Gold Nanoparticles: Synthesis and Polymeric Encapsulation Using Double Emulsion Solvent Evaporation Process

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The objective of this study was to synthesize and characterize gold nanoparticles in aqueous medium. For this purpose, tetrachloroauric acid was reduced by sodium tetrahydroborate and sodium hydroxide with and without poly(vinyl alcohol) (PVA). The prepared gold nanoparticles were encapsulated by biodegradable polymer (polycaprolactone) via modified double emulsion evaporation technique. Transmission electron microscopy morphology study of gold nanoparticles was performed before and after encapsulation. The encapsulated particles were also observed through scanning electron microscopy. In addition, hydrodynamic size of the prepared dispersions was investigated. An increase in gold nanoparticle size was observed with the increase in reducing agent concentration. The presence of PVA led to size reduction of gold nanoparticles.

Keywords: Gold Nanoparticles, Chemical Reduction, PVA, Polymer, Encapsulation, Polycaprolactone.

RESEARCH ARTICLE

1. INTRODUCTION

Owing to the special optical, electronic, and molecular-recognition properties of gold nanoparticles (AuNPs), they are the subject of extensive research, with applications in a wide variety of areas, such as nanoelectronics, nanotechnology, biosensors, biological labeling, radiotherapy, optoelectronic, and catalysts materials.[1–7] Gold nanoparticles (AuNPs) are also used in diagnostic, detection and drug delivery applications.[8, 9]

Different approaches such as chemical, electrochemical, irradiation method, sonochemical, photochemical, and seed-mediated methods have been used for the preparation of gold nanoparticles.[4, 10–17] Diverse synthesis methods of gold nanoparticles based on the reduction of chloroauric acid in the presence of a stabilizer have been reported. These methods lead to formation of several types of gold nanoparticles such as: nanorods, nanoshells, nanocages, nanospheres and surface enhanced Raman scattering (SERS) nanoparticles, with different particle size

(2–100 nm).[2, 18–21] The size and shape of nanostructures are the key parameters controlling diversified properties like electronic, optical, thermal and magnetic properties of materials at nano length scale.[9, 10]

The earliest scientific investigation for the synthesis of colloidal gold was carried out by Michael Faraday. The author reported a method for preparation of colloidal gold by electrochemical reduction of aqueous tetrachloroaurate ions ($AuCl_4^-$).[22] Almost a century later, Turkevich developed a method for synthesis of gold nanoparticles via citrate reduction of tetrachloroauric acid ($HAuCl_4$).[23] The advantage of the citrate reduction method was its simplicity and it produces particles with a relatively monodispersed distribution of particle sizes. A conventional gold nanoparticles synthesis method by Turkevich uses sodium citrate as reducer as well as stabilizer and results in water-soluble spherical particles with diameters of about 15–20 nm. Citrate-stabilized particles are still widely used due to their uncomplicated preparation method and excellent visibility of images in microscopy. However, they are not stable in dry form and their long-term stability in

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solution is also limited. This method has been refined by G. Frens, he controlled the over average particle size by varying the ratio of gold salt to citrate reducing agent.[24, 25] The Brust method can be used to produce gold nanoparticles in organic liquids like toluene that are not miscible with water. It involves the reaction of a tetrachloroauric acid solution with tetraoctylammonium bromide (TOAB) solution in toluene and sodium borohydride as stabilizing agent and a reducing agent, respectively.[26]

Recently, in 2013, the polyphenols EGCG (epigallocatechin gallate) in PVA was used by Zhu[27] to prepare small, monodisperse and size-controlled AuNPs.

Generally, inorganic nanoparticles have high polarity and they display very low dispersion stability in organic media. However, by treating of the surface of inorganic nanoparticles with organic compounds can overcome low dispersibility problem.[28] After nanoparticles formation, they need to be coated with a suitable stabilizing layer/capping agent to inhibit the growth of the particles and prevent their aggregation and to increase their stability for further use. Various reagents have been reported to serve as stabilizing agents. These include polymers such as different kind of PEG (polyethylene glycol), polyvinyl alcohol, Polyvinyl pyrrolidone and surfactants such as tetraoctylammonium bromide, Tween 20 and polysaccharide like chitosan.[9, 25, 29–33] Furthermore, most commonly used surface modification methods include grafting thiolated surfactants or polymers, adsorption of charged surfactants, charged ligands or polymer brushes, attachment of biological molecules such as DNA, peptides, proteins, antigens or coating a continuous polymer film on nanoparticles.[3, 25, 26, 34–40]

The encapsulation of colloidal nanoparticles has received great attention in recent years as a method for producing multifunctional materials. In this process a protective layer is built up around the core material (nanoparticle) to increase their chemical stability, to improve dispersibility or to confer specific properties (e.g., optical, magnetic, or mechanical properties) to the colloid dispersions. Among the different synthetic pathways, heterogeneous polymerization (i.e., emulsion, miniemulsion, and microemulsion polymerizations), physical encapsulation (polymer adsorption, layer-by-layer assembly, etc.) and double emulsion-like process have been used for the encapsulation of nanoparticles. Miniemulsion polymerization has been found to be very suitable for the production of nanoparticles with organic–inorganic nanostructures of different chemical compositions and morphologies.[28] Polymeric materials used for encapsulating nanoparticles for biomedical applications, such as drug delivery, diagnosis of disease or targeted chemotherapy must be biodegradable.[6] Among natural polymers, proteins or polysaccharides tend to be degraded rapidly. Polysaccharides have been mainly used in the preparation of drug delivery systems.[28]

The purpose of this study was to synthesize gold nanoparticles by chemical reduction technique. Tetrachloroauric acid was reduced by sodium tetrahydroborate in the presence and absence of polyvinyl alcohol (PVA). This reaction was also realized by PVA in presence of sodium hydroxide. The sodium tetrahydroborate and PVA concentration were changed and their effects on particle size and size distribution were investigated. The prepared particles were characterized in terms of particle size, particle size distribution and morphology by using photon correlation spectroscopy and transmission electron microscopy, respectively.

2. EXPERIMENTAL SECTION

2.1. Materials

Tetrachloroauric acid (HAuCl$_4$, 3H$_2$O) (≥99.999%) and polycaprolactone (PCL) ($M_W = 14000$ g/mol) were purchased from Sigma Aldrich. Sodium tetrahydroborate NaBH$_4$ (98%) was obtained from Acros Organics. Polyvinyl alcohol (PVA) ($M_W = 31000$ g/mol) and dichloromethane (DCM) were purchased from Sigma Aldrich. Sodium hydroxide, nitric acid and hydrochloric acid were obtained from BDH Prolabo-VWR International. Milli-Q water was used for the preparation of aqueous solutions, which was purified by Milli-Q purification system (Aquadem® from Veolia Water, France). All commercially available reagents were used as received without further purification. Ultrasonic homogenizer system "CY-500" ivymen® (500 W, 20 kHz) was obtained from SELECTA GROUP, Switzerland

2.2. Methods

2.2.1. Preparation of Gold Nanoparticles

The AuNPs were synthesized by the following three methods:

i. Reduction of tetrachloroauric acid by sodium tetrahydroborate (NaBH$_4$).

ii. Reduction of tetrachloroauric acid by sodium tetrahydroborate in presence of PVA.

iii. Reduction of tetrachloroauric acid by PVA in the presence of NaOH.

2.2.1.1. Reduction of Tetrachloroauric Acid by Sodium Tetrahydroborate. Gold nanoparticles were synthesized as reported in the literature.[41] The proper cleaning of glassware and stir magnetic bars is very necessary before the synthesis of AuNPs. Thus, surfactant and aqua regia (3 parts hydrochloric acid to 1 part nitric acid, v/v) were used to clean all the glassware, followed by rinsing with deionized water. For the synthesis of AuNPs by manual stirring, initially, 100 mL of Milli-Q water was poured into a flask and 10 mg of HAuCl$_4$ was added, the aqueous solution was then shaken to mix the solution properly. Then, NaBH$_4$ was added as a reducer to obtain gold nanoparticles. By using a micropipette, 2 mL of NaBH$_4$

(0.1 M) solution was added dropwise to the gold solution. Initially, it was added slowly to prevent aggregation, which was followed by relatively fast addition of $NaBH_4$ and the flask was shaken well after each small volume of reducing agent added. The color of solution in the flask changed from yellowish to ruby red indicating to the formation of gold nanoparticles (Fig. 1). In case of magnetic stirring, all other components were the same except stirring mode and the reducing agent solution was added to gold salt solution dropwise with continuous magnetic stirring.

2.2.1.2. Reduction of Tetrachloroauric Acid by Sodium Tetrahydroborate in Presence of PVA. Gold nanoparticles were also prepared by using $NaBH_4$ reduction method in the presence of poly vinyl alcohol as stabilizer. 100 ml of $AuCl_4$ solution (0.25 mM) was mixed with 100 ml of PVA solution in a conical flask under continuous magnetic stirring for three min., then, 0.1 M $NaBH_4$ solution was added dropwise to the flask. The color of dispersion in the flask was changed from yellow to ruby red, which indicates to the formation of gold nanoparticles. The solution was stirred for further 15 min for complete homogenization.

2.2.1.3. Reduction of Tetrachloroauric Acid by PVA in the Presence of NaOH. The AuNPs were obtained by simply heating an aqueous solution of gold salt (100 ml, 0.25 mM) in the presence of a mixture of 50 ml NaOH (0.1 M) and 100 ml PVA (0.01%) under magnetic stirring. The formation of particles was indicated by gradual change in color of the solution (from light yellow to red) over a time frame of 30 min. After preparation, the AuNPs were stored in the dark to minimize the photoinduced oxidation.

2.2.2. Encapsulation of Gold Nanoparticles

Gold nanoparticles (AuNPs) were encapsulated by the double emulsion solvent evaporation technique via two steps emulsification process using ultrasonic homogenization as already reported.[42] In brief, the inner aqueous phase (W1) was consisting of 1.5 ml distilled water, while oil phase was prepared by dissolving 3 g of PCL in 12 ml of dichloromethane appropriately to form a clear solution. Similarly, the outer aqueous phase (W2) was consisting of 0.5% PVA solution. In the first step of emulsification, inner aqueous phase was homogenized with oil phase to

form primary emulsion by using sonication at 70% amplitude for 5 min. In the second step, the primary emulsion was dispersed in 150 ml of the outer aqueous phase by using ultrasonic homogenization (70% amplitude, 8 min), which resulted into double emulsion. Subsequently, the organic solvent evaporation from the prepared dispersion with the help of rotary evaporator resulted in formation of solidified particles. The above procedure was used for blank formulation. For AuNPs encapsulation, the inner aqueous phase was replaced by 1.5 ml of gold nanoparticles dispersion. All the experiments were performed in triplicate. Finally, the polymeric particles incorporated with AuNPs were then recovered by centrifugation at 10000 rpm for 10 min and washed three times with deionized water properly and further characterizations were performed.

2.2.3. Characterizations

2.2.3.1. Particle Size Measurement. The average particle size of the prepared colloidal dispersions was measured by photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000 HSA (Model-Nano ZS, Malvern Instruments limited, UK). Prior to measurement, the samples were diluted with 1 mM NaCl solution. The measurements were performed in triplicate at 25 °C.

2.2.3.2. Transmission Electron Microscopy and Scanning Electron Microscopy. The prepared gold nanoparticles and submicron PCL particles incorporated with AuNPs were observed by using transmission electron microscopy (TEM). TEM was performed with a Philips CM120 microscope at the "Centre Technologique des Microstructures" (CTμ) at the University of Lyon (Villeurbanne, France). A small drop of suspension was deposited on a microscope grid (copper support covered with carbon) and slowly dried in open air. The dry samples were observed by TEM under 100 kV acceleration voltages. For, submicron PCL particles incorporated with AuNPs, scanning electron microscopy (SEM) was also performed with a Hitachi S800 FEG microscope at the "Centre Technologique des Microstructures" at the University of Lyon (Villeurbanne, France). A drop of diluted aqueous suspension of submicron particles was deposited on a flat steel holder and dried at room temperature. The sample was finally coated under vacuum by cathodic sputtering with platinum. The samples were observed by SEM under an accelerating voltage of 15 kV.
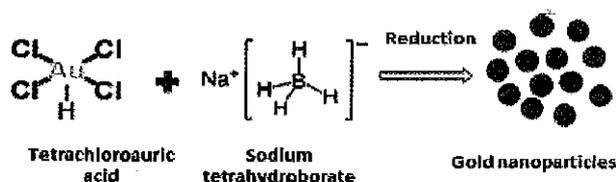

Fig. 1. Schematic illustration of gold nanoparticles preparation process by $NaBH_4$ reduction method.

Table I. Synthesis of AuNPs under different operating conditions.

Stirring mode	Formulation code	HAuCl$_4$ (ml)	NaBH$_4$ (ml)	Diameter (nm)
Manual	Au31	100	1	18.12
	Au3	100	2	22.63
Magnetic	Au33	100	1	73.00
	Au32	100	2	167.6

Table II. Synthesis of AuNPs in presence of PVA.

Formulation code	HAuCl₄ (ml)	PVA (ml)	NaBH₄ (ml)	Diameter (nm)
Au1	100	100 (0.01% wt)	1	248
Au12	100	100 (0.04% wt)	3.5	92

3. RESULTS AND DISCUSSION

3.1. Preparation and Characterization of Gold Nanoparticles

3.1.1. Reduction of Tetrachloroauric Acid by Sodium Tetrahydroborate

The AuNPs were prepared by manual stirring as well as magnetic stirring using NaBH₄ as reducing agent. As shown in Table I, in case of manual stirring, when we used 1 ml of NaBH₄ (0.1 M), AuNPs of 18 nm particle size was obtained, and when we doubled the NaBH₄ concentration (2 ml), a slight increase in the particle size (22 nm) was observed. Similarly, in case of magnetic stirring, the particles size of AuNPs there was also increased by increasing the concentration of reducing agent. The particles size of AuNPs was 73 nm and 167 nm when we used 1 ml and 2 ml of reducing agent, respectively. However, the particles size obtained by using manual method was smaller as compared to magnetic method. The increase in particles size with increase in reducing agent concentration may be due to the excessive reduction of AuNPs even after its preparation as already reported in literature[42] and this might be controlled by using proper capping agent.

3.1.2. Reduction of Tetrachloroauric Acid by Sodium Tetrahydroborate in Presence of PVA as Stabilizing Agent

The gold nanoparticles were also prepared by using NaBH₄ reduction method in the presence of different concentrations of PVA as stabilizing agent (Table II). Initially, when 0.01% of PVA solution (100 ml) and 1 ml of reducing agent were used, the particle size was found to be 248 nm. Subsequently, when the concentration of PVA was increased to 0.04% a dramatic decrease in particle

size i.e., 92 nm was detected, even with highest amount of reducing agent (3.5 ml). PVA act as stabilizing agent and prevent the aggregation of particles in liquid dispersions. The increase in PVA concentration augments the surface coating of the particulate systems thus contributes to the stability of colloidal dispersions.[43]

3.1.3. Reduction of Tetrachloroauric Acid by PVA in Presence of NaOH

Gold nanoparticles were prepared by using PVA as reducing agent in the presence NaOH under magnetic stirring. The particle size of AuNPs obtained during this process was found to be 61 nm. This reaction results in destabilization of the complex between AuCl₄⁻ and PVA at 50–60 °C, resulting in the transformation of AuCl₄⁻ ion to Au(0). These results indicate that PVA can be used successfully as reducing agent for the preparation of gold nanoparticles.

3.2. Characterization

3.2.1. Particle Size Determination

The hydrodynamic particle size of AuNPs was determined by photon correlation spectroscopy (PCS). The smallest size particles were obtained when manual stirring was used in the presence of NaBH₄ as reducing agent. The average particle sizes were 22 nm and 18 nm for sample Au3 and Au31 respectively. The size distribution of Au31 particles was in between 13 nm to 20 nm as shown in Figure 2.

3.2.2. Transmission Electron Microscopy

The morphology of the prepared gold nanoparticles was observed by using transmission electron microscopy (TEM). A small drop of suspension was deposited on a microscope grid (copper support covered with carbon) and slowly dried in open air. Afterword, various samples of AuNPs were observed with TEM. The results showed that formation of spherical gold nanoparticles (Figs. 3(A and B)), apparently TEM images particles size seem to be smaller as compared to hydrodynamic particle size determined by dynamic light scattering analysis. Additionally, the relatively large particle size measured
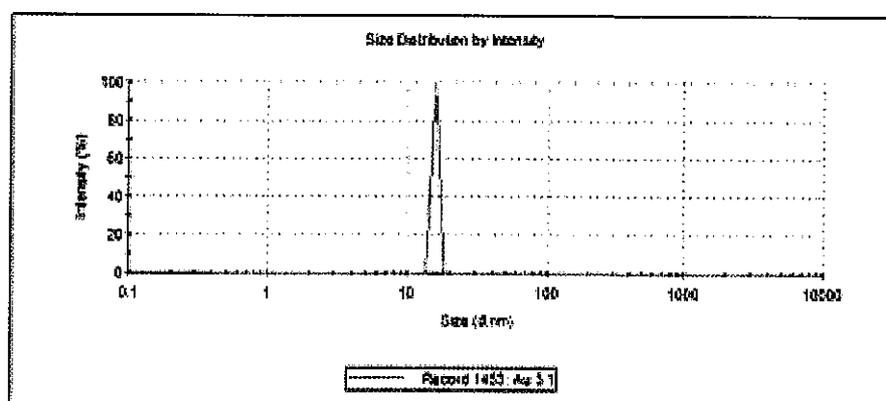

Fig. 2. Size distribution of gold nanoparticles synthesized by manual stirring in presence of NaBH₄ as reducing agent.
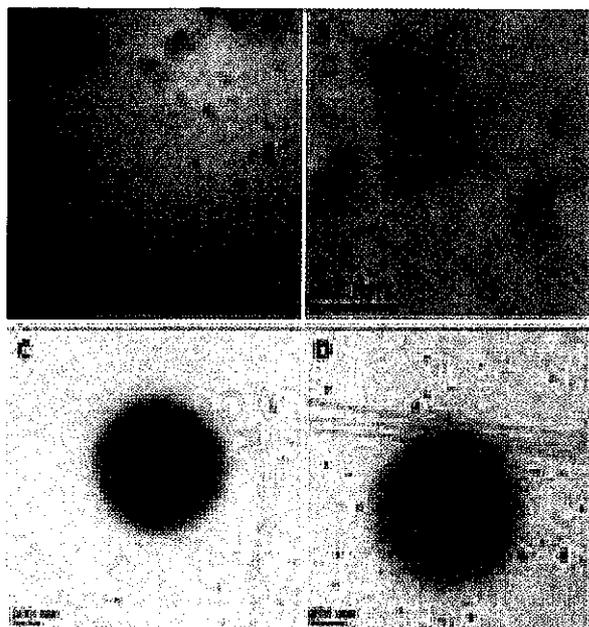
Fig. 3. TEM images of gold nanoparticles prepared with NaBH$_4$ reduction method (A, B) and TEM images of AuNPs encapsulated with PCL (C, D).

by DLS technique may be due to possible aggregation in particulate dispersion. The morphology of the polycaprolactone submicron particles incorporated with gold nanoparticles was also observed by using TEM. The results showed that the obtained particles are spherical in shape and the gold nanoparticles are well dispersed within the polymeric matrix (Figs. 3(C and D)). Some small size AuNPs can also be seen in surroundings of the polymeric particles, which represent the encapsulated gold nanoparticle in the medium (Fig. 3(D)).

3.2.3. Scanning Electron Microscopy

Scanning electron microscopy (SEM) of encapsulated gold nanoparticles was also performed in order to investigate the morphology the obtained particles (Fig. 4).
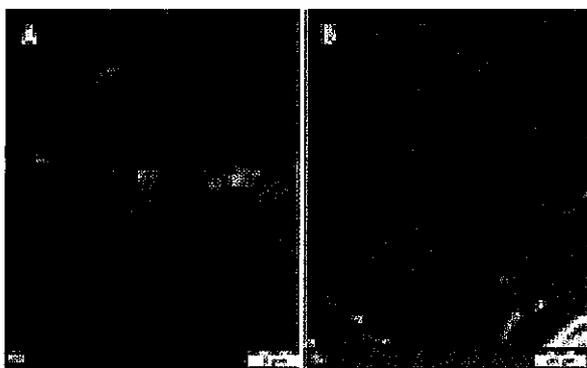

Fig. 4. SEM images of PCL particles containing AuNPs (A, B) prepared by the double emulsion solvent evaporation technique.

The SEM images showed that the polymeric particles containing AuNPs have smooth surfaces with spherical shapes. The smooth surfaces of polymeric particles support the assumption that the AuNPs release would occur through surface erosion of polymeric particle. Furthermore, some bringing among the particles were observed in the SEM images, which is possible due to presence of PVA as stabilizer during the encapsulation process, which is sticky in nature.

4. CONCLUSION

The present work was carried out in order to investigate the synthesis and characterization of gold nanoparticles and their encapsulation with a biodegradable polymer i.e., polycaprolactone. The effects of various experimental conditions on the particle size and size distribution have been studied. The formation of gold nanoparticles was evident from the change in color of gold salt solution from yellowish to ruby red with the addition of reducing agents. It has been shown that smaller nanoparticles with narrow size distribution were obtained in the case of the manual stirring method with lowest amount of reducing agent. Furthermore, the addition of PVA along with reducing agent has also significant effect on long time stabilization of dispersed system and diminishes the prospects of rapid aggregation. The gold nanoparticle size estimated from TEM images were smaller compared to the hydrodynamic particle size obtained from DLS analysis. The TEM morphology of gold nanoparticles showed their spherical morphology with uniformly dispersed distribution. TEM images of PCL particles incorporated with AuNPs have also revealed their spherical morphology with core–shell structure and the proper distribution of gold nanoparticles throughout the polymeric matrix. SEM morphology of the encapsulated particles (PCL-AuNPs) showed their smooth surfaces with spherical shapes. Some bridging among the particles was observed due to the sticky nature of the stabilizing agent PVA.

From this work, it can be concluded that reducing agents such as NaBH$_4$ and PVA can be used successfully for the preparation of gold nanoparticles and these AuNPs could be used as MRI contrast agent in various biomedical applications both *in vitro* and *in vivo*, and visualization of various tumor tissues. Functionalization of these particles would be possible for various other potential applications in different fields.

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