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Review Paper

Development of Nano structure Plasmon Gold by Green Synthesis for Fabrication of Bio/Chemical Sensor

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Abstract

More recently, metallic nanostructure particles have been fruitfully employed in chemical and bio sensors. Recent technological breakthrough in the fabrication of gold and silver nano particles has developed nano Plasmon. Usefulness of biomaterials and metal catalyst in many reactions is an urgent need of the society to fulfil our requirements. As per the environmental issue it is our responsibility to avoid hazardous effect of metal. So choice of biocompatible and micro- to nano-gram scale catalyst instead of toxic and gram scale is critical. Plasmon material suitable for the fabrication of low cost biosensors and chemical sensors based on localized surface Plasmon resonance (LSPR), can be fabricated by a simple methodology based on producing thin film of Au on commercially available transparent surfaces. This paper presents critical review of green Synthesis of Gold nano particle used in fabrication of bio and chemical sensors. Synthesis of gold nano structured materials by reduction reaction between gold salt and suitable green reducing agent is being investigated. Literature on concentration of gold solution and reducing agent, synthesis parameters and mixing rate effect to particle size have been reviewed. Fabricating these nanostructures with an unprecedented control over their sizes[1],shapes and spacing is an essential requirement for the construction of nanoscale optical and photonic devices. This inspired us to make our own contributions to this exciting and highly relevant field. It is important to develop a system, useful as a sensor that identifies the presence of gases or bio molecules at low part-per-billion concentrations in gaseous media and biomaterials. Shift of surface Plasmon resonance peak can be monitored using the Raman Spectroscopy while gold nanostructure is exposed to variable concentration of poisonous gases like ozone and CO and bio molecules like cancer cells in blood.

Keywords: Nanostructure particles, nanoplasmonics, LSPR, gas sensors, biosensors, goldnanoisland, surfaceplasmons.

Introduction

When light interacts with metal-dielectric materials, surface Plasmon are produced. Plasmonic materials are meta materials that exploit these surface plasmon. When the incident light couples with the surface Plasmon it creates propagating electromagnetic waves known as Surface Plasmon Polaritons (SPPs) which ripple along the metal-dielectric interface. Compared with the incident light the SPPs are found to be much shorter in wavelength[2]. Plasmonic meta materials are composites - combinations of metallic and dielectric materials designed to achieve optical properties not seen in nature. New approaches that simplify the fabrication process of meta materials are under development. NIST researchers are currently doing research on nanotechnology applications of these nanostructures including microscopy beyond the diffraction limit[3]. West, P.R et.al., mentioned in Laser and Photonics Reviews about exhibition of Negative real permittivity by Plasmonic materials[4]. As it is known, Gold and silver can be made most common plasmonic nano structured materials. However, there are many other materials which show metal-like optical properties in the specific wavelength ranges as indicated by Boltasseva, A.; Atwater, H.A[5]. Fabrication of plasmonic materials that exhibit lower-losses and tuneable optical properties are experimented by various researchers with different approaches. Plasmonic metamaterials are also known as negative index materials. These exhibit optical properties opposite to those of glass, air. It is understood from literature that surface Plasmon resonance at the surfaces of gold nanostructures has created great interest in fabrication of gold nanoparticles. The study of nano optical branches such as nanoplasmonics is of much interest to material engineers, physicists, chemists and biologists.

The spread of nanotechnology is strictly due to the improvement of characterization and synthesis techniques on nano meter scale. In the present paper gold and AuNP play a significant role. There is a favourable combination of physical-chemical properties and advances in chemical synthesis. The surface plasma absorption (SPA) is the main characteristics of AuNP. Frequency of gold SPA can also be tuned from visible to near infrared depending on shape, size or nano particle assembly. They have high chemical stability and photo stability, especially AuNP are non toxic for living organism. Because of their physicochemical stability, bright colour and biocompatibility, recent years have faced sensible progress in AuNP synthesis with tailored shape and size. This with engineered properties, opens the access of nanotechnology to manifold applications.

Optical properties of metal nanoparticles deposited on a dielectric/ transparent support makes plasmonic system understandable. In the conventional Surface Plasmon Resonance (SPR) spectroscopy, the response of surface Plasmon polaritons (SPP) (i.e.; electromagnetic waves propagating at metal surfaces coupled with electron motion) to changes in the refractive index of the surrounding medium are used for monitoring and sensing a variety of phenomena occurring at the interface. The optical properties of thin metal layer surfaces due to the excitation of SPP can be widely exploited for the development of label-free sensing devices[6-7]. This technology is very versatile. Biological and Chemical sensors with high sensitivity and selectivity are required for many strategic applications. Suitable nano structured material is required to be developed and characterized and tested as sensors. In life science, engineering, medicine, chemistry and biotechnology, chemical sensors and biosensors are becoming more and more indispensable tools. Sensor-related aspects of thin film and interface techniques, chemistry, biochemistry, physics, optoelectronics, measurement sciences and signal processing are benefited from material research work on chemical sensors and biosensors. Continuous inspiration for new research and new trends and brand new applications in this field is also very important.

As mentioned by Mansoori GA et al., nanoparticles are considered as the fundamental building blocks of nanotechnology[8-9]. The synthesis and characterization of nanoparticles and their applications represent an emerging trend and rapidly growing concept in science and technology[10,11]. In the recent years, there has been much concern about the synthesis of environment-friendly nanoparticles that do not produce toxic wastes in their process of synthesis. This can only be achieved through synthesis processes of biological nature. This process is an alternative to conventional physical and chemical methods[12].Also these processes are considered to be safe and ecologically sound for nanomaterial fabrication.

Silver and gold nanoparticles are reported to be nontoxic to human and most effective against bacteria, viruses, and other eukaryotic micro-organisms at very low concentration and without any known side effects[13]. This is their advantages over other nano materials. In the recent years, much advancement is brought to the technology for synthesis and characterization of nanoparticles. It is an important necessity to produce nanoparticles in an eco-friendly manner. Biological organisms such as plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner[14-17].

The present paper provides a comprehensive review on the recent technological advances brought into methodology for biological and eco-friendly synthesis and characterization of herbal and medicinal plants mediated nanoparticles followed by our initial research work on synthesis of plasmonic gold nano particles from lemon grass extract and their characterisation. The main aim of this work is to develop Nano structured plasmonic materials for a bio/chemical sensors by using non toxic and ecofriendly method of novel synthesis route.

Material and Methods

After reviewing in detail the materials and methods for synthesis of nano gold particles, the bio fabrication of pure metallic gold nanoparticles was carried out by reduction of Au^{3+} to Au^0 with the aqueous extract of lemon grass and their characterization. For the synthesis of gold nanoparticles, lemon grass extract was used as reducing and capping agent.

Plant material and preparation of extract: Sumit S Lal et al[18] have studied twenty-five medicinal herbs, extract of which were reported to be the reducing agents for synthesis of gold and silver nano particles. After their selective parts were washed and cleaned and dried with wet filter paper, they were cut into small pieces and crushed with mortar and pastel dispensed in sterile distilled water and boiled for 10 minutes. Then extract were filtered and centrifuged at 5000 r.p.m , then by standard sterilized filtration method extract were collected in separate conical flasks and were stored at 4°C. Linga Rao et al[19] have reported the biological synthesis of silver nanoparticles using leaf extract. In their study, they collected the fresh and healthy leaves of S. Hyderabadensis. They made fine powder of leaves after washing, cleaning and pressing with blotting paper and drying in shade and grinding it.100 ml of distilled water was taken in 250ml conical flask and 5g of powder was added to it. The mixture was boiled at 100°C for 10min. The prepared leaf extract was separated by standard filtration methods. Another study was carried out by Masurkar et al[20], who reported rapid biosynthesis of silver nanoparticles using *Cymbopogan citratus*. About 50 g of fresh leaves of *C. citratus* were washed thoroughly, cut into fine pieces, dipped into a beaker containing 200ml of distilled water and boiled for 10-12min. The prepared extract was filtered through Whatmann filter paper and stored for further studies. Prasad et al[21] reported the biogenic synthesis of silver nanoparticles using *Nicotiana tobaccum* leaf extract. In this study, about 5g leaves of *N. tobaccum* were collected and washed thoroughly. The leaves were cut into small pieces and subsequently mixed in 20ml buffer of Tris-HCl of pH 8.0 with the help of mortar and pestle. The thick slurry thus recovered was then centrifuged at 10,000 rpm for 7 min at 5°C. The supernatant obtained was preserved in refrigerated condition. This is used as precursor for synthesis of silver nanoparticles.

In the present research work chloroauric acid ($HAuCl_4$) , and lemon grass were used as Materials for the synthesis of gold nano particles. Both materials are commercially available. The chloroauric acid was purchased from Sigma Aldrich and the lemon grass from specialized shop. 100 gram of *Lemon grass* leaves were thoroughly washed and then finely cut and mixed in 100 ml of distilled water. The mixture was then boiled and decanted. Thus grass extract was prepared.

Green Synthesis of Gold Nanoparticles: Sumit.S.Lal and P.L.Nayak reported green synthesis of gold nano particles using various plant extracts and spices extracts in which extracts reduces aqueous $HAuCl_4.3H_2O$ to Au° and stabilized by itself at certain crystalline phase by adding 1% of chitosan and 1% of PVA. Aqueous chloroauric acid ($HAuCl_4$) solution was added to extract with ratio of 1:5. Change in colour from light yellow to various different colours obtained within a particular time, which depends upon the extracts of plants and spices. Synthesis of silver nanoparticles have been carried out by different researchers. The colour change of the solution was checked by them periodically. The conical flask was incubated at room temperature for 48 hours. The change in colour of the solution to the dark brown indicates the synthesis of silver nanoparticles from the plant extract.

Present research work is done to synthesise gold nano particles where lemon grass extract is used as reducing agent for chloroauric acid ($HAuCl_4$) at room temperature, resulting in a dark ruby pink-red coloured solution indicating the formation of gold nano particles[22-24]. 0.1mM of Au^{3+} chloroauric acid is prepared in deionised water. Leaf extract of Lemon grass is added with constant stirring at 85°-95°C temperature. After 5 minutes a colour change is observed due to reduction of Au^{3+} to Au^0. Gold nano particles are sputter coated on to a transparent substrate.

Characterisation: UV-Vis Spectra analysis: A number of studies have reported monitoring of bio-reduction of silver ions in aqueous solution by UV-Vis spectrophotometer[25-27]. It is understood from these studies that samples are diluted into distilled water and after 3 to 5 hours of diluting the samples reduction of Ag^+ ions is monitored.

In the present research work absorption spectra were recorded on a Shimadzu spectrophotometer and reduction of Au^{3+} to Au^0 was observed.

Fourier Transform-Infra Red (FT-IR) spectroscopy: Fingerprint of a material is represented by an infrared spectrum (IR). Obtained absorption peaks show the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms. No two compounds produce the exactly same IR. Therefore, IR results in a positive identification (qualitative analysis) of every different kind of material. IR is an excellent tool for quantitative analysis with modern software algorithms. Several investigators have reported FT-IR analyses of herbal and medicinal plant mediated silver nanoparticles. According to a study carried out by K. Mallikarjuna *et al*, reduced silver nitrate solution was centrifuged at 10,000 rpm for 20 min and the dried and then was ground with KBr pellets. The powder obtained was then subjected to FT-IR analysis.

In our work dry powders of the nanoparticles were prepared for Fourier transform infrared (FTIR) spectroscopy measurements. After 24 h of reaction of the salt solutions with the lemon grass extract Au nanoparticles were synthesized and were centrifuged at 10,000 rpm for 30 min and then the pellet was redispersed in sterile distilled water to ensure better separation of the uncoordinated biological molecules from nanoparticles. The purified pellets were then dried and the powders subjected to FTIR spectroscopy measurement. GX Perkin Elmer Fourier transform infrared spectrometer used to carry out the experiment.

Transmission Electron Microscopy (TEM): TEM is commonly used for imaging and analytical characterization of the nanoparticles to assess the shape, size, and morphology. The outstanding resolution achieved by TEM is an excellent fit for these extremely challenging studies. According to several studies carried out by different investigators, before taking TEM, thin films of the samples were prepared on carbon coated copper grids. Blotting papers were used to remove extra solutions. The films prepared on the TEM grid were then allowed to dry under a mercury lamp for 5 min.

Scanning Electron Microscopy (SEM): External morphology, chemical composition, crystalline structure and orientation of materials making up the sample are revealed by SEM. Data are collected over a selected area of the surface of the sample, and a two-dimensional image is generated. For this purpose, thin films of the samples were prepared by the investigators on carbon coated copper grids by same manner as in case of TEM[28-29].

Atomic Force Microscopy (AFM): For studying the morphology of nanoparticles and bio molecules AFM is an important biophysical technique. Quantitative information from individual nanoparticles can be generated from Software-based image processing of AFM data. Size information (length, width, and height) and other physical properties (such as morphology and surface texture) can be measured for individual particles. Atomic Force Microscopy can be performed both in liquid or gas mediums. This capability can be very advantageous for nanoparticle characterization. AFM has several advantages over SEM and TEM for characterizing nanoparticles. It is possible to measure the height of the nanoparticles quantitatively since images from an AFM represent data in three dimensions[30-31]. According to Satyavani *et al.* samples are prepared by spreading it on a glass cover surface mounted on the AFM stub and then dried with nitrogen flow at room temperature. Minimum of five images for each sample are obtained with AFM and analyzed to ensure reproducible results.

X-Ray Diffraction (XRD): XRD is a non-destructive analytical method which identifies and determines various crystalline forms of materials. According to studies, the solution of nanoparticles obtained was purified by repeated centrifugation at10,000 rpm followed by re-dispersion of the pellet of nanoparticles into distilled water. After freeze drying of the purified particles, the structure and composition of nano particles were analyzed by XRD.As waves interact with a regular structure the diffraction occurs.

In the present research work XRD was carried out for synthesised gold nano materials using instrument with Cu*Kα* radiation.

Energy Dispersive X-Ray Spectra (EDS): The analytical technique EDS identifies the elemental composition of the specimen. EDS utilizes x-rays that are emitted from the specimen when bombarded by the electron beam. When the sample is bombarded by the electron beam of the SEM, electrons are ejected from the atoms on the surface of specimen. A resulting electron vacancy is filled by an electron from a higher shell, and an X-ray is emitted to balance the energy difference between the two electrons. The number of emitted X-rays versus their energy is measured by the EDS X-ray detector. The energy of the X-ray is characteristic of the element from which the X-ray was emitted. A spectrum of the energy versus relative counts of the detected X-rays is obtained and evaluated by Herguth WR et.al for qualitative and quantitative determinations of the elements[32]. According to studies conducted by a few investigators, after placing one drop of the solution on carbon coated copper grid and exposing it to infrared light for 45min EDX analysis of the nanoparticles is performed.

Dynamic Light Scattering (DLS): One of the most popular techniques which is used to determine the size of particles is Dynamic Light Scattering (DLS). When a monochromatic light beam, such as laser is shined onto a solution with spherical particles in Brownian motion Doppler shift is produced. When the light hits the moving particle, wavelength of incoming light is changed. This change is related to the size of the particle. Using DLS, it is possible to compute the sphere size distribution and give a description of the particle's motion in the medium measuring the diffusion coefficient of the particle by using autocorrelation function as shown by Saxena A, Tripathi RM, Singh RP.

Results and Discussion

Results: Reduction of the metal ions from the aqueous solution by *lemon grass* extract is confirmed from the appearance of a distinctive reddish colour. It is well known that gold nanoparticles exhibit a specific colour, in water, arising due to excitation of surface Plasmon vibrations in the metal nanoparticles. Figure-1 shows the UV–Vis spectra recorded for the aqueous chloroauric acid- *lemon grass* mixture for sample of the best concentration for gold nanoparticles synthesis.

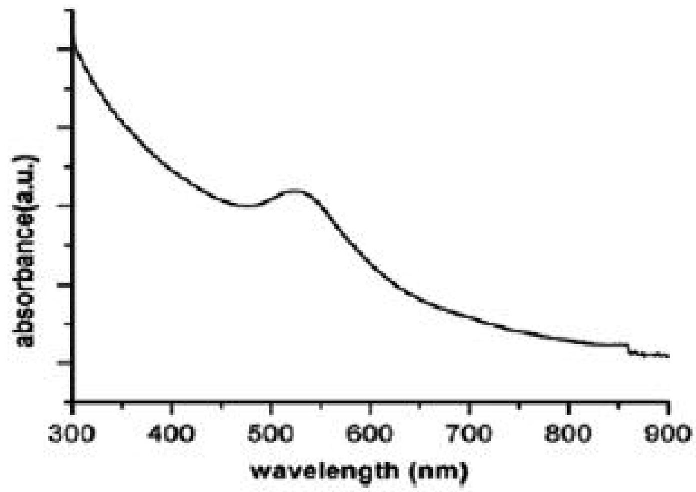

Figure-1
UV-Vis spectra of gold nano particle

UV–Vis spectra recorded even 4 weeks after their synthesis and there was no observable variation in the optical properties of the nanoparticles solutions with time. This observation shows the metal particles were stable in solution.

Figure-2 shows the XRD patterns obtained for gold nanoparticles synthesized in present research work. The crystalline nature of the gold nanoparticles is clearly shown in XRD pattern. Bragg reflections corresponding to lattice planes (111), (200), (220),(311), (222) are observed in XRD pattern.

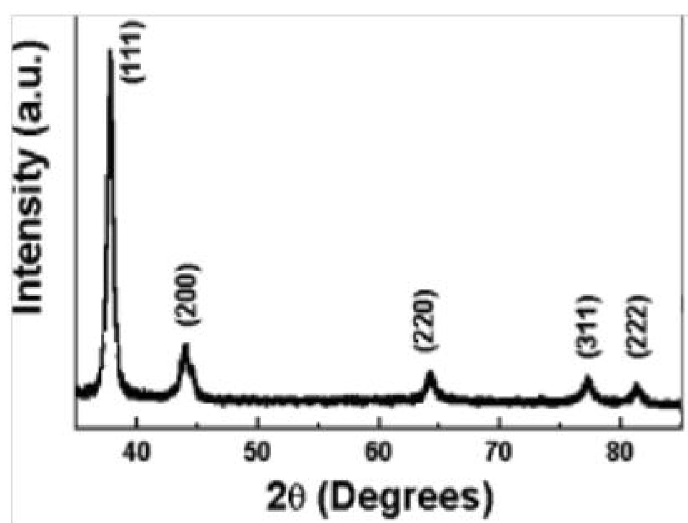

Figure-2
XRD pattern of gold nanoparticles

FTIR spectra of the stabilized gold nanoparticles solution is shown in Figure-3.

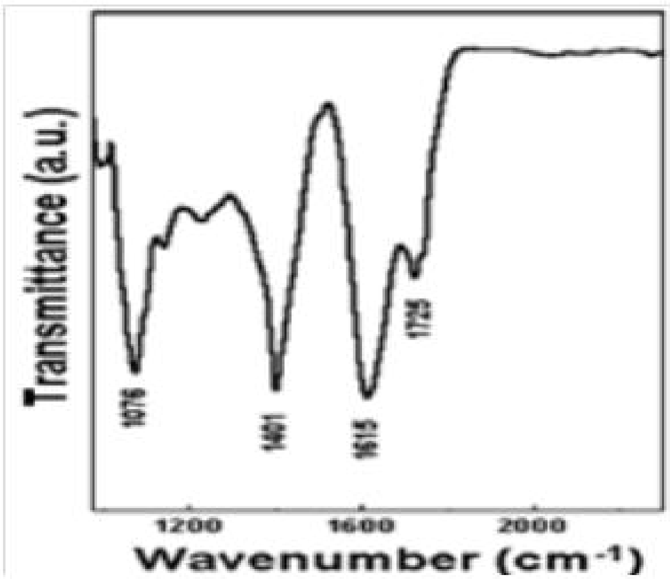

Figure-3
FTIR spectra of the stabilized gold nano particles

Conclusion

How various extract of plants and spices are capable of producing gold nano particles has been reviewed in this paper. In present work good stability of nano particles in solution is shown. In the UV-Visible wavelength nano particles have shown quiet good surface Plasmon resonance behaviour. Nanoparticles synthesized in present research work could find applications in the field of nano optics as optical sensors specially as Bio/Chemical sensors. It also finds applications in Bio- Sensors that track tuberculosis, botulism and other neurotoxins and in Diagnostic devices that monitor a patient's health, including cancer detection. Work on chemical sensors as Hydrocarbon detectors that safeguard susceptible children and adults from entering harsh atmospheric environments. This work targets the design and deployment of System-on-a-Chip (SOC) chemical and biological sensors that function in handy, portable packages. This technology will enable healthcare professionals to perform critical and inter-related functions that meet current and next generation medical standards. By detecting hydrocarbons in soil, air and water, it serves homeland security needs.

Green synthesis of metallic nanoparticles is an successive alternative to chemical synthesis protocols for synthesizing gold nano particles. Present work is certainly based on furthering fundamental knowledge on nano sensors and devices. Testing the plasmonic materials as sensor by Raman scattering experiments using Raman Spectrometers will be carried out in future.

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