Green synthesis of gold nanoparticles using glycerol incorporated nanosized liposomes

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ABSTRACT

There has been enormous interest in method development for the inorganic synthesis of metallic nanoparticles of desired size and shape in the last decade, due to their unique properties and extensive application in catalysis, electronics, plasmonics and sensing. Here we report on an environmentally-friendly, one-pot synthesis of metallic nanoparticles, which avoids the use of organic solvents, and only requires mild experimental conditions. The developed method uses liposomes as nanoreactors, where the liposomes were prepared encapsulating chloroauric acid and exploited the use of glycerol, incorporated within the lipid bilayer as well as in its hydrophilic core, as a reducing agent for the controlled preparation of highly homogenous populations of gold nanoparticles. The effect of temperature, the presence of capping agent and the concentration of

glycerol on the size and homogeneity of the nanoparticles formed was investigated and compared with solution based glycerol mediated nanoparticle synthesis. Well-distributed gold nanoparticle populations in the range of 2-8 nm were prepared in the designed liposomal nanoreactor with a clear dependence of the size on the concentration of glycerol, temperature and presence of capping agent whilst large and heterogeneous populations of nanoparticles with amorphous shapes were obtained in the absence of liposomes. Particle morphology and sizes were analyzed using transmission electron microscopy imaging and liposome size was measured using Photon Correlation Spectroscopy.

1 Introduction

Metal nanoparticles of diverse sizes and shapes have garnered great interest in the last decade due to their exceptional unique optical, electronic and chemical properties, which are not displayed in the bulk state of the metal.[1, 2] Among those metals, gold (Au) based nanoparticles (NPs) have been of particular interest due to their widespread application in catalysis, plasmonics, sensors, and biomedical technologies (e.g. drug delivery) as well as electronics.[3,4] The development of synthesis methods to obtain homogenous and tunable sized and shaped nanoparticles for specific application requirements is a priority in nanoparticle based technology development.[1] In the majority of the reported chemical synthesis methods of gold NPs, the basic principle is the reduction of Au(III) to Au(0), often exploiting sodium citrate and sodium borohydride as reducing agents in an aqueous solution. Subsequently, reduced gold atoms assemble in small clusters and finally these clusters provide nucleation sides for other molecules to adhere to and grow forming nanoparticles.[5] To avoid uncontrolled aggregation into larger particles, stabilizing or capping agents are usually added to the mixture. [6] Addressing

environmentally friendly methods of producing nanoparticles, renewable reagent sources such as alcohols[6,] bacteria,[7] plant extracts[5], and polyols[8] have been demonstrated as successful reducing agents and/or capping agents.

Glycerol is a known polyol used as a moistening and preservative agent to extend shelf life, as well as a sweetener in food technology and in the manufacture of many drugs. Oxidation products of glycerol are also of great interest and the development of new cost-effective methodologies for their production, using gold or palladium nanoparticles as catalysts have been reported.[9-12] However; there are very few attempts exploiting the reverse reaction, where the metals are reduced by glycerol, thus forming nanoparticles. To date, the synthesis of nanoparticles with polyols (most often ethylene glycol) is based on heating a polyol-inorganic salt mixture, typically to high temperatures over 100ºC depending on the melting temperature of the polyol, under continuous stirring conditions.[13] In their recent study, Grace and Pandian reported on the synthesis of gold nanoparticles and nanoprisms using glycerol as a reducing agent, both under reflux and microwave conditions, where the glycerol- $HAuCl_4$ mixture was heated to boiling point, resulting in the synthesis of spherical or prism-shaped nanoparticles, depending on the reaction time.[14] Nisaratanaporn and Wongsuwan prepared silver powders of larger than 63 nm from silver alkoxide using glycerol as a reducing agent, again heating the metal and undiluted glycerol solution to high temperatures (150 - 180 ºC),[15] whilst, Sarkar et al achieved the glycerol-mediated reduction of silver to form nanoparticles of 25 nm at room temperature but reduction required the addition of NaOH.[16] To the best of our knowledge, there is no report to date demonstrating the formation of extremely small nanoparticles using glycerol as a reducing agent at low temperatures and not requiring any additional reactants.

A widely reported method for the preparation of nanoparticles is the reverse micelle method, which exploits water-in-oil droplets stabilized by a surfactant (most often AOT (Aerosol OT, sodium bis(2-ethylhexyl) sulfosuccinate)).[17,18] They have been used as nanoreactors for the synthesis of structures having the same shapes as the micelle nuclei, such as metal nanoparticles [19] or metal hybrids,[20] ceramic materials, and [21] quantum dots[22] as well as polymer composites. [23] Reverse micelles tend to fuse and disperse randomly due to Brownian motion and the content exchange between two fused reverse micelles results in the formation of nanosized particles with their size being defined by the micelle volume.[24]

As an alternative to reverse micelles as nanoreactors, liposomes are promising candidates for the synthesis of metal nanoparticles as they provide a controllable environment, not only in the core, but also within the lipid bilayer.[25] However, the preparation of nano-sized liposomes is labor intensive. In a previous study carried out in our group, nanosized liposomes have been prepared using a one-step preparation method based on a pH jump,[26] which was not only environmentally friendly as it avoids the use of organic solvents, but is also extremely rapid as it requires no homogenization steps such as extrusion and sonication, with the preparation of a highly uniform population of nano-sized liposomes being achieved in less than an hour.

In this work, we report a new environmentally friendly, low-temperature method to obtain a homogenous population of ultrasmall gold nanoparticles using liposomes incorporating glycerol. The glycerol, which is incorporated on both the external and internal polar surface of liposomes encapsulating chloroauric acid, $HAuCl_4$, facilitates the reduction of Au(III) to form Au(0) atoms and subsequent nanoparticles. The effect of parameters such as temperature, use of capping agent and glycerol concentration was investigated in terms of particle size and monodispersity. The

resulting nanoparticles were characterized by transmission electron microscope (TEM). Highly monodisperse Au-NPs in a size range of 2-7 nm were obtained after 1 day of incubation at room temperature depending on the conditions used.

2 Experimental Sections

2.1 Materials

PBS buffer (10 mM, pH 7.4) supplied as a sachet of prepared lyophilised buffer, glycerol, $HAuCl_4$ and 6-mercapto-1-hexanol (MCH) were purchased from SIGMA. Liposomes were prepared using phospholipids supplied by Avanti® Polar Lipids Inc. All lipids were supplied as powders and were used without further purification. Sodium hydroxide and hydrochloric acid, reagent grade, ACS, were also purchased from Scharlau Chemie SA.

2.2 Preparation of encapsulating nano-liposomes Twenty mg $HAuCl_4$ or MCH/ $HAuCl_4$ encapsulating liposomes were prepared via the curvature tuned preparation method as reported previously.[26] Briefly, 50 mg of a phospholipid formulation of 1,2-dioleoyl-sn-glycero-3-[phosphor-rac-(1-glycerol)] (DOPG) and 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (lyso-PPC) in a 88:12 molar ratio was dissolved in 10 mL of previously prepared $HAuCl_4$ solution (2 mg/mL) in the presence or absence of 6-mercapto-1-hexanol (MCH, 1:50 $HAuCl_4$: MCH molar ratio) in PBS buffer (10 mM, pH 7.4) at various concentrations of glycerol. The solution was stirred at room temperature under argon.. The mixture was then treated with a rapid pH jump (pH 7.4 → pH 11 → pH 7.4) followed by an equilibration period of 25 min, where lipid

clusters curl into encapsulating liposomes of 20 nm in diameter. The resulting liposomes were purified using a Sephadex G-25 column and used freshly prepared.

2.3 Synthesis of gold nanoparticles in the presence of liposomes Solutions of nanoliposomes prepared as explained in section 2.2 were incubated at predetermined temperatures under shaking conditions. Following incubation, nanoparticles were purified by centrifuging at 1000 rpm for 5 min, 3 times with a methanol/ethanol mixture (1:4 v/v), and the collected pellet was re-suspended in toluene and stored at 4 °C for further characterization studies.

2.4 Synthesis of gold nanoparticles in the absence of liposomes I) 20 mg of $HAuCl_4$ was re-suspended in constant concentration of glycerol solution (3 % v/v to 15% v/v) in PBS (10 mM, pH 7.4) and incubated at predetermined temperatures for 24 hours. II) 20 mg of $HAuCl_4$ was re-suspended in constant concentration of glycerol solution in PBS (10 mM, pH 7.4) and the mixture exposed to a rapid pH jump from pH 7.4 to pH 11 and subsequent decrease to pH 7.4. Solution was again incubated at predetermined temperatures. III) $HAuCl_4$ and/or capping agent and 6-mercapto-1-hexanol (MCH) were mixed with a constant concentration of glycerol solution (3 % v/v to 15% v/v) in PBS (10 mM, pH 7.4) and the mixture exposed to a rapid pH jump from pH 7.4 to pH 11 and subsequent decrease to pH 7.4 and further incubated at predetermined temperatures. Solutions were continuously shaken in a temperature controlled shaker, and nanoparticles were purified by centrifuging at 1000 rpm for 5 min, 3 times with a methanol/ethanol mixture (1:4 v/v). The collected pellet was re-suspended in toluene and kept at 4 °C for further characterization studies.

2.5. Photon Correlation Spectroscopy (PCS) The mean diameter of nanoreactor liposomes

was measured using Zeta Sizer 3000H equipment from Malvern Instruments, Inc., [He-Ne laser (633 nm), detector angle of 90º] which measures the rate of fluctuation of the light scattered from the particles using photon correlation spectroscopy (PCS). Standard deviations were calculated from the mean of the data of a series of experiments (n≥3).

2.6 Transmission electron microscopy (TEM) imaging using a glass pipette, a drop of sample was added to a 200 mesh copper grid with a thin film of Formvar polymer and carefully dried using a filter paper. The sample was left at room temperature until a dried film was obtained. Transmission Electron Microscopy (TEM) analyses were performed using a JEOL 1011 transmission electron microscope operated at 80 keV with an ultra-high-resolution pole piece providing a point resolution of 2 Å. Micrographs (1024 pixels x 1024 pixels) were acquired using a Megaview III multiscan-CCD camera. Images were analyzed with an iTEM image analysis platform and the mean diameter was calculated measuring at least 100 particles from the series of experiments (n ± 3).

3 Results and Discussion

3.1 Solution based synthesis of gold nanoparticles in glycerol

We have previously reported on an environmentally friendly method for the rapid (<1h) preparation of highly homogenous spherical liposome populations, the size of which can be carefully controlled via a combination of lipid composition and temperature applied during an equilibration stage following a rapid pH jump from 7.4 to pH 11 and back to pH 7.4, which we term the "curvature tuned liposome preparation (CTLP)".[26] To demonstrate the concept of

producing gold nanoparticles by use of glycerol mediated reduction of chloroauric acid ($HAuCl_4$), solution based synthesis of nanoparticles was primarily studied at same conditions in the absence of lipids. Briefly, 20 mg chloroauric acid was mixed with glycerol in solution (15 % v/v) at 25 ^oC in PBS (pH 7.4, 10 mM), in the absence of liposomes. As illustrated in figure 1, after 1 day of incubation at room temperature, relatively large and heterogeneous particles (10-50 nm or more) with amorphous shape were obtained.

Polyol based reduction-oxidation reactions mainly depend on the reaction pH, thus one of the important parameters of the curvature-tuned liposome preparation method, which exploits a pH jump, will have an impact on reaction kinetics.[27, 28] Thus, solution based synthesis was also carried out at a constant concentration of glycerol (15 % v/v) after an instant pH jump to pH 11 and subsequent drop back to pH 7.4, followed incubation at room temperature for 24 hours. TEM images of the nanoparticle population following elimination of the glycerol excess by centrifugation with methanol/ethanol solution (1:4 v/v) (Figure 1-b) demonstrated that there was a decrease in the particle size and an increase in the number of particles (see Figure 1-b). However, the particles were still amorphous and mainly aligned as chains of several particles (inset of Figure 1-b) with a length of around 20 nm.

Further studies on the effect of capping agent at a glycerol concentration of 15 % v/v in the absence of liposomes resulted in smaller particles (5-10 nm) compared to the ones obtained without MCH (around 20 nm). These results clearly show that the reduction of the Au(III) to Au(0) at room temperature using glycerol produces relatively small nanoparticles when they are

exposed to a instant pH change compared to those formed directly in PBS solution (10 mM, pH 7.4), in the absence of capping agent.

3.2 Liposomal nanoreactor design

As an alternative to the well-established technique of the reverse micelle method for the preparation of nanoparticles, liposomes were exploited as a nanoreactor for nanoparticle synthesis. It was expected that in the presence of liposomes, the nano-environment of the interior core, would provide a semi-solid reaction environment by keeping glycerol semi-mobile, thus facilitating the formation of nanoparticles in a more controlled manner than their synthesis in solution, and we thus incorporated glycerol into the nanoliposome formulation. Glycerol is commonly used in liposomal formulations as it increases the solubility of lipids and encapsulated materials in water, as well as enhancing the stability of formed liposomes via interaction with the polar head groups of the phospholipids.[29] Furthermore, glycerol can be used to reduce chloroauric acid to form gold nanoparticles. There are many reports on polyol-mediated synthesis of metal nanoparticles, but there is not extensive information available on the underlying mechanism. Leiva et al. postulated that although the exact mechanism is not fully understood, that the gold reduction reaction in the presence of alcohols most likely occurs due to the –OH groups of the reducing agent.[30] Like all other redox reactions, the reduction of the metal is driven by the difference between the redox potentials (ΔE) of the oxidation capacity of the metal salt and the reductivity of the polyol.

The preparation of nanoliposomes encapsulating chloroauric acid, whilst also incorporating glycerol in the lipid bilayer as a reducing agent, to produce gold nanoparticles both in the lipid bilayer and in the liposome core was explored.

In the designed nanoreactor, hydrophilic Au (III) would expect to be encapsulated in the aquatic core of the membrane whilst glycerol would be located on the internal and external surface of the liposomes as well as within the aquatic core providing nucleation sites for gold nanoparticle formation (Figure 2).

Liposomal nanoreactors of 24 ± 1 nm radius, as measured by photon correlation spectroscopy (PCS) were prepared using a formulation of 1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (DOPG) and 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (lyso-PPC) in a 88:12 molar ratio using our previously reported curvature tuned preparation (CTP) method,[18] at 25 ºC in the presence of a constant concentration of $HAuCl_4$ and at varying concentrations of glycerol (0-15 % (v/v)). Figure 3 depicts the TEM images showing the stages of the reduction reaction in the presence of glycerol incorporated liposomal nanoreactors before reduction and during the reaction with a clear appearance of nanoparticles within the nanoreactor, as well as the homogenous nanoparticle population observed following the elimination of lipidic membrane by centrifugation with methanol/ethanol mixture. The particle formation occurs mostly throughout the liposome membrane where the glycerol molecules are less mobile, as well as inside the liposome core, as was expected. Although, liposomes were purified using gel chromatography, since the system is dynamic, trace amount of gold and/or glycerol could diffuse to the bulk solution. As can be seen from the TEM image of liposomal nanoreactors after 24 hours of incubation (Figure 3), some particles are located close to the liposomal membrane. Thus, it is likely that this small number of gold nanoparticles have grown from some free gold impurity

remaining after the purification. Moreover. no particle formation was observed in control experiments carried out with $HAuCl_4$ encapsulating liposomes in the absence of glycerol under the same conditions studied.

3.3 Evaluation of the effect of reducing agent concentration and capping agent.

In previous reports, 3% v/v of glycerol was found to be optimum for the long-term storage of liposomes.[26,29] However, as the reducing agent concentration is an important parameter for a well-defined method of metal nanoparticle synthesis, the effect of glycerol concentration in a range from 3-15 % v/v was studied. [Note: At higher concentrations of glycerol no liposomes were formed.] In addition to glycerol concentration, the influence of the presence of a capping agent was also evaluated using a short chain alkanethiol, 6-mercapto-1-hexanol (MCH) as a model, which was encapsulated in liposomes with $HAuCl_4$ in an excess of 1:50 $HAuCl_4$/MCH molar ratio. The formed liposomes were incubated in sealed glass bottles at room temperature under stirring conditions. In the absence of MCH, the solution colour changed from pale yellow to green-brown, with the colour intensity proportional to the glycerol concentration, which over time deepened to a very dark brown, indicative of oxidation by-products of glycerol formed due to the reaction between Au (III) and glycerol. As can be seen in Table 1, no significant changes in size were observed (from 7.7 ± 1.7 nm to 6.4 ± 1.3 nm) with increasing glycerol concentrations of 3% and 15 % v/v, (Figure 4, 1st line). However, the presence of the MCH capping agent encapsulated in the liposomes together with the $HAuCl_4$ lead to a sharp decrease in particle size, with particles of 2.9 nm to 4.9 nm obtained using MCH with glycerol concentrations of 15 % v/v and 3 % v/v, respectively (Figure 4, 2nd line). This decrease in particle size in the presence of MCH can can also be attributed to be due to the fact that MCH can, to a minor extent act as a

reducing agent due to the presence of the alcohol group. Similar correlations between the particle size and the stabilizer and/or reducing agent have been reported elsewhere.[33] In a recent study on the use of poly(e-caprolactone)/poly(N-vinyl-2-pyrrolydone) triblock copolymer as stabilizer and reducing agent for the AuNPs, a decrease in the particle size with increased ratio of copolymer to gold salt was reported.[31] In another study on the effect of Au/thiol ratio by Frenkel et al. (2005) where they used x-ray absorption fine-structure EXAFS spectroscopy technique, concluding that the mean cluster size strongly depends on the Au/thiol ratio, with the lower the Au/thiol ratio, the smaller the nanoparticles formed. [32]

3.4 Influence of Temperature

In addition to concentrations of the reactant, reaction kinetics are also governed by temperature and pH.[30] Thus, polyol driven synthesis reactions are more efficient at high temperatures, although at optimized concentrations of metal salt, slow reduction might occur at low temperatures.[33] Therefore, it is crucial to study the effect of temperature on the particle properties since the parameters influencing the reaction will control the shape and the size of the particles formed. Here, we investigated the effect of temperature (in the range of 4-50 ^{o}C), on the particles formed. MCH/$HAuCl_4$ encapsulating liposome solutions (glycerol, 15 % v/v) were incubated for 24 hours under constant stirring conditions at a defined constant temperature. As shown in Figure 5, decreasing particle sizes were obtained with increasing temperature over the range of 4 - 50 ^{o}C, with a significantly larger nanoparticle size observed at 4 ^{o}C compared to the particles obtained at 50 ^{o}C (6.3 nm, 1.9 nm, respectively). As the temperature increases, the reaction rate increases as a result of rapid nucleation, and thus the amount of metal consumed for the nucleation increases,

resulting in a decrease in the number of molecules available for the further growth of nanoparticles, thus producing smaller nanoparticles (Figure 5-e).[32,33]

4 Conclusions

The synthesis of metal nanoparticles using polyols is an environmentally friendly method which often proceeds both under reflux and microwave conditions, where the undiluted polyol-metal mixture is heated to temperatures higher than the boiling point of the polyol used achieving nanoparticles of 10-100 nm, depending on the operational conditions. In this report, glycerol, a renewable and natural polyol, was studied as a green catalyst for the reduction of gold to assemble in nanoparticles without the use of any harsh chemicals. Moreover, exploiting the ability of glycerol to be incorporated within a liposomal membrane, here we report a functional, nanosized liposomal nanoreactor exploiting glycerol incorporated in both the external and internal surface of the lipid bilayer. The liposomal membrane keeps the reducing agent semi-mobile in their nanoenviroment exposing nucleation sites for the subsequent particle growth in a controlled manner. Reaction parameters such as temperature, glycerol concentration, and the effect of capping agent were studied in terms of their effect on size and the homogeneity of nanoparticles formed and compared to solution based synthesis at same conditions studied. Increased concentrations of glycerol resulted in a slight decrease size of the nanoparticles and, furthermore, nanoparticles synthesized in the presence of capping agent showed almost a 2 fold decrease in the particle size leading to ultrasmall gold nanoparticles of around 2 nm. Moreover, a decrease in the nanoparticle size at constant concentrations of capping agent and glycerol was observed when the temperature was increased in the range of 4 ^{o}C to 50^{o}C. Comparison studies of gold nanoparticle synthesis in solution under the same conditions without the use of the

nanoliposome reactors resulted in highly heterogeneous nanoparticles with amorphous shape, where with the presence of capping agent and pH jump step relatively finer results were obtained. These results indicate that with the designed liposomal nanoreactors, with glycerol integrated in the membrane as a reducing agent, a one-pot synthesis of highly homogenous nanoparticles was successfully achieved as a result of semi-solid reaction environment provided by the liposome. The functional nanoreactors presented here could provide inspiration for the development of new and greener synthesis methodologies in order to produce metallic nanoparticles in a safer and more efficient way.

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Supporting Information

Figures of the size distribution of the particles synthesized using a liposome-free solution based method and TEM image representing gold aggregates obtained in the presence of MCH alone. This material is available free of charge via the Internet at http://pubs.acs.org.

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FIGURE CAPTIONS

Figure 1: TEM images of the particles synthesized using direct synthesis of gold nanoparticles using glycerol in PBS after 24 hours of incubation: a) Glycerol-$HAuCl_4$ mixture in PBS (10 mM, pH 7.4) incubated at 25 ^oC without capping agent, b) Glycerol-$HAuCl_4$ mixture in PBS (10 mM, pH 7.4) incubated at 25 ^oC without capping agent after an instant pH jump (arrows indicate the particle chains), and c) Glycerol-$HAuCl_4$ mixture in PBS (10 mM, pH 7.4) incubated at 25 ^oC at constant concentration of capping agent after an instant pH jump. Insets are magnified images of corresponding particles. d) UV-Vis spectra of the sample (c), band observed at 546 nm.

Figure 2: Schematic of designed liposomal nanoreactor.

Figure 3: Formation stages of gold nanoparticles inside the glycerol incorporated (15% v/v) liposomes: Liposomes before reaction (top-left) and liposomes during the reaction (middle) (scale

bars = 10 nm) and purified gold nanoparticle synthesized in the nanoreactor (bottom-right) (scale bar 20 nm). Photon correlation spectroscopy graph of liposome size distribution before the reaction, PI= 0.232 (bottom left corner) and graph of calculated nanoparticle size distribution using iTEM (top right corner).

Figure 4: Effect of capping agent and glycerol concentration: Calculated particle size distribution and corresponding TEM images of particles prepared in the liposomes in the absence of capping agent (MCH) (a-c) and in the presence of MCH (d-f) using changing concentrations of glycerol : a and d) 3 %, b and e) 10 %, c and f) 15 % after 24 hours of incubation at 25 ºC. Scale bars are 50 nm.

Figure 5: Effect of temperature on the particle size and shape synthesized in glycerol (15 % v/v) incorporated liposomes in the presence of capping agent MCH at changing temperatures: a) 4 ºC, b) 25 ºC, c) 35 ºC, d) 50 ºC after 24 hours of incubation, and e) graph presenting the calculated mean diameter of the particles, n≥3, scale bars = 20 nm.

SCHEME TITLES

Scheme 1: Structures of molecules used for the nanoreactor design.

TABLE TITLES

Tabel 1: Particle size after 24 hours of incubation at different glycerol concentration in the presence and absence of capping agent at 25 ºC Standard deviations were calculated from the mean of the data of a series of experiments (n≥3)

FIGURES

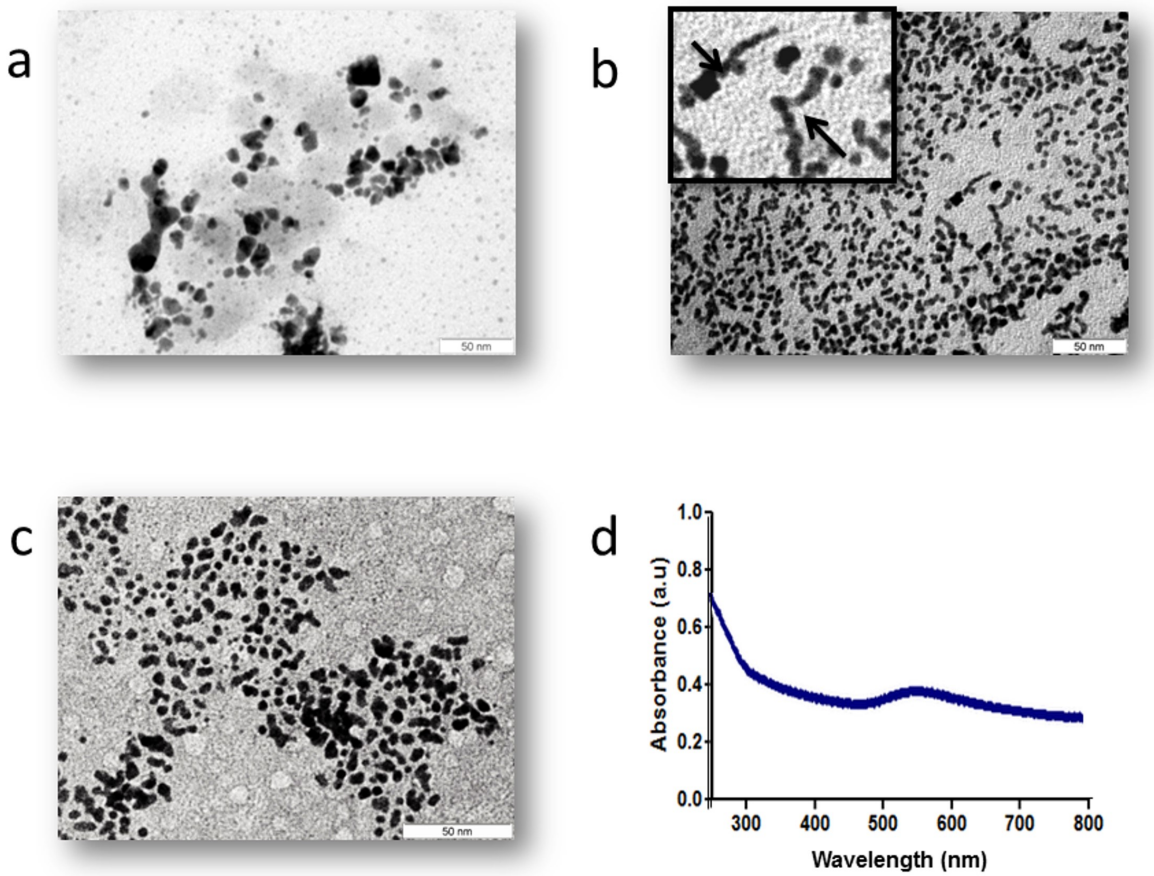


Figure 1

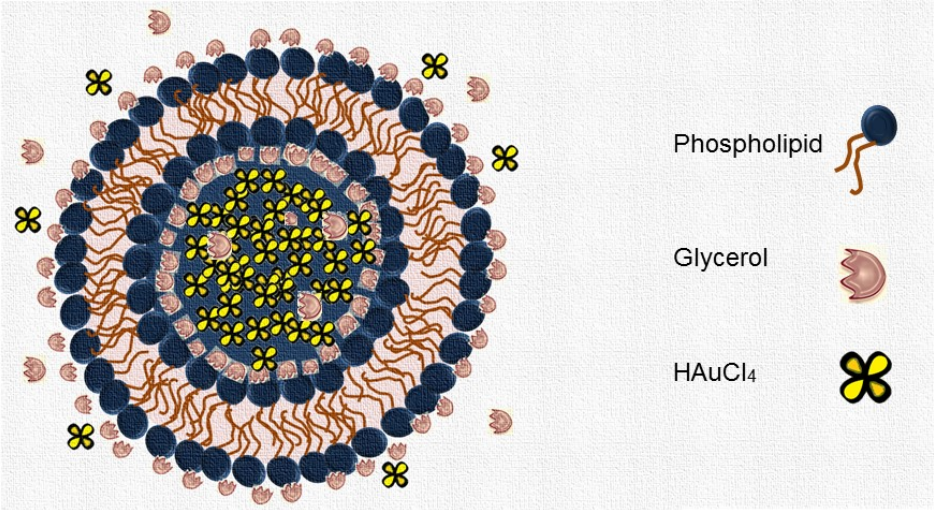
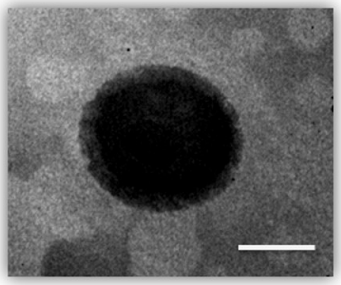
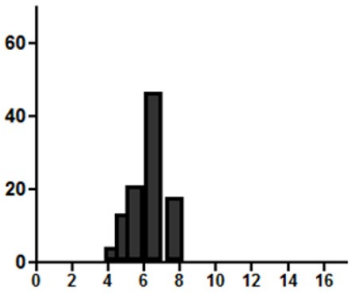
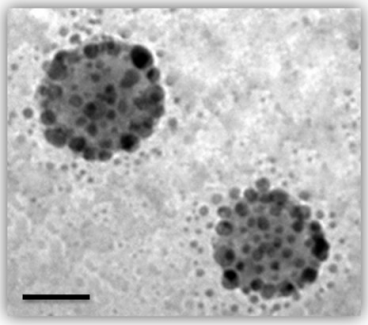


Figure 2

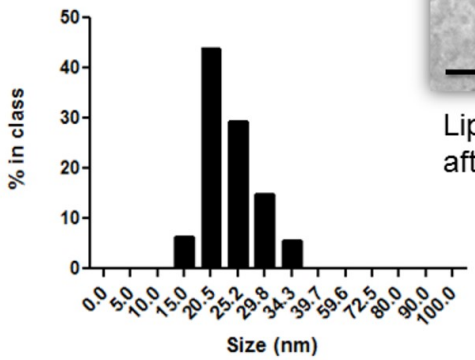


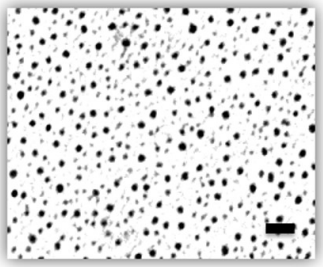
Liposomes
before reaction





Liposomes
after 24 hrs of incubation





Purified
gold nanoparticles

Figure 3

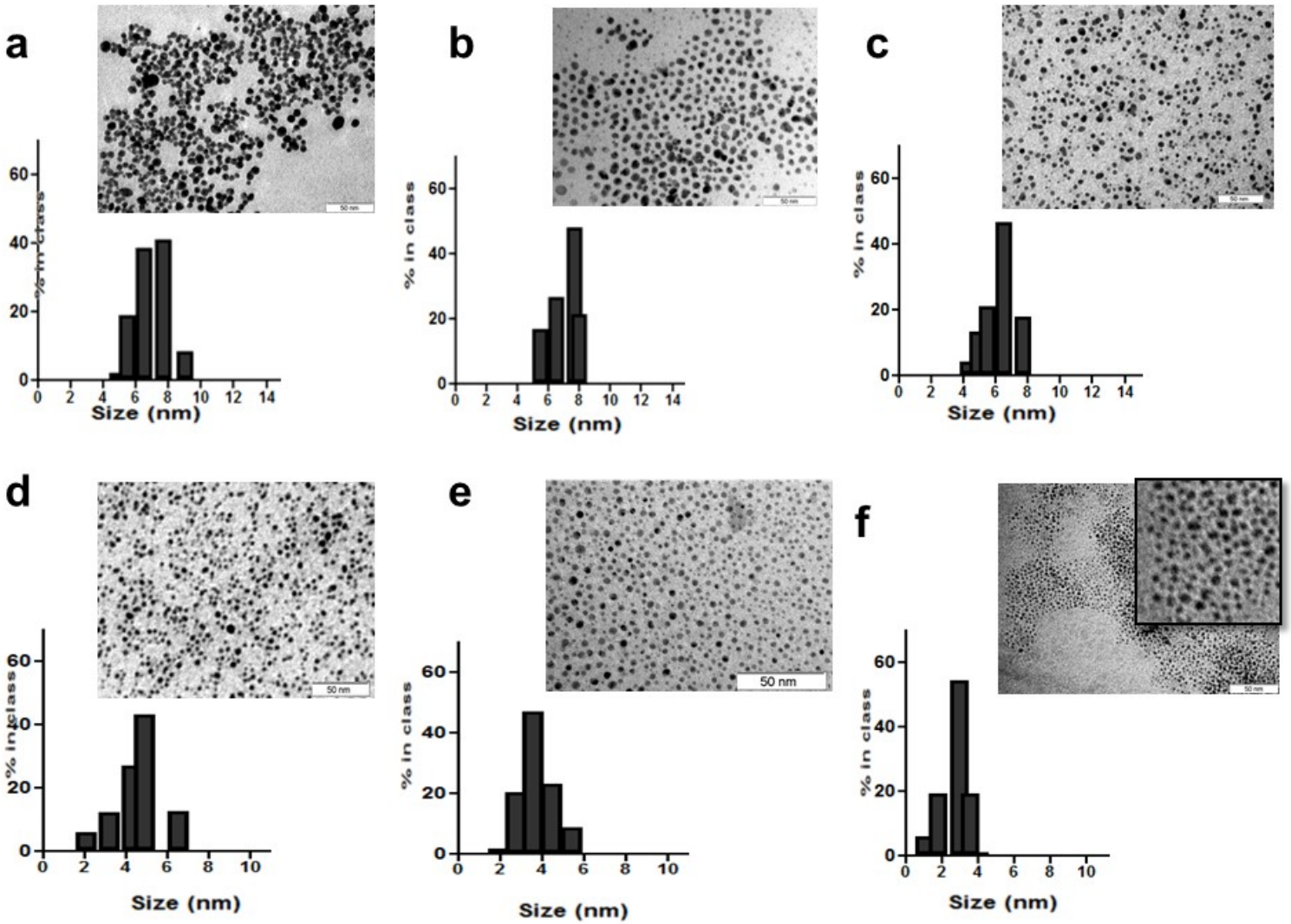
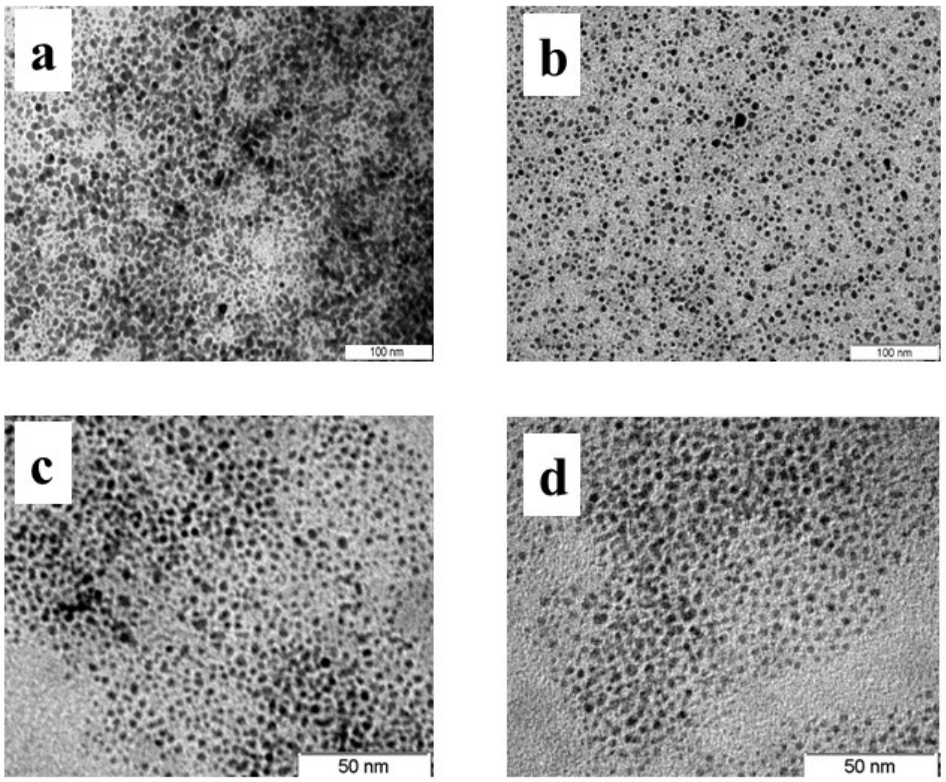


Figure 4


a
100 nm
b
100 nm
c
50 nm
d
50 nm

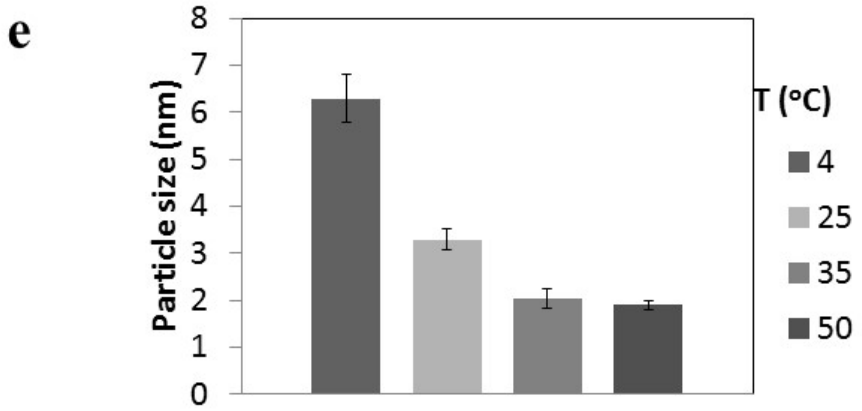
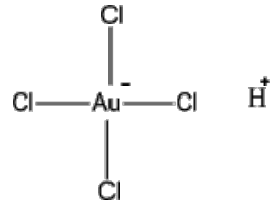
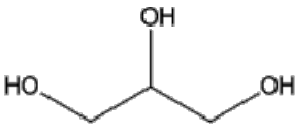

e
Particle size (nm)
0
1
2
3
4
5
6
7
8
T (°C)
4
25
35
50

Figure 5

SCHEMES

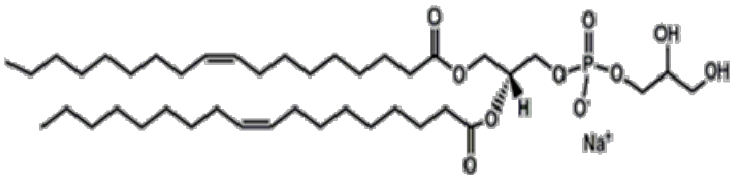


chloroauric acid

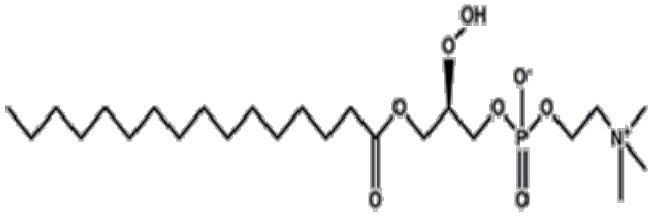


glycerol

6-mercapto-1-hexanol



1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (lyso-PPC)



1,2-dioleoyl-snglycero-3-[phosphor-rac-(1-glycerol)]

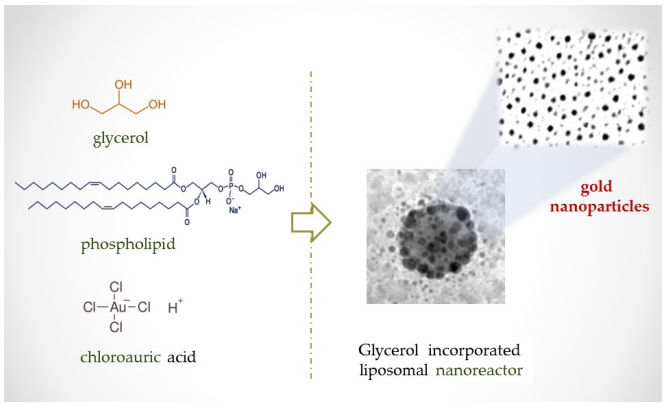
Scheme 1

TABLES

Glycerol concentration v/v	Absence of capping agent (nm)	Presence of capping agent (nm)
15 %	6.4±1.3	2.9±0.2
10 %	7.3±1.5	3.5±0.3
3 %	7.7±1.7	4.9±1.4

Table 1

TOC figure


glycerol
phospholipid
chloroauric acid
gold
nanoparticles
Glycerol incorporated
liposomal nanoreactor