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RESEARCH ARTICLE

'Inversed Turkevich' method for tuning the size of Gold nanoparticles: evaluation the effect of concentration and temperature

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ABSTRACT

In this study the influence of dicarboxy acetone (DCA), as an oxidation product of sodium citrate, was evaluated by 'inversed Turkevich' method. Gold nanoparticles (GNPs) were synthesized systematically at various sodium citrate to $HAuCl_4$ molar ratio and temperature. The GNPs were characterized by UV-vis spectroscopy, DLS and TEM techniques. According to the results, GNPs were obtained in range of 12-51 nm by inverting the reagents addition order. All of GNPs samples were monodisperse and had the same pattern of narrow size distribution in contrast to traditional Turkevich method in which GNPs larger than 40 nm became unstable. Moreover, molar ratio of Sodium citrate to $HAuCl_4$ and temperature had a significant role in size controlling and monodispersity of GNPs. By increasing sodium citrate to $HAuCl_4$ molar ratio, the size of GNPs reduced drastically. Since, temperature had a central role on the production rate of DCA, its influence on monodispersity of GNPs was more considerable than their size.

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INTRODUCTION

In recent years, by the development of nanotechnology extensive researches have been done for synthesis and characterisation of nanostructures to investigate their properties. Among these nanostructures, gold nanoparticles (GNPs) gain significant attention due to their facile synthesis, unique physicochemichal properties and biocompatibility. GNPs have promising applications in various area of technology (1) such as solar cells, electronic structures, biotechnology, drug and gene delivery, sensing, imaging and cancer therapies (2, 3). Some advantages such as tuning properties of GNPs by modifying their size, shape and composition, facilitate the use of GNPs in such fields. Thus, a giant volume of researches focuses on synthesis methods to control size, shape, and surface and core chemistry of GNPs. Among all these methods, Turkevich is the most popular one which was reported by John Turkevich et al. in 1951 (4). In this method sodium citrate was quickly added to the boiling solution of $HAuCl_4$ to produce monodisperse GNPs in sizes of 15-20 nm (5). Turkevich method is a more straightforward

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procedure (6) since, sodium citrate acts as both reducing and capping agent (7). By changing the concentration of sodium citrate, GNPs can be produced with sizes from 10 to 150 nm (1, 8). Although, in high concentration of sodium citrate small monodisperse GNPs are obtained, due to poor reducing and stabilizing abilities of it (9), in low concentration of sodium citrate Polydispersity of GNPs will increase like their size. As mentioned in some studies, for GNPs larger than 30 nm, polydipersity increases and beyond the size of 40 nm the colour of GNPs changes from brilliant red to dirty and clumped, indicating high polydispersity and agglomeration of them (8, 10). For controlling size and size distribution of GNPs some approaches have been described in literatures which, seed growth method is the common one (9). In this method, sub-10 nm GNPs are prepared to be ustilized as seeds and then they grow into GNPs with narrow size distribution in the size range of 10-50 nm (11). Recently, some literatures revealed the influence of dicarboxy acetone (DCA) on the controlling size and uniformity of GNPs. DCA is an oxidation form of sodium citrate (12), which acts as a strong reducing and stabilizing agent that increases the nucleation and growth rate of gold atoms leading to the formation of monodisperse GNPs (13). To improve the effect of DCA on the synthesis of GNPs, the order of sodium citrate addition to $HAuCl_4$ in the classical Turkevich method needs to be reversed to induce thermal oxidation of sodium citrate (6). Sivaraman and co-workers (11) studied the effects of sodium citrate/ $HAuCl_4$ molar ratio, pH of reaction solution and $HAuCl_4$ concentration on the reaction kinetics, size and monodispersity of GNPs obtained by conventional and inversed Turkevich method. They indicated that sodium citrate/$HAuCl_4$ molar ratio, pH of reaction solution and $HAuCl_4$ concentration had a considerable role in the controlling size and uniformity of GNPs. Reverse method in comparison with the standard Turkevich method gave fair monodisperse and small GNPs in range of 5–10 nm. In other study, I Ojea-Jimenez and co-worker (13) synthesized GNPs via Turkevich method and the injection of $HAuCl_4$ to hot solution of sodium citrate. They demonstrated the influences of sodium citrate/$HAuCl_4$ ratio and pH of reaction solution on the size, monodispersity and morphology of GNPs. They chose pH of 6.2 as the optimum value and showed that by inverting the order of reagent addition, the size of GNPs reduced and monodispersity of them improved significantly.

Thus, we assume that by exploiting high conversion rate of sodium citrate to dicarboxy acetone via reverse order of reagents addition, called 'inversed Turkevich' method in this study, monodisperse GNPs in sizes larger than 40 nm can be prepared. In this research we systematically investigate the role of two key parameters i.e. sodium citrate/ $HAuCl_4$ molar ratio and temperature on the size and monodispersity of spherical GNPs by 'inversed Turkevich' method.

MATERAILS AND METHOD

Materials

Hydrochloroauric acid trihydrate ($HAuCl_4.3H_2O$, > 99.9%) and trisodium citrate dehydrate ($Na_3C_6H_5O_7.2H_2O$, > 99.9%) were ordered from Shanghai Chemical Co., Shanghai, China. All glassware and Teflon-coated magnetic bars were washed with aqua regia (three parts HCl, one part HNO_3) and subsequently rinsed with deionized water. All solutions were prepared by the same deionized water.

Method

Briefly, the 'inversed Turkevich' method was done based on the method that was proposed by Sirvaraman et al. at different molar ratios of sodium citrate solution to $HAuCl_4$ solution and temperatures. Initially, appropriate volumes of sodium citrate (%1.0 w/v) were added to deionized water to set molar ratio of reactions in amounts of 3.5, 5.0, 10.0 and 15.0, while the total volumes of them were kept at 24.0 ml. The reaction solutions were heated to the boiling temperature (95 °C in 760.2 mmHg). After that 1 ml of $HAuCl_4$ stock solution (6.5 mM) was added to the boiling sodium citrate solutions under rapid stirring (Fig. 1). To ensure the completeness of the reaction, the reaction solution was maintained on the hot plate for 30 min after the color changing of the solution became stable. The additional experiments at different temperatures including 95 °C, 80 °C, 70 °C and 50 °C were done for 15.0 molar ratio.

Characterization

The UV-vis absorbance curve of GNPs samples were recorded with a Cecil CE 7250 Spectrophotometer in a 1 cm quartz quvette. The size of GNPs was calculated by Haiss and co-workers equation (14), which determined the

diameter of GNPs through UV-vis spectra (Eq. 1). The results of this equation were in agreement with HTEM, TEM and SEM results as reported in other studies (6, 15-19).

$$d = \exp(B_1 {}^{A_{spr}}\!/_{A_{450}} - B_2) \quad (1)$$

In this manner, with the utilization of fit parameters (B_1= 3.00; B_2= 2.20) and absorbance ratio of (A_{spr}/A_{450}) the diameter of GNPs was calculated. The hydrodynamic diameter and diameter distribution of GNPs were determined with a Dynamic Light Scattering (DLS) using a Particle size analyzer Scatterscope **(I).** Transmission electron microscopy (TEM) was used to determine size, uniformity and shape of GNPs with a Zeiss-EM10C Transmission electron microscopy (TEM) at 100 kV acceleration voltages. For this purpose statistical analysis was performed under 100 numbers of particles of TEM image by ImageJ software.

RESULTS AND DISCUSSION

Effect of molar ratio in 'inversed Turkevich' method

Gold nanoparticles were synthesized by adding $HAuCl_4$ to the boiling solution of sodium citrate, 'inversed Turkevich' method, at different sodium citrate/ $HAuCl_4$ molar ratios ranging from 3.5 to 15.0. After the reaction completed, we observed that by increasing the concentration of sodium citrate the colour of GNPs samples changed from purple to reddish orange which indicated the diameter alteration of GNPs as a function of sodium citrate/ $HAuCl_4$ molar ratio (Fig. 1.A) and all of GNPs samples were brilliant which was the sign of their monodispersity (20,21). For specialized characterization, UV-vis spectrum of GNPs samples was recorded. As it is indicated in Fig. 1.B the absorbance curve of each GNPs samples had a single visible peak that was positioned in range of 519-531 nm and it was related to spherical monodisperse GNPs (5, 22, 23). By increasing sodium citrate/$HAuCl_4$ molar ratios, the curves width became narrower and their peak intensity reduced (blue shift) which was the characteristic of decreasing nanoparticles diameter and increasing uniformity (3, 24, 25).

For the calculation diameter of GNPs, we used Eq.1 and the results were summerized in Table 1. These data showed that by increasing sodium citrate/ $HAuCl_4$ molar ratio, the diameter of GNPs reduced from 51 nm to 12 nm. This indicated the significant role of DCA which at high concentration led to the reduction size of GNPs, drastically. To support UV-vis spectroscopy data, DLS measurment was carried out. The hydrodynamic sizes obtained by DLS decreased in contrast to the increasing sodium citrate/ $HAuCl_4$ molar ratios which was in good correlation with UV-vis spectroscopy data; however, hydrodynamic sizes were slightly larger than the diameters determined by Eq.1, as predicted (Table 1) (26, 27). The uniformity of GNPs was measured based on the intensity of light scattering by DLS. According to Fig. 2 all GNPs samples synthesized at various molar ratios had the same narrow size distribution pattern and the same coefficient of variation (about %36), that indicates 51 nm GNPs sample had the same uniformity as similar to 12 nm GNPs sample.

TEM technique has high accuracy and reliability

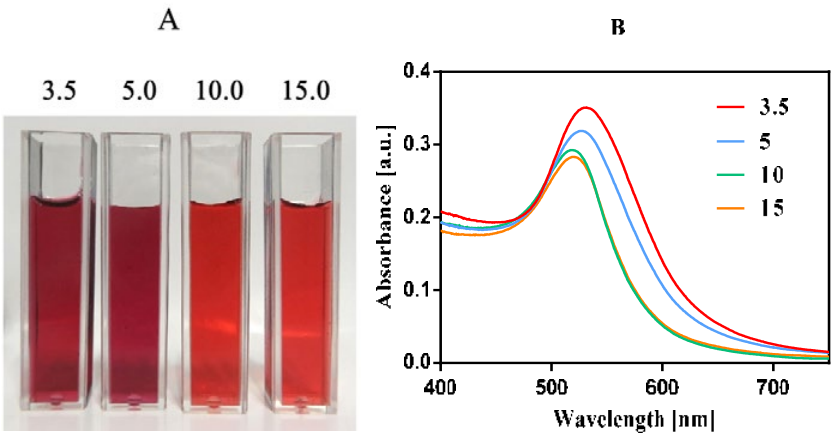

Fig.1. Image (A) and UV-vis spectra (B) of gold nanoparticles samples that synthesized via 'inversed Turkevich' method at various Sodium citrate/$HAuCl_4$ molar ratios: 3.5, 5.0, 10.0, and 15.0.

for determining size, size distribution and shape of nanoparticles so one sample which was synthesized at 15.0 molar ratio and 95 °C temperature was analyzed with TEM. The statistical analysis of TEM image (Fig. 3) revealed that spherical GNPs with the average size of 11.82±1.77 nm and narrow size distribution (coefficient of variation about %15) were obtained in this method. However, the comparison size distribution measured by DLS and TEM for GNPs sample prepared at 95 °C and 15.0 molar ratio of sodium citrate to $HAuCl_4$ indicated that size distribution measured via DLS is wider than TEM similar to the previous study, but there is suitable proportionality between them (26). There is also a great correlation between sizes measured by these three techniques, UV-vis spectroscopy, DLS and TEM (as in Table 1).

As a result, in high molar ratio large amount of DCA caused the formation of large number of nuclei in nucleation step which led to the preparation of GNPs with smaller size. Also in low molar ratio low concentration of DCA led to the formation of lower number of nuclei and thus GNPs with larger size were formed. Since DCA act as a strong reducing and stabilizing agent, in high and low concentration of it, GNPs with narrow

Table1. Summary of data that shows absorption peak of gold nanoparticles which obtained by UV-vis spectroscopy and size of gold nanoparticles samples determined via different characterization techniques: UV-vis spectroscopy (Eq. 1), DLS and TEM.

NO.	Sodium citrate/$HAuCl_4$ molar ratios	Temperature °C	Absorption peak (nm)	Gold nanoparticles size obtained by different technique		
				UV-vis (nm)	DLS (nm)	TEM (nm)
1	3.5	95	532	51.7±1.69	56.84±3.64	-
2	5.0	95	528	40.9±2.0	45.69±0.70	-
3	10.0	95	519.0	12.4±0.57	17.88±0.33	-
4	15.0	95	520.0	12.4±0.68	21.94±0.28	11.82±1.77
5	15.0	80	521.0	12.44±0.53	22.54±0.26	-
6	15.0	70	523.5	13.44±0.43	25.43±2.06	-
7	15.0	50	524.5	13.48±0.72	24.37±7.69	-

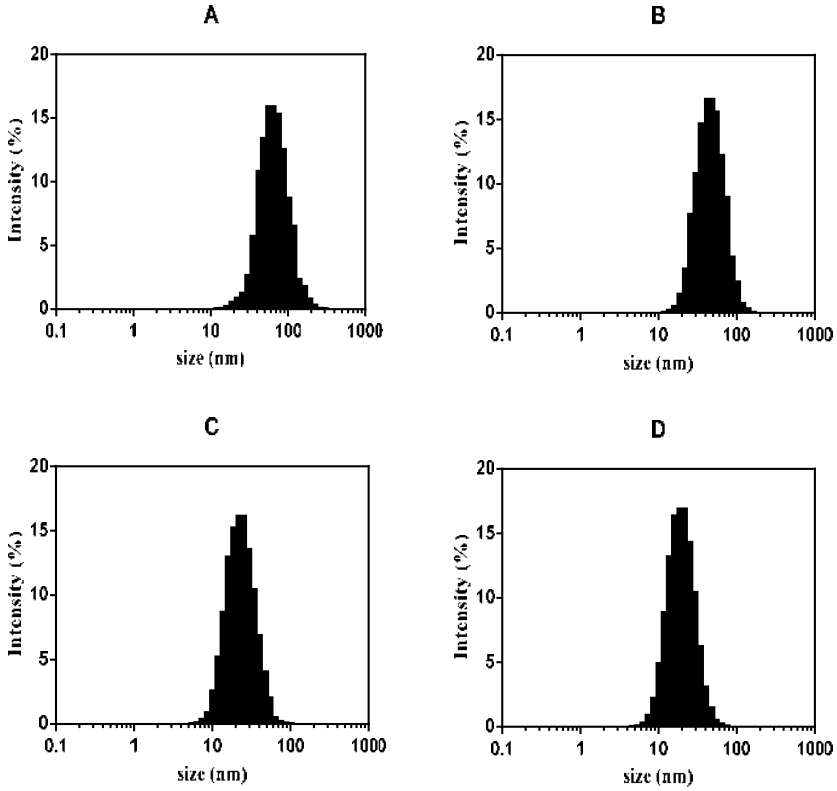

Fig. 2. Size distribution based on light scattering intensity (%) of gold nanoparticles samples that synthesized at different Sodium citrate/$HAuCl_4$ molar ratios: A) 3.5, B) 5.0, C) 10.0 and D) 15.0.

size distribution were formed (28). Thus by simple modification of Turkevich method, sodium citrate was converted to strong reducing and stabilizing agent in its oxidation form, which could synthesize monodisperse GNPs from 12 nm up to 51 nm with similar size distribution that is reported in this study for the first time.

Temperature effect in 'inversed Turkevich' method

Temperature has an important role in the Turkevich method as the main cause of thermal oxidation of sodium citrate, but in the past studies about the reverse sequence of reagents addition in Turkevich method its effect was not considered (6, 11, 13). Thus, in this research we evaluated the effect of temperature on GNPs size and uniformity by 'inversed Turkevich' method for the first time. For this aim sodium citrate to $HAuCl_4$ molar ratio was kept constant at 15.0 and reaction temperature was set at 95 °C, 80 °C, 70 °C and 50 °C, respectively. As shown in Fig.4.B by decreasing temperature, absorbance curve width and peak changed slightly. Broadening the absorbance curve and a little increase in the absorption peak indicated that reducing temperature led to the increasing size and uniformity of GNPs samples. Moreover, the colour of GNPs solution prepared in 50 °C was significantly different from GNPs samples which were prepared in other temperatures (Fig. 4.A).

Size and size distribution were measured by UV-vis spectroscopy (Eq.1) and DLS technique. As seen in Table 1 in both measurement techniques the size of GNPs increased faintly by decreasing the temperature. Fig. 3 demonstrates the size

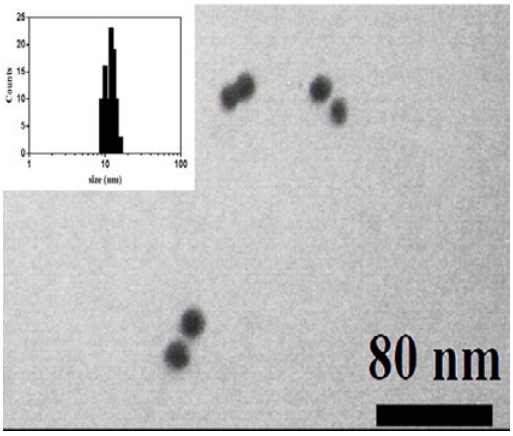

Fig. 3. TEM image of gold nanoparticles which synthesized by 'inversed Turkevich' method at 95 °C and 15.0 Sodium citrate to $HAuCl_4$ molar ratio.

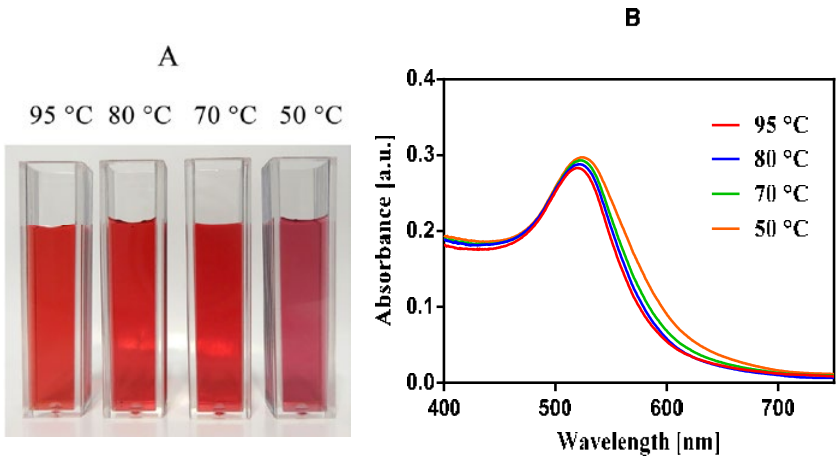

Fig. 4. Image (A) and UV-vis spectra (B) of gold nanoparticles samples that synthesized via 'inversed Turkevich' method at various temperatures: 95 °C, 80 °C, 70 °C and 50 °C.

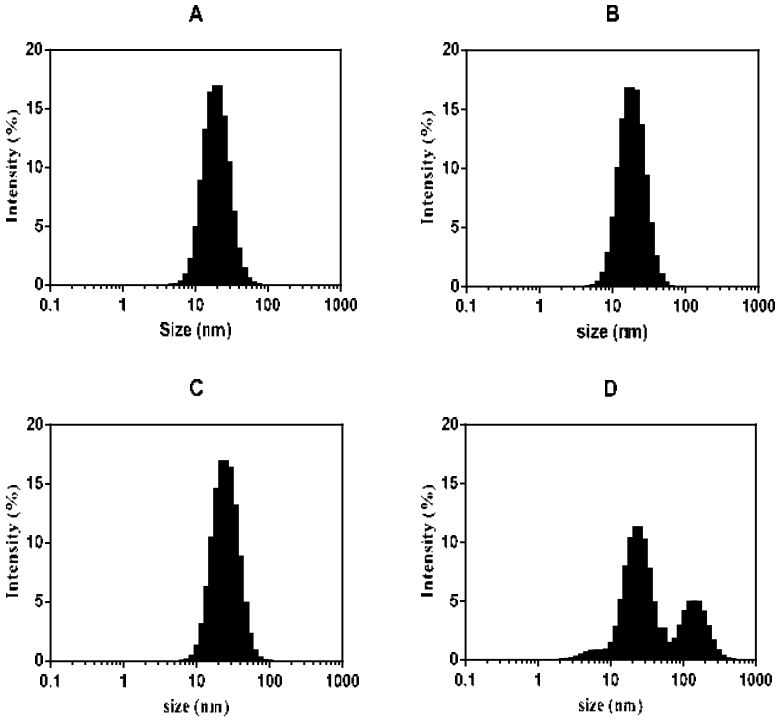

Fig. 5. Size distribution based on light scattering intensity (%) of gold nanoparticles samples that synthesized at: A) 95 °C, B) 80 °C, C) 70 °C and D) 50 °C temperatures, respectively.

distribution histograms of GNPs samples that were measured based on the intensity of light scattering by DLS All samples had uniform size distribution with the coefficient of variation about %36 except a sample which was prepared at 50 °C (coefficient of variation about %98). The size distribution histogram of this sample was divided into two peaks, one peak with higher intensity approximately around 20 nm and another peak with lower intensity around 100 nm. This size distribution histogram of GNPs sample revealed that concentration of smaller particles were significantly more than larger particles due to their higher light scattering intensity and also the GNPs sample prepared at 50 °C was polydisperse, too (Fig. 5.D).

The main reason for the obtained results is an obvious role of temperature in the production rate of DCA. In high temperature a large amount of DCA was produced which led to the fast and vast nucleation and formation of a large number of GNPs with small size. But in low temperature the production rate of DCA decreased and it happened slowly during reaction. So after the first nucleation step and during the nuclei population growth, second nucleation step occured continuously which resulted in polydispersity and wide size distribution of GNPs formed at 50°C (28). These results showed the significant role of DCA in monodispersity of GNPs that its production rate decreased in low temperature.

CONCLUSION

In conclusion, monodisperse GNPs with brilliant colour in 12-51 nm range of size can be synthesized by 'inversed Turkevich' method which confirmed our first assumption. The influence of various sodium citrate/ $HAuCl_4$ molar ratios and temperatures are studied with high precision and accurate techniques and equation. From this study it is concluded that sodium citrate/$HAuCl_4$ molar ratio in comparison to temperature, has a central role in GNPs size, also the role of temperature in the controlling size distribution and uniformity becomes significant. At low sodium citrate to $HAuCl_4$ molar ratio, large GNPs can be obtained with high monodispersity and by increasing molar ratio, size of GNPs decreases drastically, because of high concentration of DCA and its strong reducing and stabilizing potential. The temperature influence on GNPs size is not obvious but at 50 °C temperature GNPs monodispersity

has a sharp change which is duo to slow oxidation rate of sodium citrate at low temperature. Finally, 'inversed Turkevich' method can be utilized as a simple method to synthesize large size of GNPs up to 51 nm with high monodispersity in one step.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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