LIPOSOMAL NANOREACTORS FOR THE SYNTHESIS OF MONODISPERSE PALLADIUM NANOPARTICLES USING GLYCEROL

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KEYWORDS: palladium, nanoparticle, synthesis, glycerol, reducing agent, capping agent, liposome, nanoreactor.

ABSTRACT: The synthesis of highly stable ultra small monodisperse populations of palladium nanoparticles in the range of 1-3 nm in size was achieved via polyol reduction within 1,2-dioleoyl-sn-glycero-3-phosphor-rac-(1-glycerol) liposomal nanoreactors exploiting glycerol as both reducing and stabilizing agent. The liposome-based green method was compared with synthesis in solution and the reducing agent concentration and the lipidic composition of the liposomal nanoreactors was demonstrated to have a strong effect on the final size and homogeneity of the palladium nanoparticles. Glycerol molecules acting as capping agent demonstrated the ability to stabilize the palladium nanoparticles over a long period of time maintaining their homogeneity in size and shape. The obtained palladium nanoparticles were characterized using transmission electron microscopy, selected area electron diffraction, Fourier transform infrared and RAMAN spectroscopies, X-ray diffraction and dynamic light scattering to determine their morphology, size, charge, surface chemistry and crystal structure. The catalytic activity of palladium nanoparticle was also tested for a reduction reaction.

1| INTRODUCTION

The past decade has witnessed an increased interest in the development of uniform metallic nanosized particles due to their unusual size dependent chemical, electronic, magnetic and optical properties, which are not achievable in their bulk state.[1-3] Among other noble metals, palladium (Pd) nanoparticles (NPs) have been exploited in an extensive number of applications including catalysis, electronics, plasmonics, and sensors.[4-6]

The development of synthesis methods to obtain homogeneous size- and shape- tunable Pd nanoparticles is a grand challenge and different techniques, including photolytic[6] and thermal decomposition,[7] sonochemical[8] and hydrogen reduction,[9] electrochemical deposition,[10] as well as one-phase,[11] two-phase[12] reduction methods and biogenic methods,[13-14] have been reported. The most common methods utilize reducing agents such as citrates, ascorbic acid or borohydrides, often in combination with capping agents such as surfactants, polymers or organic ligands to mediate the reduction reaction and avoid aggregation.[15]

The synthesis of nanoparticles using the so-called polyol process is one of the most widely used methods to produce colloidal nanoparticles, in which a boiling polyalcohol acts as both solvent and mild reducing agent, as well as a stabilizer, for the reduction of a suitable metal salt precursor.[16-20] The most commonly used polyols for nanoparticles preparation are ethylene glycol,[21] diethylene glycol,[22] ethanol[23] and glycerol,[24] with glycerol being particularly attractive. Glycerol, a non-toxic, non-hazardous, non-volatile, biodegradable, recyclable and cheap liquid, has been reported as a solvent and reducing agent for the synthesis of many different nanoparticles including Au,[24-25] Ru,[26] Ag,[25-28] Pd,[26,29-30] Pt,[26,31] Pt-Ru,[31] Pt-Pd,[32] TiO_2,[33] $Mn(OH)_2$ and $MnCO_3$.[34]

Regarding the production of Pd nanoparticles, Y-W Lee et al. have demonstrated the ability of glycerol, together with PVP as a capping agent, to form Pd NPs at 100ºC with an average size of ~60 nm.[29] Grace et al. reported the synthesis of 7 nm Pd nanoparticles employing glycerol at its boiling point (290ºC) as a solvent and reducing agent also combined with PVP to stabilize and avoid particle aggregation.[26] In addition, Marquardt et al. achieved the synthesis of 1.5 nm sized Pt nanocrystals in glycerol without any additional stabilizer.[35]

There are several reports detailing the utilization of nanoreactors to control the reduction reaction and synthesize small homogeneous nanoparticles. An environmentally-friendly report presented by Puvvada et al. uses mild experimental conditions to produce Pd nanoparticles of 3.3 nm in size at room temperature using a controlled sponge-like structure of bicontinuous cubic phase of glycerol monooleate.[30] It has been reported that glycerol can act as a soft capping agent and Bakshi et al. have demonstrated that phospho-glycerol lipids act as stabilizers for Au and Cu nanoparticles[36] and Au-Cu bimetallic nanoparticles.[37] In another example, dendrimer mediated synthesis of nanoparticles has been reported for monometallic[38] and bimetallic[39-40] Pd-nanostructures. In addition, Coulter et al. reported the use of functional polyelectrolytes as nanoreactors to produce Pd nanoparticles using $NaBH_4$ with an average size of 5 ± 1.5 nm.[41]

A wide number of reports using reverse micelles for the synthesis of palladium nanoparticles inside the reverse micelle cores, exploiting the water-in-oil microemulsions stabilized by surfactant molecules, most frequently cetyltrimethyl-amonium bromide (CTAB),[42-44] or dioctylsulfosuccinate sodium salt (AOT)[45-47] have been reported. In a novel approach, Sanchez-Dominguez described the synthesis of Pd nanoparticles of 6 nm using $NaBH_4$ within oil-in-water microemulsions.[48]

As detailed in Table 1, most of the previously reported methodologies to produce Pd nanoparticles require the use of harsh reducing agents, lengthy preparation time or high reaction temperatures under stringent process control. There is thus a need for a straight forward method for the synthesis of highly homogeneous small Pd nanoparticles using a simple green system obviating the need for additional reactants and omitting harsh procedures with extreme temperatures or utilizing non-environmentally friendly chemicals.

In our recently reported work, liposomes were used as nanoreactors for the preparation of homogeneous small gold nanoparticles,[49] and were demonstrated to provide an excellent control over the nanoparticle synthesis process within their internal core. Moreover, the lipidic membrane of the liposomes can act as permeable nanoreactor walls offering both an enhanced and restricted mobility for different reactants of the synthesis reaction. Glycerol-incorporating liposomes are promising candidates for the synthesis of nanoparticles via glycerol reduction, as they provide a specific space inside their internal hydrophilic cavity, as well as a hydrophobic environment within the lipid bilayer, where the polyol reduction reaction can occur in a more controlled manner.

Table 1. Methodologies to produce palladium nanoparticles.

Reducing agent (Reaction conditions)	**Stabilizing / Capping agent**	**Pd NPs Diameter (nm)**	**References**
$NaBH_4$ (RT)	TOAB	~1-4	3
Glycerol (290ºC)	PVP	7	26
Glycerol (100ºC)	PVP	~60	29
Glycerol monooleate (RT)	-	~3	30
Dendrimer amino groups (50ºC)	Dendrimer	~6	38
$NaBH_4$ (RT)	Dendrimer	~2	40
$NaBH_4$ (RT, UV-light)	Poly (acrylic acid)	~5	41
Hydrazine (RT)	CTAB	~4	42
Ascorbic acid (UV-light)	CTAB	~7-50	43
Hydrazine (RT)	AOT	~10	45
Hydrazine (RT)	AOT	~6-13	47
$NaBH_4$ (RT)	Oil-in-water	~6	48

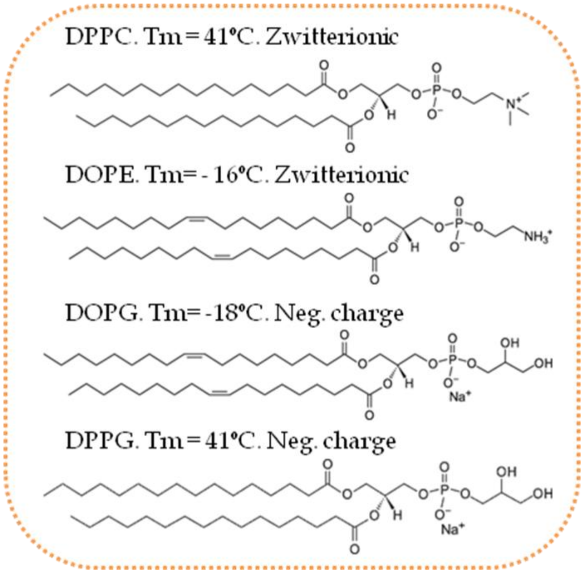
Herein, we exploit our previously reported method for the rapid preparation of highly homogeneous nanosized liposomes[50] to introduce an environmentally-friendly, solvent-free, one-pot method for the synthesis of Pd nanoparticles using glycerol as both reducing and capping agent under mild experimental conditions. The method involves the encapsulation of $PdCl_2$ with glycerol molecules within nanosized liposomes for the controlled production of highly homogeneous populations of ultra small glycerol-stabilized Pd nanoparticles. Furthermore, we have systematically investigated the effect of the experimental conditions, including the type, and concentration of reducing agent, the membrane lipid composition, as well as the effect of the addition of a capping agent on the homogeneity and size evolution of the Pd nanoparticles.

2| EXPERIMENTAL SECTION

2.1| MATERIALS

All the chemicals were used without further purification. The phospholipids 1,2-dipalmitoyl-sn-glycero-3-phospho-rac-(1-glycerol) (DPPG), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 1,2-dioleoyl-sn-glycero-3-phosphor-rac-(1-glycerol) (DOPG) were purchased as powders (Scheme 1) from Avanti Polar Lipids, USA. The phospholipid 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), as well as the palladium(II) chloride ($PdCl_2$), sodium borohydride ($NaBH_4$), p-nitrophenol, sodium citrate and phosphate buffer saline (PBS: 10 mM prepared lyophilized buffer with NaCl 0.138 M, KCl 0.0027 M, pH 7.4) were purchased from Sigma-Aldrich. Glycerol, toluene, ethanol, methanol were supplied by Scharlau. A Simplicity 185 Millipore-Water System was used to obtain Milli-Q water (1.82 MΩ.cm^{-1}) for the preparation of buffers and liposomes.

Scheme 1. Structure of the lipid molecules used for the liposomal nanoreactor study.



2.2| SYNTHESIS OF Pd NPs

Synthesis within liposomal nanoreactors

The liposomal nanoreactors were prepared employing the method previously reported by our group to produce homogeneous populations of liposomes.[50] Briefly, 20 mg of $PdCl_2$ was dissolved in 10 mL of PBS with 10% (v/v) glycerol under continuous stirring conditions and in the presence of bubbling argon gas. Due to the presence of NaCl, the suspension of non-water soluble $PdCl_2$ changed to a reddish-brown solution of $[PdCl_4]^{2-}$ after 15 minutes. Then 50 mg of different lipid formulations (DPPC, DPPG, DOPE or DOPG) was added and maintained under stirring conditions and argon at 25 ºC for another 15 minutes. The well homogenized mixture was then subjected to a rapid pH jump from pH 7.4 to pH 11 and then back to pH 7.4 within a 3 second timeframe, followed by an equilibration step of 25 minutes where lipids curl into $PdCl_2$ encapsulating liposomes. Large lipid aggregates that do not assemble into liposomes were discarded by centrifuging at 2000 rpm for 5 minutes and the nanoliposomes present in the supernatant were then incubated for 24 hours in a temperature-controlled shaker (25 ºC, 400 rpm). Following incubation, the nanoparticles formed inside the liposomal nanoreactors were purified by centrifuging 3 times at 5000 rpm for 5 minutes with a methanol/ethanol solution (1:4 v/v) to break up the liposomes and recover the pellet that contains the Pd NPs. The pellet was resuspended in toluene and stored at 4 ºC for further characterization studies.

Solution-based synthesis

Pd NPs were synthesized via reduction, in the absence of liposomal nanoreactors, in the presence of three different reducing agents, sodium citrate (1.4 M), $NaBH_4$ (0.1 M) and glycerol, which was used at different concentrations (5 %, 10 % and 20 % v/v). In all cases, 20 mg of $PdCl_2$ was mixed with each of the reducing agents in 10 mL of 10 mM PBS (pH 7.4), and then incubated for 24 hours in a temperature controlled shaker (25 ºC, 400 rpm). The nanoparticles formed were purified by centrifuging firstly at 2000 rpm for 5 minutes and the collected supernatant was then centrifuged three times at 5000 rpm for 5 minutes, as described in the previous section. Following the first centrifugation, the pellet formed was re-suspended in a methanol/ethanol solution (1:4 v/v), and after the final centrifugation, the pellet was re-suspended in toluene and stored at 4 ºC.

2.3| CHARACTERIZATION STUDIES

Dynamic light scattering (DLS) and Zeta Potential analysis

The Zeta Sizer 3000H (Malvern Instruments Inc. He – Ne laser of 635 nm, detector angle of 90º) was used to measure the mean diameter of the liposome nanoreactors via dynamic light scattering. The zeta potential was used to determine the surface charge of the liposomes and the Pd NPs. The standards deviations were calculated from the mean data of experiments (n ≥ 3).

Transmission Electron Microscopy studies

The morphology characterization and measurement of the mean diameter of the Pd NPs and liposome nanoreactors was performed using a transmission electron microscope (TEM) JEOL 1011 operated at 100 keV with an ultra-high-resolution pole piece providing 2 Å of point resolution. A drop of each sample was placed using a glass pipette onto a 200 mesh copper grid with a Formvar polymer thin film and left at room temperature to dry. The TEM images or micrographs (1024 pixels × 1024 pixels) were acquired using a CCD camera (Megaview III multiscan) and analyzed with the iTEM image analysis software. The mean diameter of the nanoparticles was calculated measuring at least 100 particles from multiple experiments (n ± 3).

In addition, the crystal structure of the Pd NPs was elucidated using the selected area electron diffraction technique (SAED), by observing the diffracted patterns of the electrons scattered from the Pd NPs.

X-Ray Diffraction investigation

X-ray diffraction (XRD) was used to reveal the crystal structure of the Pd NPs. The XRD measurements were made using a Siemens D5000 diffractometer (Bragg Brentano parafocusing geometry and vertical goniometer) fitted with a curved graphite diffracted-beam monochromator and diffracted-beam Soller slits, a 0.06º receiving slit and scintillation counter as a detector. The angular 2-theta diffraction range was between 35 and 90º. Sample was dusted on to a low background Si(510) sample holder. The data were collected with an angular step of 0.05º at 20s per step and sample rotation. Cu$_{k}$radiation was obtained from a copper X-ray tube operated at 40 kV and 30 mA.

Ultraviolet-Visible spectroscopy

The formation of the Pd NPs was monitored measuring the UV–Vis absorbance at 450 nm recorded in a Cary 100 Bio spectrophotometer (Varian) in 1 cm quartz cells.

Study of the catalytic properties

The UV-visible spectrum of a 6×10^{-5} M p-nitrophenolate solution in 0.1 M NaOH was recorded in 1 cm cuartz cell. An aliquot of a concentrated solution of $NaBH_4$ (prepared immediately before use) was added to a final concentration of 0.2 M and the spectrum was recorded again. Finally 10 µL of Pd NPs suspension were added and the spectra were recorded until the band at 400 nm disappeared. A similar experiment in the absence of Pd NPs was used as a control.

FTIR spectroscopy

FTIR spectra were recorded on a Jasco FT/IR-600 PlusATR Specac Golden Gate spectrometer. 128 scans at 2 cm^{-1} resolution were recorded.

Raman spectroscopy

Raman spectra were recorded using a Renishaw 2003 Spectrometer operating at wavelength of 633 nm of HeNe laser and using CCD camera as detector. The spectra were analyzed using Wire 3.2 version software (Renishawplc, New Mills, Wotton-underEdge, and Gloucestershire, GL12 8JR, United Kingdom).

3.| RESULTS AND DISCUSSION

3.1| SYNTHESIS OF Pd NPs IN SOLUTION

As mentioned previously, glycerol was used as a component in the liposomal nanoreactor formulation as well as for the synthesis of Pd NPs. To date, the reported methods that employ glycerol to reduce Pd(II) to form Pd nanoparticles are usually based on the use of elevated working temperatures and additional capping agent molecules.[26, 29] We have previously reported that glycerol can act as a reducing agent to produce extremely small Au NPs at low temperatures without requiring additional reagents.[49] In order to demonstrate that glycerol can reduce Pd(II) to Pd(0), palladium chloride ($PdCl_2$) was mixed with 10% (v/v) glycerol at 25 ºC in 10 mL PBS (10 mM + 0.138 M NaCl, pH 7.4) and left for 24 hours. The presence of NaCl in the buffer solution facilitates the solubilisation of Pd(II) salt by forming the complex anion $[PdCl_4]^{2-}$. The formation of Pd NPs was monitored using UV-Vis (Figure 1). The spectrum of $[PdCl_4]^{2-}$ in solution exhibited an absorption band at 425 nm and when the Pd(II) was reduced to Pd(0) the absorption band disappeared and a broad spectrum was observed, in agreement with that reported in the literature indicating the formation of nanoparticles.[26]

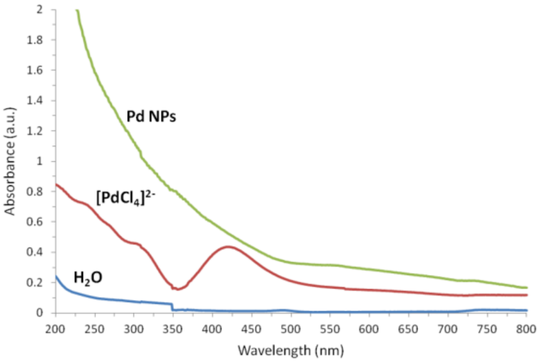


Figure 1. UV-Visible spectra of $[PdCl_4]^{2-}$ and Pd NPs produced by glycerol in solution.

The TEM characterization studies (Figure 2) clearly confirm that glycerol is able to reduce Pd(II) to Pd(0) producing small Pd NPs under mild conditions.

Once it had been demonstrated that Pd NPs can be produced using glycerol as a reducing agent, the effect of using a lower and higher concentration of glycerol (5% and 20% v/v) was investigated to observe how the ratio between glycerol/Pd(II) affects the formation of the Pd NPs. At higher glycerol/Pd(II) ratios the number of particles obtained increased and their mean size was smaller and more homogeneous (5 % = 14 ± 7; 10 % = 4 ± 1 , 7 ± 2 and 20 % = 3 ± 1). However, the particles were amorphous without a well defined shape. A 10% (v/v) concentration was selected as optimal as, whilst a marked effect on the size was observed changing the glycerol concentration from 5 % to 10 %, no considerable change was seen at higher concentrations, and above 15 % (v/v), the liposomes were not formed properly.

3.2| SYNTHESIS OF Pd NPs INSIDE LIPOSOMES

The described method for the preparation of homogeneous liposomal nanoreactors was used to provide nanoenvironments in the internal core where glycerol molecules are maintained semi-mobile and the reduction kinetics are less governed by random processes, thus facilitating the formation of Pd nanoparticles in a more controlled manner than their synthesis in solution.

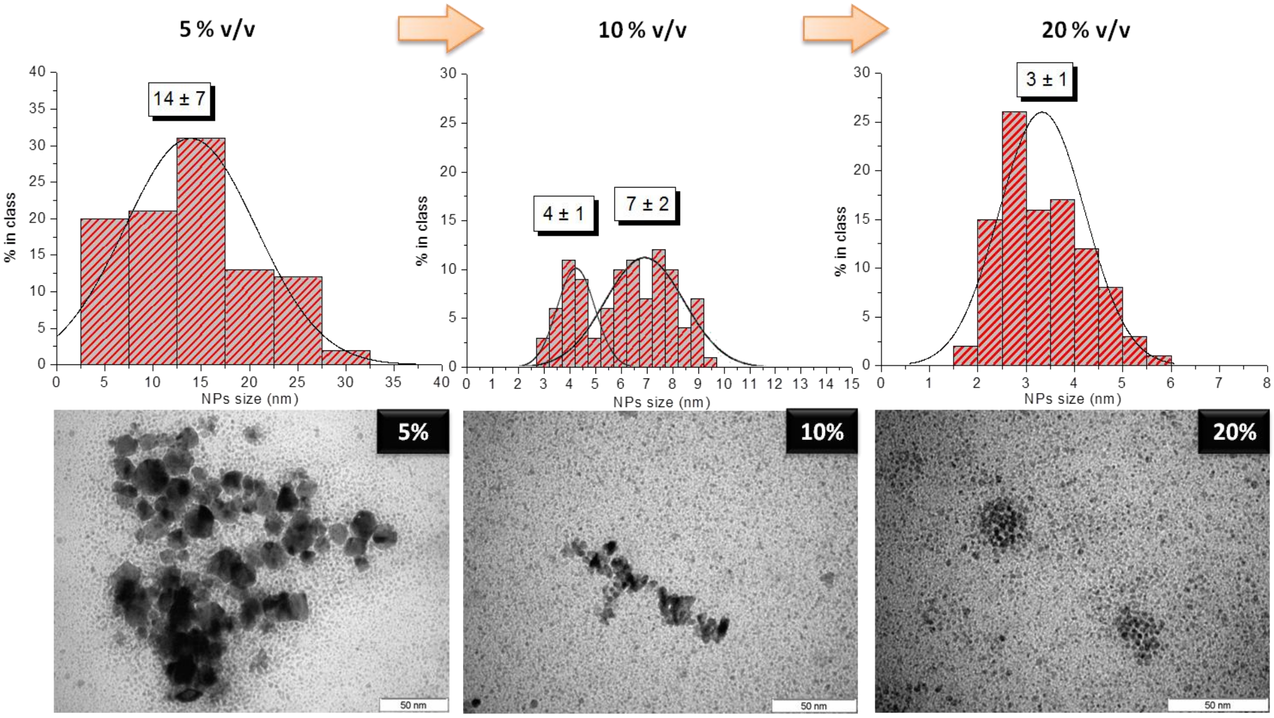


Figure 2. Effect of the concentration of glycerol on the solution-based synthesis of Pd nanoparticles. TEM images show the Pd nanoparticles produced in PBS solution containing 5 %, 10 % and 20 % (v/v) glycerol at 25 ºC and their correspondent size distribution graphs calculated using iTEM (n=100). Scale bars are 50 nm.

The formation of nanoparticles in solution is often irreproducible, as well as difficulties in restricting the freedom of components that can interact and govern the reduction kinetics in a bulky state reaction. As described in the introduction, reduction kinetics plays a key role in controlling the nucleation and growth of nanocrystals, and in the work reported here, we addressed this by providing a confined nano-environment in the liposomal internal core where the formation of nanoparticles can take place in a more controlled manner than in solution.

Taking advantage of the use of glycerol in liposomal formulations as a stabilizer, it was also exploited for the polyol mediated reduction of Pd(II) to Pd(0) within the liposomal nanoreactors. The small glycerol molecule adsorbed on the surface of the liposome could assist in increasing the solubility of the vesicle. Typically, the $[PdCl_4]^{2-}$ ions are encapsulated inside the hydrophilic core whilst glycerol molecules are distributed, in the external and internal part of the lipid membrane, as well as in the aqueous core forming small clusters from Pd atoms in the encapsulated solution. (Figure 3)

As a proof of concept, the Pd NPs produced via glycerol reduction inside DOPG liposomes were compared in terms of size, shape and homogeneity with those prepared in solution (i.e. in the absence of the liposome nanoreactors) using the same concentration of glycerol.

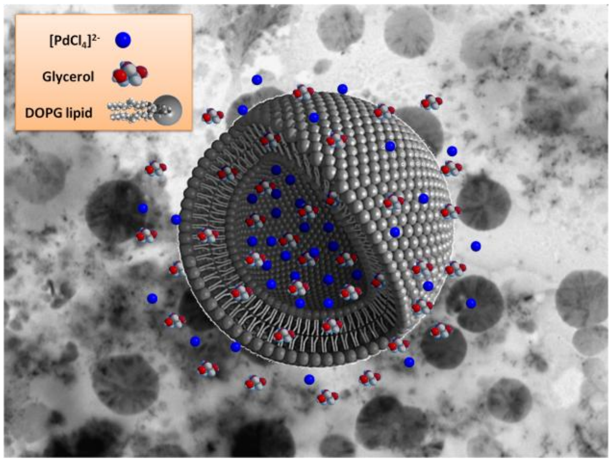


Figure 3. Schematic design of the glycerol-incorporated DOPG liposomal nanoreactor for the synthesis of Pd NPs.

As can be seen in Figure 4, the Pd NPs obtained in solution were bigger in size compared with those obtained within the liposomes. Both systems were able to produce Pd NPs. But in the case of those prepared within the liposomes, the Pd NPs were highly homogeneous, whilst in the solution based reaction the NPs appear to have a tendency to aggregate resulting in more dispersive populations of NPs with amorphous shapeless structures.

In addition, the results obtained within glycerol-incorporated DOPG liposomes were compared with standard reducing agents used for NP synthesis under the same experimental conditions. The same 10% (v/v) concentration was used with monosodium citrate (1.4 M) whilst a lower concentration of NaBH$_4$ (0.1 M) was employed as it is a much more powerful reducing agent. As expected, both reducing agents were able to form Pd NPs. Citrate-encapsulated DOPG liposomes produced highly dispersive populations of Pd NPs with heterogeneous sizes and shapes (11 ± 4 nm), and triangular- and decahedron-shaped Pd NPs were also observed which can be attributed to the citrate preferred binding sites to the (111) facets of Pd and, therefore, giving different growth rates to each of the facets.[51] When sodium borohydride was used as reducing agent in the DOPG liposomal nanoreactors, the Pd particle size synthesized was reduced (5 ± 2 nm) but dispersive and heterogeneous populations were present. However, homogeneous populations of monodisperse Pd nanoparticles of 0.9-3.5 nm in size produced with NaBH$_4$ in solution have been reported, but TOAB was required to act as capping agent and control reaction kinetics to avoid nanoparticle aggregation.[3]

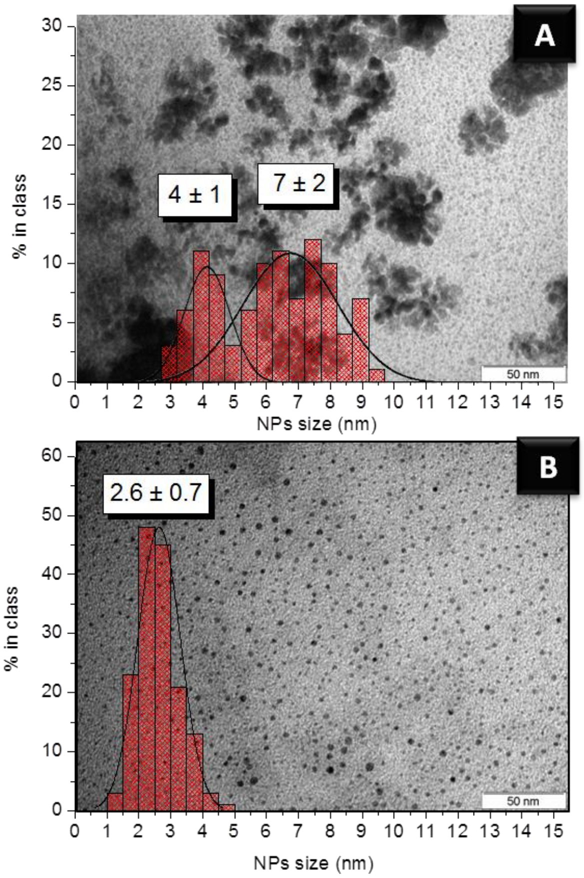


Figure 4. TEM images of Pd NPs obtained in solution (A) and within DOPG liposomes (B) using 10% (v/v) glycerol and their corresponding size distribution graphs calculated using iTEM (n=100). Scale bars are 50 nm.

Control experiments were carried out in the absence of glycerol. PdCl$_2$ was mixed with different lipids (DPPC, DPPG or DOPG) at 25 ºC in PBS (pH 7.4) under stirring conditions and argon, followed by a pH jump from pH 7.4 to 11 and back to 7.4 and left for 24 hours of incubation. It is believed that the presence of the strong acid and base reagents required for the pH jump (NaOH and HCl), even at very low concentrations, could be responsible for the presence of the small amounts of Pd NPs observed when DOPG was used (data not shown). In order to investigate if the pH jump can produce Pd NPs, control experiments were carried out mixing PdCl$_2$ with DPPC or glycerol-coated DOPG lipids in PBS (pH 7.4) at 25 ºC. TEM characterization confirmed that in the absence of the pH jump step no liposomes are formed as expected and, furthermore, no Pd NPs were produced in the case of DPPC lipids, whilst with DOPG lipids an extremely low number of NPs were formed, which can be attributed to the polyol reduction of the Pd(II) by the glycerol head groups present in DOPG lipids.

3.3| LIPID COMPOSITION EFFECT

The membrane of the liposome plays a very important role in nanoparticle synthesis, acting as route for the precursors to enter the nanoreactor. The membrane properties, such as permeability, surface charge, and functional group, can be modulated by changing the lipidic composition.[52]

The membrane permeability is a decisive parameter for the enhancement of the mobility of glycerol molecules through the lipid bilayer, providing a semi-mobile nano-environment for glycerol and facilitating access to the encapsulated palladium ions for the formation of nanoparticles in a highly controlled manner. The fluidic characteristics of the membrane are a result of the lipid melting temperature (Tm), where the lipids undergo a transition from a gel phase to a liquid phase, rendering a phase behavior to the entire bilayered system. Taking advantage of this membrane permeability given by the phase behavior, different lipidic formulations were tested with transition temperatures above and below the working temperature (25 ºC).

Liposomes prepared with DPPC and DPPG lipids (Tm 41 ºC) have a gel phase behavior that provide highly ordered and dense bilayers reducing the capacity of glycerol to flow inside the liposome core leading to a lower OH/Pd ratio and thus producing larger Pd NPs. As can be seen from the TEM images in Figure 5, the fixed number of glycerol molecules encapsulated within DPPC liposomes produces a narrow particle size distribution due to the rigid ratio of OH/Pd (16 ± 2 nm), whereas in the case of DPPG lipids, the higher OH/Pd ratio given by the glycerol-capped lipids leads to high, but irregular, reductive conditions within the liposome core producing smaller but randomly dispersed Pd NPs with irregular shapes (4 ± 2 nm and 16 ± 6 nm).

On the other hand, DOPE and DOPG liposomes have lower melting temperatures of -16 ºC and -18 ºC, respectively, and a higher degree of disorder induced by the carbon-carbon double bond in their oleic fatty acid chain. These properties confer flexible membranes where glycerol molecules can easily diffuse into the nanoreactor, producing Pd NPs smaller in size (Figure 5). The Pd NPs produced within DOPE liposomes are bigger and shapeless compared to the ones synthesized within DOPG, (9 ± 3 nm) and (2.6 ± 0.7 nm) respectively, and that can be explained due to the instability that ethanolamine head groups confer to the liposome system, promoting liposome fusion or membrane disruption.

As seen in Table 2, DOPG liposomes were optimal for the generation of small and homogeneous populations of Pd NPs. The presence of phospho-glycerol DOPG lipid provides an internal core coated with glycerol groups providing a confined reducing environment for the reaction and a semi-permeable membrane for glycerol to diffuse inside the nanoreactor core, thus forming notably smaller Pd NPs with a narrow particle size distribution. This suggests that reduction kinetics can be modulated by an optimal selection of the lipid composition of liposomal nanoreactor.

Table 2. Effect of the lipidic composition of the liposomal nanoreactor on the size and distribution of the palladium nanoparticles produced.

Lipids	Tm (ºC)	Charge	Size of Pd NPs (nm)
DPPC	41	Zwitterion	16 ± 2
DPPG	41	Negative	4 ± 2 ; 16 ± 6
DOPE	-16	Zwitterion	9 ± 3
DOPG	-18	Negative	2.6 ± 0.7

The head charge of the lipids gives both the internal and external regions a specific charge. These electrostatic forces not only prevent the fusion of liposomes into larger reactors but could also contribute to the synthesis of

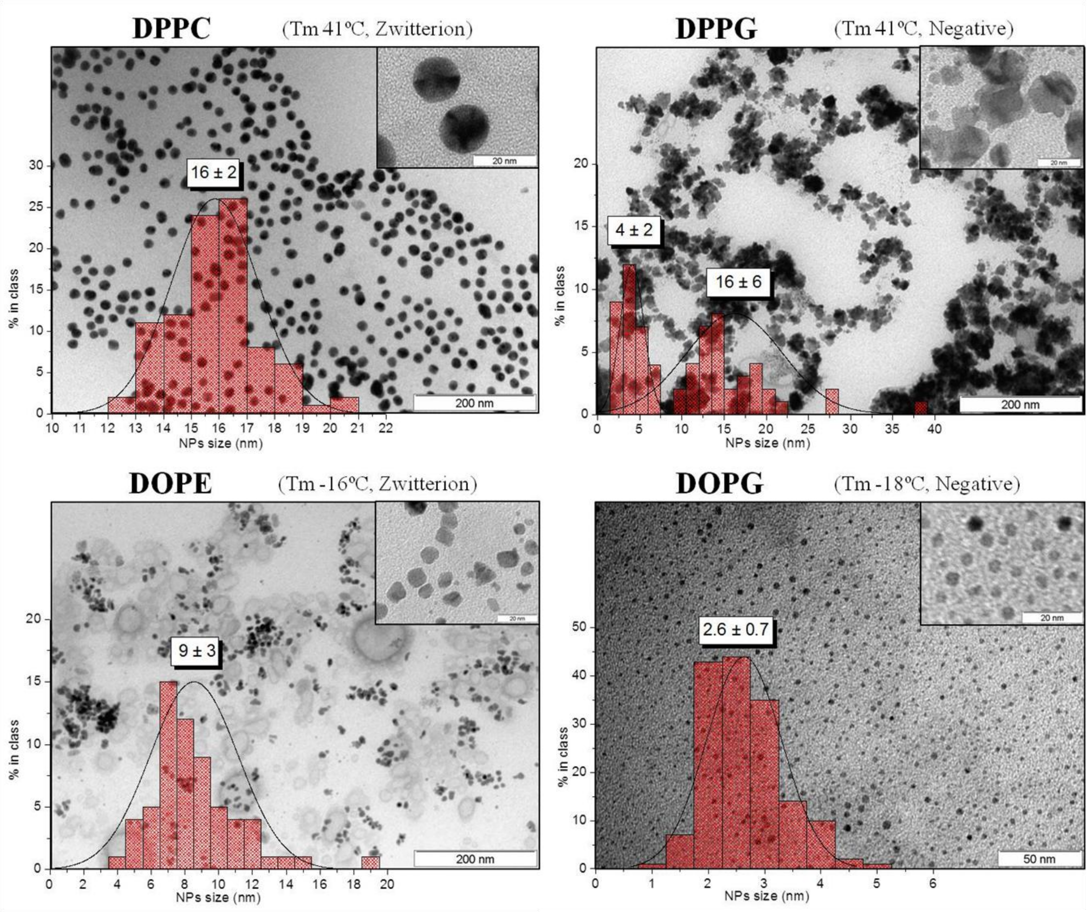


Figure 5. Lipid composition effect on the synthesis of Pd nanoparticles. TEM images show the Pd NPs synthesized inside different liposomes and their correspondent size distribution graphs calculated using iTEM. Scale bars are 200 nm in top and bottom-left images and 50 nm in bottom-right (insets scale bars are 20 nm). In the case of DPPG there are clearly two populations of NPs.

smaller sized NPs if the NPs are negatively charged as the repulsive forces between the lipids and the NPs will facilitate a tighter size control. Zeta Potential experiments were carried out to measure the charge of the synthesized Pd NPs and they were observed to be negatively charged with a Z-Pot of -23.8 ± 0.9 (n = 3). This repulsive interaction can also help in avoiding aggregations between lipids and nanoparticles, particularly in the final separation process where the liposomes are broken and the purified nanoparticles are obtained.

TEM images of the produced nanoparticles clearly show the smallest Pd NPs in size with the narrowest dispersive distribution (2.6 ± 0.7 nm) when the liposome nanoreactor is composed with glycerol head groups at the inner and outer part of the membrane, as in the case of DOPG liposomes. This can be explained by the presence of a higher number of glycerol molecules in the lipid head group which gives a higher number of available hydroxyl groups to reduce the palladium ions and stabilize the synthesized Pd NPs inside the nanoreactors. Moreover, stability studies from those nanoparticles demonstrated that were highly stable over time, maintaining homogeneity in size and shape as was observed in the TEM images from 3 months after the Pd NPs preparation (data not shown).

Since DOPG liposomes provide the most monodisperse Pd nanoparticle populations, XRD tests were carried out to confirm their crystal structure. The synthesized Pd NPs were observed to have a single-crystalline face-centered-cubic (fcc) structure which was confirmed by the XRD results shown in Figure 6. The XRD peaks correlated with the electron diffraction pattern obtained in TEM (SAED) confirmed that the nanoparticles show a cuboctahedron predominant structure with (100), (200), (220), (310) and (222) planes present in the FTT pattern.

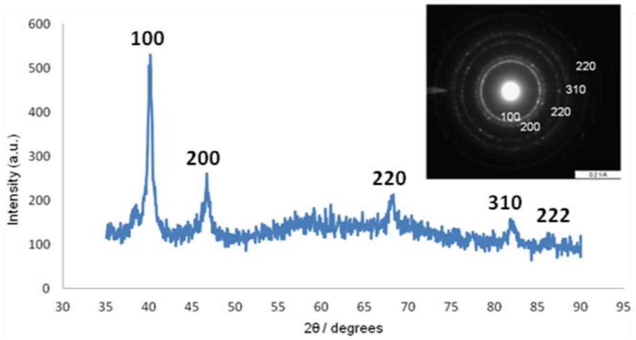


Figure 6. XRD pattern and SAED of the prepared Pd nanoparticles from glycerol-incorporated DOPG liposomes.

3.4| EFFECT OF GLYCEROL

Since the catalytic activity of nanoparticles is clearly modulated by their surface properties, we explored the role of the capping or stabilizing agent.[53,54] As previously reported, glycerol has been shown to reduce Pd(II) to form Pd(0) through the oxidation of the free hydroxyl groups. This oxidation might occur in all or in part of the alcohol groups in the glycerol molecules, and, if any hydroxyl group remains in its oxidized form they could therefore be participating in the stabilization of the Pd(0) atoms avoiding nanoparticle aggregation. We believe that glycerol-stabilized Pd nanoparticles occurs via one hydroxyl-end group or via the two hydroxyls from the ends or with the middle one, as we illustrate in Figure 7.

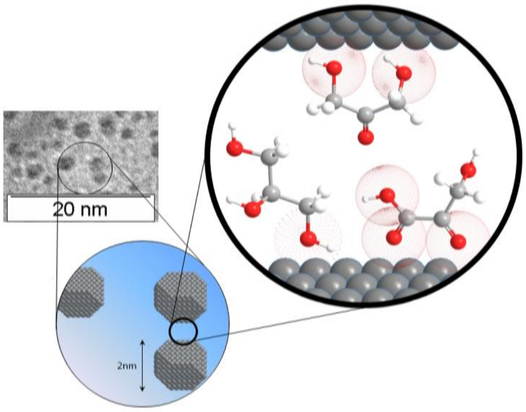


Figure 7. Schematic illustration of glycerol acting as a capping agent for nanoparticle stabilization.

To address our hypothesis, Pd NPs obtained in the absence of lipids were analyzed using FTIR (Figure 8). The FTIR spectrum demonstrated the presence of glycerol on the surface of the formed nanoparticles, as reflected by the bands corresponding to the asymmetric and symmetric C-H vibration between 2970 and 2845 cm^{-1}, as well as the bands corresponding to alcohol groups such as –OH band centered in 3300 cm^{-1} and C-O at 1035 cm^{-1}. The presence of weak bands at 1749/1724 cm^{-1} was of particular interest as it indicates the presence of C=O groups resulting from the oxidation of glycerol during the formation of nanoparticles. The low intensity of this band suggests that glycerol in a reduced form acts as a capping agent.

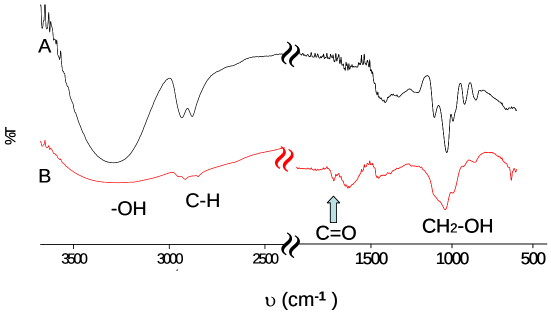


Figure 8. FTIR spectra of (A) glycerol and (B) Pd NPs.

The Pd nanoparticle surface was also analyzed by RAMAN spectroscopy taking the advantage of the already surface-enhanced RAMAN scattering (SERS) properties of these particles.[55] Figure 9 shows the RAMAN spectra of glycerol, DOPG and Pd NPs prepared in the liposomal nanoreactor. As can be observed, the prepared nanoparticles show several bands in the region where the bands of glycerol and lipid appear, making a careful assignation difficult, although the presence of both, reducing agent and phospholipid is clear. These bands are not observed when the nanoparticles were prepared in the absence of liposomes, perhaps due to a lower concentration of organic layer on the surface. This can also explain the higher stability of the particles obtained with the liposome method and the importance of the selection of lipids for the nanoreactor synthesis.

After preparation, Pd NPs were reconstituted in toluene. From that solution, they could be re-precipitated with ethanol and reconstituted again in toluene or in water, as shown in Figure S1. In the case of the reconstitution in water, some aggregation was observed. The reconstitution in water affects the stabilizing layer of Pd NPs, probably by breaking the interactions between Pd and OH groups, at least at levels non-detectable by RAMAN. It can be a method for removing the capping agent from the particles in case the presence of glycerol is not desirable, but these nanoparticles will not be as stable as those reconstituted in toluene and should be used as soon as possible.

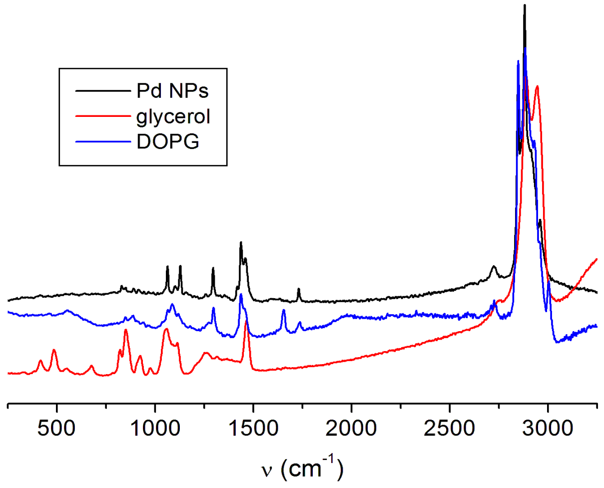


Figure 9. RAMAN spectra of glycerol, DOPG and Pd nanoparticles.

3.5| CATALYTIC ACTIVITY OF Pd NPs

Stevenson[56] has recently reported the reduction of p-nitrophenol to p-aminophenol in the presence of sodium borohydride catalyzed by bimetallic nanoparticles, demonstrating that the reaction takes place on the surface of the particles and not in the solution phase, even in the presence of an excess of reducer. We have preliminarily evaluated the catalytic activity of the ultrasmall Pd NPs obtained with the liposome nanoreactor using this model reaction.

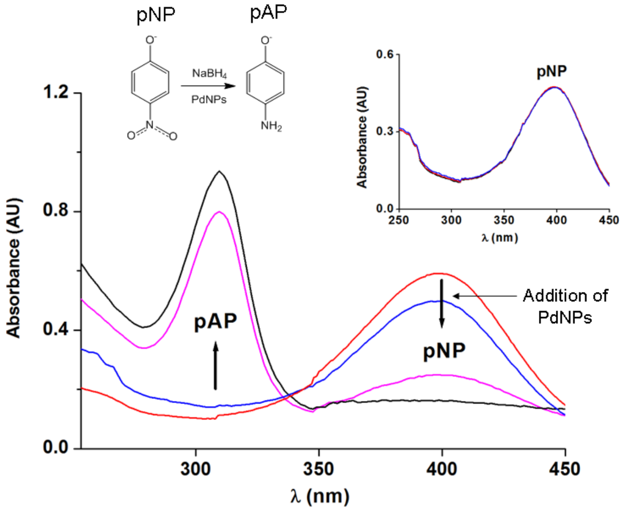


Figure 10. Reduction of p-nitrophenalate (pNP) catalyzed by palladium nanoparticles (Pd NPs) studied by UV-Vis. Inset: UV-Vis spectra of p-nitrophenalate (pNP) + $NaBH_4$ in the absence of palladium nanoparticles (Pd NPs).

Figure 10 shows the UV-Vis spectra for the reaction of p-nitrophenolate with sodium borohydride in the presence and absence of Pd NPs. The absorption band at 400 nm decreased rapidly when Pd NPs were added due to the reduction of p-aminophenolate and, at the same time an absorption band at 315 nm corresponding to p-aminophenolate appeared and increased in intensity. As can be observed in the inset of Figure 10, no reaction took place in absence of Pd NPs, demonstrating the catalytic properties of the nanoparticles. Further applications of the catalytic properties of Pd NPs are under study.

4| CONCLUSIONS

A liposomal nanoreactor system was demonstrated to provide a confined nanoenvironment in which the synthesis of palladium nanoparticles proceeds in a controlled manner. The method uses environmentally friendly conditions, such as low temperature, avoiding the use of organic solvents or harsh chemicals, only exploiting glycerol, a renewable and natural polyol as reducing agent, as well as capping agent, to produce small homogeneous palladium nanoparticles.

Moreover the importance of critical parameters in the procedure was evaluated, including type and/or concentration of reducing agent, lipid composition of the liposomes and presence of glycerol as capping agent to elucidate their effect on the homogeneity and size of the palladium nanoparticles produced in the absence and presence of nanosized liposomal reactors. In addition we have demonstrated for the first time the use of glycerol as both reducing and stabilizer agent to produce palladium nanoparticles.

Homogeneous highly stable palladium nanoparticles of ~2 nm in size were obtained without additional capping agents inside DOPG liposomes due to their contribution to form bilayers that allow glycerol to move semi-freely through the liposome membrane. The contribution of more glycerol molecules present in the DOPG formulation gives the entire system the best conditions to form extremely small monodisperse Pd NPs. The glycerol group of DOPG also participates in the stabilization of the nanoparticles.

The functional liposomal nanoreactors reported herein demonstrate that the controlled conditions created in the liposome core provide a greener, safer and more efficient system for the production of monodisperse ultra small palladium nanoparticles with catalytic properties for organic reaction.

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REFERENCES

1. Daniel, M.-C.; Astruc, D., Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology. Chem. Rev. **2003**, *104*, 293-346.
2. Grzelczak, M.; Perez-Juste, J.; Mulvaney, P.; Liz-Marzan, L. M., Shape control in gold nanoparticle synthesis. Chem. Soc. Rev. **2008**, *37*, 1783-1791.
3. Coronado, E.; Ribera, A.; Garcia-Martinez, J.; Linares, N.; Liz-Marzan, L. M., Synthesis, characterization and magnetism of monodispersed water soluble palladium nanoparticles. J. Mater. Chem. **2008**, *18*, 5682-5688.
4. Kim, S.-W.; Kim, M.; Lee, W. Y.; Hyeon, T., Fabrication of Hollow Palladium Spheres and Their Successful Application to the Recyclable Heterogeneous Catalyst for Suzuki Coupling Reactions. J. Am. Chem. Soc. **2002**, *124*, 7642-7643.
5. Xiong, Y. J.; McLellan, J. M.; Chen, J. Y.; Yin, Y. D.; Li, Z. Y.; Xia, Y. N., Kinetically controlled synthesis of triangular and hexagonal nanoplates of palladium and their SPR/SERS properties. J. Am. Chem. Soc. **2005**, *127*, 17118-17127.
6. Tobiška, P.; Hugon, O.; Trouillet, A.; Gagnaire, H., An integrated optic hydrogen sensor based on SPR on palladium. Sens. Actuators, B **2001**, *74*, 168-172.
7. Kim, S. W.; Park, J.; Jang, Y.; Chung, Y.; Hwang, S.; Hyeon, T.; Kim, Y. W., Synthesis of monodisperse palladium nanoparticles. Nano Lett. **2003**, *3*, 1289-1291.
8. Arul Dhas, N.; Gedanken, A., Sonochemical preparation and properties of nanostructured palladium metallic clusters. J. Mater. Chem. **1998**, *8*, 445-450.
9. Schmid, G.; Harms, M.; Malm, J. O.; Bovin, J. O.; Van Ruitenbeck, J.; Zandbergen, H. W.; Fu, W. T., Ligand-stabilized giant palladium clusters: promising candidates in heterogeneous catalysis. J. Am. Chem. Soc. **1993**, *115*, 2046-2048.
10. Reetz, M. T.; Helbig, W., Size-Selective Synthesis of Nanostructured Transition Metal Clusters. J. Am. Chem. Soc. **1994**, *116*, 7401-7402.
11. Yee, C. K.; Jordan, R.; Ulman, A.; White, H.; King, A.; Rafailovich, M.; Sokolov, J., Novel one-phase synthesis of thiol-fuctionalized gold, palladium, and iridium nanoparticles using superhydride. Langmuir **1999**, *15*, 3486-3491.
12. Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R., Synthesis of thiol-derivatised gold nanoparticles in a two-phase Liquid-Liquid system. J. Chem. Soc., Chem. Comm. **1994**, 801-802.
13. Srivastava, S. K.; Yamada, R.; Ogino, C.; Kondo, A., Biogenic synthesis and characterization of gold nanoparticles by Escherichia coli K12 and its heterogeneous catalysis in degradation of 4-nitrophenol. Nanoscale Res. Lett. **2013**, *8*, 70-73.
14. Srivastava, S.; Constanti, M., Room temperature biogenic synthesis of multiple nanoparticles (Ag, Pd, Fe, Rh, Ni, Ru, Pt, Co, and Li) by Pseudomonas aeruginosa SM1. J. Nanopart. Res. **2012**, *14*, 1-10.
15. Bilecka, I.; Elser, P.; Niederberger, M., Kinetic and Thermodynamic Aspects in the Microwave-Assisted Synthesis of ZnO Nanoparticles in Benzyl Alcohol. ACS Nano **2009**, *3*, 467-477.
16. Biacchi, A. J.; Schaak, R. E., The Solvent Matters: Kinetic versus Thermodynamic Shape Control in the Polyol Synthesis of Rhodium Nanoparticles. ACS Nano **2011**, 5, 8089-8099.
17. Carroll, K. J.; Reveles, J. U.; Shultz, M. D.; Khanna, S. N.; Carpenter, E. E., Preparation of Elemental Cu and Ni Nanoparticles by the Polyol Method: An Experimental and Theoretical Approach. J. Phys. Chem. C **2011**, *115*, 2656-2664.
18. Park, K. H.; Im, S. H.; Park, O. O., The size control of silver nanocrystals with different polyols and its application to low-reflection coating materials. Nanotechnology **2011**, *22*, 045602.
19. Cai, W.; Wan, J., Facile synthesis of superparamagnetic magnetite nanoparticles in liquid polyols. J. Colloid Interface Sci. **2007**, *305*, 366-370.
20. Yan, X.; Liu, H.; Liew, K. Y., Size control of polymer-stabilized ruthenium nanoparticles by polyol reduction. J. Mater. Chem. **2001**, *11*, 3387-3391.
21. Wiley, B.; Herricks, T.; Sun, Y. G.; Xia, Y. N., Polyol synthesis of silver nanoparticles: Use of chloride and oxygen to promote the formation of single-crystal, truncated cubes and tetrahedrons. Nano Lett. **2004**, *4*, 1733-1739.
22. Feldmann, C., Polyol-mediated synthesis of nanoscale functional materials. Solid State Sci. **2005**, *7*, 868-873.
23. Ayyappan, S.; Gopalan, R. S.; Subbanna, G. N.; Rao, C. N. R., Nanoparticles of Ag, Au, Pd, and Cu produced by alcohol reduction of the salts. J. Mater. Res. **1997**, *12*, 398-401.
24. Grace, A. N.; Pandian, K., One pot synthesis of polymer protected gold nanoparticles and nanoprisms in glycerol. Colloids Surf., A **2006**, *290*, 138-142.
25. Siegel, J.; Kvítek, O.; Ulbrich, P.; Kolská, Z.; Slepička, P.; Švorčík, V., Progressive approach for metal nanoparticle synthesis. Mater. Lett. **2012**, *89*, 47-50.
26. Nirmala Grace, A.; Pandian, K., One pot synthesis of polymer protected Pt, Pd, Ag and Ru nanoparticles and nanoprisms under reflux and microwave mode of heating in glycerol—A comparative study. Mater. Chem. Phys. **2007**, *104*, 191-198.
27. Ullah, M. H.; Il, K.; Ha, C.-S., Preparation and optical properties of colloidal silver nanoparticles at a high Ag+ concentration. Mater. Lett. **2006**, *60*, 1496-1501.

28. Dzido, G.; Jarzębski, A., Fabrication of silver nanoparticles in a continuous flow, low temperature microwave-assisted polyol process. J. Nanopart. Res. **2011**, *13*, 2533-2541.

29. Lee, Y.-W.; Han, S.-B.; Ko, A. R.; Kim, H.-S.; Park, K.-W., Glycerol-mediated synthesis of Pd nanostructures with dominant {111} facets for enhanced electrocatalytic activity. Catal. Comm. **2011**, *15*, 137-140.

30. Puvvada, S.; Baral, S.; Chow, G. M.; Qadri, S. B.; Ratna, B. R., Synthesis of palladium metal nanoparticles in the bicontinuous cubic phase of glycerol monooleate. J. Am. Chem. Soc. **1994**, *116*, 2135-2136.

31. Selvaraj, V.; Vinoba, M.; Alagar, M., Electrocatalytic oxidation of ethylene glycol on Pt and Pt–Ru nanoparticles modified multi-walled carbon nanotubes. J. Colloid Interface Sci. **2008**, *322*, 537-544.

32. Lee, Y.-W.; Ko, A. R.; Han, S.-B.; Kim, H.-S.; Park, K.-W., Synthesis of octahedral Pt-Pd alloy nanoparticles for improved catalytic activity and stability in methanol electrooxidation. Phys. Chem. Chem. Phys. **2011**, *13*, 5569-5572.

33. Trung, T.; Cho, W.-J.; Ha, C.-S., Preparation of TiO2 nanoparticles in glycerol-containing solutions. Mater. Lett. **2003**, *57*, 2746-2750.

34. Wang, X.; Li, Y., Hydrothermal reduction route to Mn(OH)2 and MnCO3 nanocrystals. Mater. Chem. Phys. **2003**, *82*, 419-422.

35. Marquardt, D.; Barthel, J.; Braun, M.; Ganter, C.; Janiak, C., Weakly-coordinated stable platinum nanocrystals. CrystEngComm **2012**, *14*, 7607-7615.

36. Bakshi, M. S.; Possmayer, F.; Petersen, N. O., Simultaneous Synthesis of Au and Cu Nanoparticles in Pseudo-Core–Shell Type Arrangement Facilitated by DMPG and 12-6-12 Capping Agents. Chem. Mater. **2007**, *19*, 1257-1266.

37. Bakshi, M. S.; Possmayer, F.; Petersen, N. O., Role of Different Phospholipids in the Synthesis of Pearl-Necklace-Type Gold–Silver Bimetallic Nanoparticles as Bioconjugate Materials. J. Phys. Chem. C **2007**, *111*, 14113-14124.

38. Naka, K.; Sato, M.; Chujo, Y., Stabilized spherical aggregate of palladium nanoparticles prepared by reduction of palladium acetate in octa(3-aminopropyl)octasilsesquioxane as a rigid template. Langmuir **2008**, *24*, 2719-2726.

39. Scott, R. W. J.; Datye, A. K.; Crooks, R. M., Bimetallic Palladium–Platinum Dendrimer-Encapsulated Catalysts. J. Am. Chem. Soc. **2003**, *125*, 3708-3709.

40. Scott, R. W. J.; Wilson, O. M.; Oh, S.-K.; Kenik, E. A.; Crooks, R. M., Bimetallic Palladium–Gold Dendrimer-Encapsulated Catalysts. J. Am. Chem. Soc. **2004**, *126*, 15583-15591.

41. Coulter, M. M.; Dinglasan, J. A.; Goh, J. B.; Nair, S.; Anderson, D. J.; Dong, V. M., Preparing water-dispersed palladium nanoparticles via polyelectrolyte nanoreactors. Chem. Sci. **2010**,*1*, 772-775.

42. Wang, C.-C.; Chen, D.-H.; Huang, T.-C., Synthesis of palladium nanoparticles in water-in-oil microemulsions. Colloids Surf., A **2001**, *189*, 145-154.

43. Kundu, S.; Wang, K.; Lau, S.; Liang, H., Photochemical synthesis of shape-selective palladium nanocubes in aqueous solution. J. Nanopart. Res. **2010**, 12, *2799*-2811.

44. Fan, F.-R.; Attia, A.; Sur, U. K.; Chen, J.-B.; Xie, Z.-X.; Li, J.-F.; Ren, B.; Tian, Z.-Q., An Effective Strategy for Room-Temperature Synthesis of Single-Crystalline Palladium Nanocubes and Nanodendrites in Aqueous Solution. Cryst. Growth Des. **2009**, *9*, 2335-2340.

45. Heshmatpour, F.; Abazari, R.; Balalaie, S., Preparation of monometallic (Pd, Ag) and bimetallic (Pd/Ag, Pd/Ni, Pd/Cu) nanoparticles via reversed micelles and their use in the Heck reaction. Tetrahedron **2012**, *68*, 3001-3011.

46. Chen, D.-H.; Wang, C.-C.; Huang, T.-C., Preparation of Palladium Ultrafine Particles in Reverse Micelles. J. Colloid Interface Sci. **1999**, *210*, 123-129.

47. Semagina, N.; Renken, A.; Laub, D.; Kiwi-Minsker, L., Synthesis of monodispersed palladium nanoparticles to study structure sensitivity of solvent-free selective hydrogenation of 2-methyl-3-butyn-2-ol. J. Catal. **2007**, *246*, 308-314.

48. Sanchez-Dominguez, M.; Boutonnet, M.; Solans, C., A novel approach to metal and metal oxide nanoparticle synthesis: the oil-in-water microemulsion reaction method. J. Nanopart. Res. **2009**, *11*, 1823-1829.

49. Genc, R.; Clergeaud, G.; Ortiz, M.; O'Sullivan, C. K., Green Synthesis of Gold Nanoparticles Using Glycerol-Incorporated Nanosized Liposomes. Langmuir **2011**, *27*, 10894-10900.

50. Genç, R. ; Ortiz, M.; O'Sullivan, C. K., Curvature-Tuned Preparation of Nanoliposomes. Langmuir **2009**, *25*, 12604-12613.

51. Lim, B.; Jiang, M.; Tao, J.; Camargo, P. H. C.; Zhu, Y.; Xia, Y., Shape-Controlled Synthesis of Pd Nanocrystals in Aqueous Solutions. Adv. Funct. Mater. **2009**, *19*, 189-200.

52. Drummond, D. C.; Meyer, O.; Hong, K. L.; Kirpotin, D. B.; Papahadjopoulos, D., Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. Pharmacol. Rev. **1999**, *51*, 691-743.

53. Pérez-Lorenzo, M., Palladium nanoparticles as efficient catalysts for suzuki cross-coupling reactions J. Phys. Chem. Lett. **2012,** *3*, 167–174.

54. Nalawade, P., Mukherjee, T., Kapoor, S. Green synthesis of gold nanoparticles using glycerol as a reducing agent. Advances in Nanoparticles **2013**, *2*, 78-86**.**

55. Chen, H., Wei G., Ispas, A., Hickey A. H., Eychmüller A. Synthesis of palladium nanoparticles and their applications for Surface-Enhanced Raman Scattering and electrocatalysis. J. Phys. Chem. C. **2010**, *114*, 21976-21981.

56. Pozun, Z. D., Rodenbusch, S. E., Keller, E., Tran, K., Tang, W., Stevenson, K .J. Henkelman, G. A systematic investigation of p-nitrophenol reduction by bimetallic dendrimer encapsulated nanoparticles. J. Phys. Chem. C **2013**, *117*, 7598–7604.

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