GOLD AND SILVER NANOPARTICLES: GREEN SYNTHESIS AND ANALYTICAL INVESTIGATIONS

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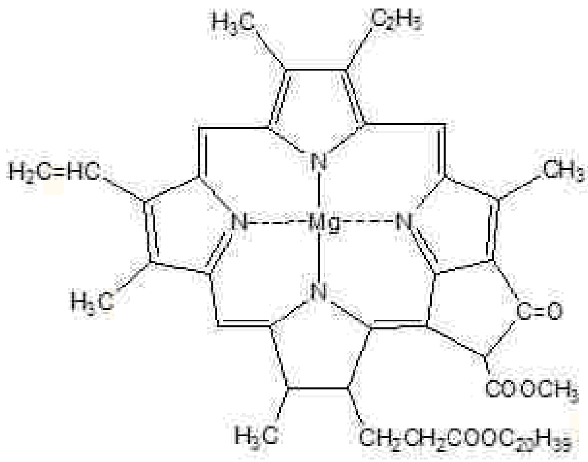
Abstract. *Nanotechnology is a most promising field for generating new applications in all areas. The process of development of reliable and eco-friendly metallic nanoparticles is an important step in the field of nanotechnology (22,23). Interaction of chlorophyll from Spinachia oleracea in aqueous* $AgNO_3$ *and* $HAuCl_4$ *was investigated for the synthesis of Ag and Au nanoparticles, respectively. Biological reduction and extracellular synthesis of nanoparticles were achieved at 37°C at pH=5.6. The nanometallic dispersions were characterized by UV-VIS at 452 nm for Ag and 550 nm for Au nanoparticles. Fourier transform infrared spectroscopic measurements (FTIR) revealed the fact that the chlorophyll pigments are the possible biomolecules responsible for the biosynthesized nanoparticles.*
Keywords: *spinacia oleracea, nanomaterials, gold and silver nanoparticles*

1. INTRODUCTION

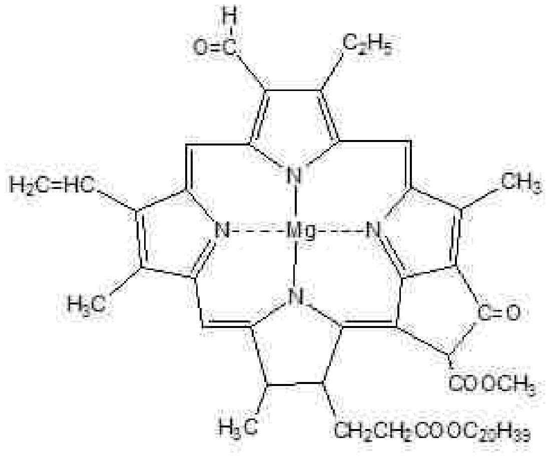
Nanotechnologies are among the fastest growing areas of scientific research and have important applications in a wide variety of fields. Nanomaterials often show considerably changed physical, chemical and biological properties compared to their macro scaled counterparts. The data suggest that in the future workers and consumers exposed to nanoparticles will significantly increase [1,2].
Nanomedicine is a new distinct scientific discipline that explores applications of nanoscale materials (1–100 nm) for various biomedical applications. At nanoscale, the physical properties of materials are altered, as are their interactions with cells and tissue. This occurs primarily because of the significant difference in the surface area/volume ratio as materials are reduced to nanosized level.1 Nanomedicine explores nanotechnology for monitoring, repair, and control of human biologic systems at cellular and molecular levels using engineered nanodevices and nanostructures [3].
Synthesis of noble metal nanoparticles for applications such as catalysis, electronics, optics, environmental, and biotechnology is an area of constant interest in the whole world. Gold and silver have been used mostly for the synthesis of stable dispersions of nanoparticles, which are useful in areas such as photography, catalysis, photonics, optoelectronics, biomedicine, etc [1].
The use of gold nanoparticles dates back to the 16th century, for both medical and staining purposes. Thereafter, gold nanoparticles have found application in analytical methods such as colorimetric techniques for the determination of heavy metal ions in aqueous solutions [4]. Gold nanoparticles are used in the field of sensors [5,6] and possess catalytic activity, and hence are used for reactions such as the water gas shift reaction and selective oxidation of CO [7,8,9].
In biology, gold nanoparticles are used for the development of biosensors, DNA labels. However, spherical gold nanoparticles have been used to generate functional electrical coatings [10].
Silver nanoparticles have found application in ecology and medicine and have showed good antimicrobial activity and therefore can be used for purification in water-filtering apparatus [11]. Silver nanoparticles also find application in topical ointments and creams used to prevent infection of burns and open wounds [12]. Another widely used application is in medical devices and implants prepared with silver-impregnated polymers. In addition, silver-containing consumer products such as colloidal silver gel and silver-embedded fabrics are now used in sporting equipment [13].
Use of biological organisms such as microorganisms, plant extract or plantbiomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner. Several plants have been successfully used for efficient and rapid extracellular synthesis of silver and gold nanoparticles. Most leaf extracts of several plants, like geranium (*Pelargonium graveolens*), neem (*Azadirachta indica*), lemongrass (*Cymbopogon flexuosus*), camphor (*Cinnamommum camphora), Aloe vera*, tamarind (*Tamarindus indica*), have shown potential in reducing Au(III) ions to form gold nanoparticles Au(0) and reducing silver nitrate to form silver nanoparticles Ag(0). Biomasses of other plants like wheat (*Triticum aestivum*), oat (*Avena sativa*), alfalfa (*Medicago sativa*), have also been used for gold nanoparticles synthesis. Nanoparticle application in catalysis, sensors and medicine depends critically on the size and composition of the nanoparticles. Thus different routes leading to the synthesis of nanoparticles of various shapes and sizes have extended the choice of properties that can be obtained. The optoelectronic and physiochemical properties of nanoscale matter are a strong function of particle size. Nanoparticle shape also contributes significantly to modulating their electronic properties [14,15, 16,17].

The leaves of plants contain a number of colored pigments generally falling into two categories, chlorophylls and carotenoids. The green chlorophylls *a* and *b*, which are highly conjugated compounds capture the (nongreen) light energy used in photosynthesis [3].
Both chlorophylls contain several polar C-O and C-N bonds and also a magnesium ion chelated to the nitrogen atoms. The distinctions between the chlorophylls, which are more polar than carotene is slight: chlorophyll a has a methyl group (-CH3) in a position where chlorophyll b has an aldehyde (-CHO). This makes chlorophyll b slightly more polar than chlorophyll a.



Chlorophyll a

Chorophyll a has a $-CH_3$ side-chain , chlorophyll b has a $-CHO$ side-chain. Chlorophyll-a is the primary pigment for photosynthesis in plants. Its structure is shown at left. It has the composition $C_{55}H_{72}O_5N_4Mg$. It occurs in all photosynthetic organisms except photosynthetic bacteria.
Chlorophyll a absorbs red light more strongly, chlorophyll b absorbs violet light more strongly. Chlorophyll b is missing from cyanobacteria (are the toxin-producing pond scum bacteria known as "blue-green algae").
Chlorophyll-b has the composition $C_{55}H_{70}O_6N_4Mg$, the difference from chlorophyll-a being the replacement of a methyl group with a CHO. It occurs in all plants, green algae and some prokaryotes. There is usually about half as much chlorophyll-b as the -a variety in plants [18,19].
The use of environmentally benign materials like plant leaf extract, bacteria and fungi for the synthesis of silver and gold nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols. Chemical synthesis methods lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical applications. Bioinspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals [20,21].



Chlorophyll b

2. MATERIAL AND METHODS

2.1 Preparation of the Extract

The chlorophylls have been extracted by fresh leaves of *Spinachia oleracea*. Leaves weighing 250 g were thoroughly washed in distilled water, cut into fine pieces and were boiled in a 500 ml Erlenmeyer flask with 100 ml of mixed solution: petroleum ether 70%: acetone30%. Then the liquid was decanted into a 50 ml round bottom flask and were filtered. The column was made using a chromatography coloumn which has filed almost to the top with petroleum ether. The stopcock has allowed slow drip of solvent. Next step was to add 10 cm alumina slowly and over about 2-3cm of sand. We took care of that column will not contain air bubbles, otherwise extraction is not done correctly. Then were added mixture pigments directly onto the sand. When the sand layer was coloured, the stopcock was open to let the liquid fall to the top of the alumina. The chlorophyll was extract using more pollar mixture (70%petroleum ether:30%acetone).

2.2. Synthesis of Silver and Gold Nanoparticles

Materials used for the synthesis of gold and silver nanoparticles are $HAuCl_4$ and $AgNO_3$ and chlorophyll of leaf extract from *Spinacia oleracea*.

2.3.UV-Vis spectra analysis

Gold and silver nanoparticles formations were carried out by taking filtrate obtained from fresh leaves of spinach. Over 100ml of pigments filtrate with 10^{-3} M aqueous $AgNO_3$ and $HAuCl_4$ and incubated at room temperature (24°C). The ph was checked during the course of reaction and it was found to be 5.6. The complete reduction of pure Ag^+ and $AuCl^{4-}$ ions was monitored by measuring the UV-VIS spectrum of the reaction medium. UV-VIS spectral analysis has been done by using a SPECORD M42 spectrophotometer.

2.4.FT-IR analysis

The silver and gold nanoparticles synthesized using chlorophylls obtained from *Spinacia oleracea* were subjected to FT-IR (Fourier transform infrared) spectrum analysis to identify whether the biomolecules are stabilizing and reducing agents. For FT-IR data, spectroscopy measurements were done on a Spectrum GX Perkin Elmer.

3. RESULTS

Addition of *Spinacia oleracea* biomass to 10^{-3} M aqueous $AgNO_3$ solution led to the appearance of yellowish brown colour in solution after 24 hour, and 10^{-3} M aqueous $HAuCl_4$ solution led to the appearance of ruby red colour in solution after 24 hour, that indicate the formation of silver and gold nanoparticles, respectively. These colours arise due to excitation of surface plasmon vibrations in the metal nanoparticles [22].

The nanometallic dispersions were characterized by UV-VIS at 452nm for Ag and 550 nm for Au nanoparticles.

In fig.3 it is show the FT-IR spectra of chlorophyll from spinach.

The gold nanoparticles, showed strong bands at 1638.09 (aromatic ring C=C functional groups), 1465.64 (antisymmetric band of CH3 functional groups), 1385.29 cm^{-1} (geminal methyls), 1126.51 cm^{-1} (ether linkages), 944.98 cm^{-1} (sugesst the presence of flavonones or terpenoids adsorbed on the surface of metal nanoparticles (fig.4).

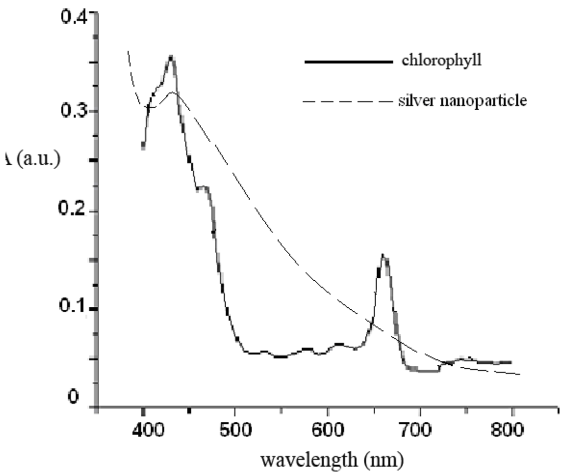


Fig. 1. UV-VIS spectra of AgNPs in chlorophyll.

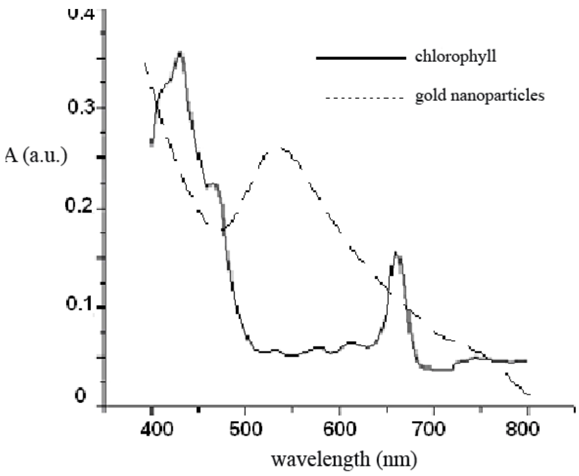


Fig. 2 UV-VIS spectra of the AuNPs in chlorophyll

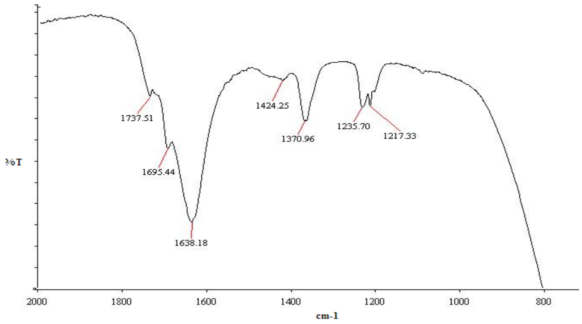


Fig. 3 FT-IR spectra of chlorophyll from Spinacia oleracea.

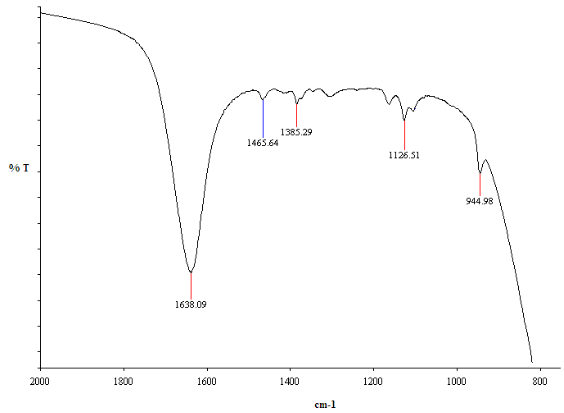


Fig.4 FT-IR spectra of gold nanoparticles by reduction of $AuCl_4^-$ ions by chlorophyll from spinach.

The silver nanoparticles synthetised using chlorophylls from spinach showed strong bands at 1737.67 cm^{-1} (esters C=O group), 1637.68 cm^{-1} (aromatic ring C=C functional groups), 1365.60 cm^{-1} (geminal methyls) and 1217.17 cm^{-1} (ether linkages), 944.98 cm^{-1} (C-H bond) (fig.5). Dates in literature reports that various species of cyanobacteria, plants and algae confirm the ability to absorb and take up heavy metal ions [22,23,24].

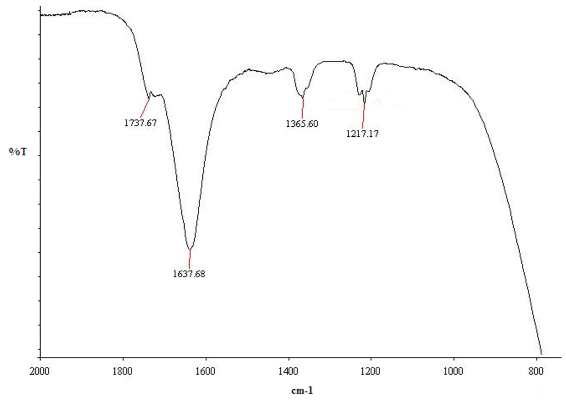
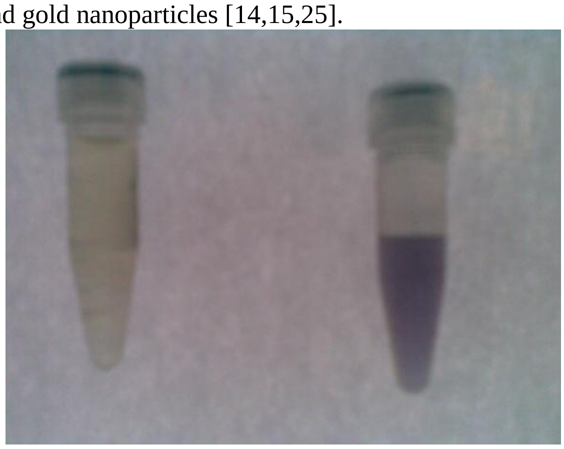


Fig.5 FT-IR spectra of silver nanoparticles by reduction of Ag^{+} ions by chlorophyll from spinach.

4. DISCUSSION

Reduction of silver and gold ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the plant leaf extract observed by the UV-VIS spectroscopy revealed that nanoparticles in the solution may be correlated with the UV-VIS spectra. As the chlorophylls from *Spinacia oleracea* extract was mixed in the aqueous solution of the silver ion complex, it started to change color from incolor to weak yellow-brown (Fig.6.a); and the color of gold ion complex, it started to change in ruby red colour (Fig.6.b), which indicated formation of silver and gold nanoparticles [14,15,25].



a) b)

Figure 6. a): an aqueous solution of silver nanoparticles (weak yellow-brown color) in diethyl ether, b): an aqueous solution of gold nanoparticles (ruby red colour) in diethyl ether.

UV-VIS spectroscopy is well known to investigate shape and size controlled of nanoparticles. UV-VIS spectrophotometry of the colloidal solution of silver and gold nanoparticles has been recorded as a function of time by using a quartz cuvette with distilled water as reference.

FT-IR measurements show the fact that the protein is the possible biomolecule for the reduction and capping of the biosynthesized Ag and Au nanoparticles [22].

5. CONCLUSIONS

The bio-reduction of aqueous Au and Ag^{+} ions by the pigments from *Spinacia oleracea* leaf extract has been demonstrated. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability. The use of this inoffensive plant *Spinacia oleracea* has the added advantage that this plant can be used by nanotechnology processing industries.

Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials).

Synthesized nanoparticles show a good stability in aqueous suspension even after several months of storage, indicating that no nanoparticle aggregation occurred. Gold nanoparticles absorb and scatter radiation in the region of visible light, which gives them the property can be used successfully in photodynamic therapy, as photothermal agents, studies in this direction is currently underway [26].

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