



Metallic Nanoparticulate Systems: Emphasis on Properties & Methodologies

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ABSTRACT

Colloidal metallic nanoparticles hold enormous interest and research potential in the areas of material sciences, biocatalysts, diagnostic & therapeutics. Researchers are interested in the nanoscale systems because at this scale the properties of materials are vary different from those at larger scale. At the nanometer regime, materials demonstrate unique optical, electronic and magnetic properties which are remarkable but not observed in their bulk counterparts which result into a wide range of applications. Metal nanoparticles (MNPs) can be synthesized by various methods. Choice of appropriate synthetic method and processing conditions is mainly based on the criteria of size, shape, surface functionalities, various properties and applications of metallic nanoparticles. The current review mainly focuses on properties and methodologies of metal nanoparticles.

Keywords: Metal Nanoparticles, Properties, Methods, Nanoparticles

1. INTRODUCTION

Now a days "Nanoscience" has been explored as a popular terminology reorientating the global arena of science and technology at the nanoscale level. It encompasses study of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale. It signifies as an interdisciplinary field of science, where the concepts derived from physics, chemistry, biology and engineering are applied to synthesize nanomaterials for numerous applications. Nanotechnology, more aptly defined as the design, production, characterization and application of structures, devices and systems by controlling their shape and size at the nanometer scale. At nanoscale dimensions, inorganic and organic components exhibit unique physicochemical properties as compared to their bulk counterparts. The instigated research and development at nano level have major direct and indirect impact on the lifestyle of human beings due to their wide array of applications in daily life [1, 2].

Nanoscience deals with the smallest possible particle, retaining size on the nanometer scale conventionally defined as 1 to 100 nm. The "Nano" is meaning "extremely small" derived from the Greek word "dwarf". When quantifiable, one nanometer is equal to one-billionth ($1 \text{ nm} = 10^{-9} \text{ m}$) equivalent to the width of 6 carbon atoms or 10 water molecules [1].

Researchers are interested in the nanoscale systems because at this scale the properties of materials are very different from those at larger scale. Nanometer scale is a size range of matter where transition of properties takes place from atomic level to bulk level. On downsizing materials to nanoscale, the small size of nanomaterials exhibits special properties like high surface area which enables more functionality with improved performance in the given space, high surface area to volume ratio and elevated surface charge when compared with bulk counterparts [3]. Thus, they exhibit distinctive features which open up new avenues and applications in a wide range of different sectors, from medicine to packaging and from sensors to bioremediation.

1.1 Metal Nanoparticles

Colloidal metallic nanoparticles hold enormous interest and research potential in the areas of material sciences, biocatalysts, diagnostics and therapeutics. The ancient civilization also recognized metallic particles in the form of bhasmas as indigenous part of the traditional system of medicine for curative purposes [4]. During the 17th century gold (Au) was widely utilized in glass painting; dark-bright shades of yellow, red, or brown were generated based on the concentration of Au. The famous Lycurgus cup, "Imperial" Fabergé eggs, decorative pigments, colourful church windows, multihued palace structures were decorated using varied amount of Au colloids [5]. Lycurgus cup can be still seen at British museum and possesses the unique feature of changing colour depending upon the light in which it is observed. It appears opaque green when viewed in reflected light, but looks translucent red when a light is shine from inside and transmitted through the glass. Analysis of glass depicts that it contains a very small amount of tiny (~ 70 nm) colloids of gold. It is presence of these nanocolloidal structures that gives the Lycurgus cup its colour display. However, the prediction that the nanoparticles formed in the range 1-10 nm would have electronic properties intermediate between those of the bulk metal and small molecules, offering exciting possibilities for novel devices or materials with promising applications. Design of experimental protocols for the synthesis of metal nanoparticles has been an important

aspect of research activities towards fabrication of miniaturized sensors, electronic components, spectroscopic enhancers, functional nanostructures and micro imaging probes [6].

In this regard, aqueous and other organic solution-phase synthetic routes have facilitated the fabrication of variety of functional inorganic quantum dots or nanoparticles [7, 8]. These nanoscale functional building blocks are expected to be useful for the bottom-up approaches to materials assembly and have therefore received consideration due to their intrinsic size dependent characteristics and subsequent relevance potentials [9].

In 1857, Michael Faraday, an English chemist and physicist discovered the synthesis of deep red coloured solution of gold colloids by the reduction of aqueous chloroaurate ions (AuCl_4^-) using phosphorus in CS_2 [10]. He reported the optical properties of thin films prepared from dried colloidal solutions and observed reversible colour changes of the films upon mechanical compression (from bluish-purple to green upon pressurizing) resembling the colour of the wine-red solutions of the particles. This probably was the first rationalized report on the purposeful synthesis of colloidal gold nanoparticles. Soon thereafter, the term “colloid” was coined by Thomas Graham (1861) for suspended particles in liquid medium [11]. During recent developments in this field, the term “colloid” has been replaced by “nanoparticles” to describe particles with size range from 1 to 100 nm [12]. The famous quote of Louis Pasteur “The role of infinitely small is infinitely large” well suits the current trends of nanotechnology research. In the 19th century, a great deal of theoretical and experimental research was conducted on many colloidal metals. In the 21st century, the number of potential applications of these colloidal particles is growing rapidly because of unique structure of nanosized metal particles and their extremely large surface areas [13].

In modern medicine, the initial utilization of gold (Au) was reported by Robert Koch (1890) for treating tuberculosis infection [14]. Commercial Au based compounds like RidauroTM, MyochrysineTM and SolgonalTM are still used today in various therapeutic applications [15]. Silver (Ag), another precious noble metal, was originally used as an effective antimicrobial agent and disinfectant for years together. Hippocrates claimed Ag in treatment of multiple maladies [14]. Earlier Phoenicians used Ag vessels for food and water storage in order to prevent spoilage. Before evolution of antibiotics, Ag compounds were established for the treatment of various diseases. In the early 20th century, nanosilver colloids like CollargolTM, ArgyrolTM, ProtargolTM were prescribed in the treatment of numerous acute and chronic infections [16]. Since 1970s, various Ag impregnated water filters were explored for safe domestic water applications while Ag nitrate amalgamated sticks were marketed for dental treatment [17].

1.2 Properties of metal nanoparticles

At the nanometer regime, materials demonstrate unique optical, electronic and magnetic properties which are remarkable but not observed in their bulk counterparts which result into a wide range of applications.

1.2.1 Optical properties

Metal nanoparticles exhibit distinctive optical properties which rely on composition, size, shape and surrounding medium of the nanoparticles from their bulk form [18-20]. Au, Ag, and copper (Cu) nanoparticles are known to display unique optical characteristics in visible and in NIR region within certain size limit of particles [19]. These effects are attributed to the interaction of electromagnetic radiation with the electron cloud present on the surface of metal nanoparticles.

1.2.2 Surface plasmon resonance (SPR)

Metallic nanoparticles of Au and Ag attain brilliant colours as a outcome of the surface plasmon resonance (SPR) band. Free electrons of the metal nanoparticle undergo a collective coherent oscillation in the presence of electromagnetic field of light. This oscillation is termed as SPR [5] and has been established by Mie in 1908, based on the Maxwell's equation of scattering [21]. Au, Ag and Cu nanoparticles represents the SPR in the visible spectrum, and are frequently used for various applications due to higher stability as compared with other metallic nanoparticles. Applications based on optical properties in the visible region have been receiving considerable scientific interest as a result of its promising technological development. Since the optical properties of metal nanoparticles are sensitive to surface modifications and environment around it. On these guidelines, DNA tagged gold nanoparticles were studied extensively for DNA base pair sequence recognition and genetic disorder studies [22].

1.2.3 Electronic properties

Metal nanoparticles lower than certain size range demonstrates remarkable electronic properties. Such as, gold nanoparticles function as quantum dot below certain size range [23]. Coulomb blockade is major consequence that occurs when nanoparticles embedded between metal- insulator metal junctions illustrate charging of differential capacitance or charging at low temperature even at zero bias. The reaction is an outcome due to extremely small capacitance of the metal nanoparticles [24].

1.2.4 Melting point

Metallic nanoparticles below 100 nm of size have low melting point as compared to its bulk form. Number of surface atoms increases with decrease in the size of nanoparticles. Since it decreases the co-ordination number of atoms, atoms on the surface can be easily rearranged than those atoms present inside the nanoparticles, thus melting process starts at lower temperature leading to decrease in melting point [25].

1.2.5 Mechanical properties

Mechanical strength of the material relies on a range of parameters viz. impurities, dislocations etc. Less mechanical strength is due to more defects in materials. Mechanical strength found to be enhanced in nano materials such as nano wires and nano rods due to small cross sections and less number of imperfections. Nanomaterials attain better mechanical strength as they eliminate imperfections in the crystal which are thermodynamically more energetic [26].

1.2.6 Scattering property of metal nanoparticles

Metallic nanoparticles have the distinguishing properties to resonantly scatter visible and near-infrared light upon the excitation of surface plasmon oscillation. The scattering light intensity is highly sensitive to the size and aggregation phase of the nanoparticles [27]. Green light is scattered by nanoparticles with a diameter of 58 nm and yellow light is scattered by particles diameter of 78 nm while red light under illumination of a beam of white light scattered by gold nanorods. [28].

Preliminary studies have described their application as contrast agents for biomedical imaging using confocal scanning optical microscopy. Sokolov et al., reported the scattering of anti-EGFR/Au nanoparticles for cervical cancer when stimulated with a laser at single wavelength [29]. Gold nanoparticle AuNPs) have many advantages for cellular imaging as compared to other agents. Gold nanoparticles scatter light intensely and are much brighter than chemical fluorophores. They do not photo bleach and they can be easily detected in as low as 10^{-16} M concentration [30].

1.3 Methods for synthesis of metal nanoparticles

Metal nanoparticles (MNPs) can be synthesized by various methods. Choice of appropriate synthetic method and processing conditions is mainly based on the criteria of size, shape, surface functionalities, various properties and applications of metallic nanoparticles. Commonly, two strategies are used to generate metal nanoparticles: -

A] 'Top-down' techniques:

In the top-down method material is removed from the bulk material, leaving only the desired nanostructures by employing milling, attrition or any other mechanical and energy input processes. However, it causes structural imperfections and difficulty in controlling particle size.

B] 'Bottom-up' techniques:

In bottom-up method, the atoms which are produced from reduction of ions, assemble to generate nanostructures. These approaches involve self-assembly of nuclei into nanostructures with comparatively lesser defects, more control over the size and therefore, more homogeneous chemical composition. The methods for nanoparticle synthesis are mainly divided in three different types-Physical, Chemical and Biological Methods

1.3.1 Physical methods

Physical synthesis of nanoparticles involves attrition, thermal quenching and pyrolysis like mechanical techniques. In attrition, starting materials are reduced to submicron size particles through planetary ball mill, media mill, etc. In pyrolysis, an organic precursor (liquid/gas) is forced through an orifice at higher pressure and burned into ash from which oxidized nanoparticles are recovered [31]. In physical vapor deposition (PVD) [32], nanosized metal films are produced by electrical heating under high vacuum. In chemical vapor deposition (CVD), where carrier gasses containing the elements of desired compound are passed over heated surfaces resulting in decomposition of carrier gas with subsequent deposition of atoms/ molecules on the surface [33,20]. Solvated metal atom deposition (SMAD) [34] method involves heating of bulk metal till it starts to evaporate under vacuum followed by co-condensation of its vapours with solvents to form nanoparticles in solution. Synthesis of Ag nanoparticles by inert gas condensation techniques involved evaporation of bulk metal in an inert atmosphere followed by subsequent cooling for nucleation and growth into nanostructures [35]. Laser ablation techniques [36-38] involve focusing of intense laser pulses on metal objects immersed in solvent containing surfactant. It results into high temperature conditions causing metal atoms to get vaporized and solvated to nanoparticles. In the ultrasonic irradiation assisted synthesis, continues bubbling at higher temperature result in generation of reactive radicals known to catalyse the synthesis process [39, 40]. In radiolytic techniques, free radicals produced by radiolysis are utilized to reduce metal ions in the presence of donor ligands to synthesize nanoparticles [41]. However, higher expense of production and hence scaling up of nanoparticles in larger quantities are major drawbacks of the physical routes.

1.3.2 Chemical methods

Wet-chemical methods are the most popular and easy route for controlled synthesis of nanoparticles involving a dual step mechanism of nucleation and successive growth [42]. Initially, nucleation of MNPs occurs by reduction and collision between ions and atoms followed by controlled growth under specific conditions of chemical concentration, mixing ratios, surfactant nature, pH and temperature. In this route, metal precursors are reduced by suitable reducing agents such as citrate [43], sodium borohydride [44], trisodium citrate [45], amino acids [46, 47], etc for concurrent reduction and stabilization [20]. The experimental conditions such as temperature, concentration of reducing agents may affect the morphology and properties of nanoparticles [48]. Easy manipulation in chemical reactivities of reactants, better control over reaction conditions during synthesis of nanoparticles and ease in surface functionalization of nanoparticles are the most attractive features of this route. It allows synthesis of nanoparticles in different solvent mediums; for

example, synthesis of nanoparticles in aqueous medium [44-47], organic medium [49], ionic liquids [50] and in supercritical fluids [51]. This particular feature is important with respect to the use of nanoparticles for different applications, which are mainly dependent on the use of medium or solvent; for example, catalysis in aqueous as well as organic medium and biological applications in aqueous medium etc. Chemical route also facilitates freedom to synthesize and assemble nanoparticles at various interfaces such as at air-water interface [52] and liquid-liquid [53] interfaces with the help of different chemical functionalities, which allow reduction and controlled growth of nanoparticles and facilitates 2-D arrangement of nanoparticles. Since the chemical route can be used to synthesize nanoparticles with minimum resources and with easy control on their shape and size of the nanoparticles, this route can be used to produce nanoparticles in larger quantities for various industrial applications.

1.3.3 Biological methods

Biological synthetic route involves biological resources such as plants, fungi, bacteria for synthesis of nanoparticles. It also involves direct synthesis using living cells, biomolecules like enzymes, peptides and biological templates. It uses microorganisms such as fungi and bacteria for the synthesis of nanoparticles [54-56]. Heavy metal ions are toxic to biological systems; micro-organisms detoxify these ions by reducing them into metal atom [56]. During this process, formation of nanoparticles takes place and these nanoparticles are stabilized by biomolecules. In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Although biosynthesis of gold nanoparticles by plants such as alfalfa [57, 58], Aloe vera [59], Cinnamomum camphora [60], neem [61], Emblica officianalis [62], lemongrass [63], and tamarind [64] have been reported, the potential of plants as biological materials for the synthesis of nanoparticles is yet to be fully explored. Fungi (*Verticillium* sp [65] and *Fusarium oxysporum* [66] and actinomycete (*Thermomonospora* sp [67] and *Rhodococcus* sp [68] were also used to synthesize nanoparticles intra- or extracellularly. Recently, Sastry and co-workers showed efficient way of synthesis of gold nanoparticles with better control on size and morphology with help of medicinal plant extracts [69-71]. Recently, Abhijit et al. developed gold nanoparticles-based enzyme device by reduction mechanism using α -amylase, where the enzyme after synthesis of gold nanoparticles retained its activity and improved its functionality after being attached with gold nanoparticles under physiological condition [72]. Kumar et al. reported the synthesis of gold nanoparticles using α NADPH dependent sulphite reductase enzyme to form stable gold nanoparticles with 20 nm particle size. The glucose oxidase synthesized gold nanoparticles have been optimized with respect to temperature, pH and method of immobilization. Here, glucose oxidase enzyme served as a reducing agent providing green route of synthesis and retaining its catalytic activity [73]. Though biological methods are simple, high-yielding and non-toxic, involvement of these natural resources fails to illustrate the exact molecule involved in the reduction-based synthesis. Additionally, the slower rate of biosynthesis hampers the use of such biological mediators. To overcome these shortcomings, the approach has been shifted towards exploring biomacromolecules and remained limited to chitosan, gellan gum, dextran [74], pullulan [75], porphyrin and hydroxyl ethyl cellulose [76].

1.4 Surface modification and bioconjugation of metal nanoparticles

1.4.1 Surface modification strategies

Stabilizing molecules maintain a shell around the colloidal nanoparticles. One of the ends of the stabilizing molecules are either adsorbed or chemically linked to the gold surface, while the other end points towards the solution and provides colloidal stability. After synthesis of the nanoparticles, the stabilizer molecules can be replaced by other stabilizer molecules in a ligand exchange reaction. As thiol moieties bind with high affinity to gold surfaces, most frequently thiol-modified ligands are used which bind to the surface of the gold nanoparticles ("monolayer-protected clusters") by formation of gold-sulfur bonds [77]. Ligand exchange allows, for example, the transfer of gold nanoparticles from an aqueous to an organic phase (and vice versa) by exchanging hydrophilic surfactants with hydrophobic surfactants (and vice versa) [78]. In this way, by choosing the surfactant molecules, it is possible to adjust the surface properties of particles. For applications in aqueous solution, typically thiol-based surfactants with carboxylic groups at the other end pointing towards solution are used. These molecules provide colloidal stability due to their negative charges; in addition they can also be used as anchor points for the further attachment of biological molecules. Often poly (ethylene glycol) (PEG) is used as a ligand as PEG reduces nonspecific adsorption of molecules to the particle surface and it provides colloidal stability. PEG brushes on the surface of nanoparticles repel each other for steric reasons [79]. The cytotoxicity results of PEG stabilized AuNPs prove the biocompatibility of this molecule. Another approach is the use of mixed monolayer stabilized gold nanoparticles. Previous reports represented one step synthesis of PEG/BSA stabilized gold nanoparticles by the photochemical process. Mixed monolayer AuNPs are ideal for drug and gene delivery purposes [80].

1.4.2 Bioconjugation

Biological molecules can be attached to the metal nanoparticles in several ways. If the biological molecules have a functional group which can bind to the gold surface (like thiols or specific peptide sequences), the biological molecules can replace some of the original stabilizer molecules when they are added directly to the nanoparticle solution. In this way molecule like oligonucleotides, peptides or PEG can be readily linked to gold nanoparticles and subsequent sorting techniques even allow particles with an exactly defined number of attached molecules per particle to be obtained [81,82]. Alternatively, biological molecules can also be attached to the shell of stabilizer molecules around the gold nanoparticles via bioconjugation.

The most common protocol is the linkage of amino groups on the biological molecules with carboxy groups of stabilizer molecules with related strategies [83], almost all kinds of biological molecules can be attached to the nanoparticle surface. Though such protocols are relatively well established, bioconjugation of gold nanoparticles still is not trivial and the characterization of synthesized conjugates is necessary, in particular to rule out aggregation effects or unspecific binding during the conjugation reaction. In many conjugation protocols, the number of attached molecules per gold nanoparticle is

only a rough estimate, as no standard method for determining the surface coverage of particles modified with molecules has yet been established [84,85]. The nanobiology toolkit has been greatly enhanced by noble metal nanostructures, which have proven to be highly versatile and tunable materials for a range of bioapplications including biophysical studies, biological sensing, imaging and medical diagnostics.

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