

Biosynthesis of gold and silver nanoparticles by natural precursor clove and their functionalization with amine group

Ashwani Kumar Singh · Mahe Talat ·
D. P. Singh · O. N. Srivastava

Received: 2 July 2009 / Accepted: 17 December 2009 / Published online: 6 January 2010
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Abstract We report a simple and cost effective way for synthesis of metallic nanoparticles (Au and Ag) using natural precursor clove. Au and Ag nanoparticles have been synthesized by reducing the aqueous solution of AuCl₄ and AgNO₃ with clove extract. One interesting aspect here is that reduction time is quite small (few minutes instead of hours as compared to other natural precursors). We synthesized gold and silver nanoparticles of different shape and size by varying the ratio of AuCl₄ and AgNO₃ with respect to clove extract, where the dominant component is eugenol. The evolution of Au and Ag nanoparticles from the reduction of different ratios of AuCl₄ and AgNO₃ with optimised concentration of the clove extract has been evaluated through monitoring of surface plasmon behaviour as a function of time. The reduction of AuCl₄ and AgNO₃ by eugenol is because of the inductive effect of methoxy and allyl groups which are present at ortho and para positions of

proton releasing –OH group as two electrons are released from one molecule of eugenol. This is followed by the formation of resonating structure of the anionic form of eugenol. The presence of methoxy and allyl groups has been confirmed by FTIR. To the best of our knowledge, use of clove as reducing agent, the consequent very short time (minutes instead of hours and without any scavenger) and the elucidation of mechanism of reduction based on FTIR analysis has not been attempted earlier.

Keywords Au nanoparticles · Ag nanoparticles · Clove extract · Eugenol · Functionalization · Nanobiotechnology

Introduction

Metal nanoparticles exhibit unique optical, electronic and catalytic properties which are primarily based on their small size and their high surface to volume ratio. The properties of these materials are often different from corresponding bulk materials of the same kind and are usually influenced by the particle size. Metal nanoparticles also show potential for bio sensing (Willems and Van Duyne 2007; Zhao et al. 2006) and cancer therapy (Jain et al. 2007). Gold nanoparticles can act as tiny heaters for enhanced rate of heating (Richardson 2006). Similarly Ag nanoparticles are known to inactivate microbes by interacting with their enzymes, proteins or DNA to inhibit the cell

Electronic supplementary material The online version of this article (doi:10.1007/s11051-009-9835-3) contains supplementary material, which is available to authorized users.

A. K. Singh · M. Talat · O. N. Srivastava (✉)
Nanoscience and Nanotechnology Unit,
Department of Physics, Banaras Hindu University,
Varanasi 221005, India
e-mail: hepons@yahoo.com

D. P. Singh
Department of Physics, Southern Illinois University
Carbondale, Lincoln, Carbondale, IL 62901-4401, USA

proliferation. Gold and silver nanotriangles in particular are promising as they could find potential applications in cancer hyperthermia (Shankar et al. 2004).

Amidst various methods for the synthesis of metal nanoparticles, one approach which is often termed as 'green method of growth of nanoparticles' relies on the use of natural precursor (Shankar et al. 2003; Mandal et al. 2006; Gardea-Torresdey et al. 2002). Shankar et al. carried out extracellular synthesis of Au and Ag nanoparticles employing fungi (Mukherjee et al. 2002; Ahmad et al. 2003; 2005). Recently, synthesis of gold nanotriangles and silver nanoparticles has been demonstrated by using *Aloe vera* plant extract (Chandran et al. 2008).

In this article, studies have been made on the gold and silver nanoparticles through reduction of Au and Ag containing salts namely AuCl_4 and AgNO_3 employing the natural precursor clove as reductant. Clove is called 'Lavang' in India and is used in Ayurveda, Chinese medicines and western herbalism and dentistry. We have used clove extract as reducing agent. Utilisation of clove is justified by its strong reducing capability. We describe and discuss the synthesis of Au and Ag nanoparticles by employing the clove extract as reducing agent for AuCl_4 and AgNO_3 . The reduction time with the clove extract is small (few minutes instead of hours). A mechanism of reduction of AuCl_4 and AgNO_3 has been suggested based on detailed analysis of FTIR spectra. It has been shown that the presence of $-\text{OH}$ in eugenol is able to release a proton and eugenol itself changes into its anionic form. Eugenol acts as a very powerful reducing agent because of the inductive effect of two powerful electron withdrawing methoxy and allyl groups at ortho and para position of $-\text{OH}$ group which enhances the proton releasing power of eugenol.

Experimental details

Preparation of clove extract

In our synthesis protocol, clove extract (*Syzygium aromaticum*) is used as a reducing agent. Clove extract is prepared by dipping 25 g of Clove in 500 mL water for 24 h and then filtering the solid residue leaving the clove extract solution. The FTIR

analysis, to be discussed later, reveals the various chemical components present in clove.

Synthesis of gold and silver nanoparticles

The required chemicals namely chloroauric acid (AuCl_4) and silver nitrate (AgNO_3) purchased from Sigma–Aldrich, were used without any further purification. For the synthesis of gold nanoparticles using clove extract, different volumes of clove extract (5–50 mL) were added to 50 mL of 0.004 M AuCl_4 solution (aqueous) during stirring at 200 rpm. The observation of faint brownish colour within few minutes indicates the formation of gold nanoparticles. After reduction, the solution was centrifuged and nanoparticles were collected and redispersed in water. The centrifugation process was repeated for several times so that other impurities may get washed out. For the synthesis of silver nanoparticles, similar procedure has been applied. Different volumes of clove extract were added to 50 mL of 0.01 M AgNO_3 solution (aqueous) with rigorous stirring at 200 rpm. Effect of the amount of clove extract on the synthesis of gold and silver nanoparticles was monitored by observing the product formed, using UV–visible spectroscopy and transmission electron microscopy (TEM). The UV visible spectra of gold and silver nanoparticles were recorded initially after an interval of 10 min and then after 1 h, on Perkin Elmer Lambda 750S instrument by taking 0.1 mL of reaction mixture, diluted with 2 mL of deionised water.

Characterization of gold and silver nanoparticles

After reduction, gold and silver nanoparticles were precipitated at the bottom of conical flask. This precipitate was washed out twice with double distilled water and then analysed by employing TEM. The samples of gold and silver nanoparticles synthesized using clove extract were prepared by placing a drop of reaction mixture over copper grid and allowing water to evaporate. TEM observations were performed on TECNAI 20 G²-electron microscope operated at accelerating voltage 200 kV. XRD pattern was recorded by X'Pert Pro X-ray diffractometer (PAN-analytical BV, the Netherland with a built in graphite monochromator). XRD patterns were recorded by operating X-ray tube at 45 kV and 35 mA. The radiation used was Cu- $K\alpha$ ($\lambda = 1.5406 \text{ \AA}$). The

details of formation and shape of Au nanoparticle were also analyzed employing Perkin Elmer Lambda 750S UV–Visible spectrometer. The FTIR spectra of clove extract, Au nanoparticles and amine functionalized Au nanoparticles were recorded using Perkin Elmer spectrum 100 instrument.

Results and discussions

Optical image of the clove plant and clove is shown in Fig. 1. The extract of the clove was used for the reduction of chloraurate ions and silver nitrate. Figure 1 also shows the as synthesized gold nanoparticles dispersed in water after the reduction of chlorauric acid with clove at different ratios (1:1, 1:0.2, 1:0.1).

XRD analysis of Au and Au nanoparticles

The as synthesized material after the reduction of AuCl_4 was characterized by X-ray diffractometer for the structural analysis. Figure 2 shows a



Fig. 1 Optical image of clove plant and clove. As synthesized Au nanoparticles solutions after the reduction of AuCl_4 with clove extract at different ratios (1:1, 1:0.2 and 1:0.1)

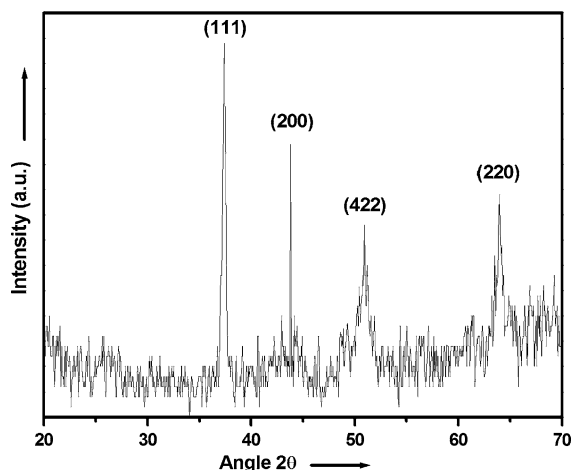


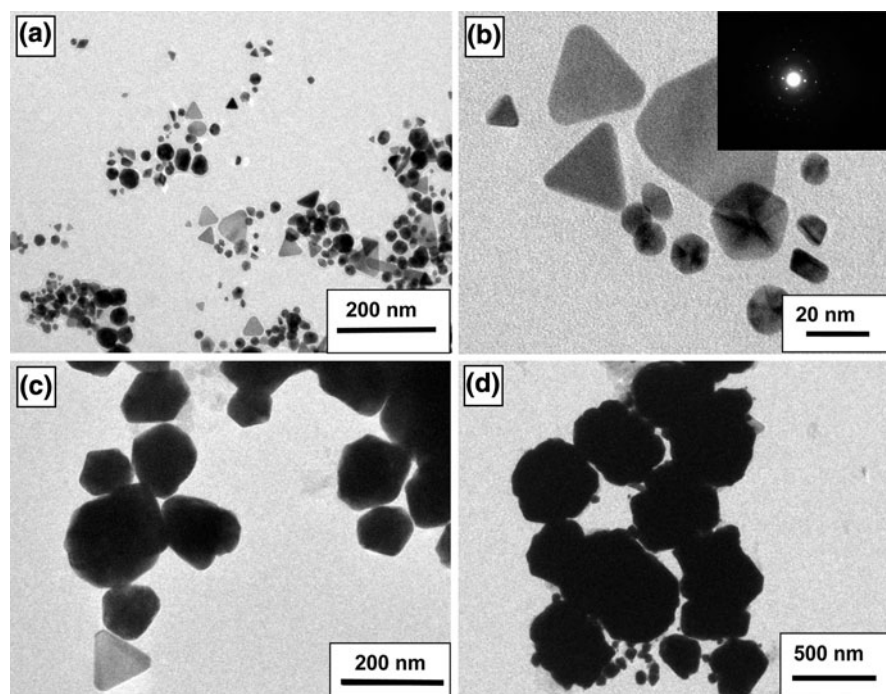
Fig. 2 XRD of Au nanoparticles

representative XRD pattern of the as synthesized material after the reduction of AuCl_4 with clove extract. This figure shows intense XRD peaks corresponding to (111), (200), (422), (220) at angles, 37.45° , 44.68° , 50.99° , 63.95° , respectively. These peaks tally quite well with FCC structure of gold with lattice parameter $a = 4.078 \text{ \AA}$ (JCPDS no. 04-0784). From the broadening of XRD peaks, it can be said that nanosized particles are getting formed.

TEM and UV–visible spectroscopic analysis of Au and Ag nanoparticles

The as synthesized materials were further characterized by Transmission Electron Microscopy for the microstructural analysis. Figure 3 shows typical TEM micrographs of gold nanoparticles synthesized after reduction of AuCl_4 with clove extract in various ratios. Figure 3a represents a typical TEM micrograph of Au nanoparticles at low magnification, synthesized with AuCl_4 to clove extract ratio 1:1. Figure 3b clearly shows the formation of triangular-shaped Au nanoparticles with various other shaped structures. Yield of triangular-shaped particles is high in comparison to the other shapes seen here. This figure also shows the equilateral triangle shaped nanoparticles with average edge lengths of 50–70 nm. Spot like SAED (inset) clearly indicates the formation of highly crystalline Au nanoparticles. On decreasing the ratio of AuCl_4 and clove extract from 1:1 to 1:0.2, the microstructural characterization through TEM reveals the formation of Au nanoparticles as shown in Fig. 3c.

Fig. 3 TEM micrographs of Au nanoparticles, synthesized in various ratios of AuCl_4 to clove extract



This figure reveals that most of the particles are polygonal shaped. Also the average size of the particles is increased in comparison to the particles synthesized with AuCl_4 to clove extract ratio 1:1. When we further change the ratio of AuCl_4 and the clove extract from 1:0.2 to 1:0.1, we found that irregular shaped, large nanoparticles of gold get formed (Fig. 3d). Average size of these nanoparticles is 100 to 300 nm which can be seen in Fig. 3d. From the above discussions, for the formation of Au nanoparticles, synthesized in various ratios of AuCl_4 and clove extract, it can be said that the shape and size of these nanoparticles are highly affected by the ratio of AuCl_4 versus clove extract. The particle size distribution of Au nanoparticles with decreasing the ratio of clove extract with respect to AuCl_4 is shown through histograms as shown in Fig. S1. The analysis of these histogram plots shows that average particle size increases with the decrease of the ratio of clove extract with respect to AuCl_4 . Similar experiments have been performed for silver nanoparticles.

UV–visible spectra of Au nanoparticle were recorded by suspending Au nanoparticle in 2 mL of distilled water. We have carried out comparative experiments to investigate the effect of different amount of clove extract on shape and size of gold and

silver nanoparticles. Figure 4 shows representative UV visible spectra recorded at different time intervals with different ratios of AuCl_4 to clove extract. The inset picture is recorded at the interval of 10 min which reveals the formation of gold nanoparticles within 10 min. In Fig. 4b, c, there are clear peaks at 534 and 564 nm. In Fig. 4a, it appears at 516 nm. These peaks strongly depend on the shape and size of Au nanoparticles. Figure 4a–c shows that there is a sharp increment in absorbance maxima at 516, 534, and 564 nm, for the ratio of AuCl_4 to clove extract, 1:1, 1:0.2, and 1:0.1. From the above UV visible spectrum, it is clear that when we decrease the ratio of clove extract with respect to AuCl_4 , the size of particles increases. This variation in size is evident from the histogram plots (Fig. S1) for various AuCl_4 versus clove extract ratios. Red shift from 516 to 564 nm with decrease of clove extract from 50 to 5 mL can be attributed to overall increment in the size of gold nanoparticles. It may be mentioned that in all the UV–visible plots, there is a discontinuity around 650 nm. This arises due to the change in the position of monochromator at 650 nm used in UV–visible spectrometer.

Similar spectral characteristics have been found for silver nanoparticles and it was found that when

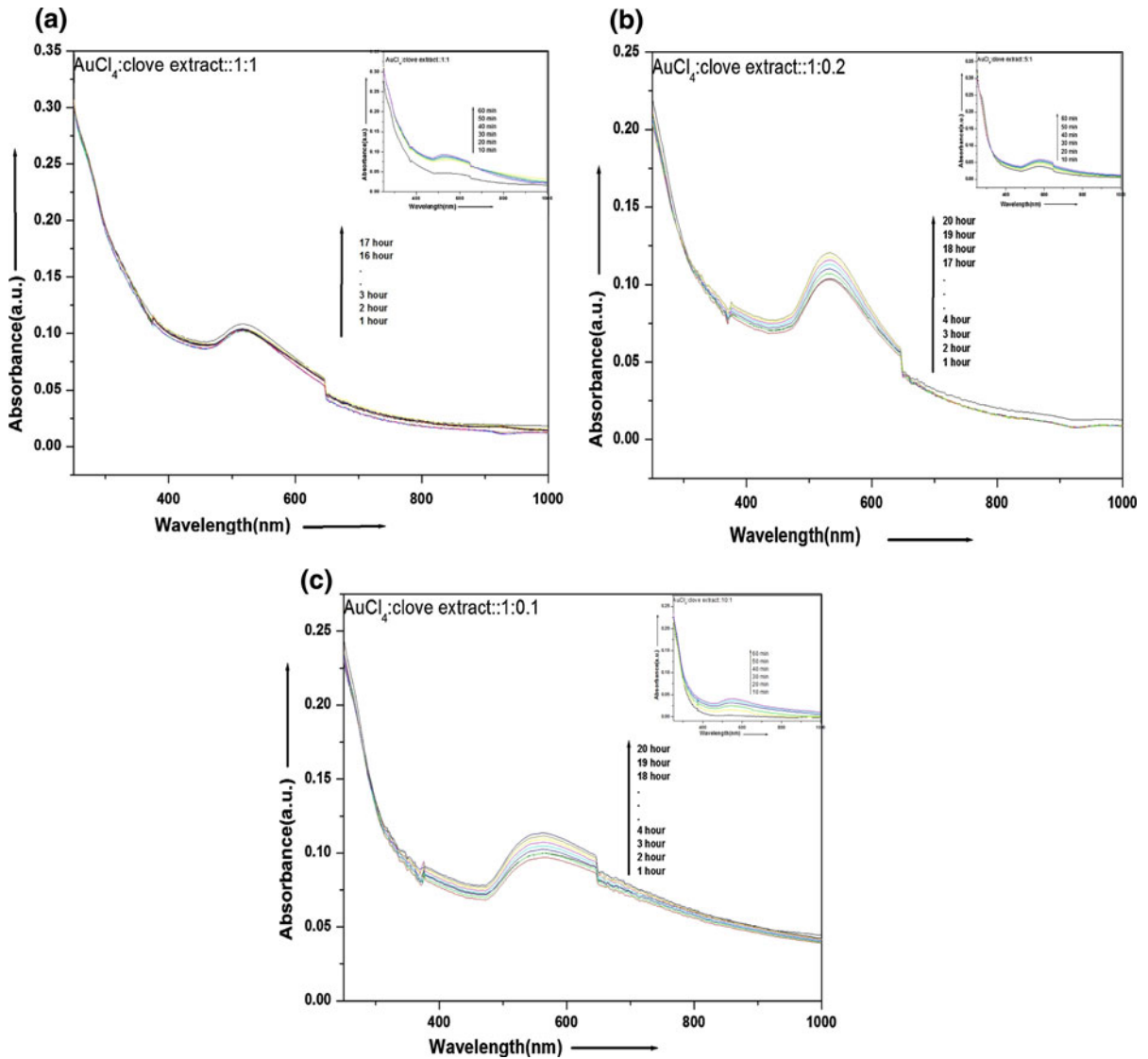


Fig. 4 UV–visible spectra of gold nanoparticles with time

we decrease the amount of clove extract from 50 to 5 mL, absorption maxima shifts from higher to lower wavelength region.

Functionalization of Au nanoparticles

It may be pointed out that chemical functionalization of noble metal nanoparticles makes them worthy of diverse applications. Since amine group is a native component of many biological, medicinal compounds, functionalization with $-NH_2$ group is of obvious importance. Keeping the above said in view,

we proceeded to develop Au NPs-(NH₂) which will correspond to a moiety ready for several applications. Au nanoparticles were functionalized through L-cysteine. For the synthesis of $-NH_2$ functionalized Au nanoparticles; 5 mL of clove extract was added to 50 mL of 0.004 M solution of AuCl₄. After 5 min of reaction, 50 mL of 0.004 M of L-cysteine was added to the reaction mixture during stirring. The sample was further centrifuged and washed. In order to check the successful functionalization, FTIR spectra of the functionalized Au nanoparticles were taken. Figure S2 shows a representative FTIR spectrum. The FTIR

spectrum exhibited intense band at $\sim 1,607$ and $\sim 1,375$ cm^{-1} , respectively which confirmed the presence of N–H in plane (bonded $-\text{NH}_2$ group) and the N=O groups. This implies that functionalization of Au nanoparticles with amine group has indeed occurred. These functionalized Au nanoparticles can readily form stable linkage with biomaterials including medicinal components. They can be covalently linked to biomolecules for various applications like drug delivery, cellular imaging, biomedical diagnostics and translocating the therapeutic molecule.

Mechanism of formation of Au (and Ag) nanoparticles

(a) FTIR analysis of clove extract before and after reduction of Au and Ag salts

On the basis of previous studies on pharmaceutical components from clove (Jirovetz et al. 2006), we found that clove, comprises of total 23 identified components. Among them the most dominant is eugenol (76.8%), followed by β -caryophyllene (17.4%), α -humulene (2.1%), and eugenol acetate (1.2%).

For unravelling the mechanism of formation of Au nanoparticles through the reduction of gold salt, FTIR spectra of the clove before and after bioreduction were taken by employing Perkin Elmer Spectrum 100 instrument.

Typical FTIR absorption spectra of clove extract before and after bioreduction are shown in Fig. 5. It gives information regarding the chemical changes occurring in the functional groups that are exclusively

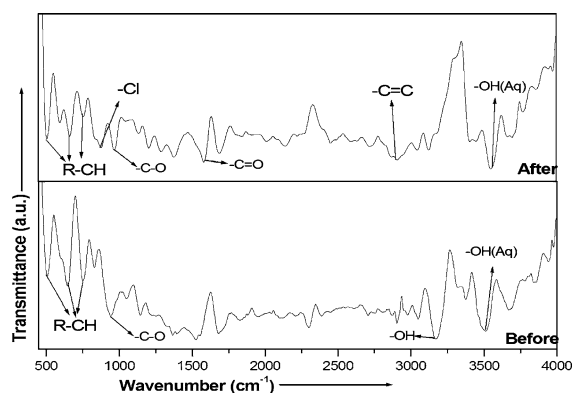


Fig. 5 FTIR spectra of clove extract, before and after reduction

involved in the bioreduction. The changes recorded before and after bioreduction were further utilized to deduce a probable mechanism of the chemical reaction taking place for the bioreduction of Au or Ag ions. One milliliter of clove extract, prepared in double distilled water was employed for recording the FTIR spectra. In order to have a detailed examination of the process occurring after reduction, another spectrum was recorded for the supernatant collected after centrifugation of the solution of the clove extract with Au or Ag nanoparticles. As is clearly visible in the FTIR spectra (Fig. 5), clove extract recorded before adding AuCl_4 or AgNO_3 solution, an intense band is observed at $\sim 3,500$ cm^{-1} . This band is also visible in spectra recorded after bioreduction of Au or Ag ions. Based on the known signature for $-\text{NH}_2$ and $-\text{OH}$, this wavenumber can be taken to confirm the presence of either $-\text{NH}_2$ or $-\text{OH}$ groups. Since the composition of clove extract does not contain any $-\text{NH}_2$ group, the possibility of the presence of this group in clove is ruled out. It can thus be taken clove embodies $-\text{OH}$ group. Other bands which are visible in both the spectra (before and after reduction) in cluster are at $\sim 513, 652, 749$ cm^{-1} . The bands visible at ~ 500 to 749 cm^{-1} signify the presence of R–CH group which is abundantly found in clove extract. Both the spectra also exhibited a band at ~ 900 cm^{-1} which is a depiction of $-\text{C}-\text{O}$, a carboxylic acid derivative which again supports the nature of the chemical composition of clove. The intense band present in the FTIR spectra before reduction at $\sim 3,200$ cm^{-1} , is absent in the spectra recorded after reduction. This is due to the $-\text{OH}$ group present in eugenol. This later disappears after reduction due to the structural changes in eugenol during bioreduction. Another significant band which appeared after bioreduction in the FTIR spectra is at $\sim 1,600$ cm^{-1} . However, this group is not exhibited in the spectra before reduction. A band at $\sim 2,915$ cm^{-1} also appeared after the bioreduction which indicates the presence of $-\text{C}=\text{C}$. Another new band visible at ~ 832 cm^{-1} shows the presence of $-\text{Cl}$ which is derived from AuCl_4 . This is again a strong supporting evidence for the mechanism to be suggested in the next section where the Au ions are reduced by eugenol thereby producing HCl in the medium. The above FTIR results can be summarised as in the following:

- (a) $-\text{OH}$ group originating from eugenol disappears on the formation of Au and Ag nanoparticles.

- (b) C=O , C=C groups appear after the formation of Au nanoparticles.
- (c) Cl group also appears after the formation of Au nanoparticles.
- (d) several other groups, e.g. R-CH , -OH (aqueous) are found both before and after the formation of Au nanoparticles.

Based on the above FTIR results, attempts were made to propose a viable chemical mechanism. This is discussed in the next section.

(b) Reduction of Au and Ag nanoparticles by clove

Chemical composition of clove extract shows the presence of eugenol, which is the main component of clove extract. The FTIR studies show bands at 500 to

750 cm^{-1} , $\sim 3,200\text{ cm}^{-1}$ which confirms the presence of methoxy group, extended conjugated (allyl group) and hydroxyl groups in eugenol. Thus, on the basis of FTIR analysis it can be proposed that in eugenol there is -OH group which is attached on benzene ring. At ortho and para position of -OH group there are two very powerful electron withdrawing groups viz., methoxy and allyl groups, respectively. This is clear from the structure of eugenol (Fig. 6, structure I). It can, therefore, be suggested that due to the presence of -OH eugenol is able to release a proton, eugenol itself changes into its anionic form (Fig. 6, structure II). Furthermore, because of the inductive effect due to the presence of two powerful electron withdrawing groups at ortho and para position of -OH group, proton releasing or reducing power of eugenol will be enhanced because

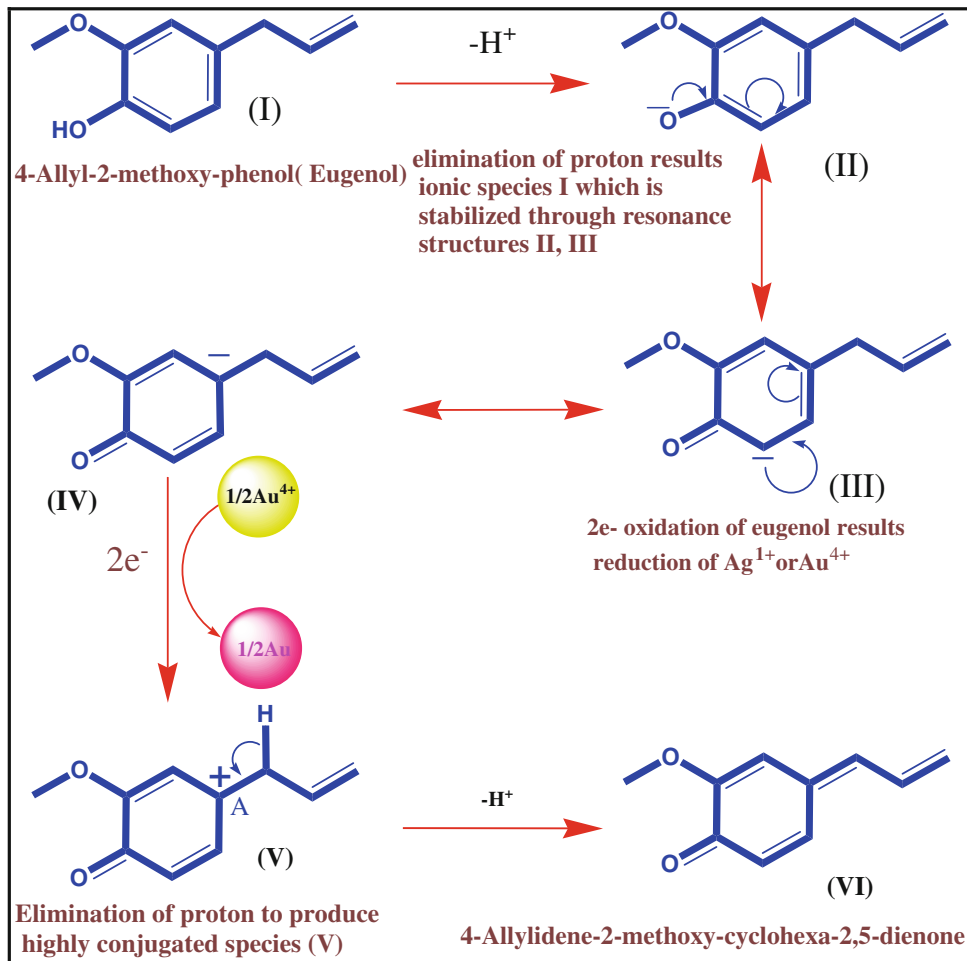


Fig. 6 Schematic representation for the reduction of Au and Ag ions

–OH is a ortho–para directing group which makes eugenol a very powerful reducing agent. In addition to this, after releasing a proton anionic form of eugenol (Fig. 6, structure II) is further stabilized by resonating structures III and IV (Fig. 6). It can thus be taken that as a result of the inductive effect and formation of stable resonating structures of an ionic ion; eugenol is able to release two electrons simultaneously. One electron which already exists at β carbon of structure IV in Fig. 6 is followed by the release of another electron which is due to the high electron cloud at the CH_2 group neighbouring to the double bonds as is clear from the structure IV in Fig. 6. These two electrons will be taken by $\frac{1}{2} \text{Au}^{4+}$ or 2Ag^+ and these ions will get reduced to $\frac{1}{2} \text{Au}^0$ or 2Ag^0 . Finally structure V gets converted into structure VI by releasing one proton (Fig. 6). Formation of structure VI is evident from the FTIR spectra of clove extract recorded after reduction. This spectra shows the signature of $-\text{C}=\text{O}$ and $-\text{C}=\text{C}$ at wavenumbers $\sim 1,590$ and $\sim 2,915 \text{ cm}^{-1}$, respectively. Above discussions is explained schematically in Fig. 6. Considerations similar to the above described for the synthesis of Au nanoparticles apply also to Ag nanoparticles.

Conclusion

There is a growing need to develop clean and nontoxic procedures for synthesis and assembly of nanoparticles in order to reduce or eliminate the use and generation of hazardous substances. But several of the methods employing natural precursors are either tedious or time consuming. We have developed a facile route to synthesize metal nanoparticles like Au and Ag of various shapes and size like spherical and triangular. The rate of reaction for the synthesis of nanoparticles by this method is rapid than the natural precursors including plants mediated synthesis reported earlier. UV–visible spectra of Au nanoparticles give the information about variation of shape and size with the change in the ratio of AuCl_4 to clove extract. The size of particles increases when we decrease the ratio of clove extract with respect to AuCl_4 . Reduction of metal nanoparticles is because of eugenol which is abundantly found in clove which is confirmed from the FTIR spectra. Mechanism for the formation of Au (and Ag) nanoparticles in terms

of ionic species and their stabilization through their resonating structures has been put forward. Metal nanoparticles by herbal approach reported in this study using clove, may have potent application, e.g. in medicine (therapeutic and diagnostics).

Acknowledgements The authors are extremely grateful to Prof. C. N. R. Rao, Prof. A. S. K. Sinha (Chemical Engineering, IT-BHU), Ashish Singh (Department of Chemistry, BHU), Kumari Bhoomika and Samantha Pynngrope (Department of Bio-Chemistry, BHU) for their encouragements and fruitful discussions. The authors acknowledge with gratitude the financial support from DST: UNANST, Council of Scientific and Industrial Research (CSIR), University Grant Commission (UGC) and Ministry of New and Renewable Energy, New Delhi, India.

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