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Small Gold Nanoparticles Synthesized with Sodium Citrate and Heavy Water: Insights into the Reaction Mechanism

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The effect of solvent isotopic replacement (H for D) on the size of gold nanoparticles (Au NPs) prepared by sodium citrate reduction has been investigated. With increasing replacement of water by deuterium oxide, smaller sizes of Au NPs are obtained, which is interpreted as a consequence of a faster reduction. A mechanism in which a substitution complex, $[AuCl_3(C_6H_5O_7)^{-2}]^-$, is formed from $AuCl_4$ and citrate ions prior to its rate-limiting disproportionation into products is suggested. This novel procedure offers an attractive alternative to the existing ones and opens a full range of possibilities for biological studies.

Introduction

Because of their unique physical and chemical properties gold nanoparticles (Au NPs) have been widely employed not only in materials science and catalysis[1] but also in biomedicine.[2,3] In the later case, some biological oriented research such as local and remote manipulation of protein aggregates,[4] where the dissipated energy is closely related to the NP size, hyperthermia,[5] or antigen scaffolding,[6,7] to cite some examples, require precise control and large choice over the shape and size of the nanoparticles with high stability and low toxicity in the biological environment. Indeed, the type of biological applications that can be carried out such as reaching the cytoplasm, targeting the nucleus, or addressing particular cellular receptors[8] are primarily influenced by the size of the NPs. For example, biological portals as the nuclear pore complex would only allow a maximal size of 8 nm for a substance to passively cross the nuclear membrane.[9] In addition, a simple modification of the surface functionalization of the citrate-stabilized Au NPs with a variety of chemical and biochemical vectors is also another important factor to be considered.

In this context, the well-known standard citrate reducing methodologies developed by Turkevich[10] and Frens[11] are limited due to the oversized resulting NPs (diameters between 8–150 nm). On the other hand, the reduction of gold salts using sodium borohydride to produce approximately 4 nm Au NPs leads to poor stability of the colloids.[12] The use of stabilizers such as hexadecyl-trimethyl-ammonium bromide (CTAB) in water,[13,14] or thiolated[15] or phosphine[16] surfactants in organic media provide stable while nonbiocompatible materials. The ability to synthesize smaller Au NPs while preserving their biocompatibility and derivability would present new alternatives for these areas of research.

An additional motivation for this work was to continue the search of ways to control the synthesis of NPs. While surfactants, precursor to reducer ratios as well as chemical species are commonly used to control the final size and shape of Au NPs, to date, isotopic replacement of the solvent has solely been explored in the case of Au nanorods seeding growth.[17] Finally, the modification of the reducing and reduced species due to isotope replacement offers unique insights into the Au NP formation mechanism. In this paper we report the synthesis of small Au NPs via sodium citrate reduction of Au (III) species using heavy water as the solvent. Since it is known that D_2O increases both the strength of the H–O bonds and the viscosity of water,[18] it was expected that these factors would have a significant impact in the generation of Au NPs.

Experimental Section

Sample Preparation. Sodium citrate, $HAuCl_4 \cdot 3H_2O$, and D_2O were purchased from Sigma-Aldrich and handled and stored under argon atmosphere. Sodium citrate (2.2 mM) was dissolved in 100% D_2O, 50% D_2O/H_2O, or 100% H_2O (150 mL) in a three-neck round-bottom flask and heated to 100 °C. A solution of $HAuCl_4 \cdot 3H_2O$ (1 mL, 25 mM) in the appropriate solvent was added, and the reaction mixture was maintained at the boiling temperature for further 3.5 min before allowing to cool to RT. Each experiment was performed by triplicate in a controlled atmosphere (Ar). Samples were stored under argon. Temporal evolution of the particle formation has been recorded as a function of time immediately after the addition of the $HAuCl_4$ (1 mL, 25 mM) to the reducing solution (150 mL, trisodium citrate, 2.2 mM) at 90 °C. Since the reaction rate is a strong function of the temperature, the reaction was quenched by cooling the samples in ice–water. To verify that quenching does indeed provide sufficient delay for sampling, two samples were collected during the reaction in which one sample was quenched and later (about 1 h) analyzed and other sample was analyzed immediately. In all cases the spectra compared well.

Instruments. UV–vis absorption spectra were recorded with a Shimadzu UV-2401PC spectrophotometer at room temperature (RT) after 2 h. To avoid shifts due to the different refractive index ($n_{H_2O} = 1.3328$ and $n_{D_2O} = 1.3283$), Au NPs were

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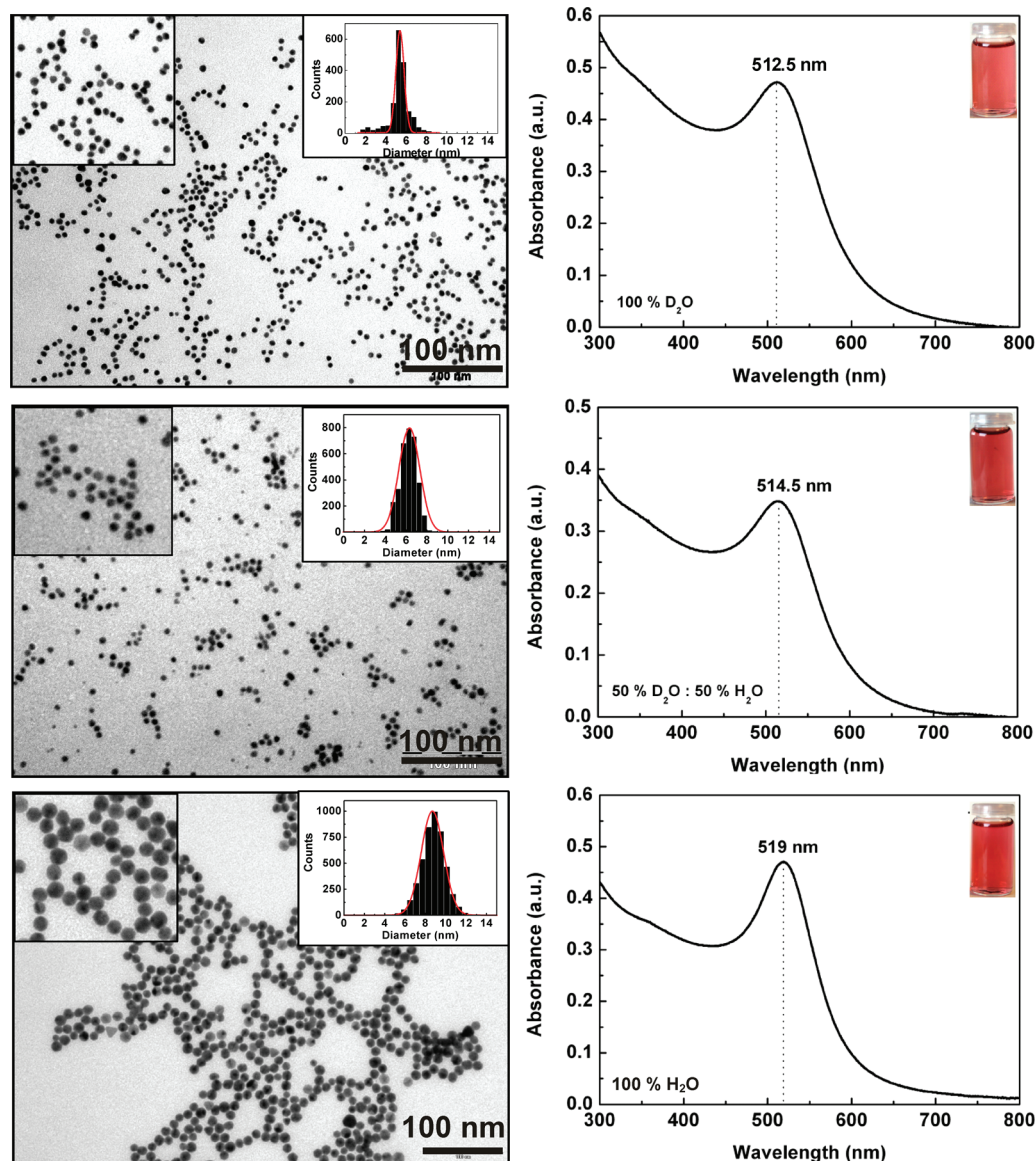∥ Institut Català de Recerca i Estudis Avançats.

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Figure 1. Optical (UV−vis spectroscopy) and morphological (TEM) characterization of Au NPs synthesized varying the solvent system: 100% D_2O (top), 50% D_2O/H_2O (middle), and 100% H_2O (bottom). Inset graphs in TEM images show size distribution measurements of Au NPs. Inset images in UV−vis spectra show colors of Au NPs solutions. These figures are representative of 3 separated experiments.

precipitated and redissolved in H_2O before analysis. Au NPs were visualized using 80-keV TEM (JEOL 1010, Japan) at an accelerating voltage of 80 kV. The sample (10 μL) was drop-cast onto ultrathin Formvar-coated 200-mesh copper grids (Tedpella, Inc.) and left to dry in air. For each sample, the size of 200 particles was measured to obtain the average and the size distribution. Z-potential and dynamic light scattering (DLS) measurements were carried out on a Zetasizer Nano ZS (Malvern Instruments). Electrospray ionization mass spectra (ESI-MS) were recorded on a Micromass Autospec M spectrometer. Fourier transform infrared (FT-IR) spectra were recorded on a Tensor27, Bruker spectrometer equipped with ATR (Attenuated Total Reflection) from MKII Golden Gate, Specac.

Results and Discussion

The synthesis of Au NPs was performed at 100 °C under identical reaction conditions but with systematically increased volume fraction of D_2O in the solvent ($\Phi_{D_2O} = 0$, 50, and 100%). Particle growth was monitored by UV−vis absorption spec-

troscopy of the solutions (Figure 1), which showed a blue-shift of the surface plasmon resonance (SPR) band from 519 to 512.5 nm as the Φ_{D_2O} increased. The shift in the SPR band can be well correlated to different particle sizes[19] and is in agreement with the reduction of the hydrodynamic diameter from 15.4 to 9.7 nm and the decrease in the negative surface charge from −43.0 to −33.9 mV (Supporting Information). These results were further corroborated by transmission electron microscopy (TEM) and image analysis, which clearly confirmed a reduction of the particle diameter as a consequence of increasing Φ_{D_2O}. Therefore, the employment of pure D_2O as the solvent lead to the production of smaller sized Au NPs (5.3 ± 1.1 nm), in comparison with the diameter obtained in the case of pure H_2O (9.0 ± 1.2 nm). It is worth noting that this synthesis is capable of producing a small fraction of Au NP down to 2−3 nm, which could be isolated using standard separation methods (Supporting Information),[20,21] and consequently higher yields of Au NPs can also be obtained.

The most popular method developed by Frens[11] for controlling the size of the gold nanocrystals by citrate reduction is based
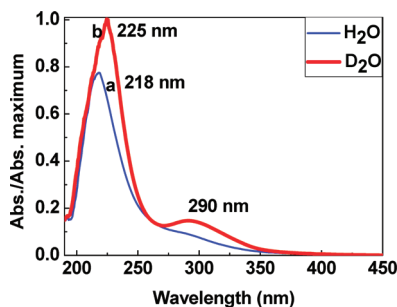
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Figure 2. UV−vis absorbance spectra of 0.17 mM HAuCl₄ and 2.2 mM sodium citrate equilibrated solutions in H₂O and D₂O.

on varying the ratio of initial concentration of sodium citrate to gold, which may cause a change in both the reduction rate and in the nucleation-to-growth ratio. It has experimentally been demonstrated that the concentration change of sodium citrate has more influence for the formation of gold nanocrystals as pH mediator than as the reducing reagent or as a ligand.[22] By variation of the pH of the system, both Au(III) complexes (reflected by their reduction potential) as well as sodium citrate itself markedly change their reactivity, thus inducing structural changes which substantially influence the reaction pathways and rates. Initially, strong tetrachloroauric(III) acid totally dissociates in aqueous solutions generating $AuCl_4^-$ complex ions of square-planar geometry.[23,24] Previous studies have proved that the progressive addition of NaOH to a concentrated solution of Au(III) leads to the rapid formation of hydroxyl containing gold complexes as a result of the stepwise substitution of Cl⁻.[25] These type of substitution reactions trigger a variation of the standard redox potential of gold species,[26] which induces a difference in the values of ΔE for the reaction conditions employed and can satisfactorily explain the trend in the primary particle size.

Organometallic compounds often show optical selectivity in the visible range as a consequence of the electron transfer between the metallic atom and the coordinating species. To study any changes in the composition of the gold complexes, the unreacted species were analyzed by UV−vis spectroscopy in D₂O and compared to those present in H₂O under identical concentrations of precursors (Figure 2). The employment of H₂O as solvent showed an absorption band at 218 nm, which could be assigned to the $p_\pi \rightarrow 5d_{x^2-y^2}$ ligand−metal transition of gold species at a pH 6.7, as correlated with similar experiments in the literature.[25] In the case of D₂O, it could be observed both a band shift toward a higher wavelength (225 nm) and the appearance of a new absorption band at 290 nm, which was indicative of a large excess of the $AuCl_4^-$ complex ions compared to other hydroxylated species.[25,27] Given the same pH conditions, the presence of higher amounts of $AuCl_4^-$ species using D₂O as solvent could be well explained by a more difficult substitution of Cl⁻ by OD⁻ than OH⁻. According to the work of Peng et al.,[22] the complex $AuCl_4^-$ is the more reactive species in the citrate synthesis of Au NPs. The bond energies of deuterated bonds are known to be greater (the bond interaction has been estimated to be $\Delta E_H \approx 3.57$ kcal/mol for H-bonds and $\Delta E_D \approx 3.81$ kcal/mol for D-bonds)[17] and therefore also the energy required breaking them.[28] It must also be noted that the pH of heavy water is 7.41. This slightly increased dissociation energy of D₂O would give account for the reduced availability of OD⁻ ions readily to be exchanged with Cl⁻ of Au(III) species, and this would imply a change in the value of ΔE and consequently of the Au NP size under the reaction conditions employed. During Au NP synthesis the rapidness of precursor decomposition and/or reduction influences the final size of the

NP.[1,12,29] A faster reduction quickly leads to a supersaturated solution and a high nucleation rate, which depletes the solution of available precursor atoms for growth, leading to smaller particles. Such an argument would explain the small particles resulting from a reaction using a strong reducing agent (i.e., sodium borohydride),[12] compared the presence of the weak sodium citrate reducer.[11]

Another determining factor is the well-known H/D exchange in the presence of D₂O, which under the experimental conditions leads to deuterated sodium citrate. Analysis by mass spectrometry (ESI-MS) of the species in solution under the reaction conditions confirmed the presence of $[C_6H_4O_7NaD_2]^-$ as the main sodium citrate isotope (Supporting Information). These results indicated that in the presence of a large excess of D₂O (solvent) and a heat source, the initial sodium citrate molecules undergo proton exchange at the alcohol group ($pK_a = 14.4$).[30] In the case of reactions involving breaking of a X−H bond in the rate-determining step, they usually exhibit a primary isotopic effect, i.e., $K_H/K_D > 1$. However, quite surprisingly we found that in our system the effect of deuterium was the opposite, $K_H/K_D < 1$. Although inverse isotope effect is rare, it has previously been observed as in the oxidation of chloroform with Au NPs.[31]

Further evidence of the modification of the reduction process between H₂O and D₂O can be obtained from the growth kinetics at 90 °C and the time evolution of the SPR peak (Figure 3). This lower temperature was chosen in order to decrease the rate of reaction and to facilitate monitoring of the Au NPs growth. It could be observed that a decrease of the temperature induced the reaction in D₂O proceeding at a slower rate, thus yielding larger Au NPs, compared to both syntheses in H₂O at 90 °C and in D₂O at 100 °C, as shown by the UV−vis peak positions. This is in agreement with what would be predicted, since it has been observed that a decrease in the temperature of synthesis overall yields larger NPs.[32] While the temperature of the reaction decreases the delivery of atoms to the solution to form NPs is also decreased. This implies that nuclei form while additional precursor is still arriving to the solution thus facilitating growth of the NPs, which contrast with the fast reactions where the entire precursor is rapidly consumed in the nucleation step leaving few amounts of monomer for growing. In addition, the enhanced temperature effect observed in the case of D₂O can be explained by the larger activation energy due to the stronger O−D bond energy.

Although the discussion of the different reaction pathways to obtain Au NPs by citrate reduction is out of the scope is this communication, we postulated a possible reaction mechanism in order to explain the reduction of Au(III) by sodium citrate, which was based on the experimental evidence and the existing literature.[33,34] Initially, Au(III) is reduced to Au(I), and this process would involve two steps (Scheme 1): (a) a fast ligand exchange with the citrate anion to form an intermediate complex, which remains unaffected by isotope effects, and (b) an equilibrium to give a ring closure followed by a slow and rate-determining step involving a concerted decarboxylation and the reduction of Au(III) species. Thus, similarly to the case of the reduction of Au(III) ion employing the oxalate anion,[33] the citrate anion is expected to coordinate equatorially substituting a planar Cl⁻ ligand and forming the corresponding complex $[AuCl_3(C_6H_5O_7)^{-2}]^-$ (Scheme 1a). Deprotonation of the alcohol group and coordination of the alcohol oxygen axially to Au(III) to give a pentacoordinated intermediate complex would take place as a rapid equilibrium, which would be followed by the axial complex disintegrating into products in the rate-limiting
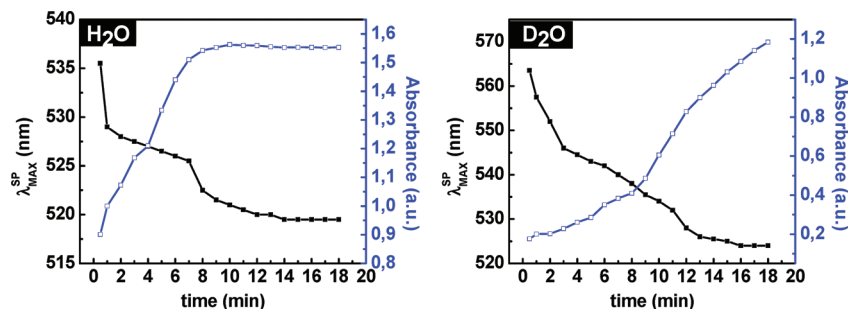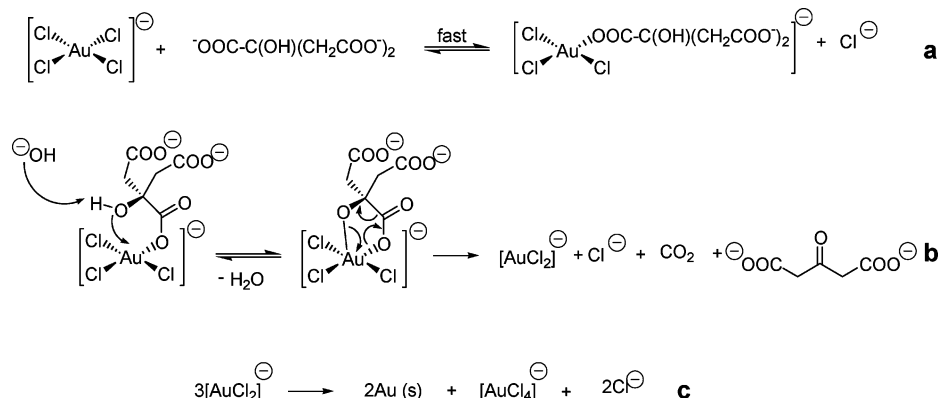
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Figure 3. Time evolution of SPR and absorbance of Au NPs solutions synthesized both in H_2O and D_2O at 90 °C.

SCHEME 1: (a) Ligand Exchange Reaction and (b) Decarboxylation and Reduction of Au(III) Species

step (Scheme 1b). The formation of the dicarboxyacetone intermediate as the oxidation product from the citrate ion has since long been postulated and overall accepted for gold colloid preparation[29,35] and also proved by ^{1}H NMR studies in the reduction of $AgNO_3$ to obtain Ag NPs.[34,36,37] However its isolation from the reaction mixture was found to be complex due to its decomposition to form either acetone[38] or acetoacetate.[36,37] In addition, FT-IR experiments provide a preliminary evidence which would reinforce the decarboxylation step (Supporting Information). The resulting Au(I) species can finally form a multimolecular complex with the dicarboxyacetone molecules present in solution which play an important role in the disproportionation of aurous species and the subsequent formation of Au (0) atoms (Scheme 1c),[39] as already described by Liz-Marzan.[29]

When the reaction is performed in D_2O as the solvent and under high energetic conditions (heating at 100 °C), the equilibrium in which the alcohol group coordinates axially to Au(III) would be slightly shifted toward the formation of the pentacoordinated intermediate complex in comparison to the use of H_2O as solvent, and this would result in an overall increase of the reaction rate. This behavior suggests that the ring formation is also involved in the rate-limiting step of the reaction. Recently, Au nanorods have also been prepared in D_2O,[17] resulting in both higher yields and a decrease in the nanorod diameter. This outcome was mainly attributed to the lower solubility of CTAB and salt species in D_2O, which induces a slowing of exchange processes that occur at the nanoparticle surface. In our case, despite the low concentrations of salt species employed (in millimoles), which were far from the saturation limits, similar assumptions could be taken given the increased viscosity of D_2O and its effect on the reaction process, which would affect to the activities of the reactants and change mass transport and association characteristics.

From these results it can be concluded that (i) the size of the final particles can be tailored by varying the solvent system and

(ii) insights into the detailed mechanism are shown by the presence of deuterium, which increases the reducing strength of the citrate molecule. The pronounced effect of solvent deuteration on the Au NP formation in aqueous solution highlights the sensibility of water-based systems in which hydrogen-bonding interactions have a dominant effect on all reaction steps. It is also important to note that the citrate/aqueous synthesis of Au NPs is the most employed recipe in today nanotechnology explosion.

Supporting Information Available: TEM image and size distribution of Au NPs after centrifugation step, dynamic light scattering and Z-potential measurements of Au NP synthesized in D_2O and H_2O at 100 °C, mass spectrometry of the species present in solution under the reaction conditions, time evolution of the UV−vis absorbance spectra and colors of the solutions of Au NP synthesized at 90 °C, and FT-IR measurements of Au NP both in aqueous solution and dried. This information is available free of charge via the Internet at http://pubs.acs.org.

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