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Article in Asian Journal of Chemical Sciences · January 2022

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# Biogenically Synthesized Silver/Gold Nanoparticles, Mechanism and their Applications: A Review

Stephen Adongo Odongo <sup>a</sup>, Solomon Omwoma Lugasi <sup>a\*</sup>,  
Fredrick Oluoch Okumu <sup>a</sup>, Martin Opiyo Onani <sup>a</sup>, Silas C. Lagat <sup>a</sup>  
and Stephen Gaya Agong <sup>b</sup>

<sup>a</sup> Department of Physical Sciences, Jaramogi Oginga Odinga University of Science and Technology, P.O.Box 210-40601, Bondo, Kenya.

<sup>b</sup> Department of Plant, Animal and Food Sciences, Jaramogi Oginga Odinga University of Science and Technology P.O.Box 210-40601, Bondo, Kenya.

## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

## Article Information

DOI: 10.9734/AJOCS/2022/v11i219116

## Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/82790>

Review Article

Received 12 December 2021  
Accepted 15 February 2022  
Published 28 February 2022

## ABSTRACT

Green nanoparticle synthesis is a vital branch in nanotechnology. These nanoparticles are synthesized with the aid of phytochemicals in plant extracts. The phytochemicals also stabilize the synthesised nanoparticles eliminating the use of toxic capping agents. Silver and gold (Ag NPs, Au NPs) green nanoparticles are common. They have wide applications in areas such as diagnosis, drug delivery and therapeutics. Despite their great applications, particle agglomeration greatly hinders their usage. As such, we explore various synthetic methods used to obtain green nanoparticles. Reaction mechanisms of the phytochemicals and precursor metals used to obtain the nanoparticles are studied in detail so as to get to the core of the problem. Use of broths obtained by boiling fresh plant leaves, stem, roots, bark or peels of fruits is the most widely used synthetic pathway. Reaction temperature, pH and metal concentration are the crucial factors controlling agglomeration and particle size. Furthermore, incorporation of sunlight in the synthetic pathway was found to be economically important. The functionality of the as-synthesized nanoparticles can be modified through careful selection of the plant material used. Specifically, use of ethnomedical plants such as *Azadirachta indica* leaves and *Hibiscus rosa-sinensis* among others

\*Corresponding author: E-mail: solomwoma@yahoo.com;

is documented to produce nanoparticles with therapeutic functions. It is desirable to obtain small size non-agglomerated green nanoparticles as the size of the nanoparticles affects their antimicrobial activity. In addition, smaller nanoparticles are more effective in drug delivery.

*Keywords: Green nanotechnology; silver nanoparticles; gold nanoparticles; phytochemicals.*

## 1. INTRODUCTION

Green nanotechnology involves the development of metal nanoparticles so as to minimize potential environmental and human health challenges associated with the manufacture and use of nano materials. It encourages replacement of existing products with new nano-products which are environmentally friendly. Various biological materials and plant parts such as leaves, stems, seeds, peels and roots act as natural source of raw chemicals which facilitate reductive synthesis of metals from a metal salt solution. Result of this is biocompatible nanoparticles which are synthesized using cost effective methods [1]. The nanoparticles of small size have a large surface-area-to-volume-ratio which give them remarkable properties such as mechanical properties, biological, optical, catalytic function, melting point, absorption, thermal and electrical conductivity as compared to their larger material counterparts. The plant extracts have phytochemicals which act as stabilizers as well as capping agents. This minimizes agglomeration of the nanoparticles synthesized. The choice of reduction mechanism determines the size and stability of the nanoparticles produced. The nature of bonding formed between the phytochemicals of the plant extract and the metallic nanoparticles determines the stability of nanoparticles [2]. The objective of this review is to provide a systematic insight into green synthesis of Ag NPs and Au NPs, mechanisms of syntheses and applications of the nanoparticles. This will provide requisite knowledge to comprehend mechanisms of biogenic syntheses of metal nanoparticles and antimicrobial activity of the synthesized metal nanoparticles.

### 1.1 Nanoparticles

A nanoparticle is any material with its dimensions in nanometer scale or at least one of the dimensions is approximately ~100 nm. Nanoparticles exist in different sizes, shapes, mono-dispersity and morphology which are different from their larger counterparts. For instance, nanoparticles develop shapes that include spherical, triangular, cubical, pentagonal,

rod-shaped, shells, ellipsoidal among others (Fig. 1 refers) [3]. Their application in various fields of medicine, electronics, chemistry as well biotechnology is based on the nanoparticles' distinctive optical, magnetic, and catalytic properties.

There are two major groups of nanoparticles; organic and inorganic. Organic nanoparticles are preferred to the inorganic nanoparticles because their synthesis processes are more friendly in terms of cost and the resulting nanoparticles are environmentally friendly. The choice of method to be used for synthesis depends on the properties of the material used for reduction, type of nanoparticles to be synthesized and the intended application of the synthesized nanoparticles. This will further influence the properties of the prepared nanoparticles [4]. The parameters that influence synthesis need to be optimized since they ultimately affect characteristics of the desired nanoparticles [4]. Carbon nanoparticles are also considered organic nanoparticles while gold nanoparticles, silver nanoparticles, magnetic and semiconductor nanoparticles are considered inorganic nanoparticles [4].

Gold nanoparticles can be used as drug-carriers and in drug-delivery because they encapsulate large volume of molecules which can be used therapeutically. For that reason, non-Newtonian nanofluid using blood with nanoparticles have shown through research study that nanoparticles can be used in drug administration [5,6]. Gold nanoparticles have shown that they can be used to inhibit or activate growth of blood vessels [5]. This has helped since drugs used to increase or inhibit growth of blood capillaries in some diseases, only work for a short period.

Addition of nanosized particles to a base fluid has been used to increase heat transfer. Various industries have applied this technique for example in microchannel cooling and in systems where heat is recovered [6-8]. In a research study, Least Square Method (LSM) was applied to solve the problem of laminar flow in nanofluid and heat transfer in walls that had been made porous in the presence of a uniform magnetic field. The results showed that the method had

good agreement with figures of results and further generalized that by applying magnetic flux, the velocity of fluid within the channel decreased while the maximum temperature went up [8]. Similarly, a study on the volume fraction of nanofluid concluded that the coefficient of convectional heat transfer when nanoparticles are added to pure working fluid increased [6]. Further, the study concluded that water-Alumina nanofluid showed that fluid velocity as well as transfer of heat had improved within the geometry that had been considered for that study [6,7].

## 1.2 Synthesis of Green Nanoparticles

There are various syntheses techniques developed for formulation of nanoparticles. These include physical, chemical as well as biological techniques. Biogenic synthesis of metal nanoparticles using various parts of the plants has been considered simple, cheap, dependable, reproducible and friendly to the environment. This has led to the genesis of green synthesis of nanoparticles which offers an alternative route utilizing the natural ingredients present in plant extracts. Green synthesis therefore provides a reliable alternative route for synthesis of metal nanoparticles.

Use of parts of plants for biogenic synthesis have been found to produce metal nanoparticles that are more stable compared to microorganisms. This makes the plant parts more useful for large scale synthesis of the nanoparticles [12]. Plants

have been found to have phytochemicals which act as reducing agents for the syntheses. The oxidants present in the phytochemical constituents in the various parts of the plants (leaves, peels, seeds, stems and fruits) are very vital during reduction of the metal salts. The use of plant parts in green syntheses of metal nanoparticles therefore creates a symbiotic relationship between the ethnobotany and nanotechnology hence green technology which is highly sustainable and environmentally safe [13].

In a study, biogenic synthesis of silver colloidal particles for sensing *dl*-alanine was done using aqueous fruit extract of *Physalis peruviana* and 1 mM Ag NO<sub>3</sub> with synthesis taking nearly 2 hrs at a temperature of 28°C [14]. In another study, biogenic synthesis of nanoparticles of silver using calyx of *Physalis peruviana* fruit was done under different light source conditions. Plants materials have also been utilized in synthesizing crystalline magnetite nanoparticles [15]. Green tea extract has also been used in synthesis of nanoparticles of iron [16]. Crystalline monodisperse magnetite (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles have been synthesized using leaf of carob [17]. Magnetite nanoparticles of average diameter of 8 nm were obtained using aqueous ferrous chloride tetrahydrate and ferric chloride hexahydrate in the ratio of 1:2. [17]. Green synthesis of different types of nanoparticles from plant extracts has also been reported, among them are plant extracts from *Aloe Vera* leaves [18] and aqueous leaf extract of *Ananas comosus* [19].

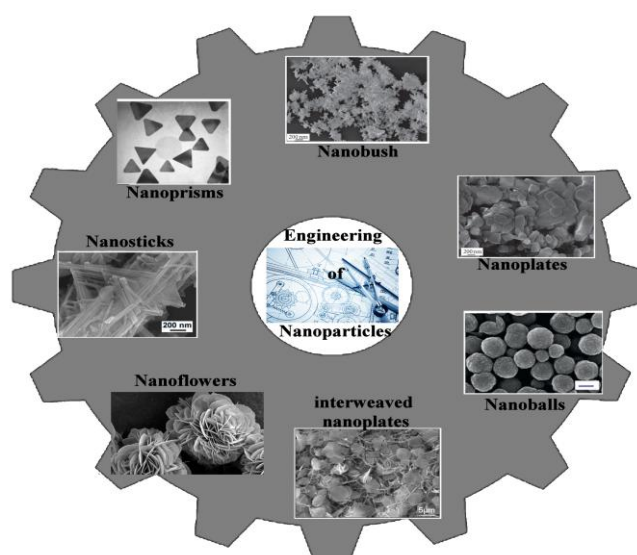


Fig. 1. Engineering of different shapes of nanoparticles [9-11]

In a study, fresh *Ixora coccinea* aqueous extract of leaves were green synthesized for Ag NPs. The particles were spherical in shape with an average size of the particle being 13-57 nm [20] while in another study, spherical small sized Ag NPs that ranged between 5-35 nm were synthesized using aqueous extract of leaves from *Ficus religiosa*. These nanoparticles were found to be effective for treatment of Dalton's ascites lymphoma in a model using mice [21].

Using biological techniques of synthesis, it's easier to control size, shape and distribution of nanoparticles by optimizing synthesis parameters. Different precursors which are mostly plant extracts and temperature ranging from ambient temperature to between 25 °C and 100 °C have been optimized for synthesis [22]. Other than that, pH optimization ranging from 3 to 11 has been used with the most preferred condition being alkaline [22]. Moreover, the concentration of plant extracts equally plays a role and in most cases is serially diluted from the stock solution. Higher concentration of metal salt during synthesis reduces the cell free extract concentration which ultimately result in reduced capping and stabilization ability of the plant extracts. In a study to determine effect of concentration on shapes and sizes of nanoparticles using two concentrations of 250 mg/l and 500 mg/l, different temperatures were used; 30, 40 and 50°C were observed with 10 % cell free extract. The results showed that smaller particles were obtained at lower concentration i.e. 250 mg/l [23].

### 1.3 Green Synthesis of Silver and Gold Nanoparticles

Metallic gold can be reduced to gold nanoparticles (Au NPs) by a variety of reducing agents. Green synthesis may involve the synthesis of Au NPs using biological means such as fungi, microbes as well as extracts of parts of the plants. Synthesis of Au NPs involving plants is preferred to others because synthesis of metal nanoparticles is considered simple, fast, eco-friendly and potentially more biocompatible.

The Au NPs have many varieties of applications, which includes cancer hyperthermia treatment, surface-enhanced Raman spectroscopy and infrared radiation absorbing optics. As a result of this, a variety of synthetic procedures for the synthesis of various shapes and sizes of Au NPs have been reported. The use of plant extracts has shown a lot of success in growing

nanoparticles. Research studies have employed use of broths obtained from boiling fresh plant leaves for green synthesis. For instance, Au NPs have been prepared using several plant extracts that include *Azadirachta indica* (Neem) leaves, *Hibiscus rosa sinensis*, *Ocimum tenuiflorum*, *Mentha spicata* leaves, and *Citrus sinensis* among others.

Studies show that the antioxidants in these plant extracts aid in reduction process during synthesis and consequently capping, nucleation as well as stabilization of Au NPs [24]. The flavonoids, phenols, alkaloids, tannins, steroids among other metabolites have been responsible for reduction and capping of the metal nanoparticles [25]. Moreover, vitamins, terpenoids, reducing sugars and metabolites with antioxidant activities have been of use in synthesis of nanoparticles [26]. Some studies have also shown that plants rich in polyols as well as hydroxyl and carboxylic groups are key players in synthesis and may also act as reducing and stabilizing agents. As much as the active components may be working synergistically, the metabolites may affect the pharmacokinetic properties, safety as well as efficacy of the plants and hence the metal nanoparticles synthesized [27].

The use of sunlight in synthesis of Au NPs has been considered novel considering that solar energy is the largest carbon free energy which is renewable, cheap and does not leave any residues in a chemical process [28]. Photochemical method has the following advantages: it reduces on excesses of reducing agents considering that reduction of the metal ions can be controlled. Even in the presence of solutes and products that absorb light, radiation could still be absorbed. There is also uniformity in the reduction process. Finally, reaction rate is defined since the reducing equivalents which are brought about by solar is specific [29]. The photochemical method does not require any specific instrument for synthesis making it cheap and reliable.

It's reported that size controlled Au NPs were synthesized using citrate under influence of sunlight in which the plant extract acted as a stabilizer [28]. Similarly, Pienpinijtham while working with his co-workers demonstrated that starch could be used as a reducing agent as well as a stabilizing agent in the synthesis of Au NPs under the influence sunlight irradiation [30]. In this synthesis, solar energy was used to reduce the gold salt and hence produce anisotropic Au

NPs. Synthesis at room temperature of gold nanoparticle has also been employed and has been considered very cheap, fast and reliable. The synthesis of the Au NPs was done at room temperature and sunlight mediated.

Synthesis of Ag NPs has been of great interest considering its stability and antimicrobial abilities [31]. Their surface chemistry, size, shape, morphology, distribution and stability among characteristics of Ag NPs influence its biological activities. Silver nanoparticles have been of use in cancer identification and therapy because of their antioxidant and antibacterial properties. [32]. Biogenically synthesized Silver nanoparticles have been found to have better antibacterial activity compared to those synthesised chemically. This is attributed to the protein materials present in plant extracts that coat the silver nanoparticles [33].

Methods such as hydrothermal, microwave-assisted, photo-irradiation and sonication have been employed when it comes to biogenic synthesis of silver nanoparticles (Ag NPs) [34]. Plant extracts of *Lantana camara* have been used in synthesis of Ag NPs using ultra sound reflux and microwave methods [34]. Other plant extracts used in a similar study include *Malus domestica*, *Bergenia ciliata*, *Zizyphus xylopyrus*, *Clitoria ternatea* and *Solanum nigrum*, *Nyctanthes arbor*, *Zelanicum* bark, *Sargassum angostifoliu*, and apple extracts [34].

#### 1.4 Nanoparticle Synthesis Mechanism

Biogenic synthesis of metal nanoparticles involves various methods with little information provided on mechanism of their synthesis. Understanding the mechanism of synthesis and biochemical pathways that result to the formation of the metal nanoparticles is necessary for understanding of the processes and hence applications of the nanoparticles. Several hypotheses have been floated for synthesis of nanoparticles. Plants have been found to have bioactive molecules. These include phenols, amino acids, carboxylic acids, aldehydes and ketones. They are responsible for reduction and capping for the stability of the synthesized metal nanoparticles [35]. Another method involves photosynthesis which have also been used in synthesis of nanoparticles, However, its mechanism is not well illustrated [36].

It's postulated that silver ions can be reduced to silver using terpenoids present in the leaf extract

and themselves oxidized to carbonyl group. The FTIR analysis supported this synthesis [37]. The same plant was used in synthesis of gold nanoparticles. The results showed that proteins present in the plant assisted in the capping of the particles. Broth of neem leaf was also used in another study to synthesize pure metallic as well as bimetallic nanoparticles. Reducing sugars in the plant reduced the metal ions to metal during synthesis with terpenoid and flavonoid being responsible for nanoparticle stabilization. Lemon grass scientifically known as *Cymbopogon flexuosus*, was also used in biogenic synthesis of gold nanoparticles. Triangular-shaped gold nanoparticles were obtained after reduction using reducing sugars (aldoses). It was also discovered that aldehydes/ketones equally affected the shapes of the nanoparticles making them liquidlike which can change to solid at room temperature [38]. Tamarind leaf broth was also used in biosynthesis of gold nanoparticles to investigate capping of nanoparticles and stabilization of the gold nanoparticles using tartaric acid [39]. In a study on synthesis of gold nanoparticles inside living plants, it was not possible to provide mechanism for the synthesis until after a year later that a study on alfalfa showed that the roots of this plant were able to absorb silver metal as Ag (0). The roots of this plant were absorbing the silver metal from agar medium and transporting to the part of the shoot with changed oxidation state [40].

The Transmission electron microscopy (TEM) analysis of the tissues of this alfalfa plant revealed that the silver atoms accumulated, underwent nucleation in the process of forming the silver nanoparticles. In a similar biogenic synthesis using *Cinnamomum camphora* leaf extract for synthesis of gold nanoparticles and silver nanoparticles, polyols were found to be responsible for reduction of the salts of those metals. However, synthesis of palladium nanoparticles using the same plant showed that heterocyclic components of the plant extract aided in the reduction and consequently stabilization of the nanoparticles synthesized [41].

Functional groups such as amino, carbonyl and sulfhydryl found in cell wall may help in synthesis of gold nanoparticles by use of *Avena sativa* (oats) [12]. In a similar study involving *Chilopsis lineasis* photo extraction using gold salt, spectroscopic methods of X-ray diffraction (XRD) and inductive coupled plasmon (ICP) spectroscopy were applied to determine the

phytoextraction by gold. According to this study, there was reduction of the transported ionic salt within the membrane of the roots of the plants to form the metal element. In extraction of silver nanoparticles using chilli extract [42], a model, “recognition– reduction–limited nucleation and growth” was proposed. In his explanation of the model, process of recognition involved the silver ions getting trapped first within the surface of the proteins in the extract of the chilli through effect of electrostatics. This follows the reduction of the silver ions by the protein molecules leading to the formation of silver nuclei and hence spherically-shaped silver nanoparticles which are stable.

For synthesis of gold and silver nanoparticles using tea leaves, kinetic studies using FTIR and cyclic voltammetry revealed that flavonoids and polyphenols were responsible for the reduction of the metal salts [43]. Shen et al. [44] in their study used polyphenols from tea to synthesize and stabilize platinum nanoparticles of diverse sizes and morphology. The reduction of the Pt ions was done by the polyphenols present in the plant. Use of flavonoids for reduction and stabilization of the nanoparticles was also witnessed when tuber extract of *Curcuma longa* was used in synthesis of Pd NPs [45]. Synthesis using guava leaf extract and fenugreek seed extract showed that flavonoids were responsible for reduction. It was also noted that the carboxylate group in the proteins attached themselves on the surface of nanoparticles as surfactants aiding in electrostatic attraction and stabilization [46].

A study showed that reduction capacity in synthesis of nanoparticles depended more on the metal ions and not on the plant [47]. In the study using *Barssica juncea* which were hydroponically grown showed uptake of the silver salt being dependent on the metals. Synthesis by use of cyclic octa-peptide enzyme in *Jatropha curcus* for reduction and curcain for stabilization was critiqued considering that plant extract are usually heated at higher temperatures that cannot allow the purported enzymes to survive [48]. The study therefore supported the presence of the plant metabolites such as terpenoids, phenolics and flavonoids among others being responsible for reduction and stabilization of the synthesized nanoparticle [49]. Studies also showed that citric acid [50], polyphenols with aromatic rings [51], saponins [52] and polysaccharides [53] have been responsible for reduction and stabilization of the nanoparticles. The amino function can be introduced from the reducing side of the polysaccharide which may assist in stabilization of the nanoparticles. The phenolic groups, amine, amide (I) group and secondary alcohols have been important components in reduction according to Vanaja and Annadurai [54].

A study involving *Garcinia mangostana* rind extract for synthesis of Pt NPs, the FTIR spectrum of the nanoparticles had bands assigned to polyols that included hydroxyl flavones and hydroxyl xanthenes and hence were responsible for the synthesis as reductant and stabilizers of the nanoparticles [55]. A schematic diagram in Fig. 2 shows synthesis of nanoparticles and its mechanism.

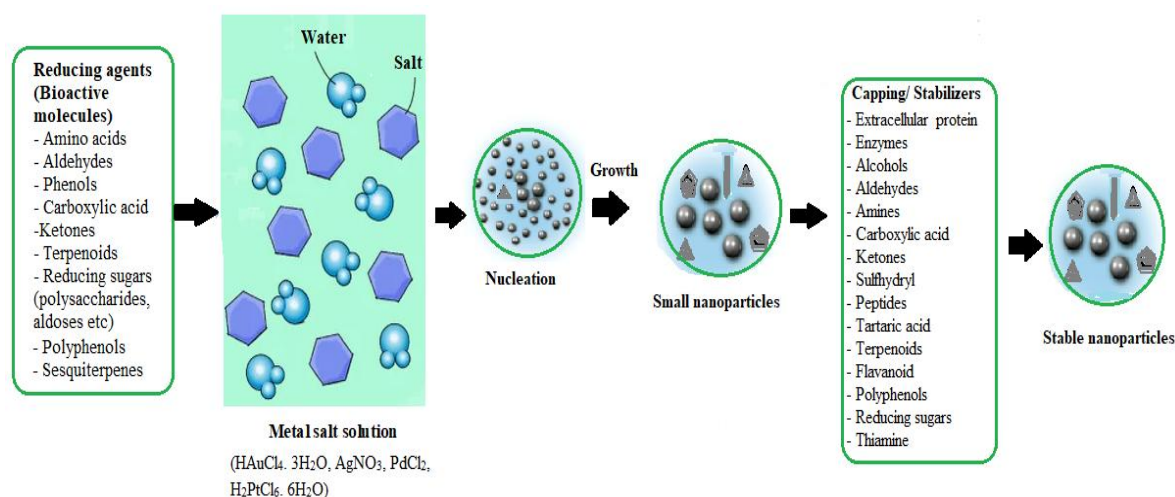


Fig. 2. Synthesis of nanoparticles and its mechanism

### 1.5 Factors Affecting Green Synthesis of Nanoparticles

Green synthesis as any other chemical reactions will always be influenced by a number of parameters. Synthetic protocols and raw materials to be used are of prime interest. The main factors include concentration of the precursor and the plant extract, pH, time of synthesis and ratio of the metallic salt to the plant extract.

### 1.6 pH Effect on Biogenic Synthesis of Nanoparticles

In synthesis of nanoparticles, pH of reaction mixture is very important [12]. Morphology of NPs produced is affected by variation in pH of reaction mixture. Variations of pH was found to influence shapes and size. For instance in a study involving synthesis of gold nanoparticles using oat, the majority of the particles were found to be of different shapes with a substantial number having 20 nm in size [12]. Of importance is the fact that nanoparticles of smaller sizes have better antimicrobial activities than the larger nanoparticles [56]. They find application also in drug delivery and because of their large surface area to volume ratio, they are capable of spreading into the target sites [57].

Different pH ranges have been used for optimization of synthesis conditions and hence synthesis itself. The pH range of 3–9, has been found to be favourable for Au NPs synthesis. Acidic environment such as pH 2 has been found unable to facilitate synthesis. High protonic solution of pH 2 or 3 causes agglomeration of the biosynthesized Au NPs. This means the synthesised Au NPs are more stable at pH range of 5-9, an aspect that is very important in synthesis of Au NPs for medical applications [58].

It was also found that different morphologies of nanoparticles were synthesised at pH of 5 and temperature of 25 °C. As the pH increased from 5 to 11, the spherically shaped nanoparticles increased in their monodispersity at temperatures of 65 °C [22]. The same variation in pH was found to lower the size of the nanoparticles from 9.9 nm to 5.6 nm but when pH of 11 was used, the size of the silver nanoparticles increased to 10.4 nm [22].

Greater absorbance of the UV-vis was measured when pH was increased from 4 to 9 an indication

that absorbance was more in a basic environment with the sizes of the nanoparticles ranging between 20 and 100 nm [59]. The absorbance may also be a measure of the number of synthesized nanoparticles [60].

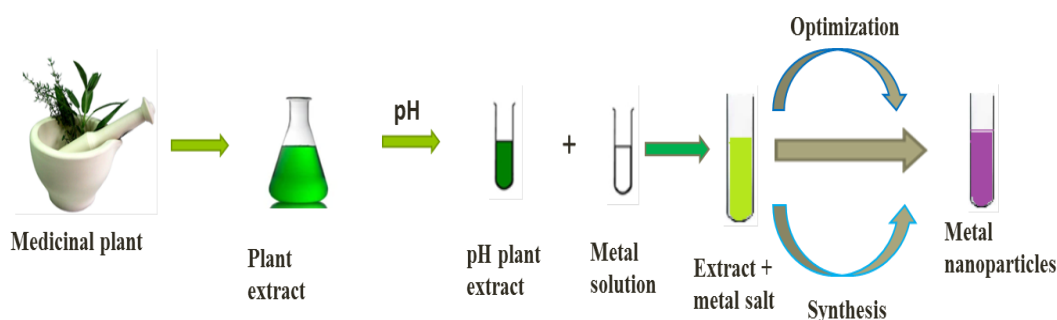
### 1.7 Effect of Concentration

Morphology of nanoparticles has been found to be influenced by the plant extract concentration. A number of research studies show that plant extract concentration affects largely the size as well as shape of synthesized nanoparticles and hence nanoparticles' biological activities [61]. In a study to investigate effect of precursor concentration using *Elaise Guineensis* (Oil palm), a set of six concentrations ranging from 0.51 mM to 4.055 mM were used to determine how it affects size and shape of Au NPs synthesized. Effect of concentration of leaf extract and that of metal salt on size and shape of gold nanoparticles synthesized was evaluated at temperature of 30°C for 60 min [62]. By varying the volume of 1 53 mM gold (III) salt by using volumes of 1 ml, 2 ml, 3 ml, 4 ml and 5 ml with constant volume of 2 ml of plant extract, it was found that ratio of 3 ml showed higher intensity in the surface plasmon resonance. However, at ratio higher than 3:1, there was a decrease in the surface plasmon resonance and this may be attributed to in availability of adequate reducing biomolecules required for reduction and stability of the synthesized nanoparticles. This leads to the red shift in the surface plasmon resonance [62] and hence unstable nanoparticles [63].

### 1.8 Effect of Time and Temperature

Other factors affecting synthesis of nanoparticles are time and temperature. The intensity of color is determined by the time of synthesis. As reaction begins, the rate of reduction of metal ions is usually slow for the first 45 minutes. In addition, the temperature of a reaction is very important when synthesizing nanoparticles since it also determines the morphology of the resulting nanoparticles. Chili *et al.* (2008) demonstrated the effect of time and temperature in the synthesis of Au NPs that are anisotropic using polymer mediated synthesis [64] while Shao *et al.* [65] demonstrated that aspartate mediated synthesis nanoparticles could take place at room temperature and this yielded nanostructures with hexagonal and triangular structures [65]. A similar reaction showed that synthesis at boiling temperatures did not form nanostructures with triangular or hexagonal shape [66].





**Fig. 3. biogenic synthesis of metal nanostructures**

Aggregation of the nanoparticles is a limitation in application of the synthesised nanoparticles [67] because they are unstable [68]. In synthesis of Au NPs using the marine macroalgae *Sargassum muticum* colloids of gold were obtained after 15 minutes with surface plasmon resonance peak around 520 nm. It's worth noting that there was a red shift in wavelength to 550 nm when the reaction time was increased leading to aggregation. In a study, temperature range of 20 – 60°C was used in determining aggregation kinetics of Au NPs. It was evident from the finding that the rate of aggregation of the particles increased with increase in temperature [68]. A study on synthesis of Ag NPs and Au NPs from *Zingiber officinale* root extract showed that the absorbance of the reaction mixture increased as the temperature of synthesis increased from 20 to 50°C [59]. This showed that higher temperatures increase the rate of reduction. The higher the temperature the greater the consumption of the metal salts and the faster the formation of nanoparticles of small size. Gold nanoparticles can equally be synthesized successfully by adopting sunlight irradiation method. Green synthesis of nanoparticles (Fig. 3) involves bottom-up approach for optimization of conditions for synthesis of nanostructures.

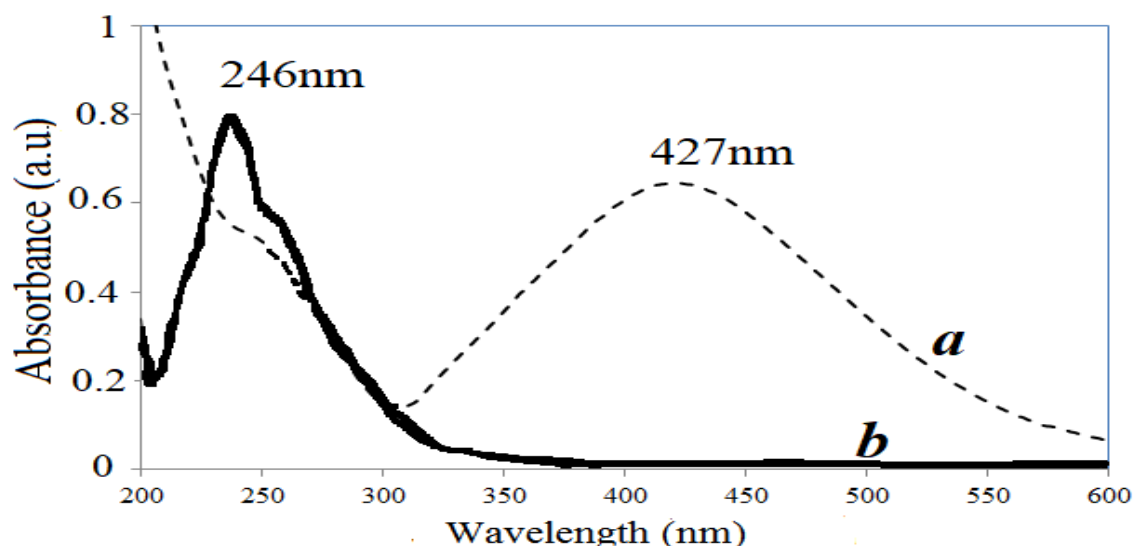
## 2. CHARACTERIZATION OF NANOPARTICLES

The safety, efficacy and bio-distribution of nanoparticles are determined by their physicochemical properties. Characterization helps to evaluate the functionalities of the synthesised nanoparticles. There are several analytical techniques used for characterization. They include UV-vis spectroscopy, transmission electron spectroscopy (TEM), scanning electron

spectroscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffractometry (XRD), dynamic light scattering (DLS), X-ray photoelectron spectroscopy and atomic force microscopy (AFM).

The UV-vis analytical technique is used for determining the synthesised nanoparticles by measuring the surface plasmon resonance by evaluating the collective oscillations of electrons in the conduction band in response to the electromagnetic waves. The UV-vis spectra provide information of stability, size, structure and aggregation of the synthesised nanoparticles. Different metal nanoparticles have different absorbance bands as provided by the spectra produced. For example, the absorbance peak of gold nanoparticles is between 500 nm and 550 nm while that of silver nanoparticles range between 400 nm and 450 nm. Synthesis of Ag NPs using *Lantana camara* employed microwave and infrared techniques and this showed that absorbance peak between 420 nm and 450 nm due to plasmon resonance. Further, the results showed that longer treatment of synthesis time increased the absorbance resulting to higher concentration of the nanoparticles [69].

Red cabbage has been used to synthesise monodispersed spherical silver nanoparticles using hydrothermal method that exhibited antibacterial and antifungal properties against *Staphylococcus aureus*, *Escherichia coli* and *Candida* [70]. Microwave technique was used to produce biogenically synthesised of Ag NPs with a mean size approximately 7 nm with the synthesis taking only 15 min [71]. Fig. 4 shows spectra of UV-visible spectroscopy of *Lantana camara* and silver nanoparticles Ag NPs found from reflux in our laboratory.

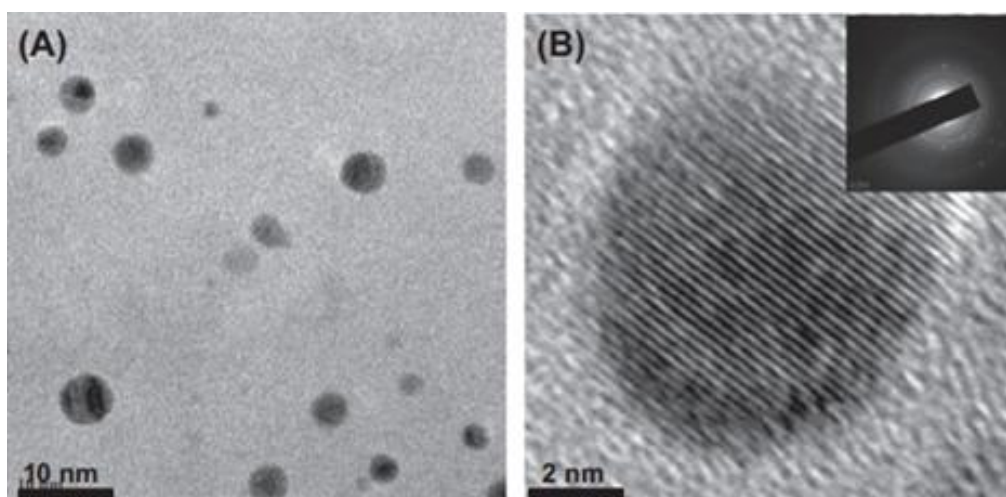


**Fig. 4. Spectra of UV-visible spectroscopy of *Lantana camara* and silver nanoparticles Ag NPs found from reflux in our laboratory (a represents *Lantana camara* extract and b represents Ag NPs) [34]**

*Lantana camara* extract showed a shoulder peak between 300 nm and 400 nm with maximum wavelength being 220 nm. Biogenic synthesis of Ag NPs using *Lantana camara* as a reductant and silver nitrate as the precursor obtained maximum absorbance between 420-450 nm as a result of the plasmon resonance [72] as shown in Fig. 4.

One of robust techniques used for characterization of nanoparticles is the transmission electron microscopy (TEM),

specifically in determination of their shapes, sizes, and morphologies. The technique enables small particles of about  $10^{-10}$  m be measured and their crystallographic structure of the particles at atomic scale obtained through images developed. Using TEM, small particles ( $10^{-10}$  m in size, which is near the atomic level) can be viewed and the crystallographic structure of a sample can be imaged at an atomic scale [73]. Fig. 5 shows transmission electron microscopy images of Au NPs obtained in our laboratory.



**Fig. 5. (A) TEM image of prepared Ag NPs and (B) high resolution TEM micrograph; inset corresponds to SAED pattern [74]. Fig. 6 shows characterization technique**

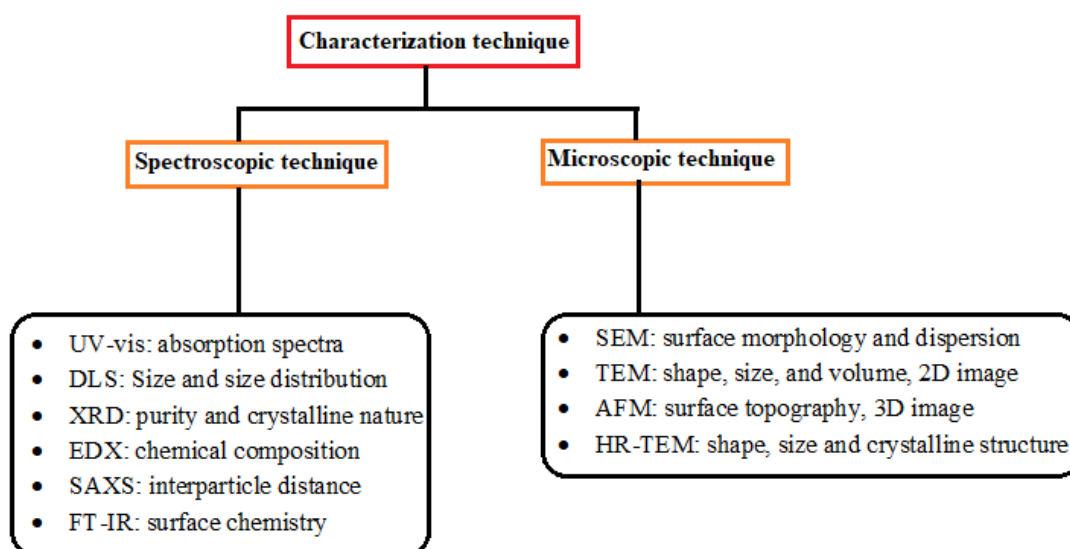


Fig. 6. Characterization technique [75]

### 3. APPLICATIONS OF GREEN NANOPARTICLES

#### 3.1 Green Nanoparticles as Antimicrobial

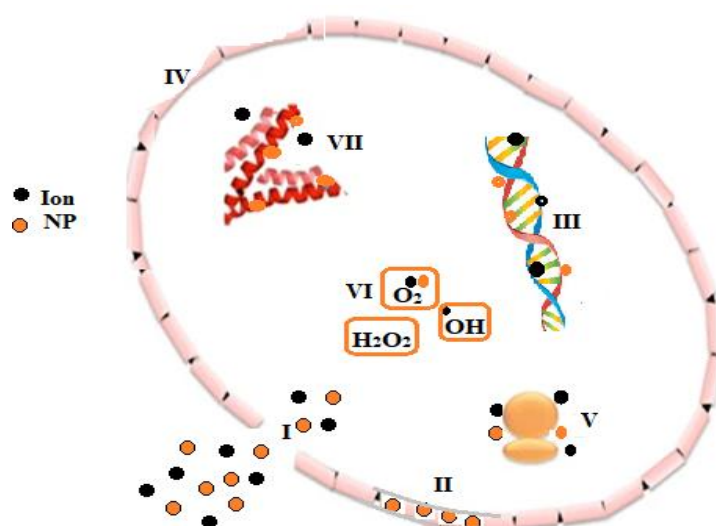
The use of gold for treatment of various disorders dates back to many years ago where gold was used in treatment of arthritis [76,77] with its first biocidal potential exploited by Robert Koch [78]. The antimicrobial activities of nanoparticles is something that has been under a lot of research [79]. As part of its mechanism, the nanoparticles are said to distort the membrane of the bacteria by impeding the electrostatic flux around the microorganism. The Au NPs show cytotoxicity against microorganisms. The functional groups within the surface of the cell of a bacteria leads to its interaction with the nanoparticles that eventually leads to its destruction and ultimate inactivation [80]. This may be caused by the bacteria coating itself on the surface of the nanoparticles or to the contaminants left on the surface of the Au NPs and not on the core of the nanoparticles [80].

There is also the enhancement of the gene expression by the nanoparticles which leads to redox processes that help in management of infections caused by microbes [81]. This is achieved by the fact that the nanoparticles have a large surface to volume ratio, polyvalent and the nature of the nanoparticles being photothermal [82].

In aerobic life of an organism, reactive oxygen species (ROS) are never welcome since they are very reactive and toxic. The reactive oxygen

species are either partially or activated derivatives of oxygen and include singlet oxygen, superoxide anion, hydroxyl radical and hydrogen peroxide. They are capable of initiating oxidative destruction of the components of the cell of the microbes [83,84]. The equilibrium between production of reactive oxygen species and how they scavenge the actual site of the cell at the required time determine their effectiveness in destroying cell of the pathogen [85]. A harmful imbalance is created when the rate of production of the reactive oxygen species overrides the rate at which they scavenge the cells. The imbalance which is referred to as oxidative burst may severely damage cells which also include peroxidation of the lipids of the membrane, important biomolecules of the cells such as proteins and nucleic acids may equally undergo oxidative damage as well as activation of programmed cell death pathway hence peroxidation of the membrane lipids which consequently makes the membranes to disintegrate and allow for the entrance of metals and NPs. Fig. 7 shows how the metal nanoparticles interact with a bacterium (*E. coli*).

For medical applications, Ag NPs are by far the most popular ones [87]. Other than being used as antioxidants and antibacterial agents, the nanoparticles have also been applied in cancer treatments [87]. Furthermore, cytotoxicity of Ag NPs against the HepG2 cells as well as their scavenging ability and antibacterial activity were investigated by measuring the inhibition zones [88]. The results showed that Ag NPs could be used in wound management [89].



**Fig. 7. Metal nanoparticles' interaction with a bacterium (*E. coli*). (I) Cell disintegration (II) Entrance of the metal NPs (III) Interaction of nanoparticles with DNA (IV) Occurrence of Cell pits (V) ROS production and inhibition of ribosome function (VI) production of ROS (VII) Nanoparticles interacting with proteins [86]**

In environmental management, silver nanoparticles have been used in water treatment, purification considering that the nanoparticles show antimicrobial activities [90]. Water treatment may also involve removal of contaminants and acting as a catalyst in managing environmental pollutants [91] and in catalytic removal of environmental pollutants [48].

### 3.2 Use of Green Nanoparticles in Wound Healing

Wound management is a challenge to mankind with two specific types of wounds; chronic and acute wounds [92]. Both types of wounds lower the self-esteem of the patient. Chronic wounds are considered silently epidemic and one is unable to predict the duration it would take for the wound to heal. This results to a lot of infections on the affected skin surface. For the case of acute wounds, they take a predictable period of time to heal which could just be a few weeks. They are caused by incisions and are with patients who pass through surgery, burns of the epithelium tissues or even abrasions. Since the skin is made open, the patient is susceptible to infections caused by bacteria [93].

Maintenance of a balanced moisture content of the wound is given prominence unlike previously where a wound would always be made to dry during healing. In modern way, wound is

managed in such a way that accumulation of exudate is prevented and at the same time a certain amount of moisture content is maintained. This allows for proper regeneration of the skin tissues without major scar developing scarring [94]. Traditional methods used in dressing wounds embraced the idea of absorbing the exudate of the wound making the wound to form a crust on the surface with a scar of reasonable size.

The degree of manifestation by the bacteria and how the body responds to the attack determines the extent to which one is infected [95]. Microorganisms that affect wounds include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococci*. They affect wound in when in a free state or in biofilms where they get attached on the surface and form extracellular network which protect them. The dressing of the wound is meant control infections and maintaining a balanced moisture content thus accelerating wound healing [96].

Nanotechnology has employed various nanomaterials for management of wound infections and thus enhancing rate of wound healing [97]. The biological properties of nanomaterials have made them be used in various fields such as tissue engineering, drug delivery, regenerative medicine among others. Gold nanoparticles and silver nanoparticles have

been found to have antimicrobial activities and hence good candidates for wound healing. The surface plasmon resonance of the Au NPs and Ag NPs are tuned to improve on their thermo-responsiveness which improves their antibacterial as well as healing efficiency both *in vitro* and *In vivo* [98]. Hydrogels and nanofibers have been found to possess surfaces that are hydrophilic which provide a moist environment for wound healing. Nanomaterials can also reduce expression of matrix metalloproteinases inhibiting collagen decomposition. This is important in the remodeling of the extracellular matrix thus promoting deposition of collagen and fibronectin. These processes are of benefit to the wound during proliferation of cell and repair, accelerating the remodeling of the extracellular matrix. This inhibits collagen decomposition. A study on *ex vivo* permeation, showed that Au NPs can also be used in wounds caused by burns preventing microbial infection and enhancing epithelialization of the wounded part [98].

When used for manage bacteria infestation, the nanoparticles attach themselves to the walls of the bacteria and bind to their DNA. During replication or transcription of the cells, the nanoparticles block the double-helix of the DNA from uncoiling thus exerting bacterial and bacteriostatic properties inhibiting the drug-resistant microbes [99]. Moreover, Au NPs are also good antioxidants which prevent the formation of reactive oxygen species which would otherwise slow the healing process of wounds [100].

Mechanistically, wound repair in its initial process involves the granulation tissues accumulating on the wounded part. There is then proliferation of the fibroblasts and new capillaries which synthesize collagen fibers and matrix components. of relies on the accumulation of granulation tissue. Nanoparticles have been found to have the capability of promoting the formation of the granulation tissues and proliferation of fibroblasts [101,102]. Then keratinocytes proliferate and form an integrated epidermis. Nanoparticles have been found to promote proliferation of keratinocytes and speed up the epithelialization of the wounded part *in vivo*. However, the growth of keratinocyte and its differentiation requires Au NPs with a low concentration since higher concentrations of the nanoparticles are cytotoxic [106]. The nanoparticles can also stimulate the production of growth factors that would enhance proliferation

and migration of fibroblasts processes that are vital for wound healing.

#### 4. TOXICITY OF NANOPARTICLES

Toxicity of nanoparticles is a concern especially that of Ag NPs and Au NPs in drug delivery systems. A number of cell/animal models have been carried out to determine the toxicity of nanoparticles [103, 104]. The toxicity concerns Au NPs were found to be insignificant and that the nanoparticles were inert biologically [104]. The toxicity however is found to be size dependent and that nanoparticles of gold below 2 nm showed reasonably high chemical activity on its surface which ultimately will be the cause for side effects as a result of biological applications of the nanoparticles [103]. Two schools of thought arise about the toxicity of nanoparticles and particularly Au NPs. In one school of thought, Au NPs are safe regardless of their size or capping agents whereas in another, the 2 nm sized cationic nanoparticles are toxic and this is dose-dependent unlike their anionic counterparts of the same concentrations [103]. A study was conducted to determine cytotoxicity of Au NPs of 4,12 and 18 nm in size stabilized with different capping against leukemia cells. It was found that Au NPs that were spherically shaped were not toxic. Conclusively, it can be reported that the toxicity of the nanoparticles depend on the dose used, ionic charge of the surface and the stabilizing agent [105].

#### 5. CONCLUSION

It is worth noting that there is no reference temperature or pH for green synthesis of nanoparticles. However, the temperature ranges from 25°C to 100°C with 30°C to 80°C being more viable and environmentally friendly. The frequent pH range utilized for synthesis equally varies from 3 to 11. However, basic pH seems to give better surface plasmon resonance based on the Ultraviolet-visible spectroscopy spectra. The Ultraviolet-visible spectroscopy complements the colours of the expected nanoparticles and helps in the optimization of parameters for synthesis on nanoparticles such as gold and silver nanoparticles. It has been documented how synthesis parameters influence the quality and quantity of the synthesized nanoparticles, their characterization and ultimate applications. Synthesis parameters, pH, temperature, pressure, time, particle size and pore size affect the nature of the nanoparticles and hence their applicability. Those parameters must be

considered when synthesizing Au NPs and Ag NPs of possible biological importance. The stability of the synthesised nanoparticles is equally important which are controlled by compounds from the plant materials. Nanoparticles applications cover many disciplines but are recently popular for disease treatment, wound management as well as in environment management.

## ACKNOWLEDGEMENTS

This study was financially supported by National Research Fund of Kenya (2014/15) and Jaramogi Oginga Odinga University of Science and Technology.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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