RESEARCH ARTICLE OPEN ACCESS

A Simple Method for Synthesis, Purification and concentration Stabilized Goldnanoparticles

Dr. Fatin F.M. AL-Kazazz[1], Kifah A. F. AL-Imarah[2], Ikteffam.Abed AL-Hasnawi[3], Agelmashotjafar L[4], Dr.Ban Abbas Abdul-Majeed[5]

[1]Assistant Professor /University of Al-Mustansiryiah/College of Science /Department of Chemistry/E.mail:
[2]Head of Chemists/ Ministry Of Science and Technology
[3]Ph.D Student / University of Al-Mustansiryiah/College of Science /Department of Chemistry
[4]Ministry of Science and technology, Iraq
[5]Professor/ University of Baghdad/College of Medicine

ABSTRACT:
The greatest barriers to biological nanoparticles in use are issues of particle stability in shape and size control. Simple process is desirable for several reasons likewide range of sample volumes, gentle conditions and inexpensive equipment.
Gold nanoparticles(GNPs) which are produced by reducing gold chloride with TSC have very limited time storage. In order to get highly stable nanoparticles, purification has been performed for industrial applications. Removal of the side product of the reaction between $HAuCl_4$ with Tri-Sodium Citrate (TSC)is considered to make GNPs more stable. The result demonstrates that GNPs are more stable after the purification process.
Low degree of polydispersity can be achieved during gold nanoparticles synthesis, however, the particles need to be purified post-synthesis. The present research relates to single process for the removal of small-molecule impurities and the isolation of small nanoparticles from larger nanostructures through dialysis and microfiltration then concentration these small nanoparticles through polymer adsorbent materials by a factor of 100 or more to storage in the room temperature prolongs the stability of the purified gold nanoparticles suspension up to one year.The size and shape of the GNps is measured by atomic force microscopy (AFM), and the optical properties by UV-Vis spectrometer.
Keywords: Gold nanoparticles (GNPs), thermal decomposition, dialysis**,** microfiltration**,** polymer absorbent

I. The claimes

The purification of water-soluble gold nanoparticles is particularly difficult because the nanoparticles and the impurities have similar solubility, often making standard purification techniques (i.e. precipitation, extraction, chromatography, centrifugation or dialysis) inadequate or inefficient. Although reasonable purity is afforded via these methods, the nanoparticles remain contaminated with precursor molecules, e.g., salts and free ligand.

1. A method for producing a population of gold nanoparticles having a narrow size distribution comprising pure GNPs which indicated through the decreasing in their particle size and stable for one year.
2. The possibility to prepare monodisperse inorganic nanoparticles of pure materials in the sub-20 nm size regime has opened an effective field of research. This field is needed to go to the next step and develop methods of scaling up the synthesis of these materials.
3. A simple method for stabilized, water-soluble nanoparticles based upon the size of the nanoparticles was found out comprises:

a) The invention didn't mix metal salts with a solution of an organic surface that had a functional group such as a thiol, disulfide, amine, oxide, or amide to prepare a stabilized nanoparticles only mixed a reducing agent was added to reduce the metal salt to the free metal.
b) The thermal decomposition method for producing gold nanoparticles in the less or 20 nm size regime from a mixture of gold salts ($HAuCl_4$) and reducing agents such as (TSC, $NaBH_4$ or both) and refluxing said homogenous mixture to a temperature above the melting point of gold salts and water not exceed 90 ^oC, but usually obtain product with high dispersity and low nanoparticle concentration which limit its application.
c) A "broad size distribution" in reference to a population of nanoparticles will refer to nanoparticles ranging in size from about 1 nm to about 100 nm, wherein the majority of nanoparticles are spread over a large range of particle sizes. For desirable size and monodispersity stabilized gold nanoparticles , the method comprises the using of dialysis membrane to an aqueous nanoparticle

solution in the presence of buffer to purify from soluble impurities and remove particles having a broad size distribution without adding a substantially water-miscible organic solvent.

d) Moreover, the concentration of as-produced gold colloidal through polymer absorbent by a factor of 100 or more without aggregating the material give stable nanoparticles for more than one year at room temperature.

II. INTRODUCTION

The physical and chemical properties of nanoparticles are critically dependent on their size. Many applications require monodispersed nanoparticles, i.e., particles of uniform size, with a defined particle size. However, chemical synthesis usually results in nanoparticles with a broad particle size distribution, i.e., polydispersed nanoparticles. Methods are known in the art for determining the size of nanoparticles and for separating nanoparticles based on their size.

The ability to tailor low-polydispersity particles is important, in several cases (e.g., using polymeric stabilizers, reverse micelles, or thermal decomposition methods. The report paper byIrshadHussain et al based on a simple *one-step protocol* for the preparation of near-monodisperse gold hydrosols in the small size regime (<5 nm). The particle size can be controlled by varying the concentration of the stabilizing polymer, which can be readily displaced by thiol ligands to yield monolayer protected clusters of the usual type [1].

NatalliaShalkevich and coworkers [2], they have developed a simple method for the preparation of nearly mono-dispersed stable gold colloids with a fairly high concentration using *two-steps procedure*. First they synthesized citrate capped (GNPs) and then exchange the citrate ions with Triethyleneglycolmono-mercaptoundecylether (EGMUDE). This leads to the immediate precipitation and formation of composite assemblies.

Samuel et al. have demonstrated millifluidicbenchtop reactor system for the gram-scale synthesis and purification of monodisperse, hydrophilically functionalized gold nanoparticles with different sizes and shapes [3].

The paper by Steinigeweg summarized the results of the separation and purification of Gold Nanoparticles in the 20–250 nm Size Range by continuous density gradient centrifugation and glycerol/water mixtures as separation media to obtain highly purified metal colloids [4].

Balasubramanianet al work demonstrates how impurities are identified and removed, and the optimal purification of 20-nm GNPs was achieved by centrifugation operating at 7000 rpm for 20 min which satisfactorily recovers ~80% of GNPs without detectable impurities. Storage in the dark at 4 °C prolongs the stability of the purified GNP suspensions up to 20 days. Purified GNPs can be a reference material to evaluate toxicity or reactivity of other engineered nanomaterials [5].

Sweeney etal.have reported studies aimed at assessing the suitability of diafiltration for (i) the purification of water-soluble thiol-stabilized 3-nm gold nanoparticles, (ii) the separation of a bimodal distribution of nanoparticles into the corresponding fractions, and (iii) the separation of a polydisperse sample into fractions of differing mean core diameter. They demonstrate that diafiltration produces nanoparticles with a much higher degree of purity than is possible by dialysis or a combination of solvent washes, chromatography, and ultracentrifugation [6]. Therefore, purification and size-based separation of nanoparticles remain significant challenges in the preparation of well-defined materials for fundamental studies and applications [7-9].

We find out a simple protocol for converting the thermal decomposition method to produce gold nanoparticles from a mixture of gold salts and reducing agents (TSC or both TSC and NaBH4) at room temperature or by refluxing said homogenous mixture to a temperature above the melting point of gold salts and water as a solvent which produced low nanoparticle concentration and highdispersity to produce as- nanoparticles of a desirable size, and monodispersity which have a great potential in large-scale manufacturing for industrial demand and other applications.

The water - based GNPs be purified from soluble impurities and removed side product using a dialysis membrane combined with microfiltration and increased the concentration of as-produced colloidal material through polymer absorbent by a factor of 100 or more without aggregating the material and remained stable for more than one year at room temperature. The size and shape of the GNPs is measured by atomic force microscopy (AFM), and the optical properties by UV-Vis spectrometer.

III. EXPERIMENTAL DETAILS

This work deals with thermal decomposition methods used for producing stable GNPs of various diameters in size. The success of the below procedure depends upon several factors including the use of clean glassware and stirrers. Most methods avoid use of chromic acid because of the toxicity and carcinogenicity of $Cr^{(VI)}$ compounds and reported cleaning with aqua regia that dissolves almost all inorganic materials including gold particles from previous preparations, followed by detergent and rinsing many times with water (the last rinses are always done with the purest water available) then with organic solvent such as acetone or ethanol to remove organic impurities. The resistivity of water used was 18 M ohm cm, which is the theoretical limit at which conductivity is due only to the dissociation of water into [H^+ and OH^-] ions. However immediately upon contact with air water absorbs CO_2. The CO_2 reacts

with water to produce carbonic acid, which ionizes to make the actual resistivity of the "pure" water about 1 M ohm cm and reduces the pH from 7 to 6 or less [10]. We use containers with narrow necks and tight fitting caps rather than beakers to reduce possible entry of contaminants from air and to keep air out and sample in during storage.

IV. Materials

All materials were of analytical grade from Sigma Aldrich and used without any further purification; ultra- pure water was provided from deionize ultra filtered system. Polymer absorbent made of polyacrylate -polyalcohol has molecular weight 20k Daltons. Dialysis tubular form membrane for sample volume 20-50 ml were used.

V. Synthesis of gold nanoparticles

Method No.1:

In this work, solutions of citrate reduced GNPs were preparedaccording to the Turkevich and Frens method [9,10]. The reaction (100 mL final volume) was performed in a 3-neck 250 mL round bottom flask with the center neck attached to a reflux condenser. The flask was set in a hot plate with a magnetic stirrer to provide continuous mixing. The temperature was precisely maintained by circulating thermostated water within the uncertainties of ± 0.1 ^{o}C. During the thermal decomposition, water is passed through condensing jacket. Vapors rising from the solvent are cooled as they pass through tube, leading to condensation of the solvent vapors. The condensed solvent then falls back into the reaction vessel. This re-condensation prevents any significant loss of volume of the solvent during the thermal decomposition reaction. Thus, the relative ratio of metal to solvent stays substantially constant throughout the reaction. As long as the temperature of the homogeneous mixture is raised to above the melting point of the metal salt, the desired thermal decomposition reaction will take place and lead to formation of metal nanoparticles.

50 mL sample of aqueous(2.5×10^{-4}M) $HAuCl_4.3H_2O$(Hydrogen tetrachloroaurate (III) trihydrate)was prepared in a flask. The solution was brought to boil at (65-70 ^{0}C) while stirring, and 1.0 mL of 5%(0.17M) aqueous Tri-sodium citrate (TSC) was added. The boiling and stirring was continued for 25 min after the final color change. After cooling to room temperature, the GNPs solution was diluted using DIH_2O to replace the water evaporated during boiling.

Method No. 2:

GNPs were prepared by the reduction of hydrogen tetrachloroaurate (III) trihydrate with reducing agent and Sodium borohydride($NaBH_4$). In brief, 1.0 mM $HAuCl_4$ (10 mL) was prepared and then 0.5 ml aliquots of freshly prepared 0.5mM $NaBH_4$ were added at 10 °C to the slowly mixing solution of gold salt until a stable orangered/purplecolored colloid was observed (≅0.8-1.2 mL $NaBH_4$). The reaction was left for 18 hours at 20 °C and finally, solutions were purified and filtered as described later and stored at 4° C. This reaction does not need high temperature to proceed. The changes of color are very fastto ruby red.

Method No. 3:

According toBrown *et al*. [11], we prepared GNPs using a seeding method to give a small nanoparticles with a diameter of ~5.0 nm. A brief description is as follows; 1.0 ml of 1% HAuCl4 was added to 90.0 ml of water at room temperature (20-23ºC). After 1 minute of stirring, 2.0 ml of 38.8 mM TSC was added. One minute later, 1.0 ml of fresh 0.075% $NaBH_4$ was added. The colloidal solution was stirred for an additional 5 minutes and stored in a dark bottle at 4ºC.

VI. Detecting the nanoparticle formation process

In the synthesis process of GNPs, a portion of the reaction mixture (0.5 to 1 mL) was taken out from the flask at different reaction time and immediately poured into 9 mL ice-cooled water at 0°C. Such an operation can basically cease the formation process of GNPs due to the low temperature surrounding and the dilution effect, so it was called here as a "sample-frozen" operation. Then, the AFM samples were prepared at the earliest time and the ultraviolet visible (UV-Vis) spectra were recorded.

Glass microscope slides (25×75×1 mm,) were cut into smaller pieces (25×10 mm) and carefully cleaned by soaking in a solution of nitric acid/water (50/50%) for 20 min. Rinsed slides were immersed in Piranha solution (70% concentrated sulfuric acid: 30% hydrogen peroxide, 7:3) for 20 min and extensively rinsed with water. Cleaned glass slides were dried under a nitrogen stream and kept in closed desiccator.

UV-Vis spectra were recorded on a UV-visible spectrophotometer to collect the surface Plasmon resonance (SPR) information of GNPs, in which the highly concentrated samples were diluted by DIH_2O to adapt the measurement limitation. AFM samples were prepared by dropping the diluted gold colloids on glass microscope slides.

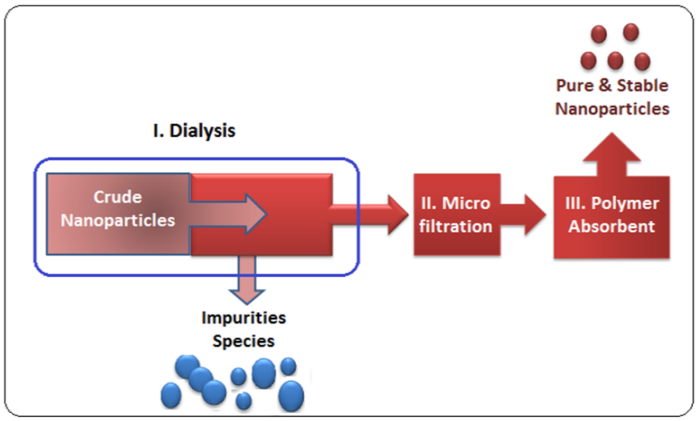
Purification Method ofGold Nanoparticles Solution

Can GNPs be purified from soluble impurities by extensively washing the nanoparticles with clean buffer solutions?

The membrane pores permit ions (or small molecules) to pass through but not the large colloidal particles (Figure 1). When a sol containing dissolved ions (electrolyte) or molecules is placed in a bag of semipermeable membrane dipping in pure water, the ions diffuse through the membrane. By using a continuous flow of fresh water, the concentration of the electrolyte outside the membrane tends to be zero.

Thus diffusion of the ions into pure water remains brisk all the time.

Figure 1: Rigid particles smaller than the pore size



will go through the dialysis membrane (Left). Operation of microfiltration to remove more impurities and sample

The dialysis tubing cellulose membrane was used in this dialysis process, the outside and inside of the dialysis tubing was rinsed with 20ml of DIH_2O for 15 to 30 minutes to remove sodium azide preservative. The water was poured out and the step repeated with another 20ml of deionized water. The dialysis is done in three days by immersing the dialysis tube containing GNPs in deionized water. The water is changed every 24 hour. In general, dialysis will be most effective when the buffer is replaced a few times over the course of a day and then left overnight at room temperature on a stir plate. A standard protocol for dialysis is 16 to 24 hours. Many factors affect the rate dialysis, including: diffusion coefficients, pH, temperature, time, concentration of species, sample volume, dialysate (buffer) volume, number of dialysate changes, membrane surface area, membrane thickness, molecular charges and dialysate agitation (stirring).

The dialysates (buffers) are commonly used in dialysis must be maintained under strict pH control to stabilize their molecular properties. The typical pH range for dialysis buffers is 6 to 8. The larger the dialysate volume, the greater the driving force for diffusion of small molecules. We generally recommend a 100:1 buffer to sample volume ratio. By replacing the buffer just as the rate of diffusion slows down and the solutions are approaching equilibrium, you can maintain the driving force and the rate of dialysis. We generally recommend two or three buffer changes over the period of 12 - 24 hrs as follows: First buffer change, after 2-3 hours; Second buffer change, after 4-5 hours and Last buffer change, prior to leaving overnight.

VII. Methodof Concentrating GNPs

For many applications it is desirable to have a high concentration solution available from which to dilute material in other solvents or buffers. Typically, the concentration of GNPs is limited by the synthesis method and ranges from (0.01 - 0.2) mg of gold per mL of solution. We have used polymer absorbent to increase the concentration of as-produced gold colloidal material by a factor of 100 without aggregating the material and keep the particle size to smaller value.

In order to concentrate the GNPs that have been prepared using Turkevich process, we tried to concentrate them down because we need to fit about 5mg into 20-50ml of space, perhaps even dry them out. Boiling reaction solution down will turn it from red to gray with black powder and gold flake like layers floating on top turn the particle size to larger value.

Polymer absorbent is a solution for easy concentration, high water absorbent capacity, made of polyacrylate-polyalcohol it has the property of expanding as it absorbs water. The high molecular weight polymer can be used with dialysis membranes of molecular weight 20k Daltons without contaminating the sample (figure 2). After dialysis process, simply remove the dialysis tubing containing the sample and coat it with absorbent. Water and smaller molecules diffuse through the membrane and are absorbed by absorbent. Concentration continues unattended until a certain volume of water is removed from the sample. The insoluble characteristic makes absorbent easy to remove after absorbency.

Table (1) and Figure (3)show the data for the "rinsing" of the gold colloid has (λmax 520, OD 1.817, as in Figure 2)with dialysate buffer solutions and microfiltration to remove all residual reactants resulting in a high purity solution that contains only nanoparticles (λmax 525.5 , OD 2.672). Further concentration with polymer absorbent results in the removal of excess water to yield an increase in its (OD 4.560).

Table 1: GNPs peak absorbance due to purification and concentration

GNPs synthesis by method No.1	**λ (nm)**	**OD**
Without [Dialysis, Filtration & Absorbent]	520.0	1.817
With [Dialysis + Filtration]	525.5	2.672
With [Dialysis + Filtration + Absorbent]	527 .5	4.560

The length of time needed for concentration will depend on the final volume, the surface to volume ratio of the membrane tubing and the amount of absorbent.

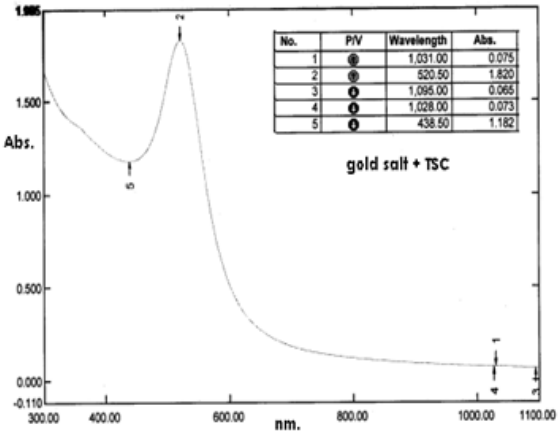


Figure2: UV-VIS spectrum for the formation of ruby red GNPs from the reaction (5%)TSC and (2.5×10^{-4}M) $HAuCl_4$ solution at (65-70)^{0}C before dialysis.

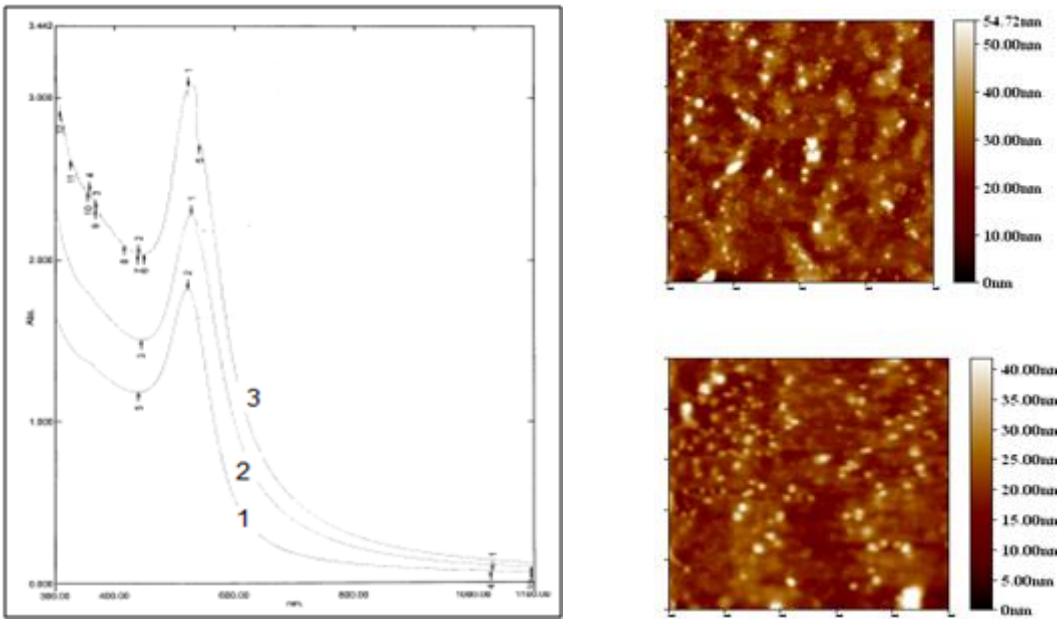


Figure 3: The influence of dialysis and polymer adsorbance on the stability of gold nanoparticles through UV-Vis spectrum and AFM images : curve (1)before dialysis , curve (2) after dialysis and absorbent {2-fold dilution with H2O} and curve (3) without dilution.

VIII. Determination the Stability of Colloidal Gold

There are several important factors that can influence the stability of colloidal GNPs. Among the most important factors are the particle sizes, concentration, reducing agent, and local environment. Different reducing agents provide differing levels of stability to salt, light, heat and pH value.

Stability of gold NPs was investigated by measuring the absorption spectra of the gold colloidal sol prepared at various times. The sample was stored at room temperature in a transparent vial and UV-Visible spectra of the sample were taken after the formation of final ruby redgold colloid sol (Plasmon absorbance at 527.7 nm) then after one week, two weeks, one month, and two months. The results indicate that there is small obvious difference in position and symmetry of absorption peak during the initial two weeks (Table 1and Figure 3) from Plasmon absorbance at 527.7 nm to 523nm. After six months, the position of the peak has a slight blue shift (from 523 to 517 nm)suggesting the formation of smaller particles without any aggregation. Even after one year the Plasmon absorbance remained at 517 nm and no aggregation was observed as in (Table 2 and Figure 4). Thus, colloidal gold can remain stable at room temperature for as long as several months. The reason for the stability of the colloid gold sol is described in the discussion section.

Table 2: The stability of gold nanoparticles **synthesis with method No.3.**

GNPs synthesis by (TSC + $HAuCl_4$)	**λ (nm)**	**OD**
[Withdialysis, filtration and polymer absorbent] After one year	**5 17**	**1.608 (2.5 -fold dilution)**

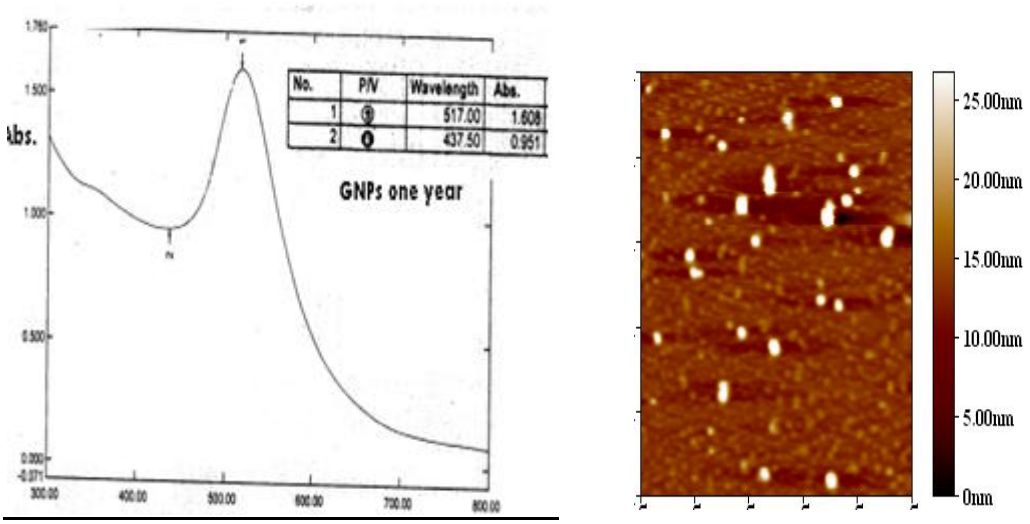


Figure 4: UV-VIS spectrum for the stability of GNPs synthesis with method No.3 and AFM image which indicate decreasing in their particle size and stable for one year after dialysis, 0.25µm filtration and polymer absorbent.

IX. RESULTS

***Decreasing reaction rate by lowering temperature*:**

Basically, the chemical reaction rate depends on temperature; the high rate of nanoparticle formation can be decreased at a low temperature. In this work, the nanoparticle synthesis was therefore performed at 80°C and 60°C.It was found that the formation rate of GNPs slowed at lower temperatures. The color of the colloids obtained at 75°C (Table 3 and Figure 6) did not differ from that of those produced under boiling state (Table 4).

Chunfang Li et al have been reported, that the reaction rate was greatly enhanced at high reactant concentration by the citrate method[12].

The first effort in our work was heated **(2.5×10^{-4}M)** aqueous chloroauric acid solution to boiling at 60 ^{0}C and the 3.3 times molar amount of sodium citrate was added, followed by continuously heating for a certain period to get the ruby red colloids.In Table3, We could find that GNPs synthesized in presence of **(2.5×10^{-4}M)** $HAuCl_4$have an average size of 15-20 nm with large size distribution, while at a high $HAuCl_4$ concentration,1.0mM, the particle size was decreased 8-10 nm with a narrow distribution.

Table 3: Color and peak absorbance change for the reaction(5%) TSC and (2.5×10^{-4}M) $HAuCl_4$ and increase boiling with time to synthesis (AuNPs) solution at 60 ^{0}C.

Time, min.	λ, nm	OD	Color change
10.0	549.50	0.090	Faint violet
20.0	531.00	0.121	Faint violet
25.0	528.00	0.130	Pink
32.0	526.00	0.221	Pink
36.0	524.50	0.307	Bright pink violet
42.0	523.50	0.557	Red
52.0	523.00	0.815	Ruby red
79.0	**523.00**	**0.914**	**Ruby red**

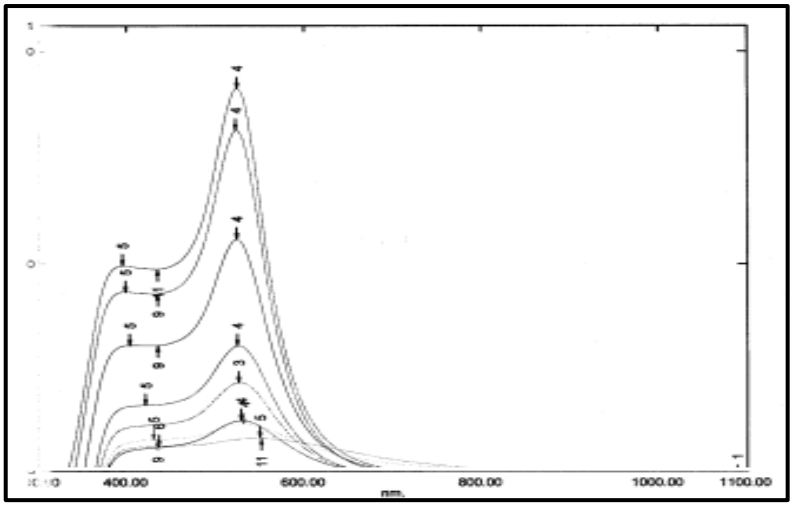


Figure 6:UV-VIS spectrumfor the reaction (5%) TSC and (2.5×10^{-4}M) $HAuCl_4$ and increase boiling with time to synthesis GNPs solution at 60 ^{0}C.

The SPR peaks of the gold colloids taken-out from the reaction mixture at different time were also studied by UV-Vis spectroscopy. We found that the colloidal samples prepared at **(2.5×10^{-4}M)** show SPR peaks around 523 nm, and the reaction time should be controlled at 30 to 40 min, although longer reaction time (~70 min) did not cause aggregation.

The color and the SPR peaks of these colloids do not show obvious differences, and no obvious difference is found in the full width at half maximum of these peak profiles. GNPs show that the size polydispersity varies with the reactant concentration increase although the particle average sizes are all located in a range of 10 to 20 nm. The large size distribution of GNPs at high reactant concentration will limit further applications such as size-related bioassays. Moreover, the as obtained gold colloids from high gold salt concentration and low TSC concentration (1%) give gold sols with double OD value (1.82) when compare with (0.91) value for higher TSC concentration (5%).

Table 3 : Color and peak absorbance change due to the reaction (1%) TSC and (1×10^{-3} M) $HAuCl_4$ and increase boiling with time to synthesis GNPs solution at 75^{0}C.

Time, min	λ nm	Abs. (OD)	Color change
10.0	530.0	1.208	Violet
20.0	530.0	1.382	deep violet
25.0	528.0	1.659	Red
32.0	520.0	1.817	ruby red

Aggregation mechanism of gold nanoparticles:

UV-Visible spectroscopy can be used as a simple and reliable method for monitoring the stability of nanoparticle solutions. In second method, solution of 18 ml DIH_2O, 0.5ml (0.01M) TSC and 0.5ml (0.1M) $NaBH_4$ was shaken and them added dropwise to 0.5 ml of $HAuCl_{4t}$ the color change to ruby red.When added (1.0ml or 2.0ml) from $HAuCl_4$(0.01M)the color change to deep violet and then precipitate black particles.

The optical properties of GNPs change when particles aggregate and the conduction electrons near each particle surface become delocalized and are shared amongst neighboring particles. When this occurs, the surface Plasmon resonance shifts to lower energies, causing the absorption and scattering peaks to red-shift to longer wavelengths. This is agree with the determination of an optimal set of conditions for the synthesis of GNPs is described in the work of Yuri et al. [13].They found Submicrometer "dense" liquid domains and aggregates (in the gray and blue solutions) of globules about 30–50 nm in diameter as intermediates in the citrate synthesis of GNPs. With decreasing nanoparticle diameter, the λmax shifts to a shorter wavelength.

As shown in Table 3 and Figure 5, the particles destabilize, the original extinction peak will decrease in intensity (due to the depletion of stable nanoparticles), and often the peak will broaden or a secondary peak will form at longer wavelengths (due to the formation of aggregates). The rapid change in the extinction spectrum as $HAuCl_4$ concentration is increased and clearly demonstrates that the nanoparticles are agglomerating.

UV-Visible spectroscopy can be used as a characterization technique that provides information on whether the nanoparticle solution has destabilized over time. Un aggregated GNPs will have a red color in solution, If the particles aggregate, the solution will appear blue/purple and can progress to a clear solution with black precipitates.

Table 3: Color and peak absorbance for the reaction (0.01M) TSC, (0.1M) $NaBH_4$ with different volumes of (0.01M) $HAuCl_4$ in order to study the nanoparticles aggregation process

Volume of $HAuCl_4$	λ max	OD	Color change
0.5 ml	520 nm (sharp peak)	0.775	Ruby red
0.75ml	520 nm 971 nm (broaden peak)	0.2915 0.493	Blue - Purple
1.0 ml	~ 551 nm (broaden peak)	~ 1.076	Deep violet
1.5 ml	~ 563 nm (broaden peak)	~ 0.447	Black pptn.
2.0ml	No peak	No peak	Black pptn.

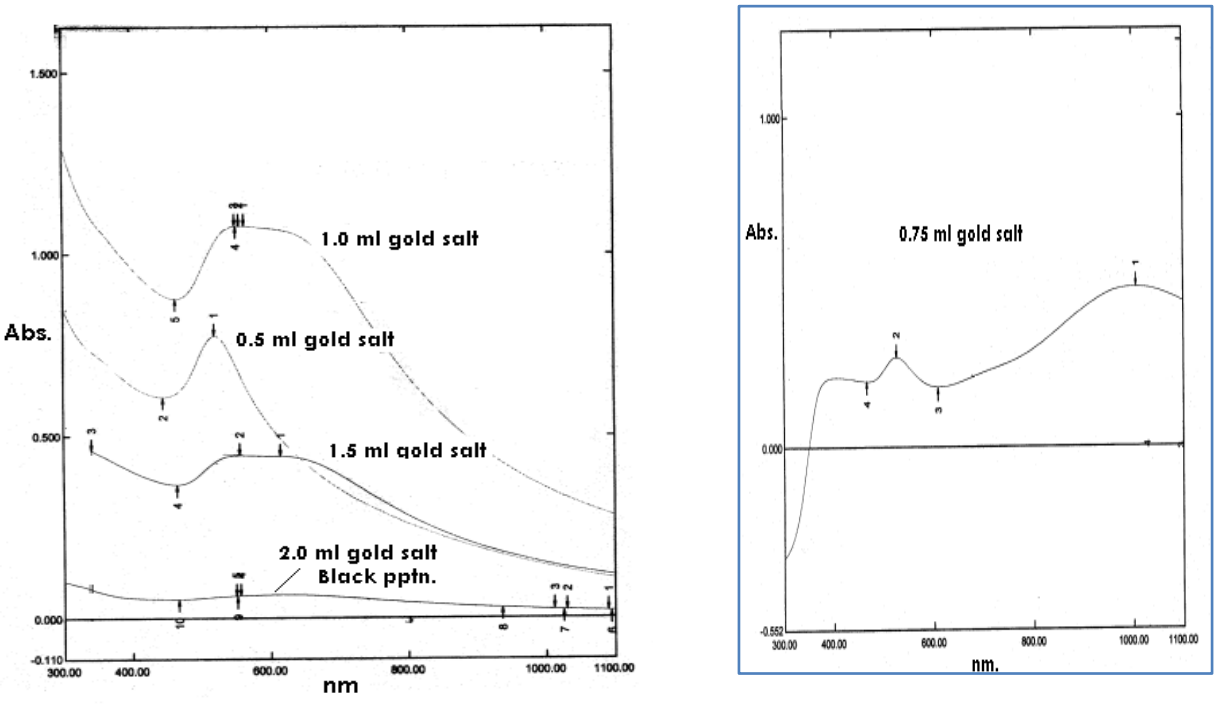


Figure 5: UV-VIS spectrum for the reaction (0.01M) TSC, (0.1M) $NaBH_4$ with different volumes of (0.01M) $HAuCl_4$ in order to study the nanoparticles aggregation process.

Figure (6) shows the third method data, ruby red colloidal gold has been obtained upon reaction gold salt with freshly prepared ice-cold sodium borohydride without using TSC. The gold particle sizes are 12 ± 2 nm due to the Plasmon absorbance near 519 nm with 0.820 OD.

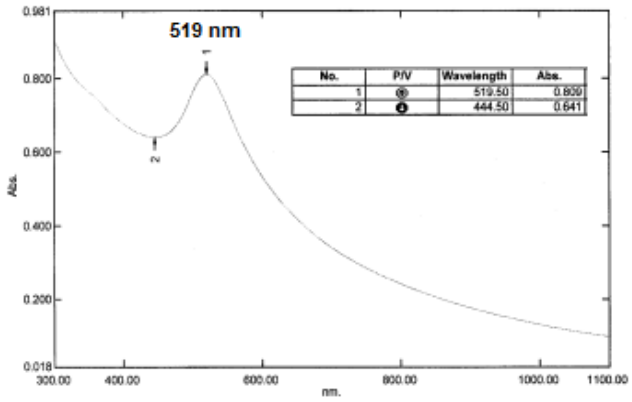


Figure 6: **UV-VIS spectrum for the reaction of 0.5ml (0.5 mM) $NaBH_4$ with 10ml (1.0 mM) $HAuCl_4$ to produce gold nanoparticles .**

An excess of $NaBHn_4$ is needed both to reduce the ionic gold and to stabilize the (GNPs) that form. The chemical reaction is the $NaBH_4$ reduction of gold salt as follow:

$$HAuCl_4 + 3e^- = Au^0 + H^+ + 4Cl^- \quad (8)$$

$$HAuCl_4 + 4\,NaBH_4 \rightarrow Au^0 + 5/2\,H_2 + 2B_2H_6 + 4NCl \quad (9)$$

** Where the core of Au^0 nucleates when reducing agents provide e^-*

Adsorption of borohydride plays a key role in stabilizing growing GNPs by providing a particle surface charge as shown in Jennifer and Mozhganworks [14,15]. There must be enough borohydride to stabilize the particles as the reaction proceeds. However, later in the reaction too much $NaBH_4$ increases the overall ionic strength and aggregation will occur.

The aggregation can also be brought about by addition of electrolytes such as NaCl. Nanoparticles are kept in suspension by repulsive electrostatic forces between the particles owing to adsorbed borohydride (Figure 7). Salt shields the charges allowing the particles to clump together to form aggregates. The colloidal gold solution turns blue. A Red shift peak around 530-550 nm appears along with a decrease in the intensity of the Plasmon absorbance which indicates onset of aggregation.

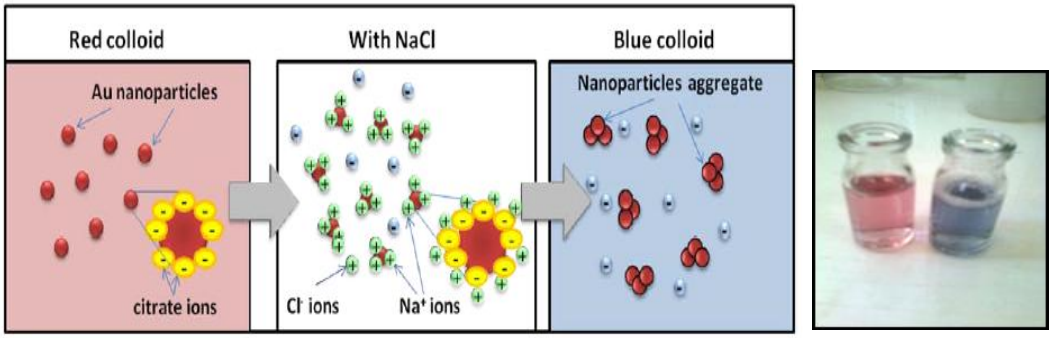


Figure 7: The aggregation of GNPs due to the addition of electrolyte

Therefore, reaction conditions, including stirring time and relative quantities of reagents (both the absolute number of moles of each reactant as well as their relative molarities), must be carefully controlled to obtain stable colloidal gold. It was also found that the initial concentration of $NaBH_4$ must be twice that of gold salt: [NaBH4] / [$HAuCl_4$] = 2.0. When concentration of borohydride was varied from 2.0 mM while using 1.0 mM gold salt, breakdown of the product took place in less than an hour. Small GNPs were prepared by the procedure in (No.2) due to the reaction of gold salt with sodium borohydride. Synthesis GNPs using $NaBH_4$ is found difficult to be stopped. However once the reaction is started, the GNPs grow along the time. After red color has been achieved which shows the formation GNPs, the solution becomes dark, and continues growing and growing, and finally the particles settle down in the bottom of the flask. This method is not suitable for industrial preparation purpose which need fast and stable product for a long periods.

X. CONCLUSION

A thermal decomposition method for producing GNPs from a mixture of gold salts and reducing agents (TSC, NaBH4 or both) at room temperature and refluxing said homogenous mixture to a temperature above the melting point of gold salts and solvent (Boiling range: 100°C for solvent (water)), wherein the concentration of the gold salts and reducing agent is selected according to the chemical reaction equation.

GNPs dialysis rate protocol is affected by diffusion coefficients, pH, temperature, time, concentration of species, sample volume, dialysate (buffer) volume, number of dialysate changes, membrane surface area, membrane thickness, molecular charges and dialysate agitation (stirring).
We have used a simple method to synthesize pure GNPs of a desirable size, and monodispersity which have a great potential in large-scale manufacturing for industrial demand and other applications. The GNPs be purified from soluble impurities and removed side product using a dialysis membrane.

Increased the concentration of as-produced colloidal material through polymer absorbent by a factor of 100 or more without aggregating the material and remained stable for more than one year at room temperature.

The size and shape of the GNPs is measured by atomic force microscopy (AFM), and the optical properties by UV-Vis spectrometer.

XI. Acknowledgements

The authors thank Dr.SamirChadaat general manager of material researchdepartment ;Dr.ZarhaAzat manager of advance material department/Ministry of Science and technology , Baghdad, Iraq) for their helpful in atomic force microscopy (AFM) measurement and assistantin UV-Visible spectrometer .

REFERENCES

1. IrshadHussain, Susan Graham, Zhenxin Wang, Bien Tan, David C Sherrington, Steven P Rannard, Andrew I Cooper, Mathias Brust ,Size-controlled synthesis of near-monodisperse gold

nanoparticles in the 1-4 nm range using polymeric stabilizers. Journal of the American Chemical Society 11/2005; 127(47):16398-9.

2. NatalliaShalkevich , Investigation on Syntheses of Nanocolloids and Their Thermophysical Properties ,These presented to à la Faculté des Sciences Institut de Physique ,Université de Neuchâtel ,2009 .
3. Samuel E. Lohse , Jonathan R. Eller , Sean T. Sivapalan , Michael R. Plews , and Catherine J. Murphy , A Simple MillifluidicBenchtop Reactor System for the High-Throughput Synthesis and Functionalization of Gold Nanoparticles with Different Sizes and Shapes, *ACS Nano*, Article ASAP , 2013.
4. Steinigeweg, D., Schütz, M., Salehi, M. and Schlücker, S. , Gold Nanoparticles: Fast and Cost-Effective Purification of Gold Nanoparticles in the 20–250 nm Size Range by Continuous Density Gradient Centrifugation, Small,2011,Volume 7, Issue 17, page 2406.
5. Balasubramanian SK, Yang L, Yung LY, Ong CN, Ong WY, Yu LE, Characterization, purification, and stability of gold nanoparticles, Biomaterials. 2010 , 31(34):9023-30.
6. Scott F. Sweeney , Gerd H. Woehrle ,† and James E. Hutchison, Rapid Purification and Size Separation of Gold Nanoparticles via Diafiltration, *J. Am. Chem. Soc*., 2006, 128 (10), pp 3190–3197.
7. Matthias Hanauer , SebastienPierrat , Inga Zins , Alexander Lotz , and CarstenSönnichsen ,Separation of Nanoparticles by Gel Electrophoresis According to Size and Shape, *Nano Lett.*, 2007, 7 (9), pp 2881–2885.
8. A.J. deMello, Microfluidic Reactors for Nanomaterial Synthesis. In: J C Schouten, editor, Advances in Chemical Engineering (Volume 38). Academic Press, 2010, p. 195.
9. Frens, G., *Nature Physical Science* 1973, *241*, 20-22.
10. Turkevich J.; Stevenson P. C.; Hillier*J.*, Synthesis of Gold Nanoparticles; *Disc. Faraday Soc.* 1951, *11*, 55-75.
11. K. R. Brown, D.G. Walter, and M.J. Natan., Chem. Mater., 2000. 12(2): p. 306
12. Chunfang Li, Dongxiang Li, Gangqiang Wan, JieXu and WanguoHou, Facile synthesis of concentrated gold nanoparticles with low size-distribution in water: temperature and pH controls ;Nanoscale research letters, 2011, 6: 440.
13. Mikhlin Yuri; Karacharov Anton; LikhatskiMaxim;et al, Submicrometerintermediates in the citrate synthesis of gold nanoparticles: New insights into the nucleation and crystal growth mechanisms, Journal of colloid and interface science, volume 362(2) ,2011.
14. Jennifer A. Dahl, Bettye L. S. Maddux, and James E. Hutchison, Toward Greener Nanosynthesis, Chem. Rev. 2007, 107, 2228-2269]
15. MozhganBahadory, Synthesis of Noble Metal Nanoparticles, 2008, Ph. D.Thesis, Drexel University.