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Short communication

Simple spectrophotocolorimetric method for quantitative determination of gold in nanoparticles

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ABSTRACT

A simple spectrophotocolorimetric method devoted to the measurement of gold content in nanoparticles (NPs) was developed. It includes two steps: (i) metal gold NPs (Au NPs) are oxidized into the $AuCl_4^-$ anion using a 5×10^{-2} M HCl–1.5×10^{-2} M NaCl–7×10^{-4} M Br_2 solution, next (ii) $AuCl_4^-$ concentration is measured using a spectrophotometric assay based on the reaction of $AuCl_4^-$ with the cationic form of Rhodamine B to give a violet ion pair complex. This latter is extracted with diisopropyl ether and the absorbance of the organic complex is measured at 565 nm. The method is linear in the range 6–29 μM of $AuCl_4^-$ with a limit of detection of 4.5 μM.

The analytical method was optimized with respect of bromine excess to obtain complete Au NPs oxidation. The method was applied to two types of Au NPs currently under investigation: citrate-stabilized Au NPs and Au NPs capped with dihydrolipoic acid (Au@DHLA). Both the gold content of Au NPs and the concentration of NPs (using NP diameter measured by transmission electron microscopy) have been calculated.

1. Introduction

Bulk gold is one of the most noble of all the metals, being unreactive to oxygen, sulphur, concentrated acids or bases even at elevated temperatures. However, gold reacts readily with halogens and dissolves in solutions containing or generating chlorine such as aqua regia to form tetrachloroauric acid ($HAuCl_4$). The preparation of biologically active gold (oxidation states: +I and +III) species for the treatment of diseases including rheumatoid arthritis and various cancers has been reported earlier [1]. Mild reduction of $AuCl_4^-$ solutions by various reducing agents such as sodium citrate or sodium borohydride gives highly coloured solutions containing gold NPs (Au NPs) [2]. The ease of synthesizing Au NPs and their affinity for binding many compounds make them attractive candidates as carriers for the delivery of various biologically active molecules such as proteins, DNA, RNA, drugs and antioxidants. Gold NPs became the most intensively studied nanosized materials and their potential for application in a wide spectrum of areas that include biology and medicine has been reviewed [2–8].

Among the numerous parameters needed for the full characterization of Au NPs (physical and hydrodynamic diameter, morphology, surface functionalization, zeta potential, ...), determination of their gold content is of main importance [9]. However, there is a lack of simple assays easily available in most laboratories working within this nanotechnological area. The most frequently reported method for the measurement of gold content in NPs needs a hyphenated and expensive technique: inductively coupled plasma associated or not with mass spectrometry or optical emission spectroscopy (ICP, ICP-MS, ICP-OES) [9–11]. The present report concerns the development of a simple and low-cost assay coupling chemical oxidation of gold in NPs and spectrophotocolorimetric measurement of gold; this alternative method was applied to two common kinds of Au NPs-stabilized either with citrate or dihydrolipoic acid.

2. Experimental

2.1. Chemicals

All solvents were of analytic grade and used without further purification. Sodium citrate tribasic dihydrate, tetrachloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium borohydride, α-lipoic acid, Rhodamine B, and bromine were purchased from Sigma–Aldrich (France), diisopropyl ether was obtained from Carlo Erba Reactifs-SDS (France). Phosphate-buffered saline (PBS) solution was prepared as follows: $[Na_2HPO_4] = 4 \times 10^{-2}$ M, $[KH_2PO_4] = 4 \times 10^{-3}$ M,

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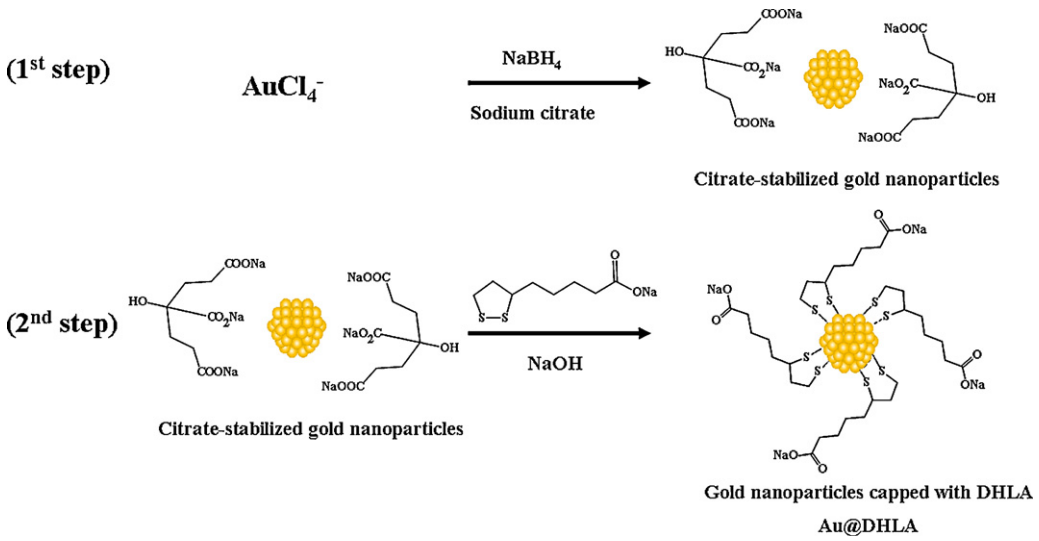

Fig. 1. Schematic representation of synthesis of gold nanoparticles stabilized with citrate (1st step) and capped with dihydrolipoic acid (DHLA) (2nd step).

and [NaCl] = 1×10^{-1} M, final pH was adjusted to 7.4. Ultrapure deionized water (>18.2 MΩ cm^{-1}) was used for all solution preparations. All glassware was cleaned with 37% HCl (w/v) and rinsed thoroughly with ultrapure deionized water prior to use. All handling conditions required to prevent any risk of toxicity and injury during NPs synthesis and oxidation (use of gloves, glasses, fume hood) were taken into consideration.

2.2. Synthesis of citrate-stabilized gold nanoparticles

Gold NPs were prepared as previously reported [12]. Briefly, at room temperature, to 90 mL of H_2O, 1 mL of 1% (w/v) $HAuCl_4 \cdot 3H_2O$ in H_2O was added. After 1 min of stirring, 2.0 mL of a 38.8 mM sodium citrate solution was added. One minute later, 1.0 mL of freshly prepared 0.075% (w/v) $NaBH_4$ in 38.8 mM sodium citrate solution was added. The reaction medium was stirred for an additional 5 min period and the resulting deep red colloidal solution was stored in the dark at 4 °C for a maximum period of 2 months.

2.3. Synthesis of gold nanoparticles capped with dihydrolipoic acid (Au@DHLA NPs)

A 600-μmol portion of α-lipoic acid in 10 mL of NaOH 0.5 M solution was added to 25 mL of freshly prepared citrate-capped Au NPs under stirring at room temperature (20–23 °C). After 24 h, Au NPs capped with dihydrolipoic acid (DHLA), obtained from α-lipoic acid reduction, were dialyzed against PBS for 48 h using a 10 kDa cut-off dialysis bag (Interchim®, France). The dialysis medium was changed once to fresh PBS after 24 h. The resulting Au@DHLA NPs solution was stored in the dark at 4 °C for a maximum period of 2 months.

2.4. Instrumentation

An UV–visible spectrophotometer (model Uvikon 932, Kontron) was used for spectra recordings and absorbance measurements. The transmission electron microscopic (TEM) images were recorded using a Philips CM20 instrument with a LaB_6 cathode operating at 200 kV. Gold NP solutions were deposited onto a 400-mesh carbon film copper grids. The average of gold core diameter was calculated for each Au NPs sample by counting 200 particles from the TEM images.

2.5. Determination of gold content in nanoparticles

One millilitre of Au NPs was mixed with 1 mL of 5×10^{-2} M HCl and 1.5×10^{-2} M NaCl solutions containing Br_2 at a concentration ranging from 1 to 7×10^{-4} M to ensure that the Au NPs oxidation was complete. The mixture was incubated for 20 min at room temperature, next it was placed at 60 °C for 30 min to remove any remaining bromine. Then the colorimetric assay was performed as previously described [13]: 0.5 mL of the solution resulting from the oxidation process, 0.5 mL of water (blank), or 0.5 mL of a standard solution were mixed with 9.5 mL of water, 2.5 mL of 20% (w/v) HCl solution, 5 mL of 30% (w/v) NH_4Cl solution, and 2.5 mL of 0.84×10^{-3} M Rhodamine B solution prepared in water. The result-

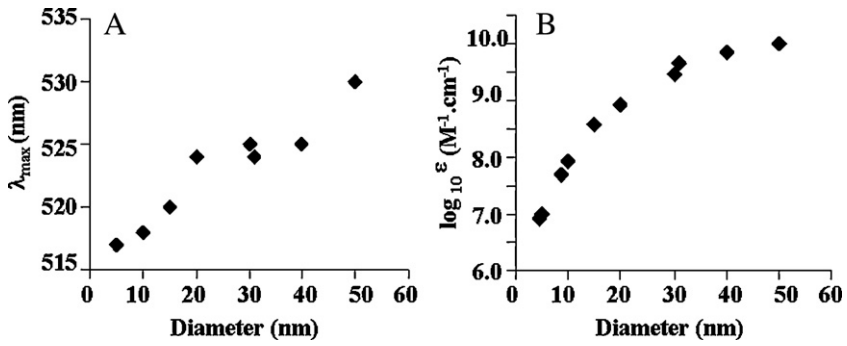

Fig. 2. Variation of λ_{max} (A) and molar absorbance (B) of citrate-stabilized gold nanoparticles as a function of diameter according to data literature [14,18–21].

ing mixture was shaken for 30 s with 5 mL of diisopropyl ether. The organic phase was separated and its absorbance was measured at 565 nm using 1-cm path glass cuvettes.

Calibration curve was operated using a series of dilutions of the $HAuCl_4{\cdot}3H_2O$ stock solution (1 mg mL^{-1}; 3 mM) prepared in water, in the range 60–290 μM.

2.6. Calculation

The number of gold atoms per AuNP (n_{atom}) was determined using the average size of the particle determined by TEM. Assuming a spherical shape and a face-centered cubic AuNP structure (fcc) [11,14,15], n_{atom} for each type of Au NPs was calculated using Eq. (1), where N_A is the Avogadro number (6.022×10^{23}), ρ is the density for fcc gold (19.3 g cm^{-3}), D is the diameter of the Au NPs in cm, and M stands for atomic mass of gold (197 g mol^{-1}):

$$n_{atom} = \frac{N_A \pi \rho D^3}{6M} \quad (1)$$

The number of NPs per liter (nAuNPs) was calculated according to Eq. (2), where $CAuCl_4^-$ corresponds to the result of colorimetric determination:

$$\text{nAuNPs} = \frac{CAuCl_4^- \times N_A}{n_{atom}} \quad (2)$$

This concentration was then divided by Avogadro number to get the final molar concentration of Au NPs, i.e. CAuNPs.

The molar absorbance ε of each type of Au NPs was determined according to Lambert–Beer law (Eq. (3)) where A is the Au NPs maximum absorbance at λ_{max}, l is the path length, and CAuNPs is the Au NPs concentration (M):

$$A = \varepsilon \times l \times \text{CAuNPs} \quad (3)$$

3. Results and discussion

Citrate-stabilized Au NPs were prepared by reduction of $AuCl_4^-$ using sodium borohydride in the presence of citrate ions [12,16]. The citrate-capped Au NPs were further functionalized with dihydrolipoic acid (DHLA), obtained from α-lipoic acid reduction, using a [DHLA]/[Au] ratio of 222/1 to yield water soluble Au NPs capped with DHLA (Au@DHLA) (Fig. 1). The NPs stabilized by DHLA offer better stability than citrate-stabilized Au NPs and can be coupled to biomolecules to obtain new delivery platforms [17].

Accurate determination of the concentration of NPs is essential to fully characterize the prepared solutions. Besides, quantifying the amount of gold reduced during the synthesis of Au NPs is also important to evaluate the stability of these materials [11]. Since Au NPs have strong plasmon absorption bands in the visible domain, the use of their molar absorbances should be the easiest way to estimate Au NPs concentrations. Several authors estimated the size and concentration of Au NPs by their UV–vis absorbance spectra, mainly focusing on the position of the λ_{max} and molar absorbance, respectively (Fig. 2A and B) [14,18–21]. Although increase of core diameter of Au NPs introduces continuous increase of molar absorbance (Fig. 2B), the estimation of the diameter using λ_{max} is imprecise. When the Au NPs diameter increases from ca. 5 to 50 nm, a narrow and imprecise bathochromic shift of λ_{max} from ca. 517 to 534 nm is observed [14,19,20]. The position of λ_{max} cannot therefore be used as a conclusive and accurate diagnostic for determining the diameter and the concentration of NPs (Fig. 2A). Thus, in most cases, this approach gives unreliable results because the position of the plasmon resonance is affected by multiple factors, like environment dielectric properties, physical or chemical interactions on particles surface, surface charge, interparticle distance, and aggregation [2].

The accurate and simple quantification of gold contained into Au NPs is still a challenge. In the present work, a spectrophotocolorimetric assay is reported as an interesting low-cost alternative to more hyphenated techniques [9–11]. It permits calculation of gold content in Au NPs, yield of NPs obtained after each synthesis step, and accurate concentration of NPs (using NP diameter measured by TEM).

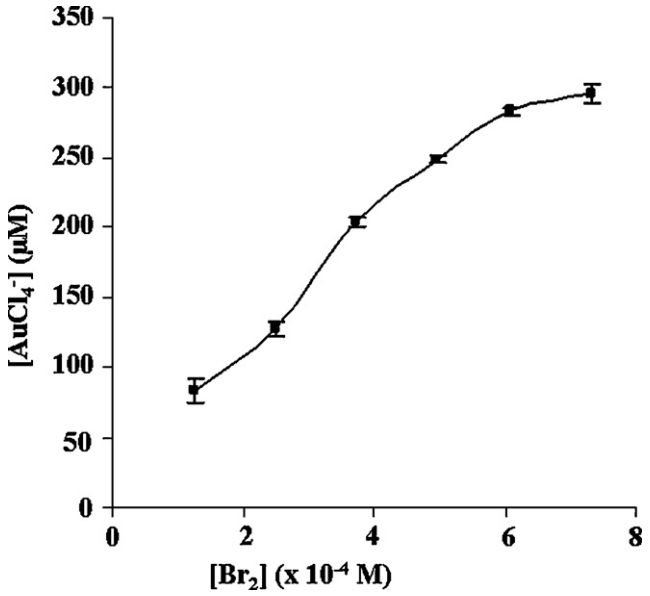

Fig. 3. Effect of bromine concentration on conversion of gold nanoparticles into the anionic species $AuCl_4^-$ (each point is the mean ± standard deviation of three independent measurements).

3.1. Optimization of bromine oxidation

A HCl–NaCl–Br_2 solution, which proved to be more efficient than other solutions such as HNO_3–HCl, NaCl–Br_2, HCl–NaCl or HCl–Br_2 solutions [21–25], was selected to oxidize gold NPs into $AuCl_4^-$. Further studies were carried in order to optimize the concentration of Br_2 required for complete conversion (Fig. 3). Using citrate-capped Au NPs, a plateau was obtained at concentrations higher than 6×10^{-4} M and the retained Br_2 concentration was 7×10^{-4} M. The complete dissolution of Au NPs was also verified by the full disappearance of surface plasmon resonance peak observed at around 520 nm (Fig. 4). Similar data were obtained using Au@DHLA NPs (data not shown). Full recovery of an $AuCl_4^-$ solution treated with bromine solution was observed (105 ± 5%, n = 6), confirming the accuracy of the present assay.

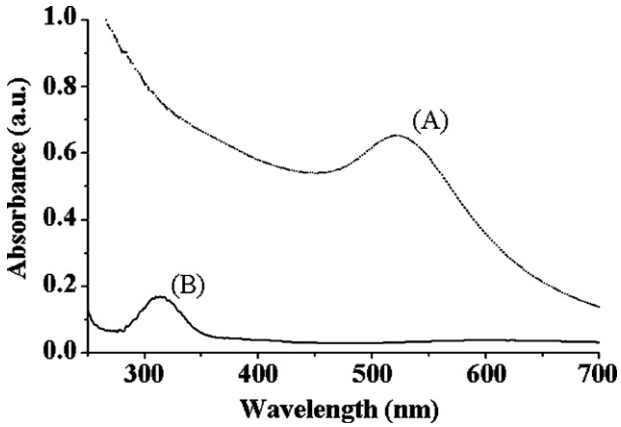

Fig. 4. UV–visible spectra of citrate-stabilized gold nanoparticles before (A) and after (B) oxidative treatment using the optimized bromine/chloride solution (5×10^{-2} M HCl–1.5×10^{-2} M NaCl–7×10^{-4} M Br_2).

Table 1
Different characteristics of synthetized gold nanoparticles (values are expressed as mean ± standard deviation of *n* independent measurements).

	Citrate-stabilized Au NPs	Au@DHLA
Diameter (nm) ($n=200$)	5.3 ± 1.1	3.5 ± 1.0
$CAuCl_4^-$ (μM) ($n=3$)	304 ± 13	116 ± 7
Yield (%) ($n=3$)	104 ± 5	56 ± 3
Number of NPs per liter (nAuNPs) ($n=3$)	$(4.0 \pm 0.2) \times 10^{16}$	$(5.0 \pm 0.3) \times 10^{16}$
CAuNPs (nM) ($n=3$)	66 ± 3	85 ± 5
ε ($M^{-1}\,cm^{-1}$) ($n=3$)	$(1.2 \pm 0.1) \times 10^7$	$(0.5 \pm 0.2) \times 10^7$

3.2. Method validation

Operating conditions previously reported [13,21], especially HCl concentration on which the intensity of the ion-pair colour is very dependent, were strictly observed. The linearity of the method was checked in the 6–29 μM (concentrations expressed in organic layer where absorbance is read) range and the corresponding equation of the regression line was: $A_{565\,nm} = 0.0569\,[AuCl_4^-] \pm 0.0017 - 0.2085 \pm 0.0439$; coefficient of determination, $r^2 = 0.9992$ (6 points, $n=4$). The inter-assay precision was determined using six different citrate-stabilized Au NPs batches: the value obtained was equal to 301 ± 7 μM.

The limit of detection (LOD) was calculated as follows [11]:

LOD = 3 sa0 (sa0: standard deviation of blank solution) ($A_{blank} = 0.041 \pm 0.016$, $n=6$, inter-day measurements). The value found for LOD was 4.5 μM, providing enough sensitivity for measuring gold content in different synthesized batches of Au NPs. LOD of the present assay at the micromolar level is 10,000-fold higher than LOD value obtained when using ICP-MS [10]. The main advantage of ICP-MS remains very low sample volume. Nonetheless, the present method represents an easy to use alternative that can become a routine protocol in a laboratory.

Otherwise, cetyltrimethylammonium bromide (CTAB) is frequently used as capping reagent of Au NPs [26] and its potential interference was presently tested with the colorimetric method. This hydrophobic cationic species could interfere during ion-pairing formation between $AuCl_4^-$ and the cationic form of Rhodamine B. We added CTAB in the protocol at two concentration levels, 200 and 1000 μM, corresponding to a [CTAB]/[$AuCl_4^-$] ratio of 1× and 5×, and we observed no interference.

3.3. Quantification of Au NPs

It is generally assumed that the reduction of $AuCl_4^-$ by $NaBH_4$ in the presence of citrate ions is complete [11,14,19]. As can be seen in Table 1, the gold concentration in citrate-stabilized Au NPs is 304 ± 13 μM, and it is consistent with the initial gold concentration used in the synthesis (292 μM), thus confirming the accuracy of this method. Indeed, the measured concentration of citrate-stabilized Au NPs is in line with what has been reported by others authors [12,14,20]. For example, the molar absorbance value for 5.3 nm diameter citrate-stabilized NPs obtained in this study is $1.2 \times 10^7\,M^{-1}\,cm^{-1}$, and similar NPs (diameter = 5.0 nm) described by Pellegrino et al. is $1 \times 10^7\,M^{-1}\,cm^{-1}$ [20].

From Table 1, another important result to notice is that the increase in core diameter of Au NPs introduces decrease of Au NPs concentration and increase in the molar absorbance of Au NPs, the same tendencies were previously observed [7,14].

4. Conclusion

In the routine laboratory practice, it would be desirable to have a simple, fast, and low-cost method for rapid monitoring of the NPs concentration during all preparative stages. The present spectrophotocolorimetric assay meets these goals and could be easily applied to all types of Au NPs varying by capping ligands or active molecules attached to their surface.

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References

[1] C.J. Murphy, A.M. Gole, J.W. Stone, P.N. Sisco, A.M. Alkilany, E.C. Goldsmith, S.C. Baxter, Acc. Chem. Res. 41 (2008) 1721–1730.
[2] M.C. Daniel, D. Astruc, Chem. Rev. 104 (2004) 293–346.
[3] D. Astruc, M.C. Daniel, J. Ruiz, Chem. Commun. 23 (2004) 2637–2649.
[4] D. Pissuwan, T. Niidome, M.B. Cortie, J. Control. Release, in press.
[5] C.-K. Kim, P. Ghosh, V.M. Rotello, Nanoscale 1 (2009) 61–67.
[6] H.M. Joshi, D.R. Bhumkar, K. Joshi, V. Pokharkar, M. Sastry, Langmuir 22 (2006) 300–305.
[7] B. Duncan, C. Kim, V.M. Rotello, J. Control. Release 148 (2010) 122–127.
[8] P. Ghosh, G. Han, M. De, C.K. Kim, V.M. Rotello, Adv. Drug Deliv. Rev. 60 (2008) 1307–1315.
[9] L. Yu, A. Andriola, Talanta 82 (2010) 869–875.
[10] A. Scheffer, C. Engelhard, M. Sperling, W. Buscher, Anal. Bioanal. Chem. 390 (2008) 249–252.
[11] R. Allabashi, W. Stach, A. de la Escosura-Muñiz, L. Liste-Calleja, A. Merkoçi, J. Nanoparticle Res. 11 (2009) 2003–2011.
[12] K.R. Brown, D.G. Walter, M.J. Natan, Chem. Mater. 12 (1999) 306–313.
[13] B.J. Macnulty, L.D. Wollard, Anal. Chim. Acta 13 (1955) 154–158.
[14] X. Liu, M. Atwater, J. Wang, Q. Huo, Colloids Surf. B Biointerfaces 58 (2007) 3–7.
[15] J.H. Lin, C.W. Chang, W.L. Tseng, Analyst 135 (2010) 104–110.
[16] J.M. Abad, S.F. Mertens, M. Pita, V.M. Fernandez, D.J. Schiffrin, J. Am. Chem. Soc. 127 (2005) 5689–5694.
[17] S. Roux, B. Garcia, J.L. Bridot, M. Salome, C. Marquette, L. Lemelle, P. Gillet, L. Blum, P. Perriat, O. Tillement, Langmuir 21 (2005) 2526–2536.
[18] P.K. Jain, K.S. Lee, I.H. El-Sayed, M.A. El-Sayed, J. Phys. Chem. B 110 (2006) 7238–7248.
[19] R.C. Mucic, J.J. Storhoff, C.A. Mirkin, R.L. Letsinger, J. Am. Chem. Soc. 120 (1998) 12674–12675.
[20] T. Pellegrino, R.A. Sperling, A.P. Alivisatos, W.J. Parak, J. Biomed. Biotechnol. 2007 (2007) 26796–26805.
[21] D. Hu, H. Han, R. Zhou, F. Dong, W. Bei, F. Jia, H. Chen, Analyst 133 (2008) 768–773.
[22] M.T. van Meersbergen, L. Lorenzen, J.S.J. van Deventer, Miner. Eng. 6 (1993) 1067–1079.
[23] A. Pal, J. Photochem. Photobiol. Chem. 142 (2001) 59–65.
[24] M.S. El-Shahawi, A.S. Bashammakh, S.O. Bahaffi, Talanta 72 (2007) 1494–1499.
[25] J. Zheng, W. Huang, S. Chen, Z. Niu, Z. Li, Electrochem. Commun. 8 (2006) 600–604.
[26] M.A. Sobhan, M.J. Withford, E.M. Goldys, Langmuir 26 (2010) 3156–3159.