Single-Step Biofriendly Synthesis of Surface Modifiable, Near-Spherical Gold Nanoparticles for Applications in Biological Detection and Catalysis

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SINGLE-STEP BIOFRIENDLY SYNTHESIS OF SURFACE MODIFIABLE, NEAR-SPHERICAL GOLD NANOPARTICLES FOR APPLICATIONS IN BIOLOGICAL DETECTION AND CATALYSIS

A Thesis
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Master of Science

By
Vivek D. Badwaik

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SINGLE-STEP BIOFRIENDLY SYNTHESIS OF SURFACE MODIFIABLE, 
NEAR-SPHERICAL GOLD NANOPARTICLES FOR APPLICATIONS IN 
BIOLOGICAL DETECTION AND CATALYSIS

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1. Ingredients in the aqueous reaction medium used for the synthesis of GNP$ \ldots \ldots \ldots 14$
There is an increased interest in understanding the toxicity and rational design of gold nanoparticles (GNPs) for biomedical applications in recent years. Such efforts warrant reliable, viable, and biofriendly synthetic methodology for GNPs with homogeneous sizes and shapes, particularly sizes above 30 nm, which is currently challenging. In the present study, an environmentally benign, biofriendly, single-step/single-phase synthetic method using dextrose as a reducing and capping agent in a buffered aqueous solution at moderate temperature is introduced. The resulting GNPs are near-spherical, stable, catalytically active, place exchangeable, and water-soluble within the size range of 10-120 nm. The added advantage of the biologically friendly reaction medium employed in this new synthetic approach provides a method for the direct embedment/integration of GNPs into biological systems such as the E. coli bacterium without additional capping ligand or surface modification processes.
INTRODUCTION

Nanotechnology is, broadly speaking; the study of physical phenomena on the nanoscale.\(^1\) It is the study of manipulating matter on an atomic and molecular scale, which deals with structures sized between 1 to 100 nanometers. One nanometer (nm) signifies to one billionth or \(10^{-9}\) of a meter thus nanotechnology deals with a very small particle size. This very small size of the materials is one of the reasons for unique qualities of nanoparticles.\(^2\) The properties that materials have in a ‘nano’ form are much different than they are when in a bulk substance. The most common and simple example of this is gold. It was found centuries ago that gold dissolved into a liquid had different properties than the gold in bulk form.\(^3\) At the nanoscale, gold physically changes color from its shiny finish and gains magnetic and semiconductance properties as well. Because particles like gold act differently as a nanomaterial, it opens up an immense number of options that were not previously available. This very small size of substances opens up a new door for the science.

Today's nanotechnology finds wide applications in chemistry, physics, materials science and biotechnology to create novel materials that have unique properties (Figure 1).\(^4\) Also, some of the applications of nanotechnology are based on the interphase of above mentioned fields.

Nanotechnology is a hope for solving the following critical challenges facing humanity.\(^5\)

- Providing renewable clean energy
- Supplying clean water globally
- Improving health and longevity
• Healing and preserving the environment
• Maximizing productivity of agriculture
• Making information technology available to all
• Enabling space development
Figure 1: Schematic representation showing various applications of nanotechnology. Among all the applications, most of the applications affect the environment and human body. Accordingly there is always a need of environmentally friendly approach towards nanotechnology.
In recent years, different metals have been used as nanomaterial for various purposes. However, when coming towards biomedical applications, there is an increased interest in understanding the toxicity and rational design of gold nanoparticles (GNPs).\textsuperscript{6} The reason for GNPs to be so important in the biomedical field is due to its special properties such as:

- Rigidity and chemical stability
- Biodegradability
- Bio-inertness
- Nontoxicity
- Cellular imaging ability
- Tunable functionality

Owing to all the above properties, GNPs find very important applications in the field of medicine, known as ‘nanomedicine’. It is the application of nanotechnology in the field of medicine. Nanomedicine gave us a new hope for the formulation of new medicine which can be used to treat various human diseases and disorders.

The application of GNPs in nanomedicine are:

- Drug delivery
- Drug targeting
- Imaging
- Bioprobe

GNPs are currently being used as a drug to cure various diseases, including certain life threatening diseases like Cancer, Alzheimer’s disease, etc.
Apart from nanomedicine, GNPs play a crucial role in:

- Separation sciences
- Nano devices
- Electrochemistry.

Thus, GNPs are useful for a wide variety of environmental, biomedical and industrial chemical applications.\(^7\)

To obtain GNPs, various wet chemical methods employing various polar and nonpolar solvents have been used.\(^8,9\) The most common method is the reduction of tetrachloroauration acid (HAuCl\(_4\)) by excess sodium borohydride (NaBH\(_4\)) or sodium citrate in the presence of stabilizing/capping ligands such as citrate, thiolates, amines, phosphanes, carbonyls, dendrimers, and surfactants (Figure 2).

![Figure 2: Schematic representation of general mechanism of GNP synthesis](image-url)
These methods have produced GNPs with sizes ranging from 2-10 and 10-100 nm, respectively.\textsuperscript{10,11} A limitation of such methodology is that the GNPs produced by citrate methods beyond \(\sim 50\) nm are nonspherical and polydispersed. Hydroquinone has also recently been employed as a reducing agent to make relatively monodispersed GNPs with particle size up to 175 nm from GNP seeds synthesized by a traditional citrate method.\textsuperscript{12,13} However, the use of hydroquinone, a suspected carcinogen, in conjunction with traditional citrates methods leaves the product GNPs with trace amounts of organic solvents. This raises environmental concerns and also limits the biocompatibility and biomedical application of GNPs, for which avoidance of cellular toxicity is essential.

There is accordingly a need for a reliable, clean, and bio/ecofriendly “green” chemical process for the synthesis of GNPs. Utilization of nontoxic chemicals and solvents is a most important aspect of such a “green” nanoparticle synthesis process.\textsuperscript{14,15} Additionally, it is to be desired that such a process obviate any need for highly laborious size-sorting or seeding of any preformed crystals.\textsuperscript{10,16} It should be noted in this regard that nanotechnology requires the synthesis of nanomaterials of different sizes, shapes, and controlled disparity for various life science related applications.\textsuperscript{17,18} In particular, the synthesis and subsequent linkage of GNPs with various biological and chemical materials find a wide array of applications in gene transfer, bioprobes for tissue analysis, catalysis, information storage, imaging, and drug delivery. In this context, exploiting GNPs in the size scale of \(>50\) nm is crucial for many biomedical applications including biomimetics of biological molecules (protein, DNA) and structures (viruses, bacteria).\textsuperscript{19,20}

Efforts focused on green synthesis of GNPs using different biological systems
such as human cells, fungi, microorganisms, different types of plants, proteins, peptides, fatty acids, different types of sugars like glucose, mannose, sucrose, maltose, lactose, starch, and cellulose, and employing different experimental conditions (including high temperature, pressure, long experimental time, the presence of other reducing agents, and other capping agents) have been reported.\textsuperscript{21-23} However, the reported green synthetic methods thus far yield GNPs with a narrow size range (30 nm) with minimum monodispersity.\textsuperscript{24-26} To our knowledge, we report for the first time a controllable synthesis of near-spherical, larger GNPs of the size range of 10-120 nm in advantageous yields without any secondary capping or surface modification. Thus, the need for a highly laborious size-sorting or seeding of any preformed crystals or the multiple step non ecofriendly reaction processes is eliminated.
MATERIALS AND METHODS

Reagents and Materials:

Chemicals including KAuCl\textsubscript{4} / HAuCl\textsubscript{4} and dextrose were purchased from Aldrich, St. Louis, MO. E. coli was purchased from Invitrogen, Carlsbad, CA. Analytical grade chemicals were typically used.

Synthesis of GNPs:

GNPs were synthesized by the reduction of Au\textsuperscript{3+} ions in aqueous dextrose dispersion, prepared from a medium (pH \(\sim 6.9\)) (Table 1). In a typical preparation, an aqueous stock solution of KAuCl\textsubscript{4} or HAuCl\textsubscript{4} [appropriate concentrations (0.05-0.75 mM) after proper mixing and centrifugation to avoid any aggregates] was added to the aqueous medium containing different concentrations of dextrose. The samples were kept in an orbital shaker with a stirring speed of 150 rpm at room temperature for about 6 h. The progress of the GNPs formation was monitored by the color change of the medium or by UV/vis absorption spectrophotometry. Size-controlled GNPs were synthesized as described above by varying the concentration of KAuCl\textsubscript{4} (0.05-0.75 mM) and the concentration of dextrose (0.5 - 250 mg/mL) at a constant pH of about \(\sim\) pH 6.9 \(\pm\) 0.2 (without addition of any acid or base). The aqueous GNPs dispersion was centrifuged for 10 min at a speed of 12,000 rpm, which deposited all of the GNPs at the bottom of the tube. The precipitated GNPs were washed and resuspended several times with sterile water and then used for further analysis.

Characterization of GNPs:

Transmission electron microscope (TEM) images were obtained to characterize the morphology and size distribution of GNPs. 4 \(\mu\) L of a 1:4 (GNP stock: water) dilution
of the particle solution was added onto carbon-coated copper grids. The samples were further air-dried and then imaged on a JEOL-TEM. Elemental compositions including carbon of the GNPs were detected by energy dispersive X-ray spectroscopy (EDS) using a JEOL JSM-5400 LV with IXRF system. For the EDS analysis, the sample was washed several times with sterile water to get pure GNPs. Next, the sample was allowed to dry in the desiccator onto the aluminum stub without any adhesive or other carbon coating material and was analyzed. Particle diameter was determined by EDS 2000, version 2.6 software. Briefly, images were observed under TEM and obtained with the same area (7 x 11cm²). The scale marker (size of the grid x magnification = size on negative film) of 200 nm (obtained from the ruler, scanned under same number of pixels as that of negative film scan) was placed on the particle picture file. Briefly, images were imported into EDS 2000, version 2.6 software, and then the particle analysis option was chosen to get the particle diameter distribution on the basis of size (under the assumption that all of the particles are spherical in shape). The output file was imported into Kaleidagraph and plotted to determine the exact size distribution. The absorption spectra of the synthesized GNPs were measured using Perkin Elmer’s LAMBDA 35 UV/vis spectrophotometer. The infrared spectra (FTIR) for the air-dried samples were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer at 4 cm⁻¹ resolution with a single reflection diamond ATR accessory.

**Estimation of free Au³⁺ ion concentration:**

To estimate the unused Au³⁺ ion concentration (to thereby determine the efficiency of the increase in the concentration of dextrose), the reaction was allowed to go to completion. The GNPs then were separated from the unreacted Au³⁺ ions by
centrifuging the reaction mixture for 10 min at a speed of 12,000 rpm, and then the supernatant was collected. The concentration of Au$^{3+}$ ions in the supernatant was measured by the absorbance at 290 nm by using a UV/vis spectrophotometer. Appropriate background corrections were made for each measurement with reaction free medium.

**Embedment/ integration of GNPs into bacteria:**

To achieve the integration of GNPs in bacterium, *E. coli* cells were grown in 500 mL of sterile medium containing 4 mg/mL dextrose overnight at 37$^\circ$C. Cells were harvested by centrifugation at 6000 rpm for 10 min and washed several times with sterile water. Equal density of harvested bacterial cells was resuspended in medium in the presence of 4 mg/mL of dextrose. Appropriate concentrations of aqueous solution of KAuCl$_4$ were added to this suspension. The mixture was kept in the orbital shaker at room temperature (or in the temperature range from 25 to 37$^\circ$C), and the reaction was carried out for a period of 3 h. The progress of formation of GNP integrated *E. coli* cells were monitored by visualizing the change of color of the medium from orange to purple.

The aqueous GNP- *E. coli* dispersion was then centrifuged for 10 min at 12,000 rpm, whereby all of the GNP integrated *E. coli* cells were deposited at the bottom of the tube. The precipitated GNP-*E. coli* dispersion was washed several times with sterile water and for use in further analysis.

**Preparation for cross-section of the bacterial cells:**

The detailed ultrastructural changes induced by the GNPs treatment in embedded bacterial cells were examined. The cell pellets of *E. coli* were fixed with 16 % v/v paraformaldehyde and 10 % v/v glutaraldehyde in 175 mM sodium cacodylate buffer (pH
~ 7.4) for 2 hrs at 25 °C. The pellets were then washed 5 times with 50 mM sodium cacodylate buffer. The specimens were postfixed with 1% v/v osmium tetroxide in the same sodium cacodylate buffer for 1 hr at 25 °C and washed with nanopure water twice. The postfixed specimens were dehydrated in a graded ethanol series (once in 25%, 50%, 75%, and 95% and thrice in 100 % ethanol for 10 min each). The dehydrated specimens were then embedded in epoxy resin and heated at 60 °C for 18 hrs. Ultrathin sections (approximately 60 nm thicknesses) were cut using a triangle glass knife using an ultramicrotome (Leica EM UC6) and mounted on bare copper grids of mesh size 300. They were stained with 2 % uranyl acetate and lead citrate for 15 min and 7 min respectively, followed by examination with the transmission electron microscope (JEOL-TEM).

**Scale-up process:**

The scale-up process of both the synthesis and the embedment of GNPs was achieved by increasing the volume of the media in the presence of appropriate concentrations of dextrose, KAuCl₄ and appropriate nutrients as shown in Table 1.

**Catalytic activity of GNPs:**

The catalytic reduction reactions of p-nitrophenol by GNPs were monitored by UV/ vis spectrophotometry using a 1 mL quartz cell with a path length of 1 cm. The reaction was carried out by adding a NaBH₄ solution (250 μL of 20 mM) to a p-nitrophenol solution (200 μL of 0.5 mM). It leads to a color change from light yellow to green-yellow and a red shift from 315 to 400 nm. This was followed by an immediate addition of GNPs of appropriate size. The resulting time-dependent absorption spectra were recorded at room temperature.
Extraction and surface modification of GNPs:

The ligand solution (dodecanethiol, DDT) was prepared in an organic solvent such as hexane. GNPs synthesized in the aqueous medium were centrifuged for 10 min at a speed of 12000 rpm, whereby all of the GNPs were deposited at the bottom of the tube. The precipitated GNPs were washed several times, dispersed in sterile water, and then mixed with the ligand solution. The mixture was then agitated vigorously until all of the GNPs were extracted into the organic phase with new capping ligand.
RESULTS AND DISCUSSIONS

Different polyhydroxylated molecules including dextrose are well known for their limited reducing capacity. Many studies have demonstrated their ability to form dynamic supramolecular structures when dissolved in aqueous medium and how they act as a template for the reduction and stabilization of nanoparticles in smaller size range. In this report, we demonstrate that dextrose can effectively reduce \( Au^{3+} \) ions into \( Au^{0} \) and produce GNPs with near homogenous shapes and sizes within the range of 10-120 nm in aqueous medium, prepared with appropriate ingredients, as in Table 1.
<table>
<thead>
<tr>
<th>Ingredients</th>
<th>100ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH$_2$PO$_4$</td>
<td>2.6 grams</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>2.0 grams</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$</td>
<td>1.8 grams</td>
</tr>
<tr>
<td>K$_2$SO$_4$</td>
<td>0.48 grams</td>
</tr>
<tr>
<td>Dextrose</td>
<td>0.1 grams</td>
</tr>
<tr>
<td>KAuCl$_4$</td>
<td>Appropriate concentrations</td>
</tr>
</tbody>
</table>

**Table 1:** Ingredients in the aqueous reaction medium used for the synthesis of GNPs
The medium is a minimal growth medium which is used to grow various types of organisms including bacteria. It possesses many advantages including that it is eco-friendly, cost effective, has very low autofluorescence, and low absorbance properties. The straight forward single-step/single-phase method could be stated as follows (details are provided in the experimental procedure). Different concentrations of KAuCl$_4$ were dissolved in a required volume of medium in the presence of different concentrations of dextrose, thus forming a reaction medium in a vessel (Figure 3A). The vessel containing the reaction medium was then placed in an orbital shaker. Dextrose, the reducing/capping agent, was included in excess with respect to Au$^{3+}$ in order to ensure that all Au$^{3+}$ ions were converted into GNPs and also for efficient capping, to produce stable GNPs (Figure 3B). Reactions were typically carried out at room temperature, although the reaction could be carried out at a temperature of 37 ºC or even greater. The synthetic procedure was found to be scalable to larger volumes.
Figure 3: Schematic representation showing (A) the strategy to synthesize GNPs by completely green, ecofriendly, single step, single phase efficient method, (B) synthesis of GNPs in test tube with the help of dextrose as a reducing as well as capping agent. The color of the whole solution changes form yellow to red within 3 hrs, which indicates the formation of GNPs.
Dextrose concentration dependent synthesis of GNPs:

To study the dextrose concentration dependent synthesis of GNPs, ten samples with corresponding concentrations of 0.5, 0.75, 1, 4, 8, 20, 40, 100, 200 and 250 mg/ml of dextrose were prepared in media. After complete dissolution, a 0.5 mM solution of KAuCl₄ was added typically to 10 ml of media. Samples were incubated at room temperature for three hours in an orbital shaker. The color of the samples gradually changed within one hour. The UV/vis absorption spectra of GNPs prepared with different concentrations of dextrose are shown in Figure 4a. The λₘₐₓ values were observed in the range 520 nm to 580 nm. The shape and position of the surface plasmon resonance band are closely related to the shape, size, and dispersion of the GNPs. In particular, the longer absorption λₘₐₓ values are typical for increasingly larger GNP diameter.⁹ GNPs were further examined using TEM. Figures 4b-4g illustrate representative TEM images of the GNPs. TEM observation indicates that the size of the GNPs could be altered in a controlled manner by changing the concentration of dextrose while keeping the gold concentration constant. In the dextrose concentration range of 0.5 to 250 mg/ml, the size of GNPs obtained was within the range of 25 to 120 nm. It was evident that the average particle size of GNPs prepared with high concentration of dextrose (200 mg/ml) was much larger (120 nm) and had a near-spherical shape (Figure 4h & Figure 5). With increasing concentration of dextrose (> 200 mg/ml), signs of solid clusters or wire formation resulted (Figure 4g). These clusters were very complex and it was sometimes difficult to distinguish one aggregate from another. From this it was inferred that particle growth is facile in the presence of dextrose concentration up to 200 mg/ml. The results
accordingly showed that increasing dextrose concentrations produced GNPs with increased size, likely due to efficient nucleation and capping.
Figure 4: Absorption spectra, TEM images and average particles sizes of GNPs synthesized with various concentrations of dextrose. (a) Absorption spectra of different sizes of GNPs, (b-g) representative TEM images (scale bar = 200 nm), and (h) average sizes of GNPs synthesized.
**Figure 5:** Representative TEM images of GNPs of three particles sizes along with the corresponding histogram of the particle size distribution. (a) 10 nm (scale bar = 100 nm), (b) 60 nm (scale bar = 200 nm), (c) 120 nm (scale bar = 200 nm)
Elemental analysis:

Energy-dispersive spectroscopy (EDS) was used to identify the elemental composition of GNPs. Area-profile analysis of thoroughly washed GNP sample showed strong peaks of Au at 2.138 keV, characteristics of GNPs, along with carbon (elemental composition remained the same in the whole area of the sample, as shown in Figure 8a). The interaction between the dextrose and the GNPs was further analyzed by FTIR spectroscopy. Figure 8b shows a comparison of the FTIR spectra of dextrose and GNPs synthesized using dextrose as a reducing agent. The strong absorption peak corresponding to hydroxyl group shifted from 3240 to 3338 nm\(^{-1}\) due to the interaction between the surfaces of GNPs and the hydroxyl group of dextrose.\(^2\)\(^6\) These results demonstrate that the GNPs were associated with and stabilized by the capping ligand dextrose.
Figure 8: EDS spectrum and FTIR. (a) EDS spectrum of dextrose stabilized GNPs, (b-I) FTIR spectra of neat dextrose, (b-II) FTIR spectra of dextrose stabilized GNPs.
Efficiency of dextrose as a reducing agent:

To understand the efficiency of dextrose as a reducing agent the unused Au$^{3+}$ ions were quantified in reaction mixtures containing various concentrations of dextrose. GNPs were separated from samples by centrifugation followed by measurement of the concentration of supernatant Au$^{3+}$ ions using the UV/vis-absorption peak at 290 nm. The absorption peak at 290 nm disappeared with increasing concentrations of dextrose (0.5 to 250 mg/ml). For concentrations above 200 mg/ml of dextrose, the percentage of Au$^{3+}$ remained relatively constant (less than 20 %), indicating the high efficiency and optimum concentration of dextrose (Figure 6). These results were consistent with the role of dextrose as a reducing agent, leading to controlled nucleation/capping, followed by controlled crystal growth, which depended on the concentration of both the gold as well as the reducing agent to produce dextrose-stabilized, homogeneous GNPs of different sizes.
Figure 6: Absorption spectra of Au$^{3+}$ ions remaining in the reaction mixture (used for the synthesis of GNPs) after 3 h of reaction with various concentrations of dextrose (0-300 mg/mL). The inset shows the kinetic efficiency of various concentrations of dextrose on the absorption intensity of Au$^{3+}$ at 290 nm.
**Gold concentration dependent synthesis of GNPs:**

In order to study the effect of different concentrations of gold on the synthesis of GNPs, samples with concentrations of 0.05, 0.1, 0.15, 0.25, 0.5, and 0.75 mM of KAuCl$_4$ solutions were added to media containing 200 mg/ml of dextrose to form a mixture. The mixture was then incubated at room temperature for three hours. The color of the samples gradually changed within one hour. Figure 7a shows the UV/vis absorption spectra of GNPs prepared with different concentrations of KAuCl$_4$ solution. The $\lambda_{\text{max}}$ values red shifted from 516 nm to 580 nm.

Also, the plasmon resonance bands of the GNPs thus obtained were altered with the concentration of KAuCl$_4$ solution. TEM observation indicated that the size of the GNPs could be altered in a controlled manner by varying the concentration of KAuCl$_4$ solution while keeping the dextrose concentration constant. At the concentration range of 0.05 to 2.5 mM of KAuCl$_4$ solution, the size of GNPs obtained was within the range of 10 to 120 nm (Figure 7b-g). It was apparent that the average particle size of GNPs prepared with high concentration of KAuCl$_4$ (0.5 mM) was much larger (120 nm) and had a near spherical shape with narrow distribution (Figure 7h and Figure 5). With higher concentration of KAuCl$_4$ solution (> 0.5 mM), signs of complex clusters or wire formation resulted (Figure 7g). From this it was inferred that particle growth is facile in the presence of KAuCl$_4$ solution concentration of up to 0.5 mM. These results showed that increasing Au$^{3+}$ concentrations produced GNPs with increased size, likely due to increased nucleation with controlled capping.
Figure 7: Absorption spectra, TEM images, and average particles sizes of GNPs synthesized with various concentrations of KAuCl₄. (a) Absorption spectra of different sizes of GNPs, (b-g) representative TEM images (scale bar = 200 nm), and (h) average sizes of GNPs synthesized.
**Direct embedment/ integration of GNPs into *Escherichia coli* bacterium:**

Several sophisticated GNP based bacterial detection and drug delivery systems, including chemically modified carbohydrate and polymers based GNPs, are available. In such systems, there is a prerequisite for substantial surface modification of GNPs or the bacteria by wet chemical processes. In this context, the versatility of the synthetic method presented in this paper enables synthesis of GNPs of different sizes and also enables achievement of uniform integration of GNPs into *E. coli* bacteria without any secondary capping group, surfactants or surface modification. In order to embed the GNPs, medium was prepared in presence of 4 mg/ml of dextrose, followed by the addition of bacterial cells and varying concentrations of KAuCl$_4$ (0.05 - 0.5 mM). The integration process of the reaction solution was monitored by visual inspection as well as measurements by TEM. The color of the samples gradually changed within a time period of an hour (Figure 9 A). TEM micrograph shows several representative images of direct and uniform integration of GNPs of different sizes (from 5 nm to 50 nm) on the surface of the bacterium (Figure 9B). A control experiment without addition of dextrose resulted in leaching of bacteria without formation of GNPs. The morphology of the GNPs integrated on the surface of the bacterium was near spherical shape. The samples treated with varying concentrations of KAuCl$_4$ solution in presence of higher concentrations of dextrose resulted in serious aggregation/cluster formation, which eventually protruded away from the outer membrane of the cell. The random aggregation/cluster formation is likely due to the uncontrolled reduction of Au$^{3+}$ by dextrose and due to the intrinsic reduction ability of the bacterium in which lipopolysaccharides, proteins, phospholipids are present in the cell wall and membrane.
Figure 9: Biological applications of synthesized GNPs (A) Digital image for the colorometric indication of GNP formation along with *E. coli* (B) Series of TEMs resulting from the direct and uniform embedment of near spherical GNPs of various sizes (5-50 nm) on *E. coli* bacteria.
**Intracellular delivery by GNPs:**

After observing the exciting phenomenon of integration of GNPs on the surface of the cell, we were eager to know the intracellular changes made by GNPs. *E. coli* cells treated with GNPs were collected at different time points and processed for cross section. TEM analysis of cross sections of untreated cells showed that the cell membrane integrity remained intact at different time points even after the stationary phase (Figure 10A-i, ii). Bacteria which were integrated with GNPs showed histological changes. Initially the GNPs were observed to anchor onto the surface of the cell at several sites (Figure 10B-i). Gradually, these GNPs penetrate into the cell membrane, and were found eventually to form a circular domain inside the cell (Figure 10B-ii, iii). Occurrence of GNPs from external surface of the cell to deep inside the cell in a sequential fashion shows that the GNPs are formed outside the cell and then delivered inside rather than forming inside the cell. Also the size of the GNPs inside the cells was same as that of GNPs outside the cell, which proves the NPs to be stable in contact with the intracellular environment of the cell. In sum the results validates the successful intracellular delivery of GNPs.
**Figure 10:** Visualizing GNP induced histological changes of in *E. coli* cell via TEM. (A-i) Morphology of the untreated *E. coli* cell at 0 hour, (A-ii) Cross-section of the untreated *E. coli* cell after 12 hours, (B-i) Integration of GNPs with *E. coli* cell at 0 hour, (B-ii) Cross-section of the treated *E. coli* cell after 3 hours showing the insertion of GNPs from the surface (B-iii) entry of GNP domain after 6hrs, (B-iv) formation of GNP domain deep inside the cell which confirms the intracellular delivery of GNPs.
Catalytic activity of GNPs:

Catalytic activity of GNPs of different sizes was demonstrated in a model system of the reduction by NaBH$_4$ of $p$-nitrophenol, a toxic pollutant. An aqueous solution of $p$-nitrophenol shows an absorption peak at 317 nm. Upon addition of NaBH$_4$, the peak red shifts to 400 nm due to nitrophenolate ion formation. Addition of GNPs of different sizes (10, 60, and 120 nm) resulted in a reduction reaction. The intensity of the 400 nm peak dropped and resulted in fading of the color. Progress of the reaction was monitored by recording the absorbance at 400 nm (Figure 11). A control experiment with addition of 200 mg/ml of dextrose to the $p$-nitrophenol / NaBH$_4$ reaction mixture resulted in no change in absorbance in the absence of GNPs. In this context, pseudo-first-order kinetics was used to evaluate the rate constant of the catalytic reaction. Among the reactions catalyzed by different sizes of GNPs, GNPs with a size of 10 nm showed the fastest reaction rate ($k \approx 3.501 \times 10^{-3} \text{s}^{-1}$) and the shortest time of absorption as compared to other sizes. These results clearly indicate that the catalytic activity of GNPs could be manipulated by controlling the size of the nanoparticles. The results also show that GNPs of different sizes are useful for the removal of toxic pollutant such as nitrophenols from the environment.
Figure 11: (A) Representing the reduction of toxic pollutant p-nitrophenol by NaBH₄ using GNPs as catalyst, (B) Absorption spectra, indicating the reduction of p-nitrophenol by NaBH₄, in the presence of GNPs; the inset shows absorption at 400 nm (for p-nitrophenol) as a function of time in the presence of three different sizes (10, 60, and 120 nm) of GNPs.
**Surface modification or ligand exchange:**

Some nanoparticle-based applications in chemistry demand modifications of the surface ligands of GNPs (Figure 12A).\(^{27-28}\) The GNPs of different sizes synthesized by the eco-friendly method presented in this paper could be completely dispersed in aqueous medium without any irreversible aggregation and could also be transferred into organic medium with ligands like dodecanethiol (DDT). Thus, the GNPs synthesized are made suitable for various applications in chemistry (Figure 12B).
Figure 12: Chemical application of GNP (A) Schematic representation showing the phenomenon of surface modification of a GNP using ligand dodecaine thiol (DDT), (B) digital image of surface modification/extracted GNP from the aqueous to the organic phase with DDT.
CONCLUSION

For the first time we have disclosed a single-step/single-phase method for the synthesis of stable, place exchangeable, and catalytically active GNPs in aqueous medium by using dextrose as a reducing/capping agent. The GNPs synthesized have shape consistency within the size range of 10-120 nm in large quantities. Tunable activity of both the metal precursor and the reducing agent is an added advantage of this methodology. With this bio-friendly methodology, synthesis/ integration and intracellular delivery of different sizes of GNPs on a bacterium without any seeding or other secondary capping ligand is made possible (Figure 13).
Figure 13: Overall strategy for the green synthesis method of GNPs and its applications in various branches of science.
**FUTURE STUDIES**

The advancement of nanotechnology, which is considered as a most promising technology in the future, requires use of completely ecofriendly methods to prepare nanoparticles. The method disclosed in this research work is completely green and environmentally friendly. Therefore, this method can find a variety of applications in chemistry. Moreover, because of the biocompatibility, it has wide applications in the field of biology including, nanoparticle based *in situ* detection of many biological agents, antibacterial activity, drug delivery, etc.
REFERENCES
