

## Size tunable gold nanoparticle and its characterization for labeling application in animal health

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### Abstract

**Aim:** The aim of the present study was to synthesize different sizes of gold nanoparticles (GNPs) and their characterization for use as a label in lateral flow assay particular for the detection of bluetongue in small ruminants.

**Materials and Methods:** Size controlled synthesis of GNPs was done by using different concentration of sodium citrate. In this study, five different types of GNP were synthesized by using trisodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7\cdot 2\text{H}_2\text{O}$ ) that reduces 20 mM concentration of gold solution ( $\text{HAuCl}_4$ ). These different types of GNPs were characterized in terms of morphology, size, shape and  $\lambda_{\text{max}}$  by transmission electron microscopy and ultraviolet-visible spectroscopy respectively.

**Results:** In the present work, it was found that the size of GNP mainly depends upon the concentration of sodium citrate. By use of 0.09375%, 0.1875%, 0.375%, 0.75% and 1.5% of sodium citrate solution, GNPs were synthesized. In our study, the size of GNP was found ranging from 25 nm to 230 nm. The size was found large with less concentration of sodium citrate (i.e. with 0.09375%) and small with large concentration of sodium citrate (1.5%) and  $\lambda_{\text{max}}$  was found to be 450-530 nm in all size of GNP.

**Conclusions:** The size of GNPs is mainly dependant on the concentration of trisodium citrate, gold salt concentration, optimum pH and temperature. The GNP synthesized by this method has been used as a label for the development of lateral flow assay against diagnosis of bluetongue disease in small ruminant.

**Keywords:** gold nanoparticles, size controlled synthesis, sodium citrate, tetrachloroauric acid, transmission electron microscopy.

### Introduction

Nanotechnology is an interdisciplinary branch of science that deals with the materials of nano sizes (1-100 nm). Gold nanoparticles (GNPs) are probably most extensively studied nanoparticles that are used as labels in lateral flow assays due to its easy preparation, nontoxicity and small particle size [1]. GNPs are most compatible for preparation of nano-platforms in smart sensing devices [2]. So they are extensively used from the field of nanomedicine to the field of nanosensing [3]. GNPs as colorimetric sensor or probe have been widely used for several analytes such as heavy metal ions mainly  $\text{Hg}^+$  ion [4] and biomolecules mainly DNA [5]. GNPs conjugated oligo-nucleotides were used for detection of DNA, RNA and proteins [6-8] by exploiting their surface plasmon resonance properties. Due to their high surface enhanced Raman scattering properties, GNPs can be used as a quencher to detect a single base DNA mismatch [9]. These properties of GNPs are mainly determined by their size, shape, composition and structure [10]. GNPs with diameter of 15-20 nm can be synthesized from tetrachloroauric acid with citrate reduction method [2,11]. The GNPs with 30-230 nm

size have attracted attention for fabrication of smart sensing devices in biomedical sciences as diagnostic tools [12]. GNPs are very much compatible with antibody or antigen and other biomolecules due to negatively charged citrate capping on the surface [13,14]. Therefore, surface functionalization of GNPs is now the main concern in various immunoassays [15]. GNPs can be used as diagnostic reagents by exploiting several conjugation chemistries [16]. By utilizing gold-thiol interaction, the functionalized GNPs with bluetongue virus (BTV) multiple peptide antigens were developed to diagnosis this disease [17]. GNPs based transfection agents have the potential target to deliver the gene for selective killing of cancerous cells both *in-vitro* and *in vivo* [18].

To fulfill all the above advantages, GNPs should be produced with most effective synthetic strategies [19,20]. GNPs of various shape and size can be synthesized in both aqueous and organic medium [21]. But due to inexpensive and nontoxic property of citrate, the citrate reduction method has remained the best option for synthesis of GNPs [22]. It has been shown that the size variation of GNPs can be controlled by changing the concentration of sodium citrate [23].

The aim of this study is to know the effect of the concentration of citrate to the size of the GNP. Here, different sizes of GNPs, i.e. from 25 nm to 230 nm were synthesized by changing the concentration of citrate.

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## Materials and Methods

### Ethical approval

The primary objective of this study was to synthesize different GNPs for labelling application in animal health. So not necessary for ethical approval, as this experiment was conducted from various chemicals.

### Materials

The various chemicals used in this study were tetrachloroauric acid trihydrate ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ) was procured from CDH (India), trisodium citrate from Sigma (USA), bovine serum albumin (BSA) from Amresco (USA), polyethylene glycol (PEG) from Merck (India), sodium azide ( $\text{NaN}_3$ ) from Spectrochem (India), sodium chloride ( $\text{NaCl}$ ) and tris hydrochloride from SD Fine Chemicals (India) and Milli Q water.

### Size controlled synthesis of GNPs

In this study, GNPs were synthesized by citrate reduction method [2,11]. 1 g of tetrachloroauric acid (49%) was dissolved in 49 ml of Milli Q water. From this, 400  $\mu\text{l}$  of gold solution was taken in a beaker and adjusted to 50 ml by adding Milli Q water to make the concentration the gold solution of 20 mM. Different concentrations of sodium citrate solution i.e. 2 ml of 1.5%, 0.75%, 0.375%, 0.1875% and 0.09375% were prepared by taking appropriate amount of it with Milli Q water. The above 20 mM prepared gold solution was heated into boiling point on the plate cum magnetic stirrer. After 15 min of its boiling, the above prepared sodium citrate solution was added and continuously stirred while for boiling. After 5 min of boiling, the colour of solution was changed from yellow to white, and then to black. The boiling was continued until the colour was changed to the deep red colour. This procedure was followed for preparation of the above GNP solution using above five concentrations of sodium citrate solution. After the preparation, the five different sizes of GNP solution were packed in labelled air tight beaker after cooling under water bath. The GNP solutions were kept at 4°C for further use. The colour variation of five different sizes GNP solution was shown in Figure-1a-b.

### Characterization of GNP

The size and shape of synthesized GNPs were characterized using transmission electron microscopy (TEM), and absorption spectra of gold solution were taken by ultraviolet-visible (UV-VIS) spectroscopy. The TEM was done on Phillips CM10 at All Indian Institute of Medical Sciences, New Delhi. The above five GNP samples were first sonicated for 10 min to prevent the particle aggregation. A drop of each sample was put on the carbon coated copper grid. The film was allowed to dry for 15 min, and excess solution was removed using blotting paper. The size of each nanoparticles was measured from enlarged images of TEM by taking different counts for each angle.



**Figure-1:** (a) Freshly prepared gold nanoparticles (GNPs) solution from 1 to 5(l) using 0.09375%, 0.1875%, 0.375%, 0.75%, and 1.5% of sodium citrate used respectively, (b) Colour variation of different types GNPs solution from 1 to 5(l) using 0.09375%, 0.1875%, 0.375%, 0.75%, and 1.5% of solution citrate used respectively.

The absorption spectrum of above five GNP solution was done to know their  $\lambda_{\text{max}}$ . The optical properties of GNPs were performed by single beam U/V spectrometer (Spectromax) at the different wave length (200-700 nm) in central instrumentation facility, IVRI, Izatnagar, Bareilly, UP (India).

### Concentration of gold solution

The concentration of gold was kept constant. The concentration of gold used in this study was 20 mM. This concentration of gold was optimum to get GNPs of uniform in size [2].

### Concentration of sodium citrate solution

In this study, the effect of the concentration of sodium citrate on the GNPs by keeping the concentration of gold solution constant, was investigated. Here we used five different types of sodium citrate solutions i.e. 0.09375%, 0.1875%, 0.375%, 0.75%, 1.5%. The effect of concentration of sodium citrate on the size of GNP was investigated in this part of study.

### Conjugation of GNPs

In this study, the above synthesized GNPs were conjugated with antisheep immunoglobulin G (IgG) by physical adsorption method which is a pH dependant method. First, the antisheep IgG 50  $\mu\text{g}/\text{ml}$  was prepared by diluting it with 5 mM  $\text{KH}_2\text{PO}_4$  (pH 7.5) to a final volume of 100  $\mu\text{l}$  i.e. 20  $\mu\text{l}$  of antibody was diluted with 80  $\mu\text{l}$  of  $\text{KH}_2\text{PO}_4$  to make it 100  $\mu\text{l}$  in 1 ml appendoff tube. 900  $\mu\text{l}$  of previously prepared GNP was added to it and kept it for incubation at room temperature for 15-30 min. This time allows the GNPs for conjugation. By blocking with BSA and PEG, the solution was centrifuged twice for removing residual chemicals and the pellets obtained at bottom were dissolved in the preservative solution for further use.

## Results and Discussion

### Synthesis and characterization

The size of the GNP is mainly dependant on the synthesis procedure of GNPs. The synthesized gold GNPs was stable due to their high negative zeta potential. The colloidal gold solutions with 5-60 nm particle size are stable for long duration in absence of any special stabilizing agent [24]. These GNPs were characterized for their size and  $\lambda_{\text{max}}$  to define their optimum properties.

### Size of GNP

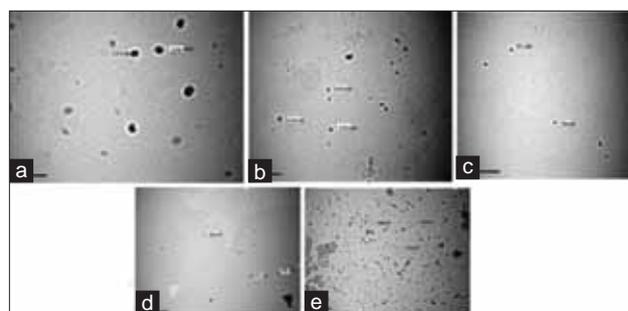
The size of the above prepared GNP solution was determined by TEM. The result of the transmission of electron microscopic measurement of the above five GNPs was shown in Figure-2a-e.

These TEM images gave the size, shape and the distribution of the GNP in well-dispersed suspensions. The size of GNPs has been determined by measuring the diameter of whole particles on TEM images. The average diameter of GNP was found from 25 nm to 230 nm depending on the concentration of sodium citrate solution. Earlier, this protocol was used to prepare spherical sizes from a range of sizes from 16 to 147 nm [21] because sodium citrate adsorb to the GNPs surface preventing the aggregation by repelling particles from each other.

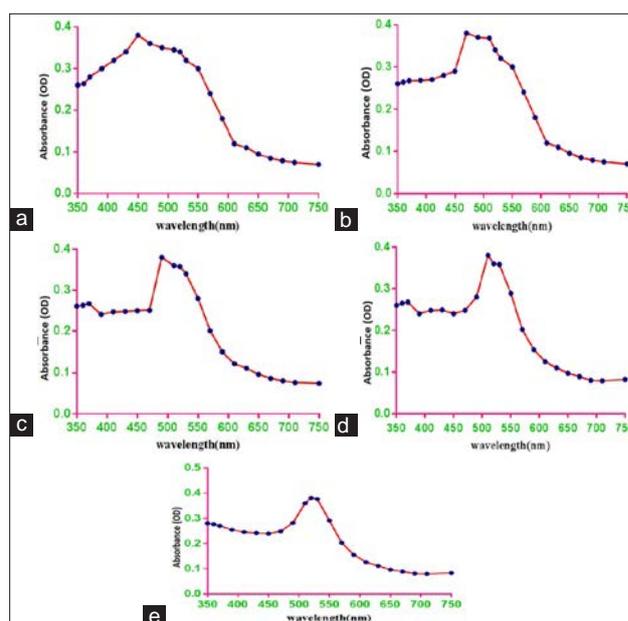
Here, it is observed that sodium citrate having concentration 1.5%, 0.75%, 0.375%, 0.1875% and 0.09375% produced GNPs of approximate sizes of 27 nm, 42 nm, 76 nm, 145 nm and 230 nm of GNP respectively. This variation is mainly due to capping of citrate on the GNP surfaces, due to capping of citrate ion on the surface of GNP, the GNP surface become negative charge [13,14]. This negative charge is the main factor for the size of the GNP due to repulsion of the oppositely charged particle. So the particles are not aggregated due to more citrate capping results in small size, but when concentration of sodium citrate is low, there is an aggregation, results in large size. The negative charge is mainly dependant on the concentration of the sodium citrate used. The TEM image show that the GNP is in monodispersional state, due to citrate capping on the surface of GNP. Here it was seen that the surface of GNP is uniform and in round or spherical in shape.

### Absorption spectrum GNP

The above five synthesized GNP solutions of different sizes were characterized in respect of their absorbance by UV-VIS spectrophotometry. The absorption spectrum was measured from 350 nm to 700 nm of each of the GNPs and the  $\lambda_{\max}$  of each GNP was measured, in this study it was found that the GNP of different sizes, absorb light maximum at 525-550 nm. The absorption spectra of five GNPs were shown in Figure-3a-e. It was seen that, with an increase in particle size, the absorption peak shifts to shorter wavelength and the Width of absorption spectra is related to the size distribution range. Due to the absorption spectra of GNP fall in the visible region, the change on the surface of the GNP can be detected in the naked eye. In this study, it was seen that, the colour of GNP varies according to their size of the GNP. In this study, it was seen that, with increase in size of GNPs there will be broadening of the absorption spectrum supported by Safenkova *et al.* [24], it may be due to the additional resonance that is occurred at longer wave length. Similar to this, Verma *et al.* [2] observed increased colour intensity due to increased size of the GNP.



**Figure-2:** (a) Transmission electron microscopy (TEM) images of gold nanoparticle (GNP) (using 0.09375% sodium citrate), (b) TEM images of GNP (using 0.1875% sodium citrate), (c) TEM images of GNP (using 0.375% sodium citrate), (d) TEM images of GNP (using 0.75% sodium citrate), (e) TEM images of GNP (using 1.5% sodium citrate).



**Figure-3:** (a) Absorption spectrum of gold nanoparticle (GNP) (using 0.09375% sodium citrate), (b) absorption spectrum of GNP (using 0.1875% sodium citrate), (c) absorption spectrum of GNP (using 0.375% sodium citrate), (d) absorption spectrum of GNP (using 0.75% sodium citrate), (e) absorption spectrum of GNP (using 1.5% sodium citrate).

The above formed anti-sheep IgG-GNPs conjugate was employed as the detector agent for the development of a rapid assay for diagnosis of blue-tongue in small ruminants. This gold based immunochromatographic assay is now the emerging platform for diagnosis of various animal's diseases at the field level without the need of any highly skilled personnel. At present, this gold based immunochromatographic strip test against JD, peste des petits ruminants, BTV, and babesiosis are being tried at different department of IVRI for rapid diagnosis.

### Conclusion

The present study was carried out to investigate the size controlled synthesis of GNP and their characterization in terms of size, shape, and their absorbance. The effect of concentration of sodium citrate on size

of GNP was investigated. Here it was concluded that, large size GNPs are produced from 0.09375% sodium citrate, and small size GNP are produced from 1.5% of sodium citrate solution. In this study, GNPs of 30-226 nm were obtained. These different sizes of GNPs may provide a new platform for diversified application not only in biomedical but also in bio sensing fields.

#### Authors' Contributions

PRS and PS designed the work plan. PRS did all the laboratory work. PS and PRS drafted the manuscript and revised it. Both authors read and approved the final manuscript.

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#### Competing Interests

The authors declared that they have no competing interests.

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