nm for gold and silver nanoparticles respectively.
Figure 7.6: XRD patterns of XG stabilized (A) gold and (B) silver nanoparticles.
7.4.1.5 Thermo-gravimetric analysis (TGA)

TGA studies were carried out to study the thermal stability of XG and prepared Au- and Ag-NPs. Equal amounts of gum and nanoparticles were heated in alumina crucibles and the thermograms were recorded at 50-900°C (Figure 7.7). Within this temperature range, three successive weight losses were observed for both the samples. The first weight loss could be owing to the loss of entrapped water molecules from the gum polymer. The second weight loss might be due to thermal decomposition of the polymer and polymer stabilizing the nanoparticles, whereas the third weight loss might be due to the thermal degradation of left over polymer to carbon remains. The observed decomposition scheme for the polymer is in best agreement with the previous studies [143]. These findings showed that gum stabilized Au- and Ag-NPs are thermally more stable than the gum alone and is in accordance with previous reports [211].
**Figure 7.7:** TGA spectra of XG stabilized (A) gold and (B) silver nanoparticles.

### 7.4.1.6 FTIR analysis of XG stabilized gold and silver nanoparticles

FTIR studies were carried out to determine the various functional groups that are exclusively involved in the biosynthesis of Au- and Ag-NPs. Figure 7.8 illustrates the FTIR spectrum of gum before and after nanoparticles synthesis. The main absorption bands observed in the gum spectrum were at ~3313, 2901, 1606, 1409, 1325, 1255, 1020 cm$^{-1}$, while the spectra of Au-NPs showed characteristic absorption bands at ~3309, 1597, 1313, 1311, 1247, 1018 cm$^{-1}$. Similarly, spectra of Ag-NPs showed characteristic absorption bands at ~3306, ~2931, 1595, 1114, 1024 cm$^{-1}$. The broad band appeared at 3313 cm$^{-1}$ might be ascribed to stretching vibration of O–H groups. The bands at 2901 cm$^{-1}$ correspond to C–H and C-OH stretching. The peak at 1606 cm$^{-1}$ arises from the carbonyl stretching vibrations. The band present at 1409-1020 cm$^{-1}$ can be attributed to the symmetrical stretching of carboxylate group. After nanoparticles synthesis, a change in the absorption peak was noted from 3313 to 3309 cm$^{-1}$ (Au-NPs) and 3306 cm$^{-1}$ (Ag-NPs) with reduced band intensity, signifying the attachment of Au and Ag ions with hydroxyl groups of gum. The absorption band at 2901 cm$^{-1}$ disappeared for Au-NPs. Similarly, absorption peaks at 1606 was shifted to 1597 for gold and 1595 for silver nanoparticles. Significant changes were noted in the absorption peaks of hydroxyl and carbonyl groups and thus indicating their involvement in the reduction and stabilization process. The huge number of hydroxyl groups along with the available glucuronic acid groups of the gum complex the metal cations.
7.5 Stability study of XG stabilized gold and silver nanoparticles

7.5.1 Effect of long term storage, temperature and sodium chloride

Xanthan stabilized Au- and Ag-NPs were stored at room temperature for about six months and no change in color and visual aggregation was observed during the entire storage time. UV-Vis spectra were recorded after storage and no major change was noted in the position of SPR peak and absorption values. Thermal stability was determined by heating the nanoparticles solution at different temperatures. Nanoparticles were stable up to boiling temperature and no microscopic precipitates were observed in the colloidal solutions after heating. Formed nanoparticles were also stable in different salt concentrations for a long time.
7.5.2 pH effect on XG stabilized gold and silver nanoparticles

Optimized samples of both gold and silver nanoparticles were slightly acidic having a pH value of 6-7, and showed maximum absorption in the UV-Vis spectra (Figure 7.11). It was noted that nanoparticles were stable in acidic medium as there were less significant shifts in the UV-Vis absorption spectra. However, in basic medium, the nanoparticles were unstable as peak broadening was observed in their UV-Vis spectra.
CHAPTER 7 METAL NANOPARTICLES STABILIZED WITH XANTHAN GUM

Figure 7.9: UV-Vis spectra showing the effect of different pH on XG stabilized (A) gold and (B) silver nanoparticles

7.6 Antibacterial assay

All reagents, media and glassware used were sterilized in an autoclave at 121°C for 20 min. The nanoparticles used in the experiments were synthesized with 0.1% gum solution
having 1mM chloroauric acid and silver nitrate solutions heated for 1h. For determining the antibacterial activity, gold and silver nanoparticles with a size of (5-20) and (5-30) nm were used. Three separate experiments were conducted for every bacterial strain with streptomycin as standard. 5µg of both dried nanoparticles samples were dissolved in DMSO and incubated at 30°C for 24 h. Growth suppression was noted in plates loaded with XG stabilized Au- and Ag-NPs, while the negative control plates with gum did not create any zone of inhibition. The positive control plates loaded with streptomycin antibiotic discs showed greater ZOI as expected. XG stabilized Ag-NPs exhibited excellent activity against the selected bacterial strains compared to XG stabilized Au-NPs. For Au-NPs zones of inhibition measuring 9.2 mm to 10.5 mm were recorded, while their silver counterparts recorded zones ranging between 11.2 mm and 18.5 mm. Gram negative strains E. coli and P. aeruginosa recorded less ZOI compared to gram positive S. aureus, and is consistent with earlier studies [151].

Table.7.1 Mean inhibition zones (mm) of synthesized nanoparticles

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Au NPs</th>
<th>Ag NPs</th>
<th>Positive control</th>
<th>XG</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus ATCC 25923</td>
<td>10.5 ± 0.3</td>
<td>18.5 ± 0.5</td>
<td>22.5 ± 0.5</td>
<td>NA</td>
</tr>
<tr>
<td>E. coli ATCC 25922</td>
<td>9 ± 0.5</td>
<td>11.5 ± 0.8</td>
<td>20.2 ± 0.3</td>
<td>NA</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27853</td>
<td>8.2 ± 0.3</td>
<td>11.2 ± 0.3</td>
<td>15 ± 0.5</td>
<td>NA</td>
</tr>
</tbody>
</table>
Conclusion

Gold and silver nanoparticles with different sizes were synthesized using selected gums as reducing and stabilizing agents. Formation of Au- and Ag-NPs was confirmed from the surface Plasmon resonance centered at 500-600 and 400-500 nm respectively. Synthesized gold and silver nanoparticles were mostly spherical with an average particle size between 7 and 30 nm (Au-NPs) and 5–30 nm (Ag-NPs) respectively. Au/Ag-NPs maintained their colloidal stability and nanoscale characteristics against variations in physicochemical factors, such as pH variation, Long term storage and extreme of temperature. Gold and silver nanoparticles stabilized with different gums possessed mild to moderate antibacterial activity as observed from their zone of inhibition against Staphylococcus aureus (10 ± 0.3 mm, 18 ± 0.5 mm), Escherichia coli (9 ± 0.5 mm, 10.2 ± 0.8 mm) and Pseudomonas aeruginosa (7.9 ± 0.3 mm, 11.2 ± 0.3 mm). Gold nanoparticles significantly alleviated the acetic acid induced writhes at much lower doses of 40 mg/kg (P < 0.01) and 80 mg/kg (P < 0.001) compared to that of gum extract at 200 and 400 mg/kg (P < 0.001). Gold nanoparticles also significantly inhibited the carrageenan induced paw edema during the 1st h (P < 0.05) and 2–5 h (P < 0.001) of the study duration. Gold and silver nanoparticles showed potential inhibitory susceptibility towards human cervical cancer cells (HeLa), and the anticancer result was analogous to that of the standard cisplatin. Gold and silver nanoparticles showed preferential inhibition against jack-bean urease. Gum stabilized silver nanoparticles can sense heavy metals in water up to mM level.