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One-step synthesis of star-like gold nanoparticles for surface enhanced Raman spectroscopy

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HIGHLIGHTS

- Star shaped gold nanoparticles are produced with a single step procedure.
- Raman enhancement of nanostars is 50 times higher than the one given by nanospheres.
- Micromolar concentrations of dyes can be detected directly in liquid.
- Apomorphine was detected at micromolar concentration directly in liquid.
- Duoscan drastically decrease the variability of SERS signals from solid substrates.

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ABSTRACT

In this paper we present a new protocol for the synthesis of Star-Like Gold Nanoparticles (SGNs) by a simple one-step, room temperature procedure not involving the use of seeds or surfactants, that can be performed in seconds in any laboratory without the need of special technologies. These particles exhibited excellent properties for Surface Enhanced Raman Spectroscopy (SERS) and, when compared with spherical nanoparticles with similar size and concentration, showed enhancing factors from 10 to 50 times higher depending on the dye and on the wavelength employed. SGNs could be used directly in suspension as single, non-aggregating particles and were shown to be active in a remarkably broad range of the light spectrum from green to near infrared. Moreover, SGNs were adsorbed on the surface of a silicon slide to prepare SERS active solid substrate. Despite the fact that the surface of the solid substrate was not perfectly homogeneous, the signals recorded from different positions acquired through DuoScan averaging mode show excellent reproducibility, demonstrating how this simple and cheap protocol can be applied in order to generate reliable and homogeneous SERS substrates.

1. Introduction

Surface Enhanced Raman Spectroscopy (SERS) is a popular technique in bioanalytical chemistry and a potentially powerful enabling technology for *in vitro* diagnostics. In fact, SERS combines the excellent chemical specificity of Raman spectroscopy with the good sensitivity provided by enhancement of the signal that is observed when the analysed molecule lies over (or very close to) the surface of metal nanoparticles [1].

A number of SERS-based bioanalytical assays have been reported in literature; however, most of them are based on solid surfaces produced by a top-down approach [2,3] or by the generation of "hot spots" randomly distributed through the passivation of positively charged surfaces with negatively charged nanoparticles in order to generate the required vicinity of metallic nanostructures [4,5]. Instead, the use of nanoparticles in suspension offers several advantages as collected SERS signals registered are more stable over time required for the analysis, and spatially homogeneous. Traditionally, this approach has been mainly based on silver nanoparticles as gold colloids usually provide just a weak enhancement of Raman signal. However, gold nanoparticles are superior to silver in terms of chemical stability and suitability for use in biological media [6,7]. Recently, multibranched or Star-Like Gold Nanoparticles (SGNs) have been proposed as a reliable nanostructure for SERS experiments as they shown peculiar plasmonic properties and are generally considered as the most efficient

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nanostructure for this kind of experiments [8–11]. As a consequence the synthesis of this kind of nanostructure attracted many efforts and several protocols are present in literature. Despite of these efforts, the synthesis of SGNs is still complex and requires multiple steps or the use of surfactant or polymers that are difficult to remove from the surface [12,13]. Recently a single step easy protocol has been reported; yet this protocol still requires a tight control of the temperature at which the process is performed [14].

In this paper, we present a novel, simple, room temperature and surfactant-free method for the synthesis of SGNs with excellent SERS properties. The present method is based on the reduction of gold ions (Au^{3+}) by hydroquinone, a weak reducing agent, which acts preferentially on the [111] surface of gold colloids and which has been previously proven able to create branched nanoparticles yet only in presence of a co-reducing agent or in presence of seeds [15,16].

According to the stoichiometry of the reaction (Fig. 1) reported by a previous study [17] we decided to use an excess of hydroquinone to change the reaction kinetics. This would shift the gold reduction reaction to a more kinetically controlled regime and create a preference for the growth of kinetically favourable particles morphology, as star-like particles, on the more thermodynamically favourable nanospheres [18].

Our procedure allowed us to obtain SGNs with enhancing factors up to 5×10^3 in one-step and at room temperature with a good control of the dimension. SERS behaviour of SGNs was compared directly with the enhancing capacity of conventional spherical gold nanoparticles with hydrodynamic diameter in the same range at equivalent particle concentrations. In order to study the potential of SGNs to be functionalized with various organic molecules for bioanalytical applications, we also tested the ability of SGNs to be isolated and redispersed without the formation of aggregates. The possibility to modify their surface with thiols has been investigated as well.

2. Materials and methods

2.1. Materials and synthesis methods

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO) and used as received. Water was deionized and ultrafiltered by a MilliQ apparatus from Millipore Corporation (Billerica, MA) just before use.

SGNs were produced as follows. A solution of tetrachloroauric acid (99% $HAuCl_4 \cdot 3H_2O$, 50 μL, 10 mg mL^{-1}) was diluted in a 50 mL backer containing 10 mL of freshly prepared ultrapure MilliQ water under magnetic stirring at room temperature. Next, 100 μL of 11 mg mL^{-1} hydroquinone solution in water was rapidly injected. The solution turned from yellow (Au^{3+}) to light blue almost instantaneously because of the formation of gold nanostars. Particles produced by this method were stable for a few hours. However, this time could be extended up to few months by simple addition of 20 μL of sodium citrate (10 mg mL^{-1}) within 20 min from the preparation. The size of the particles prepared through this method can be controlled using different amount of the reactant used.

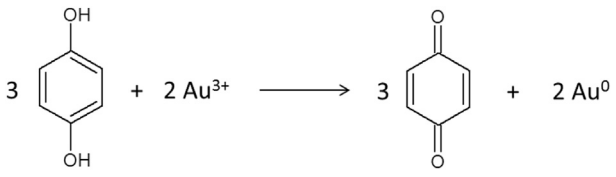

Fig. 1. Stoichiometry of the reaction between hydroquinone and gold ions.

Table 1
Resume of the dimension (DLS) of particles obtained varying the amount of the different solutions: $HAuCl_4 \cdot 3H_2O$, (10 mg mL^{-1}) and hydroquinone (11 mg mL^{-1}) mixed. Particles were prepared on a 10 mL scale except for the one with 105 nm diameter.

Diameter (DLS)	Water	$HAuCl_4$	Hydroquinone
70	10 mL	50 μL	100 μL
85	10 mL	100 μL	100 μL
140	10 mL	200 μL	100 μL
105	50 mL	500 μL	1000 μL

Moreover, some preliminary results suggest that this protocol is suitable for scale up (Table 1).

Spherical nanoparticles with a diameter of 70 nm were produced by a two-step approach as previously described [19]. Briefly, 23 nm gold seeds produced by the standard Frens method based on reduction by sodium citrate [20] were diluted in 10 mL of a solution containing a 25 mM concentration of Au^{3+} ions followed by 20 μL of a 1% citrate solution and, immediately after, by 30 μL of 30 mM hydroquinone. After addition of hydroquinone, the solution became immediately red because of the formation of 70 nm spherical nanoparticles.

2.2. Characterisation methods

Particles produced following these protocols have been characterized by dynamic light scattering (DLS), scanning electron microscopy (SEM), Raman and UV–Vis spectroscopies.

DLS measurements were performed at 90° with a 90Plus Particle Size Analyzer from Brookhaven Instruments Corporation (Holtsville, NY), working at 15 mW of a solid state laser ($\lambda = 661$ nm). Nanoparticle Tracking Analysis (NTA) was performed on a Nanosight (Amesbury, UK) apparatus equipped with a solid state green laser ($\lambda = 533$ nm) [21]. All samples used for this analysis were previously 1:100 diluted with water.

SEM images of gold nanoparticles were obtained by a FEI-Nova Nanolab 600I microscope operating at 5 kV, available at the "Nanobiosciences Unit, IHCP, Joint Research Centre–Ispra, Italy". UV–Vis spectra were acquired with a Nanodrop 2000c spectrometer in standard quartz cuvette.

Raman spectra were recorded with an Aramis Horiba Jobin-Yvon micro-Raman spectrometer equipped with solid state lasers operating at 532 nm, 633 nm and 785 nm and with a DuoScan mapping mode configuration. Raman signal of malachite-green and congo-red were recorded directly in liquid in presence of star-like or spherical particles using the three different, green, red and near infrared (NIR) laser light sources. In order to acquire the spectra, 200 μL of nanoparticles dispersion and dyes were mixed in a plastic holder and analysed with a 10× objective. The same experimental conditions were used to compare SERS spectra obtained in presence of nanostars and nanospheres. A fivefold concentrated suspension of SGNs was prepared by filtration of the original liquid using 50 mL of Vivaspin 30 kDa MWCO PES filter centrifuge tubes, centrifuging the particles at 3000 rpm in order to remove excess citrate solution. Solid substrates have been dipped in a 1 μM solution of malachite green for 20 s and then dried at room temperature. Signals have been recorded using 533 nm, 633 nm and 785 nm lasers using a 50× objective. DuoScan averaging mode was used with a 50× objective to measure areas of different size with different sizes with a 633 nm laser line. XRD patterns were recorded using a Thermo Scientific (Thermo ARL X'tra) diffractometer using a Bragg-Brentano theta–theta configuration with a maximum excursion ranging from −8° and 180°. X-Ray source is Cu Kα ($\lambda = 1.542$ Å) and the accelerating voltage can be set in the range