Synthetic Guidelines for the Precision Engineering of Gold Nanoparticles

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Abstract

Gold nanoparticles (AuNPs) are one of the most studied nanomaterials with applications spanning from catalysis to biomedicine. While numerous chemical protocols exist that allow bespoke tailoring of chemical, physical and biological properties, their translation towards industrial-scale production remains a challenge. Batch synthesis often suffers from poor reproducibility and scalability, while emerging approaches, such as continuous flow synthesis, are not widely implemented in research labs. In this review, we provide a critical overview over recent developments in the field of AuNP synthesis and provide synthetic guidelines for precision engineering of nanoparticle properties.

Introduction

After decades of intensive research, gold nanoparticles (AuNPs) now offer opportunities in a plethora of applications, ranging from the ultrasensitive detection of biomarkers in point-of-care systems [1], e.g. for naked eye ELISA [2] and lateral flow immunoassays [3], to environmental test kits [4], antimicrobial coatings [5], photovoltaics and other energy transfer systems [6]. AuNPs have been widely studied for specific interaction with biomolecules [7], as drug sensors [8] and antimicrobial agents against bacteria [9] and viruses [10]. The particle applicability typically relies on the tailoring of distinct properties, such as the surface plasmon resonance, assembly into more complex lattices [11], catalytic features [12] or biological activity [13], [14]. AuNP-based products are meanwhile entering the commercial market, with a number of startup companies and small/medium-sized enterprises offering unique solutions based on gold nanotechnology platforms [15]. We note that while out of scope herein, further implications on the widespread use of gold nanoparticles have to be considered, including the availability of resources and the environmental risk post-use [16].

Physical and chemical properties of AuNPs are strongly related to the size and shape of the metallic core as well as the displayed surface chemistry [17]. For example, in a range from sub to hundreds of nm, smaller nanoparticles exhibit a higher cell uptake than larger ones [18]. The interaction of AuNPs with their environment is often dominated by the chemical composition and charge of the NP surface, often defined by an organic ligand shell [19]. Furthermore, anisotropy in shape and surface functionalisation plays an important role in many biomedical applications [20].

The achievable precision towards control over size, shape and surface chemistry is strongly dependent on the synthetic protocol [21], [22]. Most common manufacturing routes rely on solution-based bottom-up syntheses from chemical precursors but alternative routes exist via photochemical, electrochemical, or templating principles. Despite the extensive body of research, challenges remain on the synthetic engineering of suitable protocols that allow AuNP production with precise control over the structural properties, low dispersity and high yield as well as short synthesis time and low synthesis costs, all of which at high reproducibility across laboratories [23]. Herein, we review the available synthetic toolbox and critically compare suitable batch and continuous flow protocols towards implementation in scale-up nanomaterial engineering.

Synthesis in batch

The first synthesis of colloidal gold particles dates back to 1857 when Sir Michael Faraday reduced gold chloride by phosphorus in an aqueous solution. As a milestone, this seminal experiment guided most of the colloidal synthetic methods that followed: reducing solvated gold salt in the presence of a surface passivating agent to prevent aggregation. A brief overview of some of the most common synthetic concepts for AuNP synthesis is shown in Figure 1 alongside a comparison of precursors, reducing agent, passivate ligands and resulting size range displayed in Table 1.

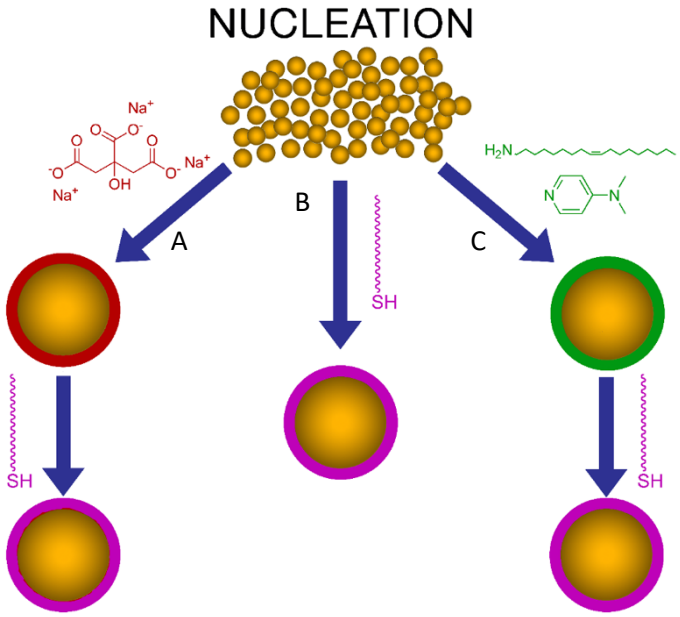

Figure 1. Schematic of the most common synthetic routes for: A) Charge-stabilized particles prepared by the Turkevich method using trisodium citrate and further exchange with a thiol, B) One-step synthesis with thiol capping directly after gold reduction, C) Two-step synthesis using labile ligand and subsequent ligand exchange with a thiol.

Gold Precursor	Reducing agent	Passivating agent	Size [nm]	References
$HAuCl_4$	Trisodium citrate	Trisodium citrate	5-150	[24], [25]
$HAuCl_4$	$NaBH_4$	TOABr, thiols	1,5-5	[26], [27]
$(Ph_3P)AuCl$	$NaBH_4$	Thiols	1-8	[28]
$HAuCl_4$	TBAB	Oleylamine	2-6	[29]
$HAuCl_4$	$NaBH_4$, CO, GSH	Thiols	0,6-1,2	[30], [31], [32]

Table 1. Comparison of precursors, reducing agent and passivate ligands and resulting size range in some of the most common synthetic protocols for gold nanoparticle preparation.

Some of the most common AuNP synthesis routes are based on the reduction of $HAuCl_4$ as gold precursor by sodium citrate, with seminal work by Turkevich and Frens. Notably, sodium citrate may act as reducing agent, stabilising agent and pH mediator, offering a versatile platform for optimisation [24]. Nevertheless, several inherent drawbacks remain, including the low yield of batch reactions as well as difficult access to monodisperse AuNPs below 10 nm in diameter. The latter challenge may be overcome by a synergistic interplay of sodium citrate and tannic acid, which offers a route to 3.5 to 10 nm-sized citrate-stabilised particles with narrow size distribution [33]. Another area of research is the so-called seeds-mediated growth technique, where sub 10 nm gold seeds serve for subsequent particle growth [25]. This process, enabling gold reduction and inhibition of secondary nucleation, may be kinetically controlled by adjusting pH, temperature and gold precursor to seeds concentration ratio [34]. The seed growth provides a versatile and reliable chemical route to the synthesis of uniform

particles up to 200 nm as well as anisotropic shapes. In this context, Mirkin and co-worker established that the formation of anisotropic nanoparticles is strictly dictated by the shape, size and the crystalline structure of the seeds [35]. Iterative seed refinement by oxidative dissolution and reductive growth enabled them to create different shapes from an identical seed source (Figure 2).

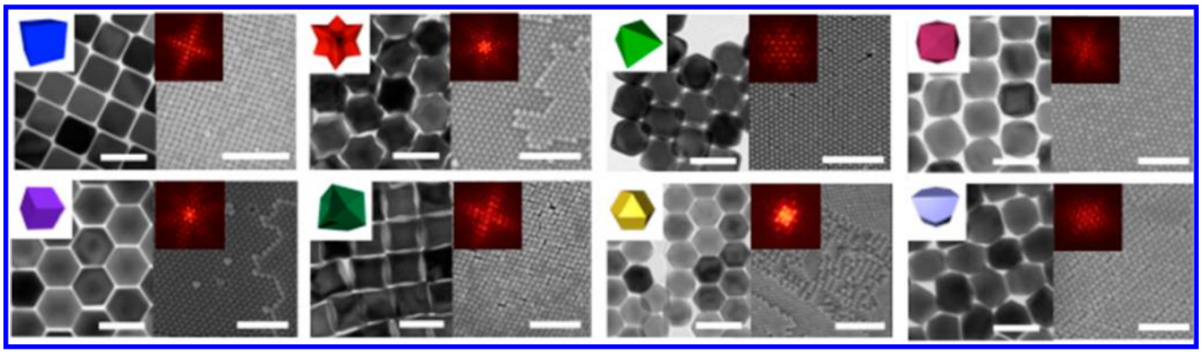

Figure 2. Relationship of AuNP seed and final shape, as manipulated by iterative oxidative dissolution and reductive growth from a single seed source. Reprinted with permission from reference [35]. Copyright 2014 American Chemical Society.

Beyond citrate-mediated electrostatic stabilisation of the AuNP surface, thiol-based ligands reliably passivate the gold core and constitute an extremely broad molecular platform for surface functionalisation, enabling the attachment of nucleic acids, proteins, peptides, lipids and a multitude of other molecules via covalent or non-covalent conjugation [36]. Several protocols offer direct access to thiol-capped AuNPs, most notably the two-phase [26] and single-phase synthesis by Brust et al [27]. and a single-phase synthesis introduced by Stucky and co-workers [28]. The former are most effective at synthesising 1-4 nm sized AuNP, the latter extends the achievable size range to 8 nm with improved size uniformity by the use of amine−borane complexes (TBAB) as reducing agent [29]. While the direct synthesis of thiol-protected AuNPs offers larger yield, shorter preparation times and smaller core diameters, the protocols are generally limited to organic solvents and non-polar thiol ligands. The replacement of the thiol capping layer via thiol-for-thiol ligand exchange is generally challenging due to high affinity of the gold-thiol bond [37].

To this end, the synthesis of AuNPs with a labile ligand as intermediate capping agent, followed by a ligand-for-thiol exchange provides a broad platform for AuNP synthesis with target functionality. Promising routes include the use of phosphines [38], 4-(N,N-dimethylamino)pyridine (DMAP) [39] and tetraoctylammonium bromide (TOABr) [27],[40]. In recent years, aliphatic amines including oleylamine have attracted particular interest [41]. In fact, we have recently shown that the use of oleylamine as stabilising agent enables the decoupled control of AuNP size and surface composition, with the former being controlled by the reaction temperature in the initial synthesis and the latter by the subsequent exchange with prescribed thiol ligand mixtures (Figure 3) [42].

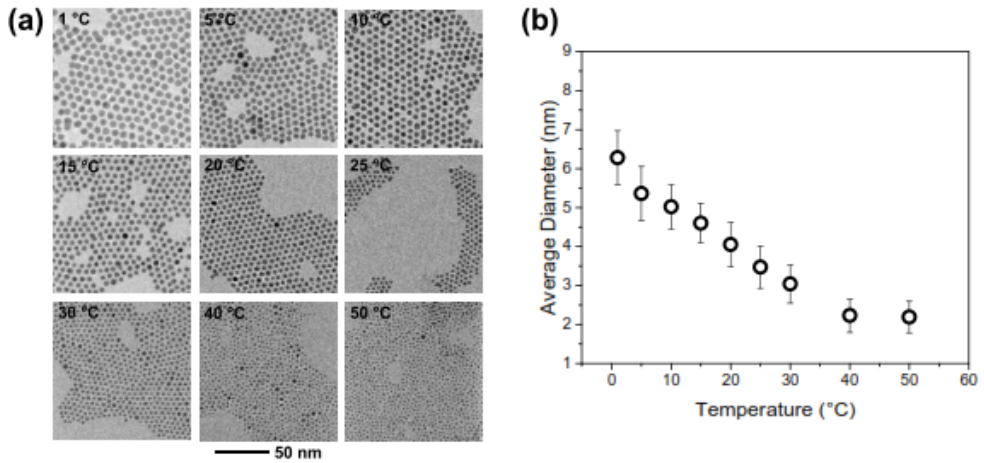

Figure 3. Size control offered by temperature manipulation in oleylamine-based AuNP synthesis. a) Representative TEM images for different reaction temperatures. b) Average size and standard deviation. Note that this approach offers a decoupled control of size and surface composition by a subsequent thiol-for-oleylamine ligand exchange. Reprinted with permission from reference [42] under Creative Commons CC-BY license. Copyright 2018 American Chemical Society.

In recent years, atomically precise gold clusters (Au_{10}, Au_{15}, Au_{18}, Au_{25}) have emerged as a particular promising nanoparticle platform [32], with distinct characteristics related to their luminescence [30] and renal clearance [43]. The formation of stable, atomically sub-1nm clusters can be effectively realised by Au(III) reduction using either $NaBH_4$, CO, glutathione (GSH) or proteins in the presence of thiol molecules [32].

While traditional bottom-up synthesis of gold nanoparticles in batch provide fast and scalable pathways for AuNP production (up to gram scale), protocols are suffering from low batch-to-batch reproducibility and limited synthetic yield [23]. The intrinsic kinetics on the mixing of gold ions with the reducing agent defines the final product, rather than the thermodynamics [44]. Thus, concentration gradients generated by ineffective mixing can lead to the formation of nuclei with variable size, which afterwards causes polydisperse particle size distribution [45]. In addition, ineffective decoupling of nucleation and growth, i.e. the continued nucleation during particle growth, promotes dispersity in size and shape [46]. To this end, Besenhard *et al.* demonstrated the influence of batch mixing of gold precursor ($HAuCl_4$) and reducing reagent (trisodium citrate) on particle size and dispersity using a fix feed 5:1 ratio (citrate/tetrachloroauric acid) [47]. In contrast to previous reports, smaller nanoparticles with reduced polydispersity were obtained by at-once addition rather than dropwise injection. This work highlights the importance not only of the molar ratio between reagents but mixing conditions and the order of reagent addition into the reaction.

Synthesis in flow reactors

Flow micro- and millireactors offer advantages over traditional batch reactors in the synthesis of nanomaterials. Their small characteristic dimensions decrease the diffusion paths, allowing faster mixing, while their high surface-to-volume ratio enhances heat transfer rates. These features facilitate tight control of critical process parameters. Furthermore, continuous operation yields product volumes significantly exceeding the reactor volume. Over the last decade, there has been an increasing number of reports utilizing flow reactors for the synthesis of gold nanoparticles of varying shape, size and surface functionality. A plethora of reactor designs was employed to-date, including radial interdigitated mixers, split-and-recombine mixers, channels with butterfly structures, Y-shape pulsating mixers, coaxial flow mixers and coiled flow inverters [48].

Many flow synthesis studies claim that, due to fast mixing and enhanced heat and mass transfer, one can synthesize smaller particles in flow than in batch, and/or flow reactors lead to faster reaction kinetics. We note that this applies only to a limited number of cases, e.g. for reactions where the mixing time is comparable to the reaction time, or when the synthesis is mass-transfer-limited. Hence, the shorter reaction times associated with flow syntheses is often related to unoptimized batch protocols [49]. In this regard, flow reactors may inform and benefit the study of nanomaterials synthesis kinetics. At the same time, the study of the synthesis kinetics could render the translation of batch protocols to flow an easier task [39]. This was recently demonstrated by Panariello et al. who translated an Au nanoparticle synthesis to flow with minimal effort [50]. Batch studies provided the relevant kinetic information on precursor conversion, which enabled the design of a flow reactor with throughput 20 mL/h (~1 mgAu/h) and a production volume limited only by the syringes used. Full exploitation of kinetic data can though only be achieved through the development of theoretical approaches that are able to utilise such information. Population balance modelling offer valuable information at limited computational cost, which can be combined with classical reactor design methodologies in order to design flow reactors synthesizing nanomaterials [51]. For gold nanoparticles, only few works on population balance modelling are available in the literature [52], [53].

Reproducibility is one of the main incentives of flow reactors, derived from increased control over experimental conditions and reduction of human intervention during the synthesis [50], [54], [55],. This can be further enhanced by improved process understanding and quality control achieved by the integration of online analysis, e.g. by the online use of SAXS/WAXS and UV-Vis [56], [57], [58]. Several techniques have been integrated in line, such as small angle X-ray scattering/wide angle X-ray scattering (SAXS/WAXS), extended X-ray absorption fine structure (EXAFS), and UV-Vis spectroscopy. The implementation in flow of new techniques, such as liquid-phase transmission electron microscopy (TEM) can bring significant advancement to the understanding of particle synthesis mechanism. Further progress in the field of process and quality control requires the integration of techniques, which allow monitoring of both size distribution and particle concentration. While this may currently be achieved by SAXS, challenges remain for more widely accessible techniques, including a relatively slow acquisition time for differential centrifugal sedimentation (DCS), unreliability for multimodal or wide size distributions for dynamic light scattering (DLS) and the relatively large limit of detection (~10 nm) for nanoparticle tracking analysis (NTA). UV-Vis can help in this direction, though the interpretation of UV-Vis spectra is not straightforward and requires a priori knowledge to obtain reliable results (e.g. particle size and surface coating) to determine concentration of spherical gold nanoparticles [59]. Atomic emission spectroscopy (AES) may provide yield values with no a priori knowledge of the analysed nanoparticles. Furthermore, there is also a need for the development and implementation of techniques that offer in situ information of the ligand shell, such as small angle neutron scattering (SANS) [60] and quartz crystal microbalance with dissipation monitoring [61].

To-date, only few reports have demonstrated the long-term robustness of flow synthesis by monitoring the reactor output over representative operating times. Instead, the common practice is to limit the operation of the flow reactor to relatively short times until a steady state is reached to collect enough sample for characterization.

One of the biggest advantages of flow reactors, the high surface-to-volume ratio, can lead to one of the biggest challenges connected to their usage in the field of nanomaterials synthesis: fouling. The accumulation of particles on the reactor walls not only reduces the synthesis yield, but also changes the synthesis conditions during operation. Fouling is in general a dynamic process, rendering the operation of the flow system dynamic rather than continuous. Segmenting liquids that preferentially wet the reactor wall are a common solution to fouling (as well as improved residence time distribution) [62]. However, while such approaches may efficiently avoid contact of the reaction solution with the reactor wall, the nanoparticle quality can suffer from interfacial adsorption [63].

Flow reactors present particular advantages for the use of gaseous reactants, where tuning of the system pressure and efficient mass transfer can be achieved more effectively in comparison to batch reactors. Examples in this context are the synthesis of AuNPs of various sizes and surface functionalities in gas-liquid segmented flow reactors, gas-liquid-liquid and membrane liquid-liquid reactors [64], [65], and the synthesis of atomically precise Au_{25} cluster in membrane liquid-liquid reactors [66], where in all cases CO was used as reducing agent.

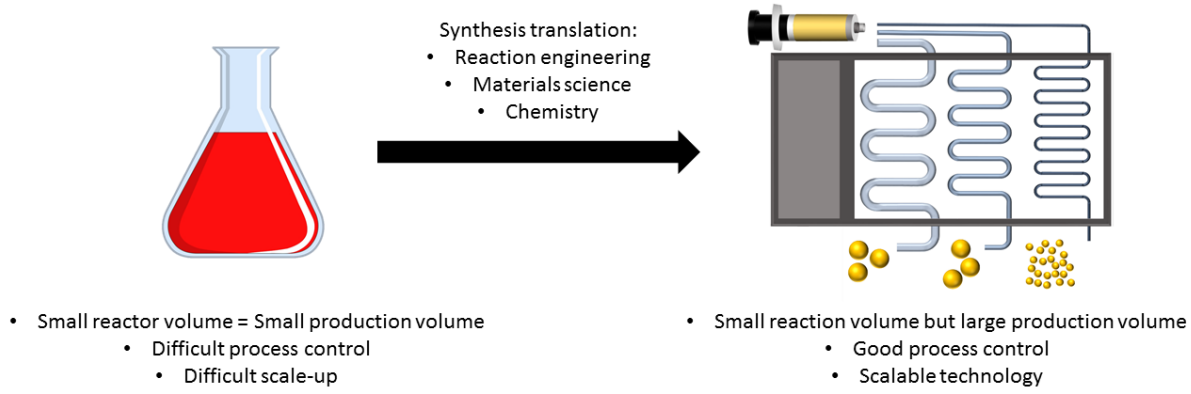

Figure 4: Translation of gold nanoparticles synthesis from batch to flow. A number of advantages derive from the use of flow reactor, though the efficient translation of the synthesis from batch to flow requires a significant interdisciplinary effort. Adapted with permission from reference [67] under Creative Commons CC-BY license. Copyright 2018 Elsevier.

Flow reactors offer the opportunity for controlled and scalable manufacturing of gold nanoparticles through available batch protocols up to g Au/day productivities [68], [69]. Some examples are already available in the literature addressing scale up of the production: Huang et al. scaled-up the production of Au25 clusters compared to the equivalent batch synthesis reported in the literature by increasing the precursor concentration by a factor of 10, increasing the reaction temperature and decreasing the reaction time from 24 h to only 3 min, while retaining full precursor conversion as in the equivalent batch synthesis [63]. In this way the production of approximately 1 g of Au25 clusters per day was achieved, with a continuous operation of the reactor over 3 days without deviation in the particles properties. Lohse et al. reported the shape-controlled synthesis of Au nanomaterials with productivity in the order of 2.5 mg/min (which projected over 24 h operation corresponds to 3.6 g/day) [68]. Gomez et al. scaled-up the production of Au nanocages both in batch and flow, demonstrating that the maximum achievable yield (without compromising the product quality) in batch was ~115 g/day, against ~570 g/day achievable in flow [70].

Optimal design of these reactors requires an interdisciplinary effort linking chemistry, material science and engineering. Research efforts are still required for a straightforward translation of existing batch protocols to flow, in particular to find innovative ways to fight fouling and to investigate synthesis kinetics [57]. The unique advantages of flow over batch reactors (e.g., operation at higher temperature/pressure, fast heating/cooling, short reaction times, controlled staged addition of reactants) are only starting to be leveraged and require the development of suitable synthetic protocols.

Summary and future directions

Recent developments on the engineering of gold nanoparticle synthesis provide pathways to scaled-up production of nanoparticles, with reproducible properties with respect to narrow size distribution,

uniform shape and surface functionality. We anticipate the following aspects to be key points for further progress in the near future:

a) While considerable improvements have been reported to-date, full control over the batch-to-batch reproducibility remains challenging. In this respect, detailed control over the nucleation kinetics and implementation of protocols that promote the decoupling of nucleation and growth are crucial. A more synergistic interplay between batch and flow synthesis may help to resolve underlying fundamentals and enable to adapt the most suitable synthetic approach for the respective application and design targets.
b) Flow technologies offer attractive opportunities towards both fundamental understanding of synthetic protocol, as well as nanomaterials manufacturing. Scalability of these technologies is still challenging, hence we anticipate that in the near future the best opportunities for flow technologies implementation in industrial settings will be in the production of high-value nanomaterials for biomedical applications, where the production scale is relatively small (below 1000 kg/year) compared to other industrial sectors.
c) In order to ensure reliable nanomaterial synthesis in flow, more robust design procedures are required, in particular to ensure full conversion of the precursor at the reactor outlet, as unreacted precursor can both hinder control over the size distribution and lower the process yield. New and more robust design procedures should be informed by principles of reaction engineering and statistics (e.g. model based design of experiments). The latter requires more effort in implementing inline analysis techniques that can provide data in real time, eventually allowing the design of closed-loop and self-optimizing systems. Furthermore, in order to make flow processes attractive from a commercial point of view, emphasis needs to be put on the implementation of downstream purification processes, which are currently generally overlooked or performed in batch-mode (e.g. centrifugation).
d) The field of atomically precise Au clusters is rapidly gaining interest, in particular in the field of biomedicine. These provide significant advantages, both with respect to their efficient use of material as well as their ability for renal clearance.

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