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Diffusion limited green synthesis of ultra-small gold nanoparticles at room temperature

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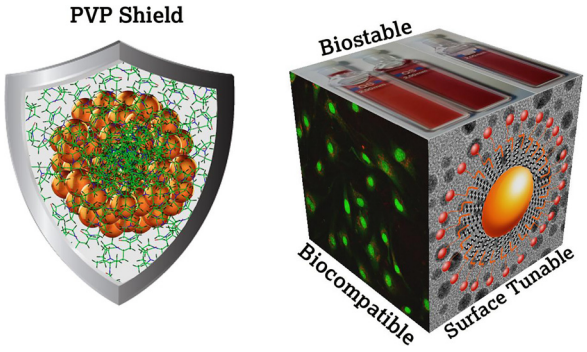
GRAPHICAL ABSTRACT



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ABSTRACT

Synthesis of biocompatible and very stable ultra-small gold nanoparticles (Au-USNPs, 1–3 nm) by green methods has posed a challenge for a long time. The well-established Turkevich method involves naturally occurring reducing agents, like citrate, but it yields particles with a mean diameter ≥20 nm. Here, we extend the Turkevich method to the low nanometer range and enables the formation of Au-USNPs by implementing a diffusion limited mechanism. We attained tight control over the particle growth by carring the process (i) at room temperature and (ii) using micro-molar amounts of poly-vinyl-pyrrolidone (PVP) in (iii) conditions of Brownian motion. Particle size distribution along with UV–vis spectroscopy data confirm that particle growth is diffusion limited and can be explained by particle migration and coalescence model. Resulting nanoparticles display (i) very high stability, (ii) biocompatibility and (iii) functionalizable surface. These Au-USNPs may easily find applications in biomedical engineering, medicine, biotechnology and related fields.

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1. Introduction

Gold nanoparticles featuring ultra-small size (Au-USNPs, 1–3 nm), biocompatibility and elevated stability are of extreme interest in many scientific and technological fields, including biotechnology, [1,2] drug [3] and gene delivery [4], sensing [5], imaging [6,7], and cancer therapies [8]. Biocompatible gold nanoparticles with a diameter greater than approximately 20 nm can be easily obtained using the Turckevich method that nevertheless is ineffective at ultra-small scales.

Currently, the most feasible route to achieve Au-USNPs was developed by Brust and Schiffrin [9]. Such a method requires $NaBH_4$ (sodium borohydride) to reduce $HAuCl_4$ (chloroauric acid) in a biphasic water/toluene system containing an alkylthiol as stabilizing and tetraoctylammonium bromide as phase transfer catalyst. However, the difficulty in separating the cited high cytotoxic chemicals limits its biological applications [10,11]. Other, more recent, procedures for the synthesis of Au-USNPs present a high control of particle size and shape with the help of nucleotides [12], ionic liquid [13], dendrimers [14], but they still require $NaBH_4$ or other chemicals with questionable safety. Seed mediated method was also found to be helpful to tune the size of gold nanoparticles but the process required additional reagents together with high pH and temperature [15]. There have also been attempts to synthesize Au-USNPS in a controlled and continous process with the help of microreacter [16], microwave assisted flow chemistry [17] or microfludic devices [18] but this makes the protocol more complicated, cumbersome, non-economical and not strictly green.

PVP is a water soluble, non-toxic polymer that binds at specific sites of lattice planes of gold nanoparticles regulating therefore their growth [19–24]. Tsunoyama and Tsukuda [24] demonstrated PVP as capping and stabilizing agent of Au-USNPs synthesized both in batch and in microfluids. However, the need of a strong reducing agent such as $NaBH_4$ in addition to an intermediate purification step (that is a crucial for preserving the NPs from coalescence) prevent the technique for being used as a high throughput, not hazardous method for Au-USNP production. Abdulkin et al. in a recent work [25] scaled the size of Turkevich Au-NPs down to the 6–10 nm dimensional range using PVP as capping agents and solvents or hybrid PVP/trisodium citrate as reducing agents.

Here, we implemented a diffusion limited mechanism to extend the Turkevich method into the ultra-small range. PVP is the key component in the proposed method because it slows down the diffusion of gold ions in conditions of Brownian motion and prevent the overgrowth of the nanoparticles. The as-produced gold nanoparticles were compared with totally different nanostructures (gold nanowires GNWs, gold foam) which were obtained in absence of PVP as in the previous protocol [26]. The behavior of the nanoparticles was investigated under different conditions of stress, such as high ionic strength, mechanical stress induced by ultra-centrifugationand, aging and drying. *in vitro* studies were carried out to test the nanoparticle bio-compatibility. In addition, the as produced nanoparticles could be easily functionalized with several functional groups. Finally, a mechanistic hypothesis was proposed to explain the mechanisms of particle growth. In comparison with other reported methods [9–18], our method is single step protocol, requires no complicated instrumentation, energy source, mechanical stirring, hazardous chemicals and occurs at room temperature. The protocol is one of the simplest and it can be used in labs with most basic facilities.

2. Experimental

2.1. Materials

Gold (III) chloride trihydrate (99.9%), trisodium citrate dihydrate, polyvinylpyrrolidone (PVP, average MW 10,000), sodium chloride, 11-mercaptoundecanoic acid (MUA), *N*-hydroxysuccinimide (NHS), *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDAC) and glucose oxidase enzyme (GOx) were purchased from Sigma Aldrich and were used as received. Milli-Q water was used in all the experiments.

2.2. PVP/citrate capped gold nanoparticles synthesis

The synthesis protocol was similar to the method already developed in our lab [26] with the difference that minute quantities of PVP (in μM amounts) were also added in the reaction mixture. Aqueous stock solutions of the starting materials (4 mM chloroauric acid, 38.8 mM trisodium citrate and 1% w/w PVP 10 kDa) were prepared and then gently mixed for about 5 s at a certain ratio. The reaction mixture was left undisturbed and reduction process was allowed to proceed at room temperature (298 K) for 8–9 h. The molar ratio $R = C_{citrate}/C_{HAuCl4}$ was fixed at 9.7 (one of the ratios studied previously [26]) and size and shape were optimized after trying several different concentrations of the reagents (Table S1). The smallest monomodal spherical particles were obtained at $C_{citrate}/C_{HAuCl4}$ fixed at 9.7 with final concentrations of C_{HAuCl4} = 2.0 mM, $C_{citrate}$ = 19.4 mM and PVP = 20 μM. PVP/citrate capped gold nanoparticles were washed with Milli-Q water by ultracentrifugation (150,000 rpm, 4 x 15 min) to remove any unreacted reagents as well as the free polymer in the solution.

2.3. Surface chemistry tailoring

MUA-AuPVP NPs. 11-mercaptoundecanoic acid (MUA) modified PVP/citrate capped gold nanoparticles (MUA-AuPVP NPs) were produced via ligand exchange reaction between citrate and MUA under the protection of PVP/citrate. 1 mL of colloidal gold (50 nM) was mixed with 2 mL of MUA solution (6.0 mM in 2:1 EtOH/H2O) and placed under stirring at least for 12 h to ensure a complete adsorption of alkanethiol on gold nanoparticles. The colloids were washed (150,000 rpm, 4 × 15 min) with PBS buffer whose pH was adjusted to pH 10 by dilute NaOH solution. This process removed the excess alkanethiol and the particles were then re-sdispersed in PBS buffer at pH 7.4.

NHS-AuPVP NPs. MUA-AuPVP NPs were reacted with EDAC/NHS to obtain NHS-terminated AuNPs (NHS-AuPVP NPs). 0.5 mL of MUA-AuPVP NPs was mixed with freshly prepared solutions of EDAC and NHS to obtain a solution (final volume of 0.6 mL) with 50 mM of EDAC and 200 mM of NHS. NHS-AuPVP NPs were washed and suspended in PBS buffer (pH 7.4, 150,000 rpm, 4 × 15 min) to remove the unreacted EDAC/NHS.

GOx-AuNPs. NHS-AuPVP NPs were incubated with GOx enzyme (0.8 mg/mL in PBS buffer pH 7.4) for more than 12 h under nitrogen atmosphere. Free GOx enzyme was removed by washing with PBS buffer (pH 7.4, 150,000 rpm, 3 × 15 min) and the final product (GOx-AuNPs) was suspended in PBS buffer pH 7.4 and stored at 252 K.

2.4. Characterization

Gold nanoparticles were characterized by ATR-FTIR, TEM and XPS. UV–vis Spectroscopy was performed on Cary® 100 UV–vis from Varian. 1 mm or 2 mm quartz cells were used to measure the absorption spectrum or perform kinetic studies. IR spectra were recorded on a Nicolet 6700 FTIR spectrophotometer (Thermo Scientific) equipped with an attenuated total reflection (ATR) accessory with a single bounce diamond crystal, a DTGS (deuterated triglycine sulfate) detector, and a standard KBr beam splitter. Samples (2 μL) were placed on diamond crystal and dried before the transmittance spectrum acquisition with a resolution of 4 cm^{-1}. TEM images were obtained using a TECNAI 20 G2: FEI COMPANY (CRYO-TEM-TOMOGRAPHY, Eindhoven) with a camera Eagle 2HS. The images were acquired at 200 kV; camera exposure time: 1 s; size 2048 × 2048. X-Ray Photoelectron spectra were recorded on a XPS Versa Probe II (PHI, Chanhassen US) by large area analysis mode where the monochromatic Al anodic beam of 100 μm, at 100 W power, normal to the surface, is rastered over an area of