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Continuous flow photosynthesis of gold nanoparticles: when nanoworld meets microfluidic technology

Abstract

Nanoparticles (NPs) are objects with one dimension of less than 100 nm (so 0.001 the size of a hair diameter) that have attracted attention for centuries because of their unique size- and shape-dependent properties. With such a small dimension, the impact of quantum effects becomes non-negligible, opening new windows on what chemists and physicists can achieve. The NPs field has deeply impacted our modern society, with numerous applications ranging from the electronic to the medical sectors. The size of such particles requires however accurate preparation methods where the NPs size and shape in a sample have to be the most possibly homogeneous. Such quality of preparation can be achieved using continuous flow technology (also known as micro- and mesofluidics) that ensures, among others, accurate control of the local parameters even at an industrial scale. This research program aimed at the photochemical synthesis of gold nanoparticles (Au NPs) under continuous flow conditions from lab scale up to industrial production [1]. The proposed protocol is robust, reproducible, and gives concrete solutions against the issues commonly encountered during the development of Au NPs synthesis. This article reports the main features of continuous flow technology applied to Au

NPs and summarizes the most important results gathered during this research program.

1. The interesting profile of gold nanoparticles

Gold nanoparticles (Au NPs) are the most famous metallic nanoparticles and have been used since the Ancient Age for staining glass artwork (Figure 1a). The discovery of their optoelectronic properties was however only attributed in 1857 by Faraday [3]. The most popular characteristic of these tiny particles is their typical red color due to their localized surface plasmon resonance (LSPR, Figure 2). This phenomenon is due to the oscillation of the electrons localized at the surface of the particle in resonance with an external oscillating electrical field, namely, a light beam [4]. After the absorption of light with a specific wavelength, the particle can return to its initial state by releasing heat or emitting a photon, which gives its characteristic color. The LSPR is characterized by a unique absorption pattern that can be followed by UV spectroscopy. This pattern depends on both the size and the shape of the particle, explaining the difference in colors between different Au NPs (Figure 1b).

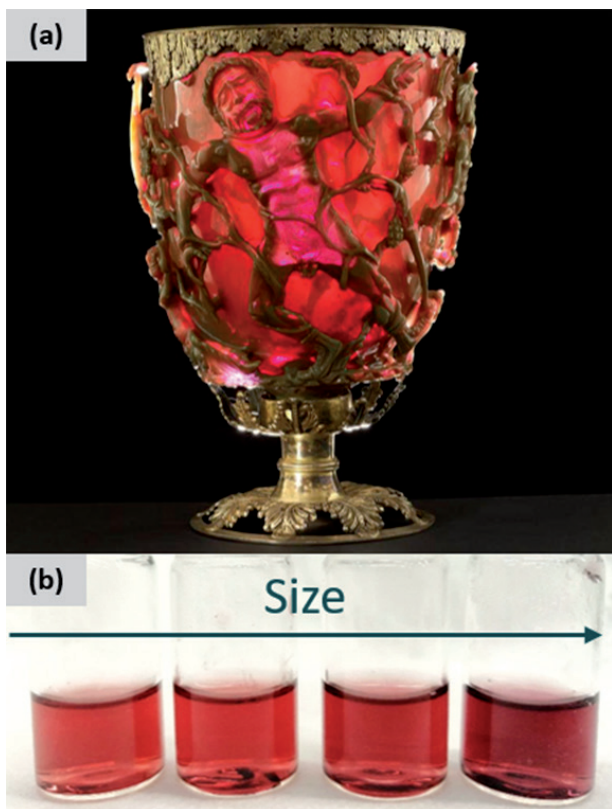


Figure 1. (a) Lycurgus cup, a 4th-century Roman glass cup partly composed of colloidal gold nanoparticles. Reproduced from the British Museum [2] under Creative Commons License (CC BY-NC-SA 4.0). (b) Samples of aqueous colloidal gold nanoparticles with different sizes.

The strong interest for these particles arises not only from their shape- and size-dependent optical properties but also from their low cytotoxicity and great stability. All these characteristics explain why Au NPs have found so many applications, even in our daily life, for example in organic catalysis, molecular sensors, surface-enhanced Raman spectroscopy (SERS), labeling, drug delivery, and cancer diagnostic, to name a few [5-7].

There are different ways to produce Au NPs, but all of them are based on the same principle: the reduction of a source of gold, typically chloroauric acid (HAuCl_4), into Au^0 by a reducing agent followed by the formation of Au^0 nanoparticles. The reduction can be chemical, biological, physical, or photochemical [8]. The last option has been chosen for this project since this method offers spatial and temporal control of the reaction. The reaction begins when an appropriate light source is switched on and

stops instantaneously when it is switched off, hence effectively reducing the size distribution of the NPs obtained in the sample. This method also involves milder conditions with lower temperatures than conventional chemical reductions. During the reaction, a photoinitiator (PI) is cleaved upon light irradiation and gives free radicals that can act as electron donors and hence as a reducing agent of HAuCl_4 . The reaction is efficient when an intense light can be strongly absorbed by the PI (related to the light wavelength and intensity, as well as the molar coefficient absorption of the PI), which is then homolytically cleaved into radical species (related to the quantum yield, namely the ratio between the number of molecules that have been cleaved and the one of absorbed photons). Different protocols have been already proposed in batch for the photochemical production of Au NPs, differing by the nature of the photoinitiator, for example, acetone, citrate, oxalate, 1-[4-(2-hydroxyethoxy)phenyl]-2-hydroxy-2-methyl-1-propane-1-one (**I-2959**), to name a few [9].

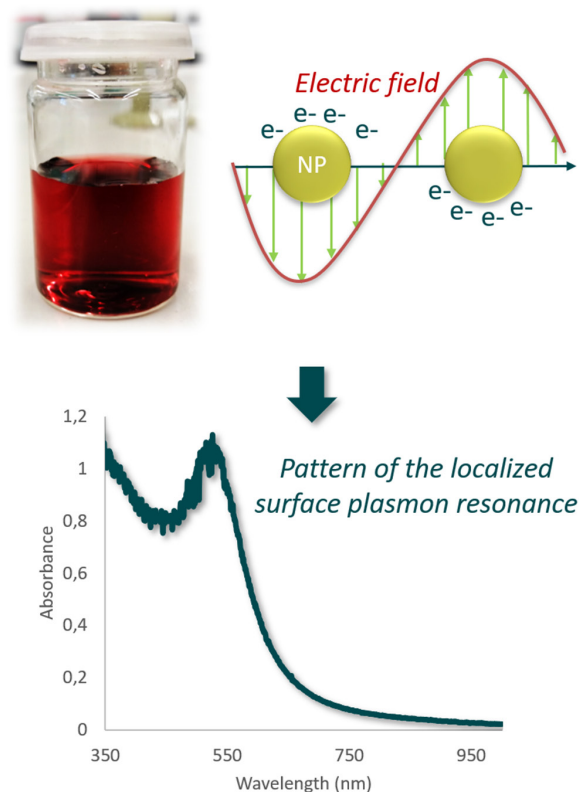


Figure 2. Localized surface plasmon resonance of a red colloidal solution of gold nanoparticles, with its characteristic absorption pattern.

However, the quality and homogeneity of the photochemical production of NPs in batch are difficult to manage as a consequence of poor mixing efficiency, poor light penetration, and inefficient control of the local parameters. This is even more pronounced on the industrial scale since the increase of the batch reactor vessel size leads to poor light penetration and inhomogeneous process conditions. These limitations can be overcome using continuous flow technology by ensuring a much better and homogeneous light penetration due to the narrowness of the reactor channels (see the next paragraph). Up to now, only four articles have been reported for the continuous photochemical production of Au NPs, but only in a lab scale [10-13].

Au NPs in a few words

- Tiny particles (<100 nm) made of gold
- Size- and shape-dependent properties
- Characteristic red color for the spheres
- Synthesized by reduction of HAuCl_4
- Spatial and temporal control of the synthesis with light irradiation

2. Continuous flow technology, a way to push towards the limits of chemical experiments

At the crossroads of preparative chemistry and chemical engineering, continuous flow chemistry aims at the continuous realization of chemical reactions in narrow channels of defined structure and typically in the submillimetric range. With a much higher surface-to-volume ratio than batch vessels, such reaction channels strongly affect fluid dynamics, mixing, heat- and mass transfer, and have a beneficial effect on light penetration. Fluidic reactors ensure accurate control of the local stoichiometry and temperature, transition to production scale, minimal footprint, potentially infinite production, and multi-step integration. This technology is therefore a strong asset for accelerating reactions and exploring hazardous or unconventional conditions while ensuring a safe environment even at production scales [14].

Depending on the chemical properties of the molecules and the conditions used, the chemist

can choose between a large array of micro- and mesofluidic reactors with specific features. A typical fluidic setup is not only composed of reactors but also of a range of critical auxiliaries including pumps, mass flow controllers, back pressure regulators, and thermocontrollers, the performance of which should be meticulously orchestrated for ensuring a homogeneous process experience. The integration of automation technologies, artificial intelligence, in-line analyses, and purification procedures has been managed to push further the limits of this technology, hence reducing the time of processing and simplifying a bit more the chemist's work.

This is not surprising to see the implementation of this technology in many branches of chemistry, including photo-, electro-, and biochemistry. From R&D to industrial scale, fluidic reactors have been used for the production of many value-added species such as active pharmaceutical ingredients (APIs) or fuel additives [15]. The nanoparticles field has not been ignored since such technology offers better monitoring of the chemical process than with conventional batch procedures [16,17]. In this context, continuous flow technology aims at the production of size-tunable and quasi-monodisperse NPs that are endowed with accurate properties.

Continuous flow technology in a few words

- Reaction in a flowing stream through narrow channels
- High surface-to-volume ratio
- Excellent mass- and heat transfers
- Overcoming of the batch limitations
- Numerous applications including nanoparticles synthesis

3. Using continuous flow reactors for the controlled synthesis of gold nanoparticles

This project aimed at developing a protocol for the large scale production of Au NPs under continuous flow conditions and upon visible light irradiation (Figure 3a). The main ideas were to use water as the solvent to provide sustainability and potential direct transposition for a use in

biological applications, as well as specific fluidic reactors. Corning® Advanced-Flow™ reactors have been designed to enable efficient mixing, heat transfer, and light penetration, as well as seamless scalability (Figure 3b). Composed of fluidic plates characterized by embedded heart-shaped static mixers that are integrated with a double-layered heat exchanger and sandwiched between two LED panels, these reactors can be found in different sizes, from the lab to the industrial scale.

The strategy was first to find the best conditions to make Au NPs on a lab scale to avoid wasteful time and resource-consuming optimizations. After optimization, the production was increased up to an industrial scale. Along with the whole development, the protocol was studied using different analytical techniques and its viability was assessed at the industrial scale.

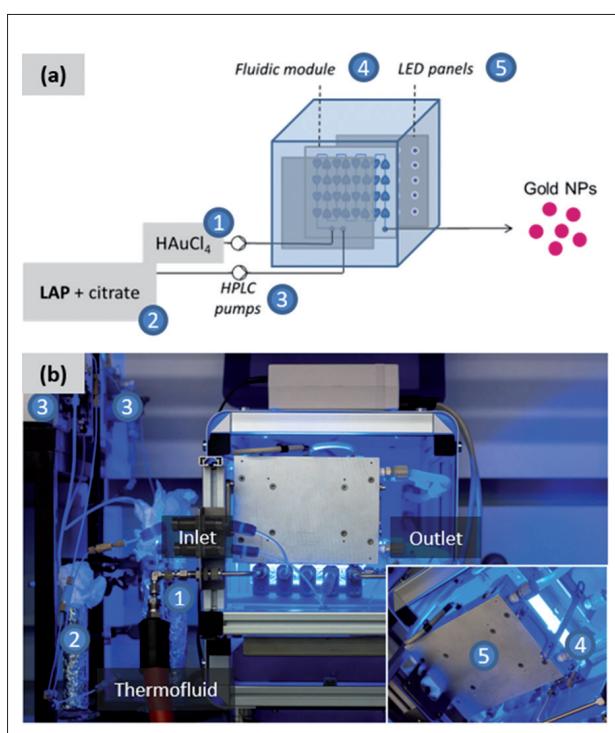


Figure 3. (a) Flow chart of the continuous flow photochemical synthesis of Au NPs; (b) Pictures of the Corning® Advanced-Flow™ photoreactor at the lab scale (2.5 mL irradiated volume, courtesy of Corning®). Reproduced from ref. [1]

4. The strategy for transitioning from conventional batch procedures to continuous flow technology

The first step of this project was dedicated to the elaboration of the most efficient conditions for the NPs fluidic synthesis without the formation of any gold coating, which included the choice of the PI, the ligand, and the medium. A preliminary screening of various PIs discriminated between two subclasses (Figure 4). Norrish type II PIs, characterized by their abstraction of a hydrogen atom from a donor (cosolvent) to form radicals, suffered from solubility issues in water and needed a sufficient amount of an organic cosolvent. Moreover, the poor stabilization of their corresponding NPs led us to choose Norrish type I PIs. Norrish type I PIs form radical species through a homolytic cleavage at the excited α -carbon and can also

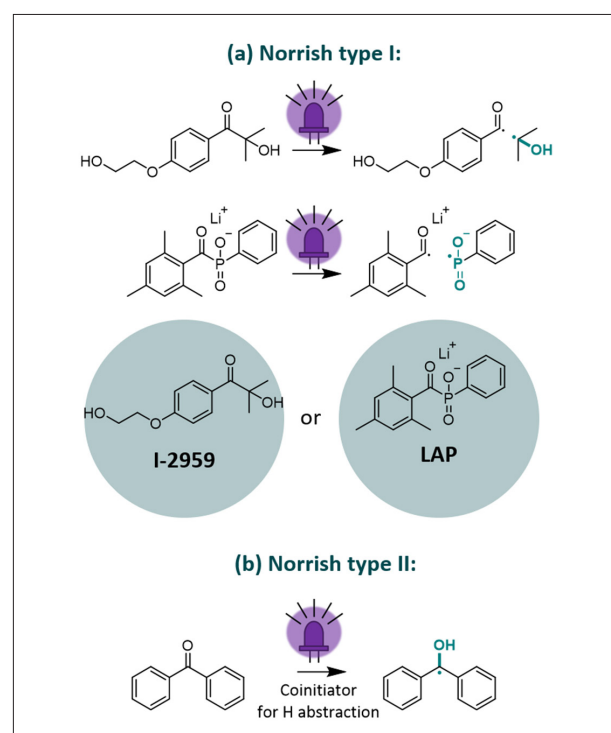


Figure 4. Examples of Norrish type I (a) and type II (b) photoinitiators for the reduction of HAuCl_4 . Free radicals able to reduce HAuCl_4 are shown in green. Among the two type I candidates (mechanisms are reported from ref. [19]), LAP has been chosen as the photoinitiator of this project because of a faster production of radical species upon visible light irradiation.

release fragments that can act as a stabilizer of the NPs, hence enabling better control of the reaction. Two Norrish type I PIs have been therefore selected: **I-2959**, a common PI in the NPs field [18], and lithium phenyl-2,4,6-trimethylbenzoylphosphinate (**LAP**) [19], unprecedentedly used before for that purpose.

However, the implementation of the synthesis under continuous flow conditions rapidly showed the higher performances of **LAP**. Its physicochemical properties, namely its higher quantum yield and molar extinction coefficient at 365 nm, ensured a faster reaction, hence reducing the likelihood of a gold coating formation on the reactor wall. In addition to the PI, an external stabilizer was required to ensure NPs stability overtime. Among the ones tested, sodium citrate was seen as the best option. Nuclear magnetic resonance (NMR) experiments confirmed the formation of citrate-capped NPs.

Nevertheless, these conditions could not prevent gold coating formation (Figure 5). The fluidic photoreactors opted for this project, the Corning® Advanced-Flow™ reactors, are composed of a specialty glass that is sensitive to pH. At $\text{pH} > 3$, most of the silanol functions that compose the glass are deprotonated, and these ionic species can bind to the gold atoms. The slower the flow rate or the nucleation rate, the higher the likelihood to be trapped by the silanoloxides along the channel surface. This phenomenon gives a pinkish/purple coating along the reactor which is not responsible for clogging but for a drastic reduction of the light penetration, hence reducing the reaction efficiency, avoiding reproducibility of the process, and precluding long production campaigns. This coating could be removed by *aqua regia* ($\text{HCl}_{\text{conc}}/\text{HNO}_{3\text{conc}}$, 3:1) after each run, but this option was not viable for industrial scale applications due to its highly corrosive nature. Up to now, no concrete solutions against this coating was proposed in the fluidic photochemical protocols found in the literature [10-13].

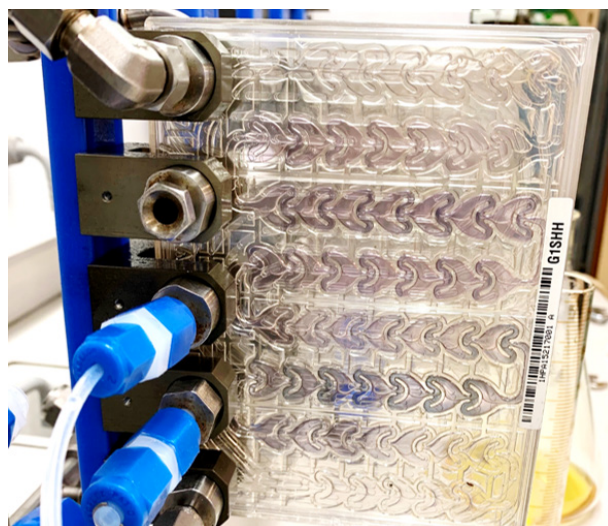


Figure 5. Picture of a characteristic pinkish gold coating

To avoid such a coating, different strategies were adopted. The use of a hydrophobic coating on the reactor channels and biphasic conditions could not provide satisfying results, but the use of repulsive electrostatic properties enabled the absence of any gold coating during the runs. Working with a neutral pH was the best compromise to have a significant proportion of deprotonated citrates that could be repelled by the negatively charged glass surface and to avoid glass deterioration. However, the complexation of OH^- anions with AuCl_4^- led to a drastic reduction of pH overtime [20]. The pH instability issue could be overcome by working under buffered conditions (phosphate buffer, $\text{pH} = \text{pK}_{a_2} = 7.2$), which also ensured NPs stability and robustness of the process.

After confirmation of the necessity to have concomitantly **LAP** and light to produce these NPs, an optimization procedure was implemented to find the best experimental conditions. This step was considerably accelerated by implementing an in-line UV spectrometer that enabled real-time monitoring. Among all the LED wavelengths proposed by the Corning® Advanced-Flow™ reactors, irradiation at 405 nm was selected for efficient tuning of the other parameters. Increasing the ligand concentration beneficially decreases the likelihood of gold coating and led to smaller but more stable NPs. Concerning temperature and light intensity, the first one led to bigger NPs while the second one had an

opposite effect. Increasing both parameters enabled a controllable NPs formation. Varying the residence/irradiation time, *i.e.* the time spent in the reactor upon irradiation, was, therefore, the most suitable parameter for tuning the NPs size. The NPs obtained were also studied by transmission electron microscopy (TEM). The corresponding images gave information about the size, shape, and size distribution of these samples. Small spherical Au NPs (~5 nm) were produced at 40 °C, with however a non-negligible size-distribution (Figure 6). But these NPs still competed with commercial samples and the ones described in the literature.

The best set of conditions was determined for the last step of this project, that is its scalability to commercial scale, by taking into account the restrictions about flow rates and residence times to fully benefit from the mixing efficiency of the Corning® Advanced-Flow™ reactors. Scalability assessment was first tested with a commercial pilot reactor (40 mL of internal volume for the pilot scale instead of 2.5 mL for the lab scale). A minor reoptimization of the light intensity, that was not directly transposable, was required before assessing the scalability with an industrial photoreactor (50 mL of internal volume for the production scale). Full light intensity was necessary to avoid gold coating at the industrial scale. The transition to larger scales showed however a slight decrease of the NPs size and increase of the size distribution, but this disparity could vanish with a better study of the mixing and flow rates at different scales.

The viability on the industrial scale, reproducibility, and robustness of the protocol was proved during a long run (nearly a half-hour) without the formation of any gold coating. At the end of this project, using a residence time of only 10 s (300 mL min^{-1} total flow rate) in the industrial reactor proposed an extrapolated daily productivity of about 360 L of aqueous colloidal spherical Au NPs (corresponding to an output of 65 g of Au NPs per day).

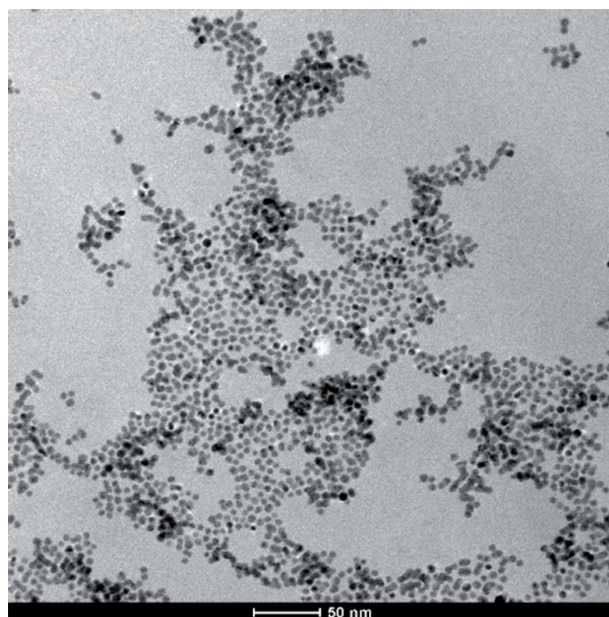


Figure 6. TEM picture of colloidal gold nanoparticles obtained with this fluidic protocol

This project in a few words

- Continuous flow photochemical synthesis of gold nanoparticles
- Discovery of a new photoinitiator
- Gold-coating free procedure
- Up to industrial scale production

5. Conclusion and perspectives

During this research program, a highly engineered strategy for the large scale production of Au NPs using photochemistry and continuous flow technology was developed. It led to the discovery of a new efficient and water-soluble photoinitiator and to the proposal of an efficient solution against gold coating using a phosphate buffer and sodium citrate as a stabilizer. Small spherical Au NPs could be produced under 405 nm irradiation at 40 °C in a fast way (10 s of residence time). This intensified photochemical process (360 L per day) is the very first one to be reported under continuous flow conditions.

Despite the need for further optimizations of the mixing efficiency along the reactor channels, the quality and the size of the NPs produced by this protocol are already in an acceptable range for industrial or academic applications: DNA

detection, biomarkers detection for microscopy imaging, radiotherapy, chemical catalysis, surface assisted laser desorption/ionization mass spectrometry (SALDI) experiments, to name a few. These NPs could also be enlarged by simply adding a fresh source of HAuCl_4 in the medium and a new PI, this process being called the “seed-mediated growth method”. This protocol opens therefore new perspectives to the nanoparticle field, by proposing Au NPs from the small to the large range with numerous industrial or academic applications, in high productivity that could be envisaged for the World demand of Au NPs (a few tons per year). This fluidic production could be also extended to other metallic (pure, alloyed, and core-shell) NPs to broaden a bit more the possibilities of production.

This project shows that continuous flow technology can be advantageously used in the nanoparticle field to overcome issues related to batch production, but its direct transposition is not straightforward. The use of fluidic reactors can raise new problems, such as the formation of a gold coating, that require a careful redesign of the process conditions. However, when the issues are overcome, chemistry can benefit from better control of the local parameters, reproducibility, fast optimization, easy scale-up, and efficient and homogeneous light penetration, even at an industrial scale.

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