RESEARCH PAPER

ICP-MS: a powerful technique for quantitative determination of gold nanoparticles without previous dissolving

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Abstract A direct and simple inductively coupled plasma mass spectroscopy (ICP-MS) method for the determination of gold nanoparticles (AuNP) with different particle sizes ranging from 5 to 20 nm and suspended in aqueous solutions is described. The results show no significant difference compared to the determination of the same AuNPs after digestion, as claimed by the literature. The obtained limit of quantification of the method is 0.15 µg/L Au(III) that corresponds to 4.40×10^9 AuNP/L, considering spherical AuNPs 15 nm sized. Spike recovery experiments have shown that the sample matrix is a significant factor influencing the accuracy of the measurement. Spike recoveries from 93% to 95% are found for AuNP samples prepared in trisodium citrate, while for deionized H_2O a spike recovery of around 80% was obtained. The sample preparation mode along with the ICP-MS parameters have been optimized and found to be crucial so as to achieve the required accuracy for the direct quantification of AuNP suspensions. The effect of the nanoparticle size upon the ICP-MS signal also was studied, and only significant differences due to the chemical environment and not to the AuNPs size were found.

Keywords ICP-MS · Gold nanoparticles · Direct detection · Colloids · Environment

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Introduction

There is a great interest for quantification of nanoparticles, either because of their presence as pollutant in aquatic media or for their interesting applications in nanobiotechnology processes. Several enzymes, DNA or protein-based sensing systems have been reported to use nanoparticles as labels or transducing platforms. They offer high sensitivity in measuring pollutants due to the bio-molecular recognition capability, coming from the use of nanoparticles.

Gold nanoparticles (AuNP), in particular, are excellent candidates for bioconjugation with interest for biosensing applications. They have been one of the most important nanoparticles used as labels in

biosensor designs in the last years. This is due to various reasons: they can be prepared in a wide range of sizes, from about 2 nm to 100 nm above. They are biocompatible, bind readily to a range of biomolecules such as amino acids (Selvakannan et al. 2003, 2004; Mandal et al. 2002), proteins/enzyme (Patolsky et al. 1999; Niemeyer and Ceyhan 2001; Gole et al. 2001a, b; 2002; Zhao et al. 1996; Keating et al. 1998), DNA (Park et al. 2001; Alivisatos et al. 1996; Kumar et al. 2001; Kanaras et al. 2003) and expose large surface areas for immobilization of biomolecules. Finally, they expose large surface areas for the immobilization of biomolecules. In addition to the above properties, a simple chemical synthesis is used for the AuNP preparation.

The attractive electrochemical properties make possible the application of AuNPs as tracing labels in several applications including DNA detection (Merkoçi et al. 2005a). Their sensitivity for long life-time along with multiplexing capability has led to extensive applications in electrochemical assays in recent years (Katz et al. 2004). Most of the reported assays have been based on the chemical dissolution of gold nanoparticle tags (in a hydrobromic acid/bromine mixture) followed by accumulation and stripping analysis of the resulting Au(III) solution. Due to the toxicity of the HBr/Br_2 solution, direct solid-state detection of silver precipitate on gold nanoparticle-DNA conjugates was reported by Wang et al. (2002). However, this method was based on direct detection of precipitated silver, not gold nanoparticle tag itself. Direct detection of colloidal gold nanoparticles, but not in connection with the detection of DNA hybridization, was reported earlier (Hernandez-Santos et al. 2002; Pumera et al. 2005a; González-García and Costa-García 1995). A novel nanoparticle-based detection of DNA hybridization based on the magnetically induced direct electrochemical detection of 1.4 nm AuNP linked to the target DNA have been reported previously by our group. The AuNP tag is directly detected after the DNA hybridization event, without the need of acidic (i.e. HBr/Br_2) dissolution (Pumera et al. 2005b; Castañeda et al. 2007).

Several optical methods are ideally suited for NP characterization. The atomic force microscope (AFM) (Jiang et al. 2002; Collins et al. 2004) offers the capability of 3D visualization and both qualitative and quantitative information on many physical properties including size, morphology, surface texture and roughness. Statistical information, including size, surface area and volume distributions, can be determined as well. Transmission electron microscopy (TEM) (Chen et al. 2006) has also been used for this purpose. Although shown advantages, the mentioned techniques cannot be used for analytical quantification of NPs, in general, and of AuNPs particularly.

The quantification of NPs at trace level is getting more and more interesting, not only for the mentioned analysis but also for further applications in nanobiotechnology as well as for environmental and toxicology studies related to nanomaterials. Novel alternatives for NP quantification are being required. In this context, inductively coupled plasma mass spectroscopy (ICP-MS) is showing to be an interesting alternative. Nevertheless, although ICP-MS is known as one of the most powerful methods for trace element analysis the possibilities of this technique on the quantification of nanoparticles are not yet deeply explored.

ICP-MS use in combination with other separation techniques, like GC or HPLC (Helfrich et al. 2006) for gold nanoparticles (AuNP) characterization is reported. In a previous work of our group (Merkoçi et al. 2005b), the ICP-MS technique was used for DNA determination at low concentrations, via detection of AuNPs. This methodology includes an acid digestion of samples (nitrocellulose membrane containing gold nanoparticles modified with antibody) at high temperature/pressure, which extends the time and costs of analysis and can be prone to additional contaminations or matrix problems.

The objective of our work was to evaluate, for the first time, the possibility of direct determination of AuNPs in colloid solutions by ICP-MS, without previous digestion/dissolution, avoiding the use of hazardous reagents and reducing the time of the analysis, for further applications in nanobiotechnology and environmental fields. A scheme of the processes involved in the ICP-MS analysis of AuNPs with (A) and without (B) previous gold dissolution is shown in Fig. 1.

Besides the optimization of the instrumental parameters for nanoparticle determination, one of the main goals was to investigate the influence of NPs surrounding area—resulting from previous preparation steps—on the ICP-MS measurement, as well as on the stability of AuNPs colloidal solutions itself.

Fig. 1 Scheme of the processes involved in the ICP-MS analysis of AuNPs with (A) and without (B) previous gold dissolution
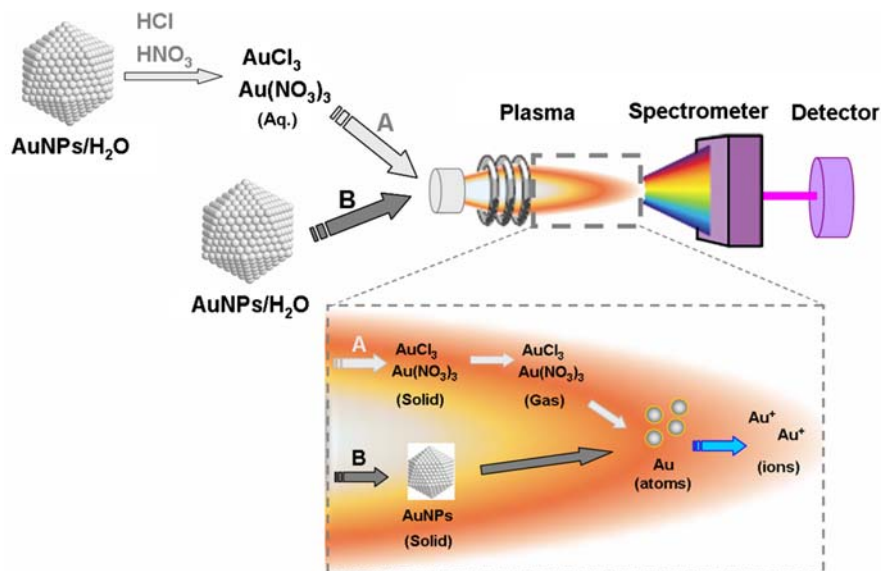
Experimental

Reagents and materials

Gold standard solution (1 g/L Au(III) in HCl, as $HAuCl_4$), mentioned in this manuscript as Au(III) solution, was obtained from Sigma Aldrich (Spain). For ICP-MS daily performance procedure, an Elan 6100 Setup/Stab/Masscal Solution from Perkin Elmer (Austria), containing 10 μg/L Mg, Cu, Rh, Cd, In, Ba, Ce, Pb and U in 1% HNO_3 was used. The indium solution used as internal standard was also obtained from PerkinElmer (Austria). Gold colloid solutions, 5 nm (G 1402) and 20 nm (G 1652)—mentioned below as commercial AuNP samples—were purchased from Sigma Aldrich (Spain). According to product information, they are produced by a modified tannic acid/citrate method. Unconjugated gold colloids contain 0.01% $HAuCl_4$ suspended in 0.01% tannic acid with 0.04% trisodium citrate, 0.26 mM potassium carbonate and 0.02% sodium azide as a preservative. Hydrochloric acid (30%, s.p), nitric acid (s.p) and trisodium citrate dihydratate (1%, p.a) were purchased by VWR Int. (Austria).

Instruments

The ICP-MS measurements are carried out with a quadrupole ICP-MS instrument (Elan DRC-e, PerkinElmer, Germany), equipped with a cross flow nebulizer and a Scott Double Pass spray chamber. The linear calibration curve is set up between 10 and 100 μg/L Au(III) in HCl 1% (v/v) and is recorded every measuring sequence. Indium is used as the internal standard, eliminating the fluctuations coming from variations on sample preparation steps and measuring conditions. Important instrumental settings, like torch position, rf power, nebulizer gas flow and lens voltage, are carefully optimized before analysis. The optimal operating parameters are summarized in Table 1.

A high performance microwave digestion unit "mls 1200 mega" from MLS GmbH, Germany, equipped with 100 ml PTFE high pressure vessels is used for sample digestion. Transmission electron micrographs are obtained with a transmission electron microscope (TEM) Hitachi H-7000 (Hitachi Ltd., Japan), with an acceleration voltage of 75 kV.

AuNP samples production

Samples 1–3 (see Table 2), mentioned below as home-made samples, are prepared at the lab, according to the method pioneered by Turkevich et al. (1951), based on the reduction of tetrachloroauric acid with trisodium citrate. Briefly, 50 mL of 0.01% HAuCl4 solution (250 μM) is boiled with vigorous stirring, and then the desired amount of 1% trisodium citrate dihydrate is added quickly to the boiling solution. When the solution turns deep red, indicating

Table 1 Operating conditions for Elan DRC-e ICP-MS

Nebulizer gas flow	0.89 L/min	Sample uptake rate	0.89 L/min
Auxiliary gas flow	1.275 L/min	Scanning mode	Peak hopping
Plasma gas flow	15.25 L/min	Detector	Dual
ICP RF power	1,250 W	Detector mode	Pulse mode
Operating frequency	40 MHz	Sweeps/reading	50
Lens voltage	6 V	Readings/replicate	1
Autolense correction	ON	Replicates	3
Analog stage voltage	−1,525 V	Dwell time	50 ms
Pulse stage voltage	750 V	Integration time	5,000 ms

Table 2 Home-made AuNP samples preparation

Sample number	HAuCl$_4$ 0.01% (mL)	Trisodium citrate 1% (mL)	AuNP size (nm)
1	50	1.25	20
2	50	3.75	15
3	50	7.50	>20

the formation of gold nanoparticles, it is left stirring and cooling down. Finally, the volume is adjusted with milli-Q water to 50 mL, having a final Au(III) concentration of 58 mg/L. The different amounts of the trisodium citrate solutions and the size of the AuNPs obtained are shown in Table 2.

AuNP samples treatment before ICP-MS measurements

While the commercial AuNP samples were just diluted in water (acid treatment was not possible due to toxic gases development), the home-made AuNP samples 1–3 were diluted in three different ways before ICP-MS measurements: (a) in deionised water, (b) in 1% (v/v) trisodium citrate and (c) in 1% (v/v) HCl. The dilution factor in all cases was 1:1000. On the other hand, an aqua regia microwave digestion is performed as sample preparation step, applying the following procedure: 5 min at 250 W, 5 min at 400 W, 5 min at 650 W and 5 min at 250 W. The same digestion procedure was applied for the preparation of calibration solutions.

Results and discussion

ICP-MS method validation

The performance characteristics of the calibration function include 21 experiments done in a period of

nearly 2 months (results not shown). Limit of quantification (LOQ) and limit of detection (LOD) are calculated as six and three time's standard deviation of blank solution, respectively. The method's precision is calculated as relative standard deviation (RSD) of an Au(III) standard solution (concentration 50 µg/L) measured twice at each measuring sequence. A very good robustness of the method is also observed from the stability of the calibration function (RSD of calibration slope: 7% for $n = 21$).

The validation of the whole procedure includes the matrix influence on performance characteristics of the method. A preliminary study (see "AuNP suspension stability") has shown that the best way for dilution of the home-made AuNP suspension samples (1–3) is 1% (v/v) hydrochloric acid. This dilution has the advantage of matrix matching with the calibration solutions, which can be the reason for the good recovery of spikes (93–95%) in this case. On the other hand, it is shown that the matrix of commercial samples 7 and 8 has a significant influence on the method accuracy (spikes recovery only 76–82%). In this case, the value of the recovery has to be considered, for calculating the real, accurate concentration in the samples. The performance characteristics of the method are summarized in Table 3.

As can be seen from the Table 3, the optimized method provides a high sensitivity (LOQ = 0.15 µg/L Au(III)) and is accurate and robust enough for performing further investigations on AuNPs in aqueous solutions. Taking into consideration, the atomic weight of gold (M = 197 g/mol)) and the Avogadro number ($N_A = 6.023 \times 10^{23}$ mol^{-1}), the previous LOQ of Au(III) (0.15 µg/L Au(III)) would correspond to 4.58×10^{14} gold atoms/L.

Assuming a spherical shape of the AuNPs and a density similar with a uniform face-centred cubic

Table 3 The performance characteristics of the method, obtained using the Au(III) standard solution

Performance characteristic		Number of experiments	Unit
Calibrating range	10–100	–	µg/L Au
Calibrating levels	5	–	–
Slope of cal. curve	0.03809	21	cps/µg L^{-1}*
Intercept of cal. curve	−0.04074	21	cps*
Correlation coefficient	0.99993	21	–
LOQ (average)	0.15	3	µg/L Au
LOD (average)	0.06	3	µg/L Au
Precision (RSD for 50 µg/L Au(III) standard sol.)	3.2	41	%
Spikes recovery for home-made samples	93.3–95.1	2	%
Spikes recovery for commercial samples	76.2–81.9	2	%

* the units for the Y axis are counts per seconds (cps) related to the internal standard (In)

Experimental conditions as explained in the text

AuNP structure (fcc) (Mucic et al. 1998), the average of gold atoms for each AuNP can be calculated by the following equation:

$$N_{at} = N_A \pi \rho D^3 / 6M,$$

where N_A is the Avogadro number, ρ is the density for fcc AuNP (1.93×10^{-20} g/nm^3) (Zhang et al. 2004; Cui et al. 2002), D is the AuNP diameter in nm and M stands for atomic weight of gold. Considering an average AuNP diameter of 15 nm, the average of gold atoms is 1.04×10^5 gold atoms/nanoparticle. Finally, dividing the gold atoms/L by the number of gold atoms/nanoparticle is obtained that the LOQ corresponds to a number of 4.40×10^9 AuNPs/L.

AuNPs suspension characterization and treatment before ICP-MS measurements

Transmission electron micrographs of both home-made samples and commercial ones are given in Fig. 2. The citrate concentration used for the home-made samples has been changed from 0.03% (sample A),

0.07% (sample B) and 0.13% sample (C). According to Kumar et al. (2007), initially the AuNP size increases by the increase of citrate concentration. After that, an increase in the concentration of citrate strongly supports the AuNPs nucleation, resulting in a decrease on the particles size, but at high concentrations of citrate the particles size becomes relatively independent of the ratio Au(III)/citrate solution concentration. This phenomenon is corroborated also by TEM images of Fig. 2. The AuNP size is decreasing from 20 to 15 nm while increasing the citrate concentration from 0.03% to 0.07% and then the size increases again (sample C). Furthermore, the solution pictures evidence that when increases the AuNPs size their solutions turn to deeper red colour which is related to the change of the plasmon absorption of colloidal gold nanoparticles by increasing the size (Link and El-Sayed 1999).

The AuNP diameters (calculated from TEM images) of around 20 nm (sample A), 15 nm (sample B) and 23 nm (sample C) along with AuNP density data from literature have been used to calculate the

Fig. 2 TEM images of the AuNPs and the corresponding pictures of the solutions for the home made: **A** sample number 1, **B** sample number 2, **C** sample number 3, and the commercial: **D** 5 nm sized, **E** 20 nm sized samples
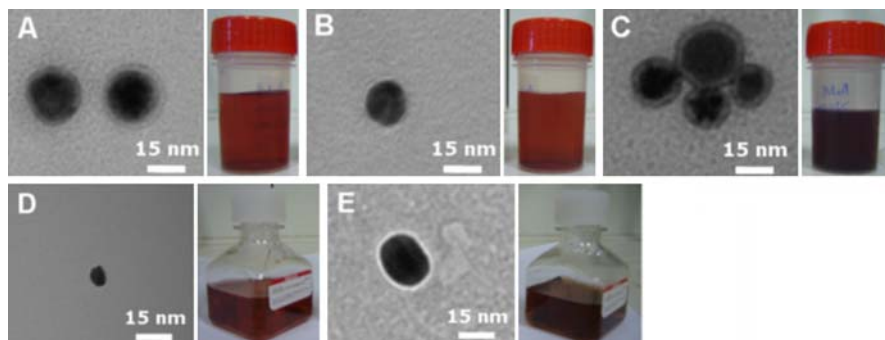
concentrations of AuNP solutions as AuNP number/ suspension volume (L).

AuNP suspension stability

It is well known that the ICP-MS measurements are strongly dependent on the matrix of the sample solution introduced. In the case of suspended nanoparticles, an additional factor influencing accuracy is the stability of the suspension, which is directly effected from the chemical modifications of particles in this suspension. Both factors are studied in this work, having as main goal the accurate quantification of gold in home-made citrate AuNP samples. Commercially available AuNP samples are used for comparison.

Three possibilities are investigated for the dilution of home-made samples: (a) deionised water, (b) 1% hydrochloric acid and (c) 1% trisodium citrate solution. Due to the lack of certified reference materials of AuNPs in the market, two criteria are considered to evaluate the accuracy of the measurement for the given AuNP samples: first, the deviation of the average Au(III)-concentration from the theoretical value and second, the recovery of spikes. The samples are spiked just before measurement with a high concentrated Au(III) standard solution, so that the final concentration of the added Au(III) in sample was 50 µg/L. As shown in Fig. 3, the recovery of spikes for all three samples diluted in citrate were very low (10–40%) and no constant value could be obtained.

For water diluted samples 1 to 3, a reduction of recovery values is observed during the measuring time, as well as a reduction of measured Au(III)
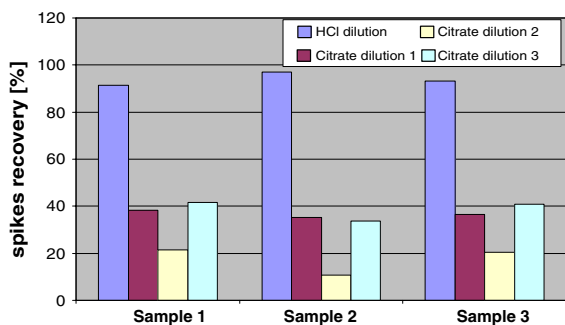

Fig. 3 Spikes recovery (%) for samples 1–3 spiked with Au(III) solution

concentration in the unspiked sample. As this effect was not observed for the Au(III) standard solution, it is obviously relating to a possible precipitation of AuNPs in vial during the measurement sequence, in the case of water dilution. According to these experience and the results seen in Fig. 3, hydrochloric acid was chosen as the most suitable solvent for the sample preparation before analysis and was used for the further investigations. On the other hand, the spikes recoveries for the commercial samples (7 and 8) are observed to be constant but slightly reduced (∼80%). This can be explained with the matrix discrepancies between these samples (diluted in water) and calibration solutions (diluted in HCl).

Following the above optimized and validated method, an extensive stability study is performed for AuNP samples with various sizes and different chemical environments. Three sizes of home-made colloid solutions (15, 20 and >20 nm) and two commercial samples (5 and 20 nm) are studied over a period of nearly two months under unchanged measuring conditions (Fig. 4). The theoretical gold concentration in the AuNP samples is 57.9 mg/L Au for both home-made samples and commercial ones.

Table 4 represents the average concentration, standard deviation, relative standard deviation and recovery (related to the theoretical concentration) for the AuNP samples analysed and presented in the above Fig. 4A and B. As can be seen from Fig. 4A and B and Table 4, in most cases although the differences between samples for both standard deviation (1.92–6.96 mg/L) and average concentration (from 36.3 to 64.9 mg/L), good stabilities ranging from 4.5% to 13.6% are observed over the whole investigation period. The observed differences can be attributed to the chemical environment of the AuNPs and not to the particle size. No significant relation was observed between the particle size and their stability in the colloid solution. Average concentration, standard deviation and RSD are calculated from the corresponding number of measurements performed over a long period of time. The recovery of the measured samples is calculated as relative percentage of the theoretical concentration of gold in solutions.

The results from sample 3 (>20 nm) show that reproducibility of the sample production step is not yet satisfactory for the home-made samples. There is a significant difference between both production
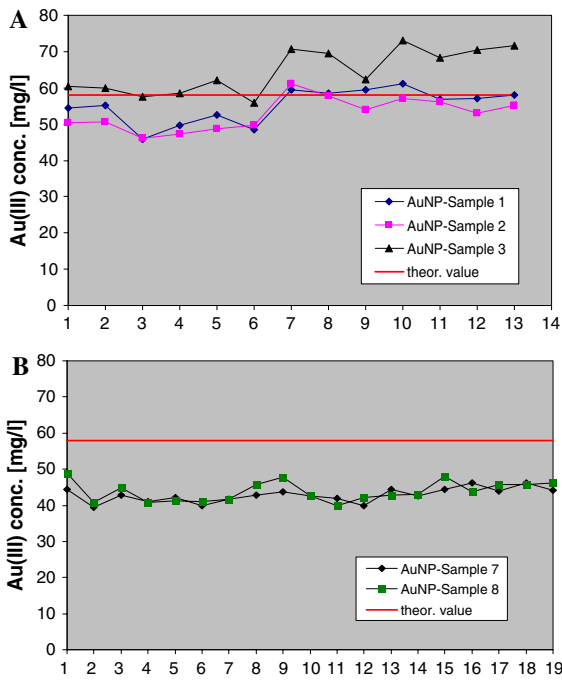
Fig. 4 Au(III)-concentration in AuNP samples, measured over a period of 2 months. Experimental conditions as described in the text. **A** Thirteen representative results for the home-made AuNP samples (1–3). **B** Nineteen representative results for commercial AuNP samples (7 and 8)

batches for this sample, concerning both average concentration and recovery. Further investigations are required to clarify if it is a special effect of the "bigger" particle size. The commercial samples show a very good stability (RSD 4.5% and 6.3%), independently of the NPs size but the recovery is significantly different from 100%, as discussed before. Taking into consideration the lower spike

recovery for this matrix (average 79%), the calculated mean concentration for samples 7 and 8 is 54.2 and 55.4 mg/L Au(III), respectively, which corresponds to recoveries 93.6% and 95.7%.

Effect of AuNP digestion upon ICP-MS signal

AuNP digestion prior to ICP-MS measurements has been reported previously as a necessary step for an accurate AuNP determination. Several drawbacks could be associated with the digestion step. First of all, an additional contamination through digestion reagents is probable, which influences the analytical performance (detection limit, reproducibility, etc.) of the method. Furthermore, an undesirable dilution of the sample occurs during the digestion, which also leads to higher LODs. On the other hand, sample throughput is much lower, because of the extended time of analysis. The effect of the digestion procedure described before, upon the accuracy of AuNP analysis, was also studied using the home-made samples 1–3 with 20, 15 and >20 nm particle sizes. The Au(III) concentrations measured by ICP-MS for these samples were 49.8, 46.4 and 30.6 µg/L for samples diluted in 1% HCl and 48.5, 44.7 and 22.2 µg/L for the same samples digested in aqua regia, according to the procedure described in "Experimental."

While no significant difference between the results for samples with and without preliminary digestion (samples 1 and 2) was shown, the discrepancy at the results for sample 3 is supposed to be addressed to the production procedure of this sample, which leads also to big discrepancies from the theoretical Au(III) concentration (see "AuNP suspension stability").

Table 4 Summary of the average concentration (Av. conc. in mg/L), standard deviation (SD in mg/L), relative standard deviation (RSD in %) and recovery (in %) for the AuNP samples analysed

Nr.	AuNP Sample/particle size (nm)	Av. conc. (mg/L)	SD (mg/L)	RSD (%)	Recovery (%)
1.	Lab—batch 1/20	59.0 ($n = 16$)	6.96	11.8	101.8
1.	Lab—batch 2/20	55.1 ($n = 14$)	4.75	8.6	95.1
2.	Lab—batch 1/15	46.5 ($n = 16$)	4.26	9.2	80.2
2.	Lab—batch 2/15	52.9 ($n = 14$)	4.49	8.5	91.2
3.	Lab—batch 1/>20	36.3 ($n = 16$)	4.94	13.6	62.6
3.	Lab—batch 2/>20	64.9 ($n = 14$)	6.36	9.8	111.9
7.	Commercial/5	42.9 ($n = 20$)	1.92	4.5	74.0
8.	Commercial/20	43.8 ($n = 20$)	2.75	6.3	75.6

Conclusions

The use of ICP-MS as a sensitive and accurate technique for the direct determination of gold nanoparticles in aqueous colloid solutions has been demonstrated. It is clearly shown that the sample matrix is the main factor influencing the accuracy and precision of gold determination in these kinds of samples. An extensive investigation of the sample matrix and its effect on the performance characteristics of the method is crucial for an accurate determination of gold nanoparticles suspensions. Different ways are shown for achieving a good accuracy, depending on the specifications and different behaviours of the sample matrix during ICP-MS measurement.

It is shown that a preliminary digestion of AuNP colloid solutions with aqua regia, as reported before in the literature, is not always necessary. Avoiding this sample preparation step leads to better sensitivity, lower detection limit and lower analysis cost for this determination.

The preparation mode of the AuNP samples, as well as the other preparation steps before analysis (dilution or digestion), is of crucial importance for the stability of colloidal solutions.

The optimized ICP-MS method offers a quantification limit for Au(III) in colloid samples of 0.15 µg/L that corresponds to 4.40×10^9 AuNPs/L considering spherical AuNPs 15 nm sized.

Taking into consideration the aggregation test for 13 nm gold nanoparticles performed earlier (Ambrosi et al. 2007), it was found that up to 10 anti-human immunoglobulin G antibodies conjugated with the enzyme HRP (anti-human IgG-HRP) can be attached to the AuNP nanoparticles. This fact allows the AuNPs to act as carriers of antibodies and enzymes, with applications in biosensing. For the obtained Au detection limit of around 0.15 µg/L given by the optimized ICP-MS, that would correspond to 4.40×10^9 AuNPs/L, a quantity of anti-human IgG-HRP of 1×10^{-5} µg/L could be expected to be involved in immunoassay reactions. This quantity would correspond to the detection limit of proteins similar or even lower than several standard methods (Ambrosi et al. 2007).

Due to the obtained analytical performance, the ICP-MS method may have significant applications for analysis of nanoparticles in aquatic mediums. Moreover, it can be extended in bioanalytical applications for the simultaneous determination of various sequences by simultaneously labelling different kinds of inorganic nanoparticles (including quantum dots like CdS, PbS, ZnS, etc.) for which better sensitivities might be excepted, since according to the literature (Merkoçi et al. 2005b) and the supplier information (PerkinElmer SCIEX) the detection limits for Cd and Pb by ICP-MS are expected to be better than for Au.

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