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Highly Stable PEGylated Gold Nanoparticles in Water: Applications in Biology and Catalysis

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ABSTRACT

Gold nanoparticles (Au NPs) with diameters ranging between 5-60 nm have been synthesised in water, and further stabilized with polyethylene glycol-based thiol polymers (mPEG-SH). Successful PEGylation of the Au NPs was confirmed by Dynamic Light scattering (DLS) and Zeta potential measurements. PEG coating of the Au NPs is the key of their colloidal stabilty, and its succecssful applications. Catalytic efficiency testing of the PEG-AuNPs were carried out on homocoupling of boronic acid. PEG-Au NPs with AuNps diameter < 30 nm were useful as catalyst in water. Finally, the PEG-Au NPs were also shown to be stable in biological fluid and not cytotoxic on B16.F10 cell line, making them attractive for further studies.

Keywords: Gold nanoparticles, Stabilisation, Polyethylene glycol (PEG), Ullmann reaction, cytotoxicty.

1 INTRODUCTION

The increasing progress of nanotechnology in the last few decades, has made nanomaterials indispensable in many areas such as material science, medicals, and cosmetics.[1] Nanoparticles (diameter 1-100 nm) exhibit particular properties that arise from quantum size effects and also from a high surface over volume ratio. [1] Their small size enable them to interact with biomolecules (such as enzymes, antibodies, DNA, Protein etc...) and cells, as such they are of appropriate dimensions for applications in nanobiotechnology and catalysis.[1,2,3] Noble metal nanoparticle, especially gold, are well known for their surface plasmon resonance band (SPR), a phenomenon associated with coherent oscillations of conduction-band electrons on nanoparticle surface upon interaction with light.[1] The wavelength of SPR band depends on size, shape and aggregation state of nanoparticles.[1] Therefore, depending on their size, shape and degree of aggregation, and local environment, gold nanoparticles can appear red, blue, or other colours. These plasmons are now the basis for many biological sensing and imaging applications of gold nanoparticles.[1] Besides their potential applications in biology, the catalytic applications of gold nanoparticles in organic reactions has seen considerable interest in recents years. Important transformations using gold catalysis, such as the selective oxidation of alcohols,[2] CO oxidation in the absence of H_2,[3] chemoselective reduction of nitroarenes,[4] and a wide variety of commercially important synthetic protocols have been studied exstensively. [5] It has also been shown that poly(2-aminothiophenol) (PATP) supported Au NPs can be used in Suzuki-Miyaura cross-coupling reactions.[6] More recently, the homocoupling of arylhalides has been accomplished using a catalytic system comprising of Au NPs supported on a bifunctional periodic mesoporous organosilica.[7] Aryl boronic acids have been homocoupled using gold on solid supports through heterogeneous conditions. Homocoupling with high selectivity was achieved using Au supported in CeO_2.[8] Applications of nanoparticles in biology and catalysis require them to be well dispersed and stable in solution. Unfortunately, the dispersion of nanoparticles are unstable due to their high surface energy. Due to this instability they suffer from losing their size dependent properties (catalytic, optical, magnetic etc…) caused by the high tendency of adhesion and aggregation.[1d] Aggregation of nanoparticles is much more pronounced in solvent with a high ionic strength (*ie*. biological fluids) and in presence of other chemicals.[1d] Therefore, dispersion of nanoparticles most be controlled for better results. Herein, we report on the synthesis of stable colloidal Au NPs in water and under physiological conditions (0.15 M NaCl), achieved by attaching polyethylene glycol-based thiol polymers (mPEG-SH) as sterical stabilizer ligand to the surfaces of Au NPs via thiol linkages. PEG was chosen because of it's importance as preventive of non specific interactions with proteins in biological applications, as well as for its good affinity to different solvents (*ie* water, DMSO, THF etc…). Au NPs–

PEG were used for the first study of PEG supported Au NPs in the homocoupling of boronic acids in water. Variation of the nanoparticle and ligand size was also investigated. Finally, the PEG-Au NPs were tested for toxicity on B16.F10 cell line.

2 PREPARATION AND CHARACTERIZATION of PEG-AuNPs

The Au NPs used were synthesised with mean diameters of 5, 15, 30 and 60 nm in water by the controlled chemical reduction of a $HAuCl_4$ solution (see section 4).[9] The physical properties of the Au NPs synthesised were further characterised using UV-visible spectroscopy and either TEM or SEM (**Figure 1)**. The UV-visible data clearly shows that the nanoparticles exhibit size dependent optical properties, resulting in a shift of the SPR band from a wavelength of 514 to 534 nm, as the mean diameter of the Au NPs increased from 5 to ~ 60 nm respectively.[1e] TEM and SEM analysis of the nanoparticles, utilising the *Image J* software, showed that the Au NPs have a size polydispersity below 20% with the exception for 60 nm AuNPs (~30%). Electrostatically stabilised nanoparticles are very sensitive to changes in pH and ionic strength of the aqueous system.[10] Therefore, to maintain an adequate dispersion of the Au NPs in high ionic strength media, they were coated with the PEG-SH capping ligand directly after synthesis.[10a] In a previous study, the stability of the PEG-Au NPs under physiological conditions (0.157 $mol.L^{-1}$ NaCl) was monitored by UV-visible spectroscopy and the number of PEG-SH ligands needed to coat the surface of a Au NP of a particular diameter was estimated. The grafting density was also determined by thermal gravimetric analysis (TGA) and TEM.[10a] Our results have shown that Au NPs-PEG hybrids were very stable when compared to *bare* citrate-stabilised Au NPs in presence of NaCl.[10] Following addition of NaCl to the citrate-stabilised AuNPs, the red colour immediately turned blue (aggregation), and then became colourless over approximately 3-4 hours due to total precipitation of the nanoparticles aggregates.[10] However, the PEG-stabilised Au NPs were only slightly affected by the addition of NaCl to the dispersion and resisted aggregation under these conditions No colour change was observed, only a slight plasmon shift 1-2 nm, detected with mPEG-SH ligands with M_w of 5400 and 10800 g mol^{-1}.[10a] Here attachment of mPEG-SH (Mw~10800 g mol^{-1}) to the surface of 5, 30 and 60 nm Au NPs through Au—S bond was also carried out. The 15 nm Au NPs were coated with different molecular weight mPEG-SH ligands (*i.e* Mw~2400, 5400, and 10800 g mol^{-1}). The successful PEGylation of the Au NPs was confirmed by DLS/Zeta potential measurements and in presence of salts. **Figure 2 (a)** shows DLS analysis for 15 nm Au NPs functionalised with various mPEG-SH ligands molecular weight at different polymer concentrations. The mean diameter of the Au NPs is observed to increase exponentialy with the PEG concentartion in all cases. This increase was more pronounced as the length of the PEG capping ligand increased, *ie*. AuNPs-Citrate size increased from 19.8 to 41 nm when coated with $PEG_{10\ 000}$. However, the size increase was slower with the shorter PEG_{2000}, indicating that more polymers were grafted at the AuNPs before reaching surface saturation (plateau). A similar trend is seen for the Zeta potential increase **figure 2(b)**. We note that thermogravimetric analysis performed in a previous study, [10b] has also shown that the number of PEG ligands grafted on ~15nm AuNPs increase when the PEG length decrease.[10b] As the hydrodynamic diameter increases with the PEG length inducing a thicker PEG layer which shields the surface charges, providing their good stability.

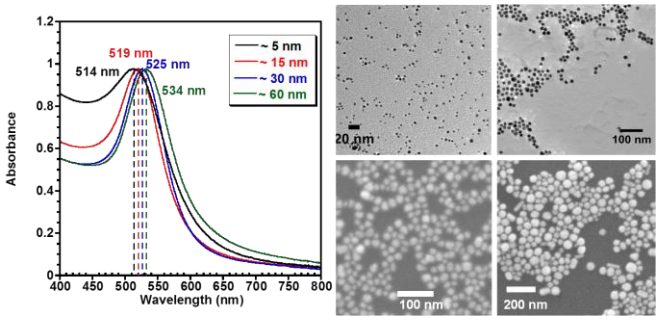


Figure 1. UV-visible spectra (left), and TEM/SEM micrographs (right) of the AuNPs-Citrate with diameter ~5 nm (up left); ~15 nm (up right); 30 nm (down left) and 60 nm (down right) obtained in this study.

In addition the PEG-Au NPs did not aggregate significantly in presence of salt and other organic chemicals. DLS/Zeta combined with TEM analysis, provided full characterisation of the colloidal dispersion for further applications.

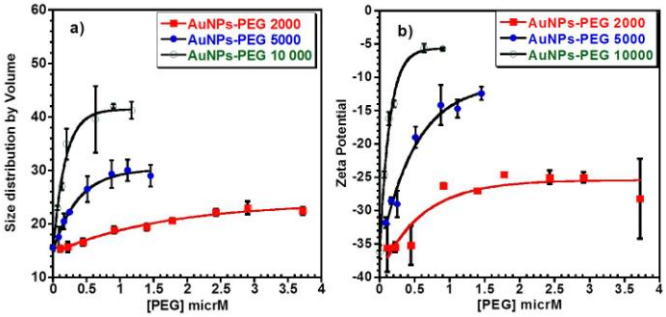
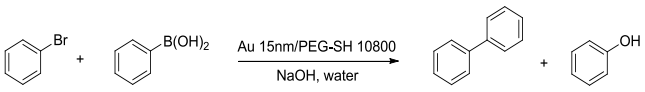


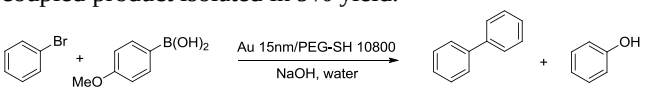
Figure 2. Size distribution (a) and zeta potential (b) of 15 nm AuNPs-PEG hybrids in function of the mPEG-SH polymer concentration expressed in µmol L^{-1}, for mPEG-SH with M_w 2100; 5400; and 10 800 $g.mol^{-1}$.

3 PEG-AuNPs CATALYTIC EFFICIENCY

We initially tested our potential catalyst Au NP size 15nm, PEG-SH 10800 in Suzuki-Miyaura reactions whereby bromobenzene and phenyl boronic acid were heated at reflux with base (NaOH) in water at 95-100°C. After 4 h the reactions were worked up. We noticed that a significant amount of phenylboronic acid remained in the NMR spectra of our crude reaction mixture.



Pure biphenyl was isolated in 2% yield. Phenol was also observed. To test if homocoupling rather than cross-coupling was occurring, we repeated the reaction using bromobenzene and *p*-MeO-phenylboronic acid. To our surprise no cross-coupled product was observed with homo coupled product isolated in 9% yield.



With these results in hand, a number of optimization experiments were undertaken as shown in the table below for the cross-coupling of phenyl bromide and *p*-MeO-boronic acid. The homocoupling of phenylboronic acid *para*-methoxy boronic acid were also carried out and the pure products were collected and characterised in section 4.

Boronic acid	PEG size	Base	% Conversion
$PhB(OH)_2$	5000	NaOH	23
$PhB(OH)_2$	10000	NaOH	0
$PhB(OH)_2$	20000	NaOH	0
[a]$PhB(OH)_2$	10000	NaOH	2
[b]$PhB(OH)_2$	10000	NaOH	2
[c]MeO-$PhB(OH)_2$	10000	NaOH	18

[a]Excess PhBr [b]PhI used [c]Excess $PhB(OH)_2$

4 MATERIALS AND METHODS

4.1 Chemicals and Materials:

Tetrachloroauric acid trihydrate ($HAuCl_4.3H_2O$), sodium citrate ($C_6H_5Na_3O_7.2H_2O$), sodium borohydride ($NaBH_4$) and ascorbic acid were purchased from Sigma Aldrich. Thiol terminated poly(ethylene glycol) methyl ether, M_w = 5400; 10800 and 20800 g mol^{-1}) were purchased from Polymer Source.

4.2 Preparation and PEGylation of Au NPs.

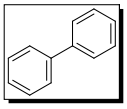
Diameter of ~5 nm: 150 mL of an aqueous solution of $HAuCl_4.3H_2O$ (0.25 mmol L^{-1}) in presence of 2.5 µmol L^{-1} of mPEG-SH (Mw = 10800 g mol^{-1}) was stirred vigorously. To this solution were added a volume of an ice cold solution of $NaBH_4$ in order to have a final concentration of 0.25 mmol L^{-1} $NaBH_4$. After addition of $NaBH_4$, an instantaneous colour change from pale yellow to deep red was noted. The AuNPs obtained with this procedure were approximately 5 ± 1.5 nm.

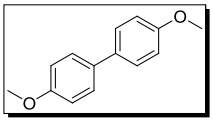
Diameter of ~15 nm: 150 mL of an aqueous solution of $HAuCl_4.3H_2O$ (0.25 mmol L^{-1}) was heated to 95°C with stirring. 0.53 mL of a 340 mmol L^{-1} sodium citrate aqueous solution was rapidly added. The colour of the solution changed from pale yellow to dark blue, and then to deep red-burgundy within about 8 minutes. Stirring and heating was maintained during 1h 30 mins after addition of sodium citrate. The heat was then removed and the solution was kept under stirring, until cooled to room temperature. The Au NPs obtained with this procedure were ~ 15 ± 1.5 nm

Preparation of ~30 and 60 nm AuNPs: For Au NPs, larger than 15 nm, a weak reducing agent ascorbic acid was used. For ~30 nm AuNPs, 150 mL of an aqueous solution of $HAuCl_4.3\ H_2O$ (0.25 mmol L^{-1}) and 1 mmol L^{-1} sodium citrate was stirred vigorously. To this solution was added a volume of ascorbic acid in order to have a final concentration of 0.38 mmol L^{-1}. After addition of ascorbic acid the colour of the solution changed from pale yellow to dark blue, and then to deep red-burgundy in less than 1 minute. Stirring and was maintained during 1h. The AuNPs obtained were 30 ± 4 nm. By increasing the concentration of ascorbic acid to 1 mmol L^{-1}, added in 2 times sequence (0.5 each), AuNPs ~ 60 nm were obtained, we note that these AuNPs were less monodisperse.

PEGylation of Gold Nanoparticles: PEGylation was performed by adding mPEG-SH to the Au NP colloidal solution under stirring. Excess ligand was removed by centrifugation and washing.

4.3 General protocol for Suzuki homocoupling: Characterization data:

Synthesis of Biphenyl[1,2,4]: In a round bottom (100 mL)  equipped with a condenser and magnetic stirrer bar, phenylboronic acid (2 mmol) and NaOH (8 mmol) were mixed with 15 nm $AuNP_0.PEG_{2000}$ (0.02 mol%) in H_2O (40 mL). The resulting mixture was stirred at 80°C for 48 h. The crude reaction mixture was then extracted with ethyl acetate (3 x 20 mL). The organic layers were combined and washed with 10% aqueous NaOH solution, dried with $MgSO_4$, filtered and concentrated under reduced pressure to afford the pure product (67 mg, 28%) as a white solid, m.p. 67-68°C (lit.[2] 67-69°C); λ_{max}/cm^{-1} (KBr)[4]:3034, 1943, 1569, 1480, 1429, 729, 696; δ_H (300 MHz) 7.61-7.57 [4H, m],7.46-7.41 [4H, m], 7.39-7.31 [2H, m]; δ_C ($CDCl_3$, 75.5 MHz) 141.26, 128.8, 127.3, 127.2.

Synthesis of 4,4'-dimethoxybiphenyl[3,4]: In a round  bottom (100 mL) equipped with a condenser and magnetic stirrer bar, *para*-methoxy phenylboronic acid (0.5 mmol) and NaOH (10% w/v, 0.8 mL) were mixed with 15 nm $AuNP_0.PEG_{10\ 000}$ (0.05 mol%) in H_2O (25 mL). The resulting mixture was stirred at 80°C for 22 h. The crude reaction mixture was then extracted with ethyl acetate (3 x 20 mL). The organic layers were combined and washed with 10% aqueous NaOH solution,

dried with $MgSO_4$, filtered and concentrated under reduced pressure to afford the pure product (40.4 mg, 9%) as a white solid, m.p. 168-169°C (lit.[3] 168-170°C); λ_{max}/cm^{-1} (KBr)[4]:2958, 1500, 1439, 1276, 1249, 1041; δ_H (300 MHz) 7.49-7.45 [4H, m], 6.98-6.93 [4H, m], 3.84 [6H, s]; δ_C ($CDCl_3$, 75.5 MHz)158.7, 133.5, 127.7, 114.2, 55.4.

5 INSTRUMENTATION

Optical spectra were obtained on a CARY uv−vis spectrophotometer with a Xenon lamp (300−900-nm range, 0.5 nm resolution).

Dynamic Light Scattering (DLS) and Zeta Potential: Measurements were carried out with the Malvern instrument (Zeta sizer Nano series) at 25°C. Measurements on each sample were performed in triplicate.

Microscopy Observation

Transmission Electron Microscopy (TEM): A drop of nanoparticles dispersion was first placed on a carbon-coated TEM copper grid (Quantifoil, Germany) and left to air-dry, before being introduced into the sample chamber of the TEM. Samples were analysed using a JEOL JEM-2100 TEM operating at 200 kV. All images were recorded on a Gatan 1.35 K × 1.04 K × 12 bit ES500W CCD camera. TEM images were analysed using Image J software.

Scanning Electron Microscopy (SEM): Citrate and PEG-stabilised gold nanoparticles were placed on a silicon wafer and dried before introducing them into the SEM. The samples were viewed using a FEI 630 NanoSEM fitted with Oxford INCA EDX detector, operating at 5 kV.

NMR Spectroscopy NMR spectra were run in *deuterio*chloroform ($CDCl_3$) using tetramethylsilane (TMS) as the internal standard, unless otherwise specified. ^1H NMR spectra were recorded at 300 MHz on a Bruker AVANCE 300 spectrometer. ^{13}C NMR spectra were recorded at 75 MHz on a Bruker AVANCE 300 instrument.

IR Spectrscopy (IR) spectra were recorded on a Perkin-Elmer FT-IR Paragon 1000 spectrophotometer.Solid samples were dispersed in potassium bromide and recorded as pressed discs.

Melting Points were measured in a Thomas Hoover Capillary Melting Point apparatus.

6. CONCLUSION

Electrostatically stabilized AuNPs-citrate has very limited applications due to its fast aggregation in complex media. However, sterical stabilisation of AuNPs with PEG polymer offers a very high stability to the AuNPs dispersion. AuNPs-PEG hybrids were shown here to be able to catalyse Ulmann reaction in water. As the AuNPs surface may contain Au(I), catalytic activity and selectivity is similar to the corresponding Pd complexes, indicating that Au(I) with the same d^{10} configuration as Pd(0) can catalyze reactions typically catalyzed by palladium. No conversion was observed with AuNPs of 60 nm in diameter. It was also noticeable that the increase in the polymer molecular weight tends to decrease the catalytic efficacy of the AuNPs, most likely by limiting the access of reactant to the AuNPs surface. Finally, all AuNPs-PEG hybrids tested here were checked for cytotoxicity on B16.F10 cell line, and were found to be not cytotoxic (results not shown), making them very attractive for further studies in biology and catalysis (in progress).

REFERENCES

[1] a)M. Haruta, *Chem. Rev.* **2003**, *3*, 75; b)M. C. Daniel, D. Astruc, *Chem. Rev.* **2004**, *104*, 293; c)A. Corma, H. García, *Chem. Soc. Rev.* **2008**, *37*, 2096; d)K. Rahme, F. Gauffre, J. D. Marty, B. Payre, C. Mingotaud, *J. Phy. Chem. C* **2007**, *111*, 7273; e) Link, S. El-Sayed, M. A. *J Phys Chem B* **1999**, *103*, 4212; f)Whitesides, G. M. *Nat Biotechnol* **2003**, *21*, 1161; g)Wang, J. Q., Sui, M. H.; Fan, W. M. *Curr Drug Metab* **2010**, *11*, 129.

[2] a)A. Abad, P. Conceptciòn, A. Corma, H. Garcia, *Angew. Chem. Int. Ed.* **2005**, *44*, 4066; b)D. I. Enache, J. K. Edwards, P. Landon, B. Solsona-Espriu, A. F. Carley, A. A. Herzing, M. Watanabe, C. J. Kiely, D. W. Knight, G. J. Hutchings, *Science* **2006**, *311*, 362; c)H. Miyamura, R. Matsubara, Y. Miyazaki, S. Kobayashi, *Angew. Chem. Int. Ed.* **2007**, *46*, 4151.

[3] a)M. Haruta, N. Yamada, T. Kobayashi, S. Iijima, *J. Catal.* **1989**, *115*, 301; b)J. Guzman, B. C. Gates, *J. Am. Chem. Soc.* **2004**, *126*, 2672; c)C. Lemire, R. Meyer, S. Shaikhutdino, H. J. Freund, *Angew. Chem. Int. Ed.* **2004**, *43*, 118.

[4] A. Corma, *Science* **2006**, *313*, 332.

[5] a)G. C. Bond, C. Louis, D. T. Thompson, *Catalysis by Gold*, Imperial College Press, **2006**; b)A. Arcadi, S. Di Giuseppe, *Curr. Org. Chem.* **2004**, *8*, 795; c)A. S. K. Hashmi, G. H. Hutchings, *Angew. Chem. Int. Ed.* **2006**, *45*, 7896.

[6] J. Hamn, Y. Liu, R. Guo, *J. Am. Chem. Soc.* **2009**, *131*, 2060.

[7] B. Karimi, F. K. Esfahani, *Chem. Commun.* **2011**, *47*, 10452.

[8] S. Carrettin, J. Guzman, A. Corma, *Angew. Chem. Int. Ed.* **2005**, *44*, 2242.

[9] a)J. Turkevich, P. C. Stevenson, J. Hillier, *Discuss. Faraday. Soc* **1951**, *11*, 55; b)G. Frens, *Nature-Physical Science* **1973**, *241*, 20; c)D. A. Fleming, M. E. Williams, *Langmuir* **2004**, *20*, 3021; d)J. Kimling, M. Maier, B. Okenve, V. Kotaidis, H. Ballot, A. Plech, *J.Phys.Chem. B* **2006**, *110*, 15700; e)X. H. Ji, X. N. Song, J. Li, Y. B. Bai, W. S. Yang, X. G. Peng, *J.Am.Chem.Soc* **2007**, *129*, 13939

[10] a)K. Rahme, T. Doody, A. Jain.K, L. Chen, M. Morris.A, J. Holmes.D, *Biomed Eng Res*, **2011**,*1*, 5. b)Sistach, S.; Rahme, K.; Perignon, N.; Marty, J. D.; Viguerie, N. L. D.; Gauffre, F.; Mingotaud, C. *Chem Mater* **2008**, *20*, 1221.