How does the size of gold nanoparticles depend on citrate to gold ratio in Turkevich synthesis? Final answer to a debated question

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Graphical abstract

**ABSTRACT**

The dependence between the size of gold nanoparticle (AuNP) and the citrate to gold molar ratio ($X$) is still a matter of debate 65 years after the seminal work by Turkevich et al. for high $X$ values. We assume that this dispersion of results is due to the variation of certain parameters that are often not mentioned in the protocols, and to the use of a single characterization technique (dynamic light scattering (DLS) or transmission electron microscopy (TEM)). To address definitely the question of this dependence, we have synthesized AuNPs with very precise protocols ensuring that the only parameters to be modified are $X$ and the sequence of reagent addition. We have then studied, for the first time, the dependence of the size with $X$ quantitatively with a multimodal approach (UV–Visible spectroscopy, DLS and TEM) for 2 synthetic routes differing only by the sequence of reagent addition. We show unambiguously that AuNPs' size decay monotonically, with $X$ whatever the order of reagent addition. It allows us to exclude the occurrence of a measurable discontinuity for a peculiar value of $X$ that prompted some authors to postulate the existence of two different reaction pathways when the citrate to gold molar ratio is around this value. In contrast, our result is in line with one reaction pathway, likely a “seed-mediated” growth mechanism, which should lead to monotonic size decrease. Also, we note that our result agrees with the sole available theoretical prediction (Kumar et al., 2007) on the whole range of $X$. Despite this apparent agreement, we point some contradictions between recent experimental results and basal hypothesis of this model.
1. Introduction

Gold nanoparticles (AuNPs) are the most widely used metallic nanoparticles and drive a variety of applications in nanomedicine, sensing, optoelectronics and catalysis [1–5]. The fine control of AuNPs individual characteristics is a prerequisite to exploit the macroscopic properties, that emerge from collective effects [6]. Although the syntheses in non-aqueous solvents were preferred so far for the synthesis of high quality building blocks, great efforts have been done in the last decade to optimize synthesis pathways directly in water [7]. The prominent member of this group of aqueous synthesis is the ‘Turkevich’ protocol introduced in 1951 [8]. This method enables to obtain monodisperse citrate-stabilized AuNPs by simply changing the relative concentration of citrate molecules that are quickly injected in a boiling HAuCl$_4$ aqueous solution. In spite of its advantages, this approach suffers from lack of reproducibility and of predictability due to a poor theoretical understanding of the mechanism of formation. This is due to the fact that citrate and gold ions exist both in different chemical forms, which can be reversibly converted into each other. This is also due to the multiplicity of roles played by citrate molecules (i.e. reductive agent, stabilizing agent and pH mediator), which results in multiple intricate steps that are hard to probe separately. However, one can attempt the following description. First, Au$^{III}$ is slowly reduced to Au$^{I}$ thanks to citrate decarboxylation into dicarboxy acetone (DCA) which leads to electron transfer. Differential functional theory simulations have shown that the most favorable reaction path is obtained at low pH where [AuCl$_4$]$^{-}$ is the dominant structure of aureate complex due to the presence of highly labile Cl$^-$/ ions around Au$^{III}$ [10,19]. We wish to underline that pH is controlled by citrate to gold molar ratio ($X$) at fixed gold concentration. As formed Au$^0$ atoms can be assumed to form multi-molecular complexes with DCA [8] but experimental proofs are lacking [11,28]. Au$^0$ could be formed in bulk when the concentration of Au$^I$ species increases locally to a ‘high enough’ level ([AuCl$_2$]$^-\approx 10$ nM) [9] to trigger homogeneous disproportionation [12]. Further disproportionation may lead to larger aggregates of Au$^0$ atoms and when the aggregate size reaches a critical radius of order 1.5 nm, a stable nucleus of gold atoms should be formed [13,15]. Disproportionation can also occur at the particle surface, leading to nucleus growth and also to the regeneration of some Au$^{III}$ [20].

The reactant molar ratio, $X$, is the eldest and probably the most frequent lever for controlling the size and the polydispersity of AuNPs. Forty years ago, Frens mentioned a steep decrease of AuNPs’ size by a factor 6 when $X$ was varied from 0.8 to 2 at [Au] = 0.25 mM. This result has been confirmed by several studies [8,21–27] together with a concomitant decrease of polydispersity. It is also recognized that this evolution of size is restricted to low values of $X$. Indeed, above a certain value, noted $X^*$ in Fig. 1c, which vary with the absolute gold concentration [23], the different results are conflicting. This is shown in Fig. 1a which summarizes the results obtained for the most popular conditions of synthesis: [Au] = 0.25 ± 0.05 mM and $T = 98 \pm 2$ °C. One can discern two families of behaviors:

(i) On one hand, several studies [14,16,23] showed a discontinuous evolution of the size with a sharp minimum at $X^* \approx 3.5$ [14,23]. Ji et al. [14] observed that this minimum corresponds to a pH at which the dominant structures of aureate complexes and citrate ions modify (Fig. 1b). These authors propose that the observed discontinuity may be related to a modification of the mechanism of formation (Fig. 1c). A two-steps process involving nucleation followed by a slow diffusion-controlled growth should dominate above $X^*$, while a three-steps process involving fast nucleation and random binding, followed by an intra-particle ripening, should dominate below $X^*$. This last mechanism may involve transient “nanowire-shaped” clusters that have been detected by TEM [17,22], but never by SAXS or in situ AFM [15,18], suggesting that they result from the reduction of Au$^I$ and Au$^{III}$ species during drying [18].

(ii) On the other hand, several studies [22,26] showed that the size decreases continuously with $X$ on the whole range of $X$, with lowest radius ranging between 2.5 nm [26] and 10 nm [15] at $X = 20$. A so-called four-steps “seed-mediated” growth mechanism, which is supposed to be congruent with a monotonic decrease of the size, was proposed on the basis of coupled in situ XANES and SAXS during AuNPs formation, for $X > X^*$ [15]. Such proposal is based on the observation that gold equilibrium is shifted from [AuCl$_4$]$^-$/to less reactive [AuCl$_{x-}\cdot(OH)_{x-1}$] during the fast seed particle formation (∼30 s after mixing), so that for $X > X^*$, the kinetics of seed formation and pH neutralisation should be

![Figure 1](https://example.com/figure1.png)

Fig. 1. Evolution of the averaged radius of citrated AuNPs as a function of (a) the molar ratio $X$ and (b) the pH for similar conditions of synthesis given in inset of Fig. 1a and summarized in Table 1 of SI. All radii are number averaged values obtained by TEM except for [23] which corresponds to intensity averaged hydrodynamic radii. The line corresponds to Kumar’s model prediction [27]. The different colors of patterns and the numbers in Fig. 1b characterize the regions of hydrodynamic radii. The line corresponds to Kumar’s model prediction [27]. The different colors of patterns and the numbers in Fig. 1b characterize the regions of hydrodynamic radii. The line corresponds to Kumar’s model prediction [27].
approximately the same irrespective of the molar excess thus explaining the size independence. For $X < X^*$, the molar excess might still be good enough to reduce Au$^{III}$ species but not to shift the gold complex equilibrium. Hence, homogeneous and heterogeneous disproportionation can also occur unselectively during the entire synthesis, increasing the polydispersity. At very low $X$, seed particles should grow up to a size that could be stabilized by the available citrate molecules. Interestingly, the continuous evolution of size with $X$ has been theoretically predicted by Kumar et al. [27] until $R \approx 7.5$ nm at $X = 20$, but with a model neglecting the dependence of the aureate complexes structure and reactivity with the pH.

In summary, we note that there is no consensus on the evolution of the size with $X$. We believe that this dispersion of results is due (i) to the variation of certain parameters that are often not mentioned in the protocols, and (ii) to the use of a single characterization technique (TEM or DLS). In this article, we want to answer experimentally at the following open questions:

- Is the evolution of AuNPs size with $X$ monotonous or not?
- What is the size of AuNPs for $X \geq X^*$? Indeed, all authors agree that the size is independent of $X$ and the polydispersity is low but the size vary on a decade between $R = 2.5$ and 19 nm.
- What is the influence on our results of the sequence of addition of the reagents?
- What is the validity of the only theoretical prediction, as checked in the light of our results?

We synthesized AuNPs with very precise protocols ensuring that the only parameters to be modified are $X$ and the sequence of reagent addition. The as-obtained batches of AuNPs have allowed us to give clear-cut answers by studying thoroughly the size dependence as function of $X$ on a large range of $X$ values (i.e. $1 \leq X \leq 20$) by combining UV–Visible spectroscopy, dynamic light scattering and transmission electron microscopy for 2 synthetic approaches differing by the sequence of reagent addition.

2. Experimental

2.1. Materials

Gold (III) chloride trihydrate (HAuCl$_4 \cdot 3$H$_2$O, ≥99.99%), trisodium citrate dihydrate (Na$_3$C$_6$H$_5$O$_7 \cdot 2$H$_2$O, ≥99%) were purchased from Sigma-Aldrich and used as received without further purification. All the content of a gold salt powder batch was used at the first opening to prepare, using a glass spatula, a mother solution at $[\text{Au}^{III}] = 0.25$ mM in a 100 mL double-necked round flask. The flask was then immersed in a temperature controlled oil bath at 96 ± 3 °C without reflux and was vigorously stirred at 900 rpm. 10 min after reaching the desired temperature, 2.5 mL of a citrate solution (final $X$ ranging between 1 and 20) preheated during 10 min at the reaction temperature were all added at once with a thermalized tip. After 15 min under vigorous stirring at 96 °C, we switch off the stirring and the oil bath. The magnetic bar is removed but not the oil bath until reaching ~70 °C (i.e. after 20 min). Then, the solution was allowed to cool down to the room temperature. The time evolution of the oil bath and of the reactive media temperatures measured during a typical synthesis are plotted in Fig. 3 of S.I. to enable easy reproduction of our synthesis as pointed in [29].

“Inverse” synthesis. We used the protocol proposed by Ojea-Jiménez et al. [16]. The desired amount of Citrate was diluted in a 250 mL double-necked round flask with water and stirred during 20 min at 25 °C. The solution was then heated during 15 min at 100 °C ($T_{\text{oil bath}} = 140 \pm 5$ °C) with reflux and vigorous stirring at 900 rpm. Then, 1 mL of mother precursor solution ([Au] = 25 mM) preheated at 100 °C during 10 min was injected all at once with a thermalized tip into the citrate solution under the same vigorous stirring. After 15 min at 100 °C, we switched off the stirring and the oil bath. The magnetic bar was removed but not the oil bath until the solution’s temperature had reached ~70 °C (i.e. after ~40 min). Then, the solution was allowed to cool down to the room temperature. We show in Fig. 1 of S.I. the evolution of pH and electrical conductivity as a function of $X$ for AuNPs prepared by this method.

Each synthesis has been reproduced at least 3 times with the same protocol, set-up and batches of chemicals.

2.3. Methods

Dynamic light scattering (DLS) experiments were carried out using a NanoZS apparatus (Malvern) operating at $\lambda = 632.8$ nm. The dependence of the characteristic relaxation times upon the scattering angle ($\Theta$) was determined with a 3D DLS set-up (LS Instruments) operating at $\lambda = 632.8$ nm in conventional autocorrelation mode. The dispersions were never filtered before measurements.

For dispersions characterized by two diffusive relaxations, the normalized electric field autocorrelation function $g^1(q, t)$ were fitted with the sum of two exponentials:

$$g^1(q, t) = A_{\text{fast}}(q)e^{-t/\tau_{\text{fast}}} + A_{\text{slow}}(q)e^{-t/\tau_{\text{slow}}}$$

where $\tau_{\text{fast}}$ and $\tau_{\text{slow}}$ represent the two cooperative characteristic relaxation times, and $A_{\text{fast}}$ and $A_{\text{slow}}$ are their corresponding amplitudes with $A_{\text{fast}}(q) + A_{\text{slow}}(q) = 1$. Electrophoretic mobility ($\mu_e$) was also measured with a NanoZS. This set-up operates with an electrical field of 25 V cm$^{-1}$ oscillating successively at 20 Hz and 0.7 Hz to reduce the electro-osmosis effect due to the surface charge of the capillary cell. The particle’s velocity is measured by LASER Doppler velocimetry.

Ultraviolet visible spectroscopy (UV–vis.) was performed on Cary 50 Scan UV–Visible Spectrophotometer of Brand Varian. The diluted AuNPs solutions were poured into a 5 mm thickness Hellma cell (quartz). The absorbance values were recorded after baseline correction.

Transmission electron microscopy (TEM) experiments were performed on a JEOL 2010 instrument operating at 200 kV. Samples were prepared by casting a single drop of a 1 g/L aqueous dispersion onto a standard carbon-coated Formvar films on copper grids (200 mesh) and drying in air for >30 min. A minimum of 300 NPs per sample was considered for the size statistics except at $X = 1$ for which 50 NPs were considered.

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2.4. Model

In this section we briefly present the main elements of the model proposed by Kumar et al. [27].

Main hypothesis. The model considers that: (i) the critical nucleus' size is about 2 nm, (ii) all the AuNPs including those formed by aggregation are spherical, (iii) the repulsive interaction between AuNPs is only due to double layer interaction, (iv) the rate of AuNPs' aggregation could be derived from the expression introduced by Reerink and Overbeek [30] and (v) AuNPs' aggregation is irreversible.

This model does not take into account: (vi) the possible disaggregation of intermediate aggregates leading to individual AuNPs, (vii) the hydrothermal oxidation of citrate and (viii) the pH evolution as a function of the molar ratio X and the inherent effect on the electrochemical potential of the dominant aureate complexes in water, (ix) the steric repulsion due to citrate adsorption on the AuNPs surface but the author balances this by overestimating the AuNPs' surface potential on purpose and (x) the influence on the stability of citrate oxidation product (DCA) as pointed out by Ref. [28]. The value of the AuNPs' surface potential is hence determined by an 'empirical' relation giving values always higher than the 90 mV derived from electrophoretic mobility measurements performed at X = 3 by Chow and Zukoski [13]. We show in Fig. 2 of the S.I. that our AuNPs have almost the same electrophoretic mobility than in the Ref. [13] in the same range of X.

Scheme of reactions. The model considers that the formation of citrated AuNPs result from the following reactions involving auric and aurous species (noted T and M respectively); citrate, dicarboxyacetone.

The number of gold particles in a given size range R to R + dR hence appears in the mass balance of aurous and auric species.

1. Homogeneous reduction auric ions by citrate:

\[ T + C \xrightarrow{k_1} M + DCA + \text{products} \]

2. ‘Degradation’ of dicarboxyacetone:

\[ H_2O + DCA \xrightarrow{k_2} A + \text{products} \]

This reaction corresponds to the hydrothermal oxidation of dicarboxyacetone.

3. Homogeneous reduction by acetone:

\[ 4T + A \xrightarrow{k_3} 4M + \text{products} \]

Note that the stoichiometric ratio of this reaction has been assigned to obtain a complete conversion of auric chloride at a stoichiometric ratio of 0.4 as observed by Frens [21].

4. Nucleation: \[ 3M + 2DCA \xrightarrow{k_4} T + \text{Nucleus} + 2DCA \]

5. Heterogeneous disproportionation:

\[ 3M \xrightarrow{k_5} T + \text{particle mass} \]

The rate of this reaction is assumed to be proportional to the AuNP surface area since the gold surface catalyzes this reaction. The number of gold particles in a given size range R to R + dR hence appears in the mass balance of aurous and auric species.

The number of particles in a size range as a function of time is obtained by solving the 'population balance equation' using a discretization technique described by the same author in [31]. The 'population balance equation' is obtained by considering that the apparition and disappearance of AuNPs in a given interval of sizes results from growth process due to surface reaction (3) and Brownian aggregation while new nuclei are formed in continuous reactions (1), (3) and (4) until the concentration in DCA is insufficient due to the degradation process (2). The rate constant \( k_1 \) was fixed to make the process time of the same order as the experimentally reported values at 100 °C while the other rate constants were adjusted to obtain the best fitting of the AuNP sizes, determined with TEM by [8,21,24,25] variation with X in the range: 0.4 \( \leq X \leq 7.5 \).

It is important to underline that these rate constants remain to be measured experimentally so that they cannot be fixed a priori. The initial gold concentration is fixed at \( [T]_{t=0} = 0.3 \) mM and the initial citrate concentration \( [C]_{t=0} \) is varied to cover an X range between 0.4 and 40.

3. Results

AuNPs' dispersions prepared by the Turkevich (i.e. “classical”) and “inverse” methods were first characterized in bulk by

\[
\begin{align*}
\text{(a) Abs. (a.u.)} & \quad \lambda (\text{nm}) \\
\text{(b) Abs. (a.u.)} & \quad \lambda (\text{nm}) \\
\text{(c) } \lambda_{\text{SPR}} & \quad \lambda_{\text{SPR}} \\
& \quad \langle R_{\text{Int.}} \rangle_{\text{Inverse method}} \\
& \quad \langle R_{\text{Int.}} \rangle_{\text{Classical method}} \\
\end{align*}
\]

Fig. 2. Absorbance (Abs.) versus wave length (\( \lambda \)) at 25°C for AuNPs dispersions synthesized by (a) “classical” and (b) “inverse” methods for different ratio X. (c) Evolution of the wave length at the surface plasmon resonance (\( \lambda_{\text{SPR}} \), full symbol) and AuNPs' intensity averaged radius (\( \langle R_{\text{Int.}} \rangle \), open symbol), determined with Haiss relation [32], for “classical” (red circle) and “inverse” (black square) methods. The dashed line is an exponential decay and must serve as guide for the eyes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
UV–vis. spectroscopy as shown in Fig. 2. Whatever the method of preparation, one observes a significant narrowing of the surface plasmon resonance (i.e. SPR) peak accompanied by a blue shift of SPR maximum from 546 nm (direct synthesis)/555 nm (inverse synthesis) down to 519–520 nm when X increases from 1 to 3.5 (Fig. 2c). Then, the SPR peak does not evolve up to X = 8. Above this value a slight red shift can be detected up to 522–523 nm at X = 20. This evolution agrees qualitatively with the picture of a transition from large and polydisperse AuNPs at X < 3.5, to small and quite monodisperse AuNPs at X ≥ 3.5 until a limit at high X values above which aggregation may occurs. We tried to derive the intensity average AuNPs’ size from these absorbance data using the following relation proposed by Haiss et al. [32]:

\[ (R)_{int} = \frac{1}{2} e^{-\frac{3}{2}(\frac{A_{SPR}}{A_{450}} - 2.2)} \]

where \( A_{SPR} \) and \( A_{450} \) are the absorbance at the SPR peak and 450 nm respectively, while the factors 3 and 2.2 were empirically determined by the authors. The as-determined radii evolution plotted in Fig. 2c give rise to a rather inaccurate result due to points noticeably scattered in the whole range of X. It appears that the Haiss’ relation is too much sensitive to uncorrelated variations of the absorbance and does not enable to extract in a quantitative manner the evolution of the AuNPs’ sizes.

To better quantify the size distributions in bulk we performed DLS measurements. The normalized electric field autocorrelation functions, \( g^{(1)} (173^\circ, \tau) \), measured one day after the synthesis are plotted in Fig. 3. For the two synthetic routes, the curves depict two relaxations for X = 1 and one relaxation for: 1 < X < 10 with the ‘classical’ method or 1 < X < 20 with the ‘inverse’ method. In these intermediate ranges, the relaxation times decrease when X goes from 2 to 3.5 and no longer varies beyond. At high X ratio,
these relaxations are stretched or associated with a slow mode of low amplitude (i.e. $X = 13$ or $X = 20$ for ‘classical’ and ‘inverse’ methods respectively). This analysis suggests again the formation of particles of smaller and smaller size until $X = 3$ after which the size does not evolve significantly except at high $X$ values where aggregation may appears.

Accordingly, the intensity weighted distributions of apparent hydrodynamic radius ($R_{H, \text{App.}}$) obtained with the Contin’ algorithm (Fig. 3c) reveal a transition from large and polydisperse AuNPs at $X \leq 3$, to small and quite monodisperse AuNPs at $X \geq 3.5$, with:

$$\langle R_{H, \text{App.}}(X) \rangle \sim 9.9 \pm 1.2 \text{ nm for the ‘classical’ method, and}$$
$$\langle R_{H, \text{App.}}(X) \rangle \sim 10.6 \pm 1.5 \text{ nm for the ‘inverse’ method.}$$

Interestingly, at high $X$ values, the presence of a second population of large objects with $R_{H, \text{App.}} > 100$ nm is not associated with a modification of the small objects’ mean size. In spite of their big size (hence large individual scattering) the large objects detected at $X = 13$ and $X = 20$ for ‘classical’ and ‘inverse’ methods respectively are always minority in% of intensity meanwhile they represent a low number fraction compared to the one of individual NPs.

The number weighted size distributions have been then determined for the two sets of synthesis by means of TEM (Fig. 4).

### 4. Discussion

Fig. 5 summarizes the results obtained during this study. It appears that the intensity averaged radii determined by DLS and TEM decrease in a monotonous manner when $X$ increases for the two batches of AuNPs. We consider it as a good support for reliability of the measurements. The size evolution is sharp at low $X$ and not significant above $X = 3.5$ with $\langle R \rangle_{\text{int.}} = 9.5 \pm 1.5$ nm. This prompts us to a more detailed focus on the slight increase of $\lambda_{\text{SPR}}$ (Fig. 5) for $X \geq 3.5$, and to consider that it should not be assigned to an increase of AuNP size. It appears that the intensity averaged radii determined by DLS and TEM decrease in a monotonous manner when $X$ increases for the two batches of AuNPs. We consider it as a good support for reliability of the measurements. The size evolution is sharp at low $X$ and not significant above $X = 3.5$ with $\langle R \rangle_{\text{int.}} = 9.5 \pm 1.5$ nm. This prompts us to a more detailed focus on the slight increase of $\lambda_{\text{SPR}}$ (Fig. 5) for $X \geq 3.5$, and to consider that it should not be assigned to an increase of AuNP size.

The comparison between our measurements and previous, experimental [14–16,21,23,26] and theoretical [27], data for comparable conditions (i.e. $[\text{HAuCl}_4] = 0.25 \pm 0.05$ mM and $T = 98 \pm 2$ °C) is shown in Fig. 6.

One observes that above $X = 3$ our results only agree, within experimental errors, with the two points extracted from [15]. Note that the monotonic character of the size decay agrees with [28] even if the measured size for $X \geq 3$ is clearly lower. This size discrepancy for a priori similar conditions of synthesis is hard to analyze with available experimental details. One can only underline that parameters often ignored in the experimental procedure, like the effect of water latent heat, could act on the final size [29]. Also, our results quantitatively agree with the theoretical prediction proposed by Kumar et al. on the whole $X$ range in contrast with all other experimental results available so far. Now, let us consider how our results enlighten the ongoing discussions about the

Fig. 4. Number weighted size distribution determined by TEM observations of AuNPs prepared by the traditional method (left column) and the inverse (right column) at different $X$ ratio indicated on the plot. Representative TEM images taken with the same magnification are shown as inset for each histogram. The width of the images corresponds to 560 nm.

Fig. 5. Evolution of the $\lambda_{\text{SPR}}$ and the intensity average radii determined by DLS and TEM, $\langle R \rangle_{\text{int.}}$, as function of $X$ for the two methods of synthesis as indicated in the figure. For the two plots, the dashed line is the same exponential decay function (i.e. $R(X)_{\text{int.}} = 176 \times e^{-x/0.8} + 8.8$) and must serve as guide for the eyes. The intensity averaged radii corresponding to TEM observations have been determined as described in [32].
Fig. 6. Evolution of the average radii of AuNPs prepared in similar conditions of synthesis summarized in Table 1 of S.I., as function of the molar ratio X. All the radii are number averaged values obtained by TEM except for [23] for which we represent $R_{\text{H, App}}$. The line corresponds to Kumar model prediction without aggregation [27].

mechanism of formation. Our study allows us to confirm the monotonic decay observed in [26] and to exclude the existence of a measurable discontinuity (i.e. $X^*$ in Fig. 1c) that motivated to postulate the existence of two different reaction pathways around $X^*$. The observed monotonic evolution has been predicted by Kumar et al. [27] and the “seed-mediated” growth mechanism proposed on the basis of measurements performed at $X > X^*$ (i.e. $X = 6.7$ and 10), is expected to be congruent whatever $X$ with this theoretical prediction [15]. For the first time we obtain experimental results in line with these assumptions, since their results at $X = 6.7$ and 10 superimpose with our data, which, beyond these two points, reveal a monotonic decrease of size that superimpose with the theoretical prediction on the whole range of $X$. Despite this observation, we prove neither the validity of the Kumar' model, nor the validity of the “seed-mediated” growth mechanism on the whole range of $X$. However, we could mention that some basal assumptions of Kumar' model appear poorly compatibles with the “seed-mediated” growth mechanism and with recent experimental findings. For instance, the hydrothermal oxidation of citrate occurring before AuIII addition, the effect of citrate concentration on pH and the correlated effect on the electrochemical potential of the dominant aureate complexes in water are not taken into account. Nonetheless, the rate constants of the reactions that are taken into account are “free” parameters of the fitting procedure since their values remain to be measured. For these reasons, we believe that the observed agreement with our experimental results is possibly fortunate. We hope that our study will serve as a new reference for the evolution of the AuNPs’ size with $X$ and that further theoretical studies will complement the beautiful seminal work done by Kumar et al. by taking into account the above mentioned experimental findings of the last decade.

5. Conclusions

The effect of citrate to gold molar ratio ($X$) on the size of citrated gold nanoparticles (AuNPs) has been studied experimentally for the most popular condition of synthesis (i.e. [Au] = 0.25 ± 0.05 mM and T = 98 ± 2 °C) by using, for the first time, a multimodal approach of characterization (UV–vis spectroscopy, dynamic light scattering and transmission electronic microscopy). This dependence is still a matter of debate at high X values [14,16,23,26,27]. We show unambiguously that the size of AuNPs decay monotonically when the molar ratio $X = [\text{Citrate}]_{t=0}/[\text{Au}^{\text{III/II}}]_{t=0}$ increases between 1 and 20. The same evolution and the same sizes have been obtained whatever the order of reagent addition. We believe that the precise protocols of synthesis which has been established has allowed to minimize the standard deviation between experiments and has ensured that the only parameters to be modified are $X$ and the sequence of reagent addition. For intermediate $X$ values, our finding allows us to exclude the existence of a measurable discontinuity for a peculiar $X$ value [14,23] that prompted authors [14] to postulate the existence of two different reaction pathways when the citrate to gold molar ratio is around this value. Our result is in line with the continuous evolution reported by Majzik et al. [26] and one reaction pathways which should lead to monotonic size decrease [15,27]. Also, we note that our result agrees with the sole available theoretical prediction [27] on the whole range of $X$. Despite this apparent agreement, we point some contradictions between recent experimental results and basal hypothesis of this model. For instance, the variation of pH with $X$ and the inherent effect on the electrochemical potential of the dominant aureate complexes in water [10] are not taken into account. For high $X$ values, we confirm the presence of a plateau at $R_{\text{number}} = 9 \text{ nm}$ in contrast with the very low size reported by Majzik et al. [26]. We hope that our study will serve as reference for the evolution of the AuNPs’ size with $X$ and that further theoretical studies could rely on it to complement the beautiful seminal work done by Kumar et al. [27]. This article may also show to the broad readership in colloid and interface science that the rationalization of metallic NPs synthesis is an actual subject which necessitates the methodology and the technical knowledge developed in this community to develop theoretical prediction tools and new strategies of synthesis based on a profound mechaistic knowledge rather than on imagination.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcis.2016.10.065.

References

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References