Sonochemical synthesis of porous gold nano- and microparticles in a Rosette cell

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\textbf{A B S T R A C T}

We report the synthesis of Au nano- and microparticles that relies on α-D-glucose (C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}) as the reducer and stabilizer in a Rosette cell under 20 kHz ultrasound irradiation. The chemical and physical effects of ultrasonic irradiation on the synthesis were investigated. The results showed that an optimum pH is required for the formation of insoluble Au(0) particles. Upon irradiation, low pH yielded Au nanoparticles while high pH resulted in microparticles. The Au surface capping by α-D-glucose hydroxyl and carbonyl groups was confirmed by Fourier transform infrared (FT-IR) spectroscopy. X-ray diffraction (XRD) analysis indicated that the Au particles crystallize within the face-centered-cubic (FCC) cell lattice. Moreover, continuous sonication reduced larger amounts of the Au precursor compared to the intermittent mode. Furthermore, tuning sonication time and mode influences the particle size and porosity as characterized by scanning and transmission electron microscopy. Our results shed a new light into the importance of the experimental and ultrasound parameters in obtaining Au particles of desired features through sonochemistry.

1. Introduction

Nanotechnology deals with the production, manipulation and use of materials ranging from 1 to 100 nm (nm). Nanoparticles can be manufactured with similar sizes and shapes to biomolecules; this makes them suitable for various applications in nanomedicine, such as targeted drug delivery, molecular imaging and biosensing [1–3].

Gold nanoparticles (Au NPs) are highly demanded for different applications in various fields, especially in nanomedicine [4,5]. For instance, Au NPs are among potential candidates to selectively attach to tumor cells through binding affinity [6,7]. Numerous approaches have been elaborated for the synthesis of Au NPs; however, only a few reports exist on the synthesis of gold microparticles (Au MPs) [8]. Microparticles are successful delivery vehicles capable of encapsulating/tethering interesting molecules to elicit efficacious and controlled release based on unique properties: particle size, shape, structure, entrapment efficiency, drug loading, porosity, targeting and release. The manufacturing of Au MPs with controlled particle size is gaining attention owing to concerns related to biodistribution and possible toxicity at the nanoscale [9].

Au NPs are known to change their color based on their size, shape, and surrounding medium; this is a physico-chemical property referred to as the surface plasmon resonance (SPR), making them suitable as thermal energy vehicles for the ablation of tumor cells [7]. Tiny Au nanospheres (Au NSs) display only a single SPR peak, occurring at a wavelength of ~520 nm [10]. On the other hand, Au nanorods (Au NRs) display two SPR bands, corresponding to the transversal (T) and longitudinal (L) modes [11,12]. The first SPR peak (T mode) is located in the visible region, centered at ~520 nm, while the second SPR peak (L mode) can be easily tuned by varying the aspect ratio of the rods (length/width) to be located in the near-infrared region (NIR), suitable for deep penetration of light into biological tissues with applications in the photodynamic or photothermal therapy of cancer [13,14]. Besides, the light scattering of Au NPs is exploited in surface-enhanced Raman spectroscopy (SERS) for cell labelling [15,16]. On the other hand, Au NRs have been shown to enhance fluorescence signals compared to Au NPs.
The sonochemical synthesis of Au NPs is influenced by the synthesis and operating parameters as well as by the geometry of the vessel. The influence of ultrasonic power was explored by Garcia et al. using a 20 kHz processor whereby an increase in ultrasonic power decreased particle size and polydispersity. At a power of 60 W, the Au NPs solution was violet with a decreased SPR intensity due to the nucleation and growth of large particles and increased polydispersity. Samples irradiated with powers of 120 W or 150 W produced ruby red suspensions with a lower polydispersity [28]. Similarly, Khani et al. observed that increased ultrasonic power reduced particle size and improved the crystallinity of Mn doped TiO₂ photocatalyst [29]. At 40 kHz, glucose produced Au nanobelts; when glucose was substituted by cyclodextrin, only spherical NPs were obtained while longer irradiation times merged Au NPs to yield nanobelts.

In general, the conditions that provide the beneficial effects of sonochemistry in Au NPs synthesis, are not widely examined in the literature [30]. Ultrasound frequencies from 20 kHz to 950 kHz have been explored for sonochemical synthesis purposes although higher frequencies require more power. Furthermore, the number of radicals generated is larger at lower frequency. Radical generation is a chemical effect of acoustic cavitation. Radicals are generated when the instantaneous local pressure is negative in a liquid irradiated by ultrasound resulting from bubbles generated by dissolved gases [31]. For this reason, typical operating frequencies in the range of 20–40 kHz are employed in benchtop processors for NPs synthesis. The physical and chemical effects of ultrasound irradiation arise from transient cavitation dominating at lower ultrasound frequency (20 kHz) [32]. Transient cavitation results in relatively more violent collapse of cavities followed by fragmentation, consequently generating new cavitation nuclei [33]. Reports show that ultrasound horns at low frequency contribute to an increase in radical production [34] while intermittent pulse sonication is favored due to a slower temperature rate increase from the resulting cavitation process, thereby avoiding excessive temperature increase [35]. The main difference between sonochemistry and thermal processes resides in the collapse of bubbles arising from acoustic cavitation [36] leading to localized high temperatures and pressures which generate highly reactive species advantageous to nanomaterial fabrication [37]. In addition to the chemical effects owing to radical generation, particle size reduction and surface area increase derive from the physical effects of ultrasound irradiation, as depicted by Stucchi et al.; both features are critical for the adsorption of drug molecules [38]. Likewise, the use of a Rosette cell was found to be more effective in reducing the particle size of sodium-exchanged vermiculite due to the additional hydrodynamic cavitation [39]. Used to produce biodiesel under ultrasound irradiation, this reactor increased the transesterification yield by 90% when compared to a round-bottom glass flask; this geometry favors the hydrodynamic cavitation which usually occurs in small orifices, herein under the form of the reactor loops. Indeed, the loops would help faster dissipate the heat delivered by ultrasound, thus limiting the temperature rise and keeping more gas nuclei dissolved [40].

In this work, we describe a novel method for the sonochemical production of Au MPs and Au NPs in a Rosette cell reactor, whereby the ultrasound waves from the probe are propelled around the typical loops of this reactor. The synthesis method is simple and eco-friendly, it produces Au MPs and AuNPs passivated by hydroxyl and carbonyl
Table 1
Seed-mediated synthesis of Au MPs.

| HAuCl₄ (mM) | NaOH (mM) | C₆H₁₂O₆ (mM) | pH |
|------------|-----------|---------------|----|
| 12.6       | 38        | 9.9           | 5  |
| 12.6       | 63        | 19.9          | 7  |
| 12.6       | 115       | 39.9          | 12 |

2. Materials and methods

2.1. Materials

Gold tetrachloride trihydrate (HAuCl₄·3H₂O, 99%), α-D-glucose (C₆H₁₂O₆, 99%), and sodium hydroxide (NaOH, 99%) were purchased from Sigma Aldrich (St. Louis, MI, USA), potassium iodide (KI, 99%) was purchased from Sigma Life Sciences (Oakville, ON, Canada) and used as received. Deionized water (DIW) (18 MΩ, 4.3 μS/cm, Millipore) was the solvent.

2.2. Equipment

All Au NPs synthesis experiments were carried out in a Rosette cell reactor (Fig. S1) (Sonics and Materials, Newton, CT, USA).

A Rosette cooling cell is used instead of a conventional beaker to achieve higher mixing efficiency provided by unique flow patterns through the reactor’s lateral loops (coils). During the sonication, the solution is circulated from the bottom to the top of the reaction vessel. Besides, the immersion in a cooling bath allows the exposure of a larger surface area of the reactor to the cooling medium, creating a more efficient heat exchange compared to a conventional beaker.

The ultrasound equipment (Sonics® & Materials, Inc., Newtown, CT, USA) included a 20 kHz VibraCell control panel operating at 130 W, a transducer (model CV18), fitted with a probe of 3 mm diameter tip (Fig. 1). The ultrasound intensity was controlled by changing the amplitude. The power density of the ultrasound processor with the 3 mm probe was calibrated with a K type thermocouple. The probe and thermocouple were dipped into DIW, and the temperature increase induced by sonication (for 9 min) was recorded to derive the power density for volumes of 10, 15 and 20 mL following the calorimetric method [41].

The experiments were carried out under either continuous or intermittent ultrasound irradiation. In the intermittent experiments, ultrasound was on for 30 s and off for 59 s. The probe was maintained at 1 cm from the bottom of the Rosette cell. The power density for the different experiments was calculated by dividing the total energy delivered (J) by the cumulative amount of time for which the probe was on and the volume of the medium. Besides, the energy absorbed by the solution was determined by potassium iodide (KI) dosimetry [42].

An isostep bath circulator (6200 R28, Fischer scientific, Pittsburgh, PA, USA) maintained a temperature of 25 ± 1 °C during the sonochemical synthesis. pH values during the experiments were measured using Orion Star A214 pH/Ise Bench Top Meter (Thermo Scientific) with a glass electrode.

2.3. Determination of the sonochemical activity in the Rosette cell

Experiments were conducted in a Rosette reactor and were compared to their beaker counterparts of the same volume (30 mL), immersed in a thermostatic bath at 25 °C. Briefly, 1 mM potassium iodide (KI) was dissolved in 15 mL DIW. Ultrasound parameters optimized in the seed-mediated protocol were applied. The solutions were sampled after continuous or intermittent sonication for 30 min at regular intervals of 5 min. The I₂ concentration was calculated using the Beer-Lambert law: A = εCl.

2.4. Seed-mediated sonochemical synthesis of Au MPs

For this experiments, α-D-glucose and NaOH were used to synthesize Au MPs. The effect of glucose concentration, pH, order of reagents’ addition and ultrasound irradiation time on the Au MP formation was investigated through a series of experiments (Tables 1 and 2).

Initially, the seed solution was prepared by mixing 10 mL aqueous solutions of 12.6 mM HAuCl₄ with NaOH at 38 mM (pH 5), 63 mM (pH 7) and 115 mM (pH 12). Next, growth solutions containing 5 mL aqueous solutions of 12.6 mM HAuCl₄ mixed with 5 mM C₆H₁₂O₆ concentrations at 9.9 mM, 19.9 mM, and 39.9 mM at pH 2 were prepared; the solutions were yellow in color. The seed solution turned from yellow to transparent within 30 s with a color change rate depending on the added amount of NaOH. Mixing HAuCl₄ with NaOH produces gold hydride (Au(OH)₄⁻) and sodium chloride (NaCl(aq)). Then, the seed solution (HAuCl₄ and NaOH) and growth solution (HAuCl₄ and C₆H₁₂O₆) were mixed to obtain final pH values of 5, 7 and 12 (Fig. 1). Following the combination of the seed and growth solutions, probe sonication was applied at a power output of 20 ± 1 W and ultrasound power density of 1.3 W mL⁻¹ for 30 min resulting in the production of anisotropic Au MPs at pH 7 and porous Au MPs at pH 12 with the porosity and size depending on the mixing sequence and sonication mode.

For the experimental design (Table 2), we focused on repeating our initial results obtained at pH 12 from the simple 3² factorial design (Table 1). This experimental design aimed at screening the operating and synthesis parameters that influenced the formation of precipitated porous Au MPs. The effect of mixing sequence (NaOH first, then HAuCl₄, and vice-versa), sonication time (30 min and 40 min with a mid-point at 35 min) and sonication mode (intermittent vs. continuous) on the particle size and morphology were explored at a constant power output of 20 ± 1 W corresponding to an ultrasound power density of 1.3 W mL⁻¹. Mixing the growth and seed solutions yielded a violet solution which, after sonication, became a clear suspension with dark particles that precipitated at the bottom of the Rosette cell. A total energy consumption of ~49,000 J was recorded for all the experimental runs. On the other hand, the difference in recorded total energy from the processor during intermittent vs. continuous sonication was negligible.

2.5. Two-step sonochemical synthesis of Au NPs

Au NPs were synthesized following a two-step sonochemical process (Table 3).

For this experiments, we initially followed the work by Zhang et al. [43] who used a 40 kHz ultrasonic processor to synthesize gold nanobelts (Au NBs) using α-D-glucose as the reducing and capping agents, respectively. During the first step, the probe sonication was continuously applied to aqueous solution-A containing 2 mM HAuCl₄ and 100 mM C₆H₁₂O₆ inside a Rosette cell reactor kept in a thermostatic water bath at 25 ± 1 °C. A digital thermometer in the reaction vessel during the sonication process recorded a temperature rise of 5 ± 1 °C. We observed no changes in the color or absorbance of this solution implying that the synthesis result/outcome did not lead to the reduction of the Au precursor. Therefore, we decided to increase the solvent volume thereby...
decreasing the concentrations of both the Au precursor and reducing agent. Solution-B was prepared by maintaining the same molar ratio of the reagents and ultrasound parameters as in solution-A; however, DIW volume was increased to 20 mL. The change in solvent volume led to a positive outcome whereby we observed a slight reduction in peak intensity which correlates with a slight reduction of the Au precursor (Au^{3+}) after sonication for 90 min. This process concluded the first step of the experiment. The power output was 10 ± 1 W for both solutions, as recorded by the digital processor. However, the power density at an amplitude of 68% was 1.0 W mL^{-1} for solution-A and 0.5 W mL^{-1} for solution-B. After 24 h storage at 4 °C, 5 mL of solution-A were mixed with 5 mL of solution-B at pH 2, followed by direct probe sonication with a power density of 1.1 W mL^{-1}. The solution remained yellow and no color change or UV–Vis absorbance peak was observed after 30 min in that case. For solution-C, 10 mM NaOH were added dropwise into the mixture of solution-A and solution-B undergoing ultrasonic irradiation with a power density of 0.4 W mL^{-1} producing a violet color at a corresponding pH of 9. A neat SPR peak at 563 nm measured by UV–Vis absorption.

| Sample  | Mixing Sequence       | Sonication Mode | Time (min) | \( \lambda_{\text{max}} \) (nm) | Yield Au^{3+} (mg mL^{-1}) | A (a.u.) | Au^{3+} (mg mL^{-1}) |
|---------|-----------------------|-----------------|------------|-------------------------------|-----------------------------|---------|---------------------|
| NG-35-I | NaOH \( \rightarrow \) C_{6}H_{12}O_{6} | 30 s on: 59 s off | 35         | 276                           | 81                          | 0.2053  | 0.0605              |
| GN-30-I | C_{6}H_{12}O_{6} \( \rightarrow \) NaOH | 30 s on: 59 s off | 30         | 274                           | 80                          | 0.2030  | 0.0601              |
| GN-30-I | NaOH \( \rightarrow \) C_{6}H_{12}O_{6} | 30 s on: 59 s off | 40         | 278                           | 78                          | 0.1931  | 0.0582              |
| GN-40-I | C_{6}H_{12}O_{6} \( \rightarrow \) NaOH | 30 s on: 59 s off | 40         | 276                           | 77                          | 0.1900  | 0.0576              |
| NG-30-C | NaOH \( \rightarrow \) C_{6}H_{12}O_{6} | Continuous      | 30         | 272                           | 75                          | 0.1829  | 0.0562              |
| NG-40-I | NaOH \( \rightarrow \) C_{6}H_{12}O_{6} | Continuous      | 40         | 276                           | 73                          | 0.1751  | 0.0547              |
| NG-40-C | NaOH \( \rightarrow \) C_{6}H_{12}O_{6} | Continuous      | 40         | 276                           | 67                          | 0.1511  | 0.0501              |
| GN-30-I | NaOH \( \rightarrow \) C_{6}H_{12}O_{6} | Continuous      | 30         | 276                           | 65                          | 0.1431  | 0.0485              |
| GN-30-C | C_{6}H_{12}O_{6} \( \rightarrow \) NaOH | Continuous      | 30         | 272                           | 63                          | 0.0149  | 0.0476              |
| NG-30-I | NaOH \( \rightarrow \) C_{6}H_{12}O_{6} | Continuous      | 40         | 276                           | 59                          | 0.1212  | 0.0443              |
| GN-40-I | C_{6}H_{12}O_{6} \( \rightarrow \) NaOH | Continuous      | 40         | 274                           | 59                          | 0.1209  | 0.0442              |

Table 2
Parameters (mixing sequence, sonication mode and sonication time) varied during the seed-mediated synthesis of Au MPs.

Table 3
Two-step sonochemical synthesis of Au NPs.

| Sample | HAuCl_{4} (mM) | C_{6}H_{12}O_{6} (mM) | NaOH (mM) | Energy (W mL^{-1}) | pH | Outcome |
|--------|----------------|-----------------------|-----------|---------------------|----|---------|
| 10 mL solution (A) | 4 | 200 | 0 | 1.0 | 2 | No reduction |
| 20 mL solution (B) | 2 | 100 | 0 | 0.5 | 2 | Au^{3+} reduction |
| 15 mL solution (C) | 8 | 200 | 6.6 | 0.6 | 9 | Au(0) |
| 25 mL solution (Exc.) | 8 | 200 | 12 | 0.4 | 12 | Au(0) |

Fig. 2. Synthesis of porous Au NPs via a two-step sonochemical process.
confirmed the formation of Au NPs. The change in color occurring during the addition of NaOH under continuous irradiation signifies a positive outcome whereby the Au precursor is completely reduced from an oxidation state of Au$^{3+}$ to Au (0). Adding excess NaOH (solution Exc.) with a power density of 0.6 W mL$^{-1}$ changed the color of the suspension from violet to blue at pH 12, and the intensity of the corresponding UV–Vis peak decreased. The solvent volume, reaction time and power delivered from the probe resulted in the formation of Au NPs at pH 9 and Au MPs at pH 12 (Fig. 2).

2.6. Characterization of gold nanoparticles

The Au ions and Au NPs were characterized using UV–Vis absorption spectroscopy (Thermo Scientific Evolution 220 UV–Vis spectrophotometer, Madison, WI, USA) in quartz cuvettes with DIW as the blank. The morphology and the chemical nature of the produced Au particles were studied using scanning electron microscope (TM3030 Plus Hitachi Tabletop Microscope, Toronto, ON, CA; JOEL JSM-7600F, Tokyo, Japan) and transmission electron microscope (JOEL 2100F, Tokyo, Japan). SEM-EDX images were performed at 15 keV with solid samples cast on carbon grids. The adsorption of glucose moieties on the surface of the as-synthesized Au particles was confirmed using Fourier transform infrared spectroscopy (Spectrum 65, Perkin Elmer, Woodsbridge, ON, CA).

3. Results and discussion

3.1. Effect of HAuCl$_4$ concentration under continuous ultrasonic irradiation

In the two-step protocol, we modified the operating procedure by using a 20 kHz ultrasound processor instead of 40 kHz and the HAuCl$_4$ precursor was diluted 100 times when compared to the one used by Zhang et al. [43]. In our case, a mixture of C$_6$H$_{12}$O$_6$ at fixed HAuCl$_4$ concentration did not yield Au NPs in the absence of the NaOH-containing seed solution. The addition of NaOH during the sonication process resulted in colloidal Au NPs after aging; similar results were obtained via the seed-mediated synthesis route.

In the two-step protocol, when aqueous Au(III) precursor solutions containing α-D-glucose were sonicated in a NaOH-free medium for 90
min, the reduction of Au(III) to Au(0) did not occur. For instance, solution A, containing 4 mM HAuCl₄ showed no visible color change and no SPR peak (Fig. 3A). However, a decrease in the Au(III) absorbance intensity occurred in solution-B containing 2 mM HAuCl₄ after 90 min of direct sonication; this might be due to the hydrolysis of HAuCl₄ (Fig. 3B). The reduction in peak intensity for the diluted solution-B can be attributed to the thermal dissociation of water molecules producing H• and OH•, which participate in the subtraction of a proton from the Au precursor. In addition, methyl groups resulting from the thermal decomposition of glucose molecules in the reaction medium could trigger the removal of a proton thereby reducing the Au precursor concentration [44].

Solvent volume is an important parameter to establish the sonochemical efficiency of irradiated solutions. This is reflected in the reduced peak intensity observed for the 20 mL solution (Fig. 3B), which remains unaffected in the 10 mL solution (Fig. 3A) even after prolonged sonication. In a 37 kHz ultrasound bath, the degradation of amoxicillin by waste sludge catalyst was dependent on its concentration [45]. Similar to our experiments, a decrease in absorbance peak was only observed for solutions with lower Au precursor concentrations at larger volume. Since no absorbance change was observed after 90 min in the concentrated solution (10 mL), the dilution of the solution allows an increase in energy dissipation across the system aiding in the reduction of the gold precursor (HAuCl₄).

Mixing 5 mL of solution-A with 5 mL of solution-B under continuous sonication caused no visible changes to the color of the solution nor to the absorbance spectra. However, increasing the pH from 2 to 9 by adding NaOH turned the solution color from yellow to violet accompanied by the appearance of an SPR peak at 556 nm indicating the formation of colloidal Au NPs. Consequently, the features of the Au NPs evolved dramatically whereby at pH 9 three-dimensional (3-D) Au NPs are formed (Fig. 3D). Excess of added NaOH under sonication precipitated Au MPs at pH 12. This resulted in the disappearance of the SPR peak observed at 563 nm and a color change from violet to blue (Fig. 3E), which became clear a day later owing to the appearance of Au precipitates at the bottom of the Rosette cell. Previous studies in a 50 kHz ultrasound bath using electron spin trapping technique to determine the radicals formed during ultrasonication showed that, at low concentrations of non-volatile solutes (e.g., C₆H₁₂O₆), H• and OH• are produced because of water sonolysis. On the other hand, solute pyrolysis leads to the formation of CH₃• due to its thermal decomposition at sufficiently high concentrations [44]. In our case, continuous ultrasonication and high pH disrupted the stabilizing properties of glucose resulting in the aggregation of Au NPs and the subsequent formation of...
3.2. Effect of C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} MPs.

In the two-step synthesis protocol, the effect of C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} concentration on the ultrasonic-mediated reduction of HAuCl\textsubscript{4} in NaOH-free reaction medium was investigated. Initially, the effect of glucose was not evident at low solvent volume. However, increased solvent volumes at 0.5 W mL\textsuperscript{-1} after 90 min of ultrasonic irradiation resulted in a decrease in the peak absorbance intensity for the solution containing 2 mM HAuCl\textsubscript{4} mixed with 100 mM C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}. This is ascribed to the chemical effect of the ultrasonic irradiation whereby the thermal dissociation of water and decomposition of glucose from cavitation lead to the production of OH\textsuperscript{-} and H\textsuperscript{+}, which take part in the reduction of the Au precursor. This is in good agreement with published data. For instance, Kondo et al. explored the radical formation in a 50 kHz ultrasound bath in aqueous solutions using a spin trapping method and showed that glucose concentrations above 0.2 M produced reducing CH\textsubscript{3}\textsuperscript{•}, rather than aldehydes were responsible for the reduction of the Au precursor in an inert atmosphere [46]. Accordingly, it was reported that ultrasound frequency between 20 and 80 kHz can oxidize carbohydrates, such as glucose, in the presence of oxidants [47].

These results indicate that C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} by itself, does not reduce Au ions under low frequency ultrasound. Furthermore, control experiments using heat did not result in Au NP formation.

Via the seed-mediated protocol, for a HAuCl\textsubscript{4} concentration of 12.6 mM and in the absence of NaOH, increasing C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} concentration of 10–40 mM did not bring changes to the reaction mixture, indicating that a suitable activation energy was required before glucose could reduce the Au ions in solution. C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} does not seem to possess any reducing property by itself as no significant reduction of HAuCl\textsubscript{4} was observed in the experiments at pH values below 7 while there is a possibility that it decomposes at higher pH under ultrasonic irradiation. El’piner and Sinclair described the decomposition of glucose in an alkaline medium under heat as a thermal effect comparable to the ultrasonic effect. The decomposition products resulting from thermal and ultrasonic processes had absorption maxima in the UV range [48].

Although a visible color change in the solution above pH 7 was noticed, no SPR peak was initially recorded. However, after aging, there was a visible SPR peak that red-shifted and increased in intensity with increasing glucose concentration, suggesting a possible synergy between glucose reduction and pH of the medium (Fig. 4). At 10 mM C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}, SEM shows anisotropic shapes for the suspension (Fig. 4A) and the precipitate (Fig. 4B) with corresponding EDS (Fig. S2) while, at 20 mM C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} (Fig. 4C) and 40 mM C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} (Fig. 4D), it displays precipitated and agglomerated Au NPs that have lost the anisotropic structures observed at lower C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} concentrations. EDS analysis confirms that the agglomerated Au NPs are chemically composed of gold (Figs. S3 and S4).

The efficiency of C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} to generate reducing CH\textsubscript{3}\textsuperscript{•} towards Au reduction and to act as a capping agent was verified in a separate experiment in an aqueous solution containing 200 mM C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}, 7.5 mM NaOH and 2.0 mM HAuCl\textsubscript{4}. This experiment, designed with optimized concentrations of precursor and reducing agent, was conducted under continuous ultrasonic irradiation and compared to a conventional chemical synthesis route (Fig. 5A). Evidently, the combination of high C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} and low Au precursor concentrations produced significantly stable Au NPs (up to 1 month at room temperature). It is noteworthy that these sets of experiments produced stable Au NPs with or without ultrasonic at pH 7 and a low Au precursor. In this respect, there was a significant difference between the observed colors and recorded absorption spectra of the ultrasonic irradiated solution vs. the conventional synthesis (Fig. 5A). The Au colloids produced via the chemical synthesis route had a red color with an SPR maximum located at 520 nm for the synthesis without heat (Ca), which red-shifted to 527 nm when heating was applied (Cb). On the other hand, Au colloids produced when NaOH was added during ultrasound irradiation (Usa) had a violet color and an SPR maximum located at 532 nm which decreased in intensity when the reagents were pre-mixed prior to ultrasound irradiation (Usb). These results corroborate our initial deduction that a molar ratio of Au precursor to C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} (1:50), high solution volume and pH values between 7 and 9 are required to reduce and stabilize Au NPs when working...
at a low ultrasonic frequency (20 kHz). As $C_6H_2O_6$ is a weak reducing agent, low frequency ultrasound (20 kHz) requires longer sonication times to oxidize $C_6H_2O_6$ and generate $CH_2O$ which reduce the Au precursor. The NaOH addition during the ultrasonic treatment resulted in a slightly red-shifted SPR peak indicating an increase in NP size. When the reagents were mixed prior to ultrasound irradiation, the SPR peak had a weak absorbance and a broad peak indicating polydispersity and agglomeration. Adding NaOH under either ultrasound irradiation or stirring improved the stability of the formed Au NPs compared to samples where NaOH had been previously added; this was based on visual observation of samples stored at 4°C for 1 week.

Glucose capping of Au NPs was confirmed using FTIR (Fig. 5B). FTIR measurements for Au MPs and Au NPs samples showed the presence of a polar covalent bond involving carbon and oxygen, ascribed to the glucose molecule. The strong absorption band of the –OH group observed at 3320 cm$^{-1}$ was attributed to O–H stretching of intermolecular bonding of alcohol stemming from adsorbed OH groups on the surface of Au particles. The characteristic C=O group at 1636 cm$^{-1}$ was attributed to the formation of an acetal from the oxidation of the anomeric carbon of $C_6H_2O_6$ molecules. The absorption bands at 1030 cm$^{-1}$ (solid $C_6H_2O_6$) and 1089 cm$^{-1}$ (Au MPs) were attributed to the C–O stretch from the oxidized primary alcohol group of $C_6H_2O_6$.

3.3. Effect of pH

In the present study, NaOH was the only contributing factor that could affect the pH of the reaction medium. The addition of $C_6H_2O_6$ to Au$^{3+}$ stock solution did not affect its pH 2 or color; besides, there was no impact on its UV–Vis absorption features. The addition of NaOH hydrolyzes HAuCl$_4$ causing changes to the pH by substituting the Cl$^-$ groups with OH$^-$ groups, as follows (Eq. (1)) [24]:

$$\text{AuCl}_4^- + 4 \text{OH}^- \rightarrow \text{Au(OH)}_4^- + 4\text{Cl}^-$$  \hspace{1cm} (1)

Our findings corroborate previous reports that highlight the critical role played by NaOH in the formation of Au NPs. At pH 12, a mixture of 12.6 mM HAuCl$_4$ and 40 mM $C_6H_2O_6$ produced a violet suspension prior to ultrasonic irradiation that evolved to brown after the irradiation due to the destabilization of the suspension through ultrasound mechanochemical effects. This further implies that there is an optimal pH required for the formation of stable Au NPs using α-D-glucose as the reducing and capping agent at 20 kHz (Eq. (2)).

$$\text{Au(OH)}_3^- + C_6H_2O_6 \rightarrow \text{Au}_{\text{colloid}}$$  \hspace{1cm} (2)

Increasing the NaOH amount dramatically impacted the morphology of the obtained Au NPs. At pH 5, the Au NPs were one-dimensional nanowires mixed with thin plates (Fig. 6A), analysis of the particles confirmed the presence of Au and carbon which was attributed to the grid used to support the dried suspension during analysis (Fig. S5). Increasing the pH to 7 resulted in spherical Au MPs that where cross-linked (Fig. 6B) and further increase to pH 12 resulted in the agglomeration of the produced Au NPs into ellipsoidal microstructures (Fig. 6C). EDS analysis confirmed the agglomerated Au MPs are chemically composed of gold (Fig. S6). Overall, the Au NP formation and stability can be tuned by optimizing the pH of the solution. In the synthesis of gold nanobelts, Zhang et al. did not change the pH, however we found that adding NaOH created a suitable environment for the formation of Au NPs and, consequently, the evolution of their morphology and optical properties.

3.4. Effect of ultrasound operating parameters

Building on the results of the seed-mediated procedure, the process and synthesis parameters were screened to determine their effects on formed Au particles. Specifically, the sequence of the growth solution (aqueous solution of glucose and Au precursor at pH 2) and seed solution (aqueous solution containing NaOH and Au precursor at pH 12) were varied, reaction times were set at 30, 35 and 40 min, and sonication mode was either continuous or intermittent to let the mixture cool (30 s on/59 s off). The probe position was maintained at 1 cm from the bottom of the Rosette vessel while the acoustic intensity, as obtained from calorimetry, was 1.3 W mL$^{-1}$. For the experimental runs, the power output as obtained from the processor was 21 ± 1 W at 100% amplitude. During ultrasonic irradiation, the acoustic cavitation gives rise to OH$^-$ and CH$_3$O$^-$ owing to water sonolysis and glucose pyrolysis, respectively. Acoustic mixing arises from macroscopic mixing and bubble collapse in the liquid medium, leading to uniform shear rates and increased reaction rates [37]. According to Wood et al., pulsed wave ultrasound increases the sonochemical active region, the amount of sonochemistry and associated degassing [34]. The concentration of unreduced Au precursor based on absorption intensity at 275 nm is lower in the case of continuous sonication mode, indicating a significant effect on the overall Au conversion at 30, 35 and 40 min, irrespective of reagent addition method (Fig. S7). Cubilana et al. evaluated the effect of ultrasound power on the Au NP concentration and observed that increasing intensity of the UV–Vis absorption spectra correlates with an increase in Au NP concentration [49]. Based on our results, the peak intensity at 275 nm is higher for intermittent sonication compared to its continuous analog; this difference is correlated to the highest efficiency of continuous sonication towards the reduction of Au(III) into Au(0) (Fig. 7A).
From a crystallographic point-of-view, the sonication mode, intermittent vs. continuous, had no impact. In fact, the XRD peaks of the synthesized Au NPs could be indexed to the face-centered cubic (fcc) cell lattice (JCPDS: 04-0784) of metallic Au. XRD analysis revealed four dominant peaks corresponding to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes observed at angles located between 30° to 80° (Fig. 7B) corresponding to the Bragg reflections obtained for metallic gold fcc crystal structure. Furthermore, the Au NPs crystallite size, calculated using Scherrer equation, were determined to be 16.4 nm and 17.8 nm for intermittent and continuously sonicated Au NPs, respectively, regardless of the sonication time, indicating that both Au NPs populations are polycrystalline. The Au (1 1 1) plane which is the dominant peak is identified as the most stable structure for the preferential adsorption of sulfur and nitrogen functional groups, making the as-synthesized Au NPs suitable for biomolecule attachment [50]. Similarly, Au NBs produced by Zhang et al. were preferentially grown along the (1 1 1) direction based on analysis of selected area electron diffraction (SAED) patterns [43].

The effect of sonication time was evident from SEM analysis of the Au MPs. When Au NPs prepared by the seed-mediated protocol were sonicated for 30, 35 and 40 min, porous Au MPs were obtained, as observed from TEM (Fig. 8). The pore size is equivalent to 200 nm and the internal structure shows protruding Au particles layered on preceding particles. At 40 min, large porous particles are formed while 30 min sonication produced smaller potato-shaped structures (Fig. 8). From high- and low-resolution SEM micrographs obtained using a back scatter detector (BSE), 30 min sonication produced microporous Au particles with more pore openings compared to samples subjected to 40 min sonication. The characterization of the microstructure using high resolution SEM shows that intermittent sonication produced layered particles with wider diameter openings for the agglomerated particles while
3.5. Chemical effect of ultrasound on radical generation

When 1 mM potassium iodide (KI) is sonicated, the generated reactive radicals oxidize the iodide ion ($I^-\cdot$) to give iodine ($I_2\cdot$) (Eqs. (3) and (4)). This $I_2\cdot$ reacts with the excess iodide ion ($I^-\cdot$) to form tri-iodide ion ($I_3^-\cdot$) (Eq. (5)–(7)). The amount of generated OH• is determined by doubling the yield of $I_3^-\cdot$ which corresponds to the amount of $H_2O_2$ measured using its absorbance at 350 nm ($\varepsilon = 26,303 \text{ L mol}^{-1} \text{ cm}^{-1}$).

The reactions occurring during the KI sonication are:

$$\text{OH}^• + I^- \rightarrow \text{OH}^- + I^- \cdot $$(3)

$$I^- + I^- \rightarrow I_2^- \cdot $$ (4)

$$2I_2^- \cdot \rightarrow I_2 + 2I^- $$ (5)

$$H_2O_2 + 2I^- + 2H^+ \rightarrow I_2 + 2H_2O $$ (6)

$$I_2 + I^- \rightarrow I_3^- $$ (7)

The generation of OH• increased linearly with sonication time. However, the number of generated radicals was higher in the beaker under either continuous or intermittent pulse sonication compared to the Rosette cell (Fig. 10). During the initial sonication using the beaker, the amount of OH• increased and then started to decrease after 20 min, indicating that there is an optimum time for radical generation which may be due to a decrease in dissolved gases in the system. Since the solution volume was the same (15 mL) and the supplied power was constant (20 W), the increase in OH• amount in the beaker compared to the Rosette cell is likely due to the difference in geometry. It may also be due to the liquid burst around the loops in the Rosette cell that might destroy some bubble nuclei and/or facilitate their migration to the surface of the liquid to finally evaporate. Interestingly, when the probe position was set at 1 cm from the bottom of the Rosette cell, the hydroxyl absorbance was 0.266. However, when the probe position was placed at 3.5 cm from the bottom of the Rosette cell, the absorbance value was 0.285. This shows the influence of the probe position in the Rosette cell.
on the OH generation. In the Rosette cell, intermittent sonication produced more radicals compared to continuous sonication (Fig. 10), which is the reverse of sonicated samples in the beaker. Intermittent pulse sonication lowers the temperature in the reactor during the off cycle, promoting the presence of dissolved gases necessary for the sonochemical activity.

3.6. Physical effect of ultrasound on Au NP agglomeration

Under the seed-mediated protocol, Au NPs were produced before sonication or conventional stirring was applied (Fig. 11A). Ultrasound irradiation efficiently minimized Au NPs agglomerates through intense pressures generated from the cavitation process. Accordingly, Stucchi et al. and Boffito et al. reported that the sonication reduces particle size thereby increasing surface area and porosity [51]. When the seed-mediated protocol was performed under conventional stirring, the obtained Au NPs formed large agglomerates (Fig. 11B). However, under ultrasound irradiation, the aggregated Au NPs were dispersed as submicronic aggregates (Fig. 11C). In the two-step protocol, the action of ultrasonic irradiation at 100% amplitude (1.3 W mL⁻¹) led to the generation of Au NPs agglomerates interconnected by a network of pores. Besides, the production of hydroxyl radicals increases with high ultrasonic power and in the presence of small particles. Therefore, we infer that the reduction in pore size at 40 min compared to 30 min sonication could be a result of further reduction of Au NPs on the surface by hydroxyl radicals generated during the extended synthesis time. In addition, high power can oxidize carbohydrates, such as glucose, as observed using FT-IR (Fig. 5B). Sandrine et al. exploited the sonophysical and sonochemical effects of ultrasounds to provide medium homogeneity and chemical activation of glucose and sucrose [47]. In the absence of a catalyst, 96% of D-glucose were oxidized to glucuronic acid after 4 h using a 550 kHz ultrasound processor. The oxidation of glucose was confirmed with FT-IR by the presence of a carbonyl (C=O) band at 1723 cm⁻¹ [52].

4. Conclusion

Our results offer a novel perspective for the synthesis of Au NPs using ultrasound irradiation. The present study aimed to expand the synthesis of Au NPs by examining the effect of different parameters, such as the order of reagent addition, sonication mode and time, glucose concentration and pH, on the sonochemical formation of Au NPs using a 20 kHz ultrasound processor in a Rosette cell.

The main outcomes of this work are: (1) high solvent volumes and low precursor concentrations are necessary to achieve the reduction of Au precursor when using ultrasound; (2) high concentrations of Au precursor in alkali environment result in the precipitation of large Au NPs; and (3) changes in pH of the reaction medium significantly affect the size and stability of obtained Au NPs. A high pH, especially above 9, corresponds to a significant redshift in the UV–Vis absorbance peak, indicating that large porous particles form from aggregated nanowires. Moreover, the synthesis yield is affected by the different sonication approaches. Unambiguously, our results support that sonication throughout the mixing process rather than only after all solutions are combined has the strongest effect in terms of optical density and hence Au NPs yield and stability. Furthermore, the formation of pores in the microstructure advocates for the use of low frequency ultrasounds in the synthesis of Au NPs as these make them suitable for the encapsulation/attachment of biomolecules of interest. Finally, our results offer an early perspective on analyzing the efficiency of different mixing methodologies in obtaining Au NPs of desired features and pave the way for the extensive comprehension of the physico-chemical phenomena occurring during the synthesis of valuable metallic nanomaterials via sonochemistry.

CRediT authorship contribution statement

Ndifreke Usen: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. Si Amar Dahoumane: Validation, Data curation, Writing – original draft, Writing – review & editing. Mamadi Diop: Investigation, Writing – review & editing. Xavier Banquy: Resources, Writing – review & editing, Project administration. Daria C. Boffito: Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2021.105744.

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