Talanta 82 (2010) 869–875

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Review

Quantitative gold nanoparticle analysis methods: A review

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ARTICLE INFO

Article history:
Received 16 March 2010
Received in revised form 8 June 2010
Accepted 9 June 2010
Available online 16 June 2010

Keywords:
Gold nanoparticle
Quantitative analysis

ABSTRACT

Research and development in the area of gold nanoparticles' (AuNPs) preparation, characterization, and applications are burgeoning in recent years. Many of the techniques and protocols are very mature, but two major concerns are with the mass domestic production and the consumption of AuNP based products. First, how many AuNPs exist in a dispersion? Second, where are the AuNPs after digestion by the environment and how many are there? To answer these two questions, reliable and reproducible methods are needed to analyze the existence and the population of AuNP in samples. This review summarized the most recent chemical and particle quantitative analysis methods that have been used to characterize the concentration (in number of moles of gold per liter) or population (in number of particles per mL) of AuNPs. The methods summarized in this review include, mass spectroscopy, electroanalytical methods, spectroscopic methods, and particle counting methods. These methods may count the number of AuNP directly or analyze the total concentration of element gold in an AuNP dispersion.

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Contents

1. Introduction

Nanotechnology and nanoscience, being an interdisciplinary subject of physics, chemistry, biology, and engineering, advanced the contemporary fundamental and application research in the past two decades. No matter which nano-sized material will be chosen first for mass production, nanotechnologies (nanotech) will be one of the carriers for the next industrial revolution. Among many nanomaterials, gold nanoparticle (AuNP) and its colloidal dispersions are promising candidates for future scientific, industrial, and domestic applications. Series of thorough reviews about the preparation, structure, properties, and applications of AuNP have been published [1,2]. The pace of nanotech development is extraordinary. According to a recent estimate from Lux Research, a consulting firm that monitors emerging technologies, $147 billion worth of nanotech based products were manufactured in 2007, while about $3.1 trillion worth of nanotech enabled products by 2015 could be expected [3].

Currently, fundamental and application research and development work is focused on discovering new nanomaterials, constructing new nanostructures, and exploring new applications. As nanotech and nanomaterials became more and more pervasive, two topics have appeared to the vision of the science community. The first topic is the potential risks to human health and to

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0039-9140/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.talanta.2010.06.017

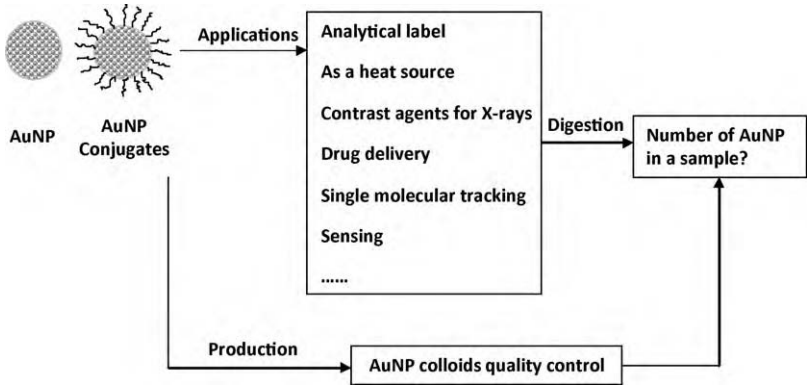

Fig. 1. AuNP quantitative analysis following its production and applications.

the environment. The second topic is the quality control of nanomaterials manufacturing. These two separate topics are strongly interrelated because it is necessary to know the population (number of nanoparticles) of the nanomaterials and then to investigate their effect. Once nanomaterials are mass consumed as food additives, cosmetics, medicine, etc., it is necessary to know where the nanomaterials are afterward and how the environment digests the "nanowaste" [4–9]. Two typical series of chemicals that have been consumed vastly by human activities, that have significantly affected human health and environment, and whose effects had not been effectively investigated before mass applications are pesticides and fluorochlorohydrocarbons.

Once colloidals of nanoparticles are mass produced, quality of the product needs to be controlled. These qualities should include the "amount" of the nanoparticles in a dispersion. The characterization includes not only the size of the nanoparticles, but also their quantity. Therefore, quantitative nanoparticle analysis methods are also very important for the production and quality monitoring (see Fig. 1).

This paper reviews the up-to-date methods that have been developed for quantitative analysis of the population of AuNP in its colloidal dispersion. The AuNP could be considered a representative of the metal or metal oxide nanoparticles. Therefore, reports about the analysis of other metals and metal oxides are not included in this review. Polymers and biopolymers, fullerenes, and single wall carbon nanotube solutions are thermodynamically "real" solutions, though the size of these molecules is in the range of nanometers instead of sub-nanometers. Therefore, the analysis of these macromolecules is considered quantitative "chemical" analysis, and hence, is not discussed in this paper. To the author's knowledge, this is the first systematic review about the quantitative analysis methods of gold nanoparticles. In this paper, we reviewed the methods that can be used for AuNP analysis. These methods include mass spectroscopy, electroanalytical methods, optical spectroscopy, and particle counting techniques. Microscopes have been used intensively for AuNP characterization. These microscopy techniques are transmission electron microscopy (TEM), atomic force microscopy (AFM), scanning tunneling microscopy (STM), and electronic scanning microscopy (SEM) [1,10–13]. Microscopy techniques are not quantitative characterization methods and, therefore, are not included in this review.

Two terms, population and concentration, are used in this review to describe the quantity of gold in a dispersion. Population refers to the number of AuNPs per mL, no matter what size the AuNP is. Concentration in molarity (mol/L) refers to number of moles of gold per liter of dispersion. The AuNP is not considered a molecular species. Therefore, the number of moles of gold equals the number of gold atoms divided by the Avogadro's constant (6.02×10^{23}), instead of the number of nanoparticles divided by the Avogadro's constant. In the cited references, authors may not state the meaning when concentration in molarity is used. To make our discussion clear and consistent, population in number of AuNP/mL will be used in this review. When concentration in molarity is used, it refers to the total concentration of gold element. Mass percentage and parts per million (ppm) are not ambiguous, and are also used in this review.

2. Challenge and opportunities of nanoparticle analysis

AuNP dispersions are not solutions. Each individual particle can be considered as a solid phase in liquid dispersion. An AuNP dispersion is, theoretically, thermodynamically not stable due to the relatively large interface energy. Traditional methods that have been used to analyze the "concentration" of a chemical in its solution may not be suitable for the analysis of the "population" of AuNP in its dispersion, especially the methods that are based on the colligative properties of solutions.

The total "amount" of gold nanoparticles in a typical colloidal dispersion is very small. For example, a 20 nm AuNP colloidal dispersion from Ted Pella Inc. contains about 7×10^{11} particles/mL [14]. This is about 57 ppm of gold, equals to about 2.9×10^{-4} mol/L. Very sensitive methods are needed to measure either the total amount (number of gold atoms) or the population (number of AuNPs) in dispersions.

Quantitative analytical chemistry analyzes chemicals at the molecular level. Usually, these species (molecules and ions) are smaller than 1 nm, except for polymers and biomacromolecules. On the other hand, there are other well-developed engineering methods in characterization of fine particles that are 0.1 μm (100 nm) or larger [15]. The AuNP usually has an average diameter between 1 nm and 100 nm, which is neither covered by the "chemical analysis", nor fine particles characterization, as shown in Fig. 2. In this review, we summarize how the current chemical analysis methods and particle characterization methods are used to investigate AuNPs with diameters over the range of 1–100 nm.

3. Mass spectroscopy

Mass spectroscopic (MS) methods analyze the mass–charge ratio (m/z) of a charged species with high resolution and large

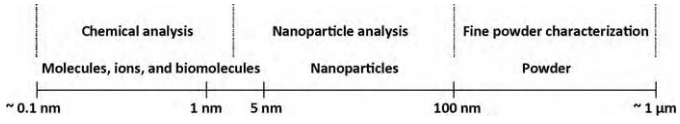

Fig. 2. Chemical analysis, nanoparticle analysis, and fine powder characterization.

range of "molar" mass. There is theoretically no absolute upper limit of m/z [16,17]. The range of detectable m/z values depends on many factors such as power of desorption laser used, acceleration potential, vacuum, and stability of species. Usually, the MALDI–TOP method can detect m/z up to hundreds of kilo-Dalton (kDa) with good accuracy and resolution. A geometrically spherical gold nanoparticle with diameter of 5 nm has "molar" mass of 754 kDa, given the density of gold is 19.3 g/cm^3. Therefore, AuNPs of 5 nm or smaller are in the theoretical detectable range of MS. A series of reports about the characterization of AuNP by MALDI–TOF MS demonstrate excellent sensitivity and resolution of results from gold clusters. These gold clusters have gold atoms less than 100, and hence, yield a m/z value of about tens of kDa [18–24]. This m/z value corresponds to an AuNP of 2 nm or smaller.

Zhang et al. reported MALDI–TOP MS detection of *N*-acetyl-L-cysteine monolayer protected polydisperse gold clusters [18]. High-resolution peaks of gold clusters with a core of Au_4–Au_{22} have been observed over the m/z range from 1436 to about 7000. Broadened peaks of larger gold clusters up to Au_{39} also obtained over ~7.5–9.5 kDa. Also with MALDI–TOF MS, Arnold and Reilly observed high-resolution peaks of alkanethiolate-coated clusters up to $Au_{71}S_{31}$, which is 15 kDa [21].

Chaki et al. observed the peak of $[Au_{144}(SC_n)_{59}]^z$, which is about 29 kDa with laser desorption ionization MS [24]. Wu et al. and Dass et al. carefully characterized the peaks of $Au_{25}(SCH_2CH_2Ph)_{18}$ cluster (around 5308 Da) [22,23]. It is also found that when different matrices are used, the resulting mass spectra of the same gold cluster $Au_{25}(SCH_2CH_2Ph)_{18}$ are dramatically different. Typical matrices may include sinapinic acid (SA), 4′-hydroxy-azobenzene-2-carboxylic acid (HABA), 1:1 mixture of dihydroxybenzoic acid and α-cyano-4-hydroxycinnamic acid (UMM), and trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) [22]. Mass spectra in matrices of SA, HABA, and UMM are dominated by fragment peaks, even at lower laser intensities. In DCTB matrix, significant amounts of intact molecular ions are produced. Based on these and other previous works [25–30], electrospray ionization mass spectroscopy (EIS) gave high-resolution results for $Au_{144/146}$ clusters with multiple charge, 10+–15+, around 29 kDa. The exact m/z value depends on the structure and coverage of the protection monolayer. The high resolution of the mass spectra peaks indicates what exactly the species are. The intensity of the peaks is at million-count level which can provide a limit of detection for counting the numbers of the particles. Meanwhile, a $Au_{68}(SR)_{34}$ nanocluster is also identified by its mass spectra peak at about 18 kDa [31].

Mass spectroscopy is a very reliable means for the identification and quantization of AuNPs at a sub-nanometer level. Owing to the high resolution and high sensitivity of MS, the size, size distribution, and abundance of AuNPs (Au clusters) can be easily obtained by analyzing a mass spectrum. However, reports of MS of lager AuNPs (diameter > 2 nm) are not currently available.

4. Direct amperometric methods

Murray thoroughly reviewed the electrochemical properties of metal nanoclusters, such as Ag nanoclusters and gold nanoclusters [32]. When the nanoclusters are small enough, they have molecular-like electrochemical voltammograms. For Au_{75} (ca. 14 kDa) and smaller clusters, the HOMO-LUMO energy gaps are larger than 0.74 V, and voltammograms of molecular-like nanoparticles were obtained [33–37]. The cyclic voltammograms (CV) and differential pulse voltammograms (DPV) of gold nanocluster dispersions showed ambiguous multiple peaks of the reversible or semi-reversible 1e oxidation/reduction at room temperature and at −70 °C. It has been observed that the electrochemical current is determined by the diffusion of the molecular-like clusters. The diffusion coefficients of these nanoclusters are around or smaller than ~10^{-6} cm^2/s, which is close to or smaller than "usual" inorganic ions in their water solutions [38]. Therefore, both the Cottrell equation and the Levich equation apply to relate the Faradic current and the population of the gold clusters. An amperometric analysis can be applied because the Faradic current is intrinsically proportional to the gold cluster population.

Interestingly, when relatively larger gold clusters (14–28 kDa) were examined, quantized double layer charging/discharging voltammograms showed distinguishable peaks of 1e transfers [32]. In these dispersions, the gold nanoclusters behave as quantum capacitors, instead of electroactive species [39–43]. Population of gold clusters with a core of Au_{147} and Au_{38} have been measured by the transition time of chronoamperometry [43]. The populations were between 90 μM and 590 μM, which are very high for gold clusters and AuNPs. Meanwhile, the diffusion coefficients were also estimated with the same dataset. The quantitative analysis was not based on a calibration curve of current and concentration, and therefore, the uncertainty of the measurement was not reported.

The direct amperometric method has been used as one of the detectors of a liquid chromatography for AuNP separation. After eluted from a HPLC column, the AuNP dispersion, with a core of Au_{38} or Au_{140}, was detected by both a spectroscopic method and a fast-scan CV method [44]. A Pt microcylinder electrode was used as the working electrode. The scan rate was 150 V/s that cannot be reached by most commercial brand potentiostats. The output currents are all in nA level [45,46]. Another significance of this method is that after the combination of the results from optical and electrochemical detection, the size of the gold clusters can be estimated by a ratiometric method. The ratio of the absorbance and the current approximately equals to $r^{11/3}$, where r is the radius of the AuNP [44,46]. This is valid for AuNPs with a diameter smaller than 1.7 nm. The results are also significantly affected by the protection monolayer.

There is no report so far about the quantitative characterization of AuNP population by their redox current. The reason is probably that the Faradic current is intrinsically small at the sub-microamper or nanoamper level, which yields a very poor sensitivity and limit of detection. When gold clusters are prepared and used for different applications, their populations are usually very low. This makes the amperometric detection even more difficult. For example, the DPV peak current of a 177 μM Au_{147} dispersion is only 0.05 nA [43]. In two reports [33,39], the DPV peak current was about 0.1 μA for a 0.30 mM 8 kDa solution; ca. 0.05 μA for 0.1 mM 22 kDa solution, 28 kDa solution, and 38 kDa solution.

The largest AuNP that has been characterized by voltammetric methods has a core size of 38 kDa. These molecular-like gold clusters are within the sub-nanometer range.

Larger AuNPs, with diameter greater than 3–4 nm [32], has Bulk-Continuum voltammetric behavior. The oxidation and reduction currents are the charging and discharging of the double layers of the AuNP [47]. However, the oxidation and reduction current strongly depends on the properties and the composition of the protective layer, usually a monolayer of alkanethiol. Therefore, the currents are not solely from the core of the AuNP.

5. Cathodic linear sweep voltammetry

When the AuNPs are larger than 5 nm, they are not considered like molecules. Each AuNP consists of a number of gold atoms. For example, assuming the AuNPs are perfect spheres and their densities are identical to bulk gold, an AuNP of 5 nm diameter, 10 nm diameter, or 20 nm diameter contains about 3.8×10^3, 3.1×10^4, 2.5×10^5 gold atoms, respectively. Therefore, when the average

diameter of the AuNPs is a known value, the number of AuNP can be estimated by analyzing the quantity of element Au. After adsorbed on a graphite electrode, the AuNP (5–72.5 nm) can be oxidized to $AuCl_4^-$ at 1.25 V vs. Ag/AgCl in a 0.1 M HCl electrolyte solution [48–50]. The so-produced $AuCl_4^-$ anions are then reduced and deposited on the surface of the electrode by linear sweep of the electrode potential from 1.25 V to 0.0 V. A cathodic peak at around 0.4 V vs. Ag/AgCl appeared, which came from the reduction of $AuCl_4^-$ to Au metal. The half electrochemical reactions are showed below:

$$Au_{NP}{}^0 + 4Cl^- \xrightarrow{H^+} AuCl_4^- + 3e \quad \text{oxidation of Au at 1.25 V}$$

$$AuCl_4^- + 3e \xrightarrow{H^+} Au^0 + 4Cl^- \quad \text{reduction of } AuCl_4^- \text{ at 0.4 V}$$

García et al. reported that the reduction peak current related to the linear sweep rate, oxidation potential, oxidation time, and the size of AuNP [48]. Like most voltammetric methods, increase in the sweep rate increased the peak current. The peak current is proportional to the sweep rate over the range of 2–100 mV/s, indicating that the electrode kinetics is not diffusion control, and the onsite-produced $AuCl_4^-$ was adsorbed on the surface of the electrode. The peak current increased with the increase of oxidation potential and oxidation time, but reached a limit value in both variances [48]. It is very interesting that the peak current was proportional to the diameter of the AuNP, from 5 nm to 71.5 nm, when the AuNP concentrations were fixed at 2.94×10^{-6} M. The reason of the linear relationship is still unclear.

When linear sweep voltammetry was used, a dynamic range of gold concentration, over 5.89×10^{-7}–2.94×10^{-5} M, was obtained with a limit of detection of 3.49×10^{-7} M based on a signal-to-noise ratio of 3 [48]. When the amperometric sensitivity was improved by the use of differential pulse voltammetry, the dynamic range was 5.08×10^{-8}–4.06×10^{-6} M and the detection limit was 1.78×10^{-8} M at the same signal/noise ratio [48]. Also by use of the same method, Merkoci and co-workers improved the dynamic range further, over a gold concentration range of 2.5×10^{-8}–2.5×10^{-5} M with a limit of detection of 9.3×10^{-9} M [49]. For 10 nm diameter AuNPs, this dynamic range and limit of detection corresponds to 4.7×10^{8}–4.7×10^{11} nanoparticles/cm^3 and 1.8×10^{8} nanoparticles/cm^3, respectively.

One advantage of this method is that the existence of biomolecules, such as immunoglobulin, does not affect the oxidation and reduction of Au and $AuCl_4^-$. Therefore, this method can be used to detect the antibody/antigen conjugated AuNP in immunoassay and other bioanalytical approaches [48,50–58]. This method required that the AuNP is pre-adsorbed on the surface of electrode by physical adsorption. Therefore, carbon paste electrode or graphite–epoxy composite electrodes were used due to their larger surface area and adsorptivity to AuNPs. The sensitivity and the limit of detection are determined by the overall coverage of the AuNP on the electrode surface. In the next part, anodic stripping voltammetry will be introduced, which does not depend on the immobilization of AuNPs.

6. Anodic stripping voltammetry

Stripping analysis is an extremely sensitive electrochemical technique for measuring trace metals. Its higher sensitivity, compared with other amperometric methods, is attributed to the electrochemical pre-concentration. Typical detection limits are as low as 10^{-9}–10^{-12} M [59–63]. When metallic gold in the AuNP is chemically oxidized to Au(III), the Au(III) ions can be deposited (pre-concentrated) on the electrode by electrochemical reduction usually at −0.8 V vs. SCE. Then, the thin layer of Au metal can be stripped by anodic sweeping the electrode potential from 0 V to 1.0 V vs. SCE. An anodic peak of Au oxidation showed up at about 0.7 V vs. SCE. The chemical oxidization was usually achieved by soaking in HBr/Br_2 solution [64–66]. The chemical and electrochemical procedures are showed below:

$$2Au_{NP}{}^0 + 2Br^- + 3Br_2 \xrightarrow{H^+} 2AuBr_4^- \quad \text{oxidation by } Br_2/HBr$$

$$AuBr_4^- + 3e \xrightarrow{H^+} Au^0 + 4Br^- \quad \text{reduction of } AuBr_4^- \text{ at } -0.8\,V$$

$$Au^0 + 4Br^- \xrightarrow{H^+} AuBr_4^- + 3e \quad \text{oxidation of } Au^0 \text{ at } 0.7\,V$$

Owing to the high oxidation potential of Br_2 in HBr media ($E^\circ_{Br_2/Br^-} = 1.066$ V vs. standard hydrogen electrode), AuNPs can be oxidized when it is conjugated with different types of biomolecules and organic protective monolayers [67–74]. In its dilute aqueous solution, Br_2 can convert to HBrO by reacting with H_2O, which is a stronger oxidizing reagent than Br_2 ($E^\circ_{HBrO/Br^-} = 1.331$ V vs. standard hydrogen electrode). The detection limit of Au(III) concentration can be as low as 5×10^{-9} M with the stripping voltammetry [60]. This concentration of Au(III) will equal to approximately 1×10^{8} 10-nm particles/cm^3 or 1.2×10^{7} 20-nm particles/cm^3. It is also reported that prolonging the deposition time with stirring will further reduce the limit of detection [64]. In most of the references, a disposable carbon paste electrode was used that made the detection become very convenient and reproducible. However, the use of bromine, that is toxic and corrosive, restricts the broader applications of this method.

The cathodic linear sweep voltammetry and the anodic stripping voltammetry methods are means of Au analysis. In order to obtain the value of population of number of AuNPs, other methods, such as TEM, are required to characterize the average size (diameter) of the AuNP.

7. ICP–MS methods

Inductively coupled plasma–mass spectroscopy (ICP–MS) offers many benefits to trace metal detections. The limit of detection of element gold can be as low as 1 part per trillion, about 1 pg/mL [75,76]. AuNP dispersions can be directly analyzed without any previous dissolving [77]. The AuNP will be introduced into the ICP torch by nebulization and then converted to plasma by the extreme high temperature, approximately 6000–8000 °C. When standard plain AuNP dispersions were used to validate the method, a good recovery of spikes (93–95%) was obtained if the AuNP was dispersed in HCl solution. The calibration range was found to be over 10–100 μg/L Au. The limit of detection was about 0.06 μg/L Au. The limit of quantification was about 0.15 μg/L Au, which equals to about 4.40×10^{6} 15-nm AuNPs/mL. Although no pre-dissolution is required, the components of matrix strongly affect the results. When 1% (v/v) HCl was used, an excellent spike recovery of above 90% was obtained with excellent reproducibility. When 1% trisodium citrate solution was used, the spike recovery was very low, 10–40%, and no constant value could be obtained. When AuNP dispersion was diluted by deionized water, a reduction of recovery values was observed, as well as a reduction of measured Au(III) concentration in the unspiked sample [77]. The results from different sized AuNPs, 5 nm– 20+ nm, showed that the results (spike recovery and uncertainty) did not depend on the size of the AuNP.

Immunoglobulin conjugated AuNPs have also been analyzed by ICP–MS [78–82]. In these reports, the 1.4–15 nm AuNP was digested by aqua regia [80], mixture of 10% HCl and 0.1% HF [79,82], or diluted HNO_3 solution [78,81] before the ICP–MS measurement. The use of the AuNP, as detection labels, significantly improved the sensitivity and detection limit of the immunoassay. The results also showed that with the pre-digestion, the matrix

does not have observable effects on the ICP-MS measurement of Au. Oligonucleotide-linked AuNPs are also detected by ICP–MS [83–85]. The oligonucleotides were linked to 1.4–15 nm AuNPs by the streptividin–biotin binding or by the thiol–Au bond.

Combined with pre-separation methods, such as liquid chromatography or electrophoresis, the eluates can also be detected by ICP–MS [86,87]. When laser ablation (LA) ICP–MS is used to detect AuNPs, an even lower detection limit can be obtained [88]. Antibody conjugated 10-nm AuNP was separated by Western Blot first, and the detection limit was about 0.449 fmol of gold, which equals to 10 particles/mL [89]. The LA–ICP–MS method showed an excellent linear calibration curve at this extremely low concentration of gold.

Following the ion-flash-intensity theory, a single-particle mode ICP–MS AuNP detection method was applied. This method requires relatively larger AuNPs, for example, over the range of 80–250 nm. Theoretically, AuNPs as small as 25 nm could be detected with a detection limit of $\sim 10^3$ particles/mL with a data acquisition of 20 s [90]. Further increasing the scan time may reduce the limit of detection to an even lower value. The AuNP can be dispersed in deionized water directed without any other pre-treatment before the ICP–MS detection. However, it may not apply to AuNPs that are 20 nm or smaller. The single-particle mode ICP–MS method has been applied to detect 20 nm, 45 nm, and 80 nm antibody conjugated AuNPs [91].

8. Spectroscopy

In the visible range of light, the optical spectrum of AuNPs of 3.4 nm or larger has an absorption band centered on the wavelength of 520–530 nm. The Lorenz–Mie theory described the surface plasmon resonance effect (the so-called Mie scattering) of spherical nanoparticles [92,93]. In a dilute AuNP dispersion, the Beer's law applies: the absorbance is proportional to the number of nanoparticles per unit volume of dispersion, as described below:

$$A = \frac{NdC_{\text{ext}}}{2.303}$$

where A is the absorbance, N is the number of nanoparticles per unit volume of dispersion, d is the path length, and C_{ext} is the extinction cross section of a single particle [92]. The value of C_{ext} depends on many factors including the particle size, shape, chemical components of the particle surface, interaction between the particles, and the circumstance of the dispersion [94–96]. For a series of AuNP dispersions, if these factors are identical, the number of AuNP per unit volume can be measured by the absorbance of the dispersion based on the Beer's law. The optical method is good to AuNPs with diameter of 5–100 nm, which is in the "nanoparticle" range. The spectroscopic method can determine both the size and the concentration of the AuNPs based on the peak position and peak intensity [97,98]. However, due to the relatively low sensitivity of UV–vis, a very low limit of detection has not been obtained. The aggregation of AuNPs also significantly affects the spectrum: both peak position and peak intensity. A number of reports used the UV–vis method to analyze the concentration of biomolecule-conjugated AuNPs. A few recent papers are cited here [99–106]. Some of the references directly used the colorimetric method for semi-quantitative analysis that is also based on the plasmon scattering of visible light [107].

9. Particle counting techniques

The laser light scattering is a very mature method for the detection of fine particles in aerosol science. This technique is based upon the amount of light that is scattered by a particle. Typical light scattering method is good for AuNPs of 0.05 μm (50 nm) or larger. However, employment of the condensation nuclei counter (CNC) technique would allow higher detection sensitivity in particle sizes down to nanometer range. The method usually combined an electrospray nebulizer for liquid samples and a dynamic mobility separator [108,109]. The CNC method has also been used for sizing and quantifying of AuNPs below 30 nm [110–112]. The light scattering strongly depends on the surface properties of the nanoparticle and the thickness of the layer surrounding an AuNP. Both the solvent to make the AuNP dispersion and the temperature of the nebulizer affect the results of the particle counting [110]. With a modified particle size magnifier, AuNPs below 2 nm can be separated and counted effectively. The counting efficiency is seen to depend greatly on the aerosol flow, the amount of vapor, and the temperature [112]. With ethylene glycol vapor under optimal conditions, the counting efficiency is 100% down to 1.6 nm. It was also found that negatively charged particles are more easily activated than positively charged particles [111]. The same method can be applied for both unconjugated AuNPs and monolayer-conjugated AuNPs. In both cases, the existence of salts in the dispersion significantly affect the measuring results because the surface of the AuNP will be covered by salt residue after evaporation of the solvent [113]. This will bring error to the size measurement of the AuNP, but will not affect the results of the AuNP counting.

Also based on the light scattering technique, a direct photon burst counter method was developed for AuNP counting. The photon burst was counted by an avalanche photodiode [114]. It requires a tiny volume of sample, less than 1 fl, and provides excellent linearity of photon burst counts vs. AuNP concentration, over a range of four decades from as low as 1.0×10^7 particles/mL. The diameters of the AuNPs tested are 17–55 nm. Increasing the measurement time can further increase the sensitivity and decrease the limit of detection. The method can be applied for counting antibody, aptamer, and DNA conjugated AuNPs, and the results agreed with traditional bioanalytical methods such as ELISA.

10. Conclusion and perspectives

AuNPs of 5–10 nm have mass at the million-Dalton level that is comparable to many macromolecules. However, they are usually not considered molecules. In fact, their exact structure and composition are not completely clear yet [93]. Each individual AuNP can be considered as a condensed phase of gold instead. Therefore, AuNPs are not considered a molecular "species", even in its absolute monodispersed colloidal. Colligative properties of AuNP dispersions based on the number of AuNP in the dispersion have not been reported due to the extremely small "molarity" (multiple of Avogadro's constant AuNP per liter) of AuNP. With the industrialization of AuNPs, the quantitative analysis of the number of AuNPs has become more and more important. There are mainly two ways to approach the quantitative analysis. One is to directly count the numbers of AuNP by MS, spectrophotometry, or light scattering methods. The other way is a combination of size characterization methods such as TEM and chemical analysis methods such as amperometry and ICP–MS. Both of these approaches are based on current materials analysis methods that can be used for the characterization of AuNPs over 1–100 nm. Direct counting methods are straightforward, but have limitations in the range of AuNP diameters. Neither MS nor CNC can cover the whole range from 1 nm to 100 nm. Chemical analysis of gold provides the lump-sum of gold atoms as a whole. However, the existence of gold Au(III) ions and complex compounds interfere with the results. Other methods are required to characterize the average size of the AuNP and size distribution.

While analytical chemistry has been established and developed during the past century, tremendous work and new methods are needed in the area of gold nanoparticle analysis. On the one hand,

new protocols of sampling and calibration will be developed specifically for AuNP based on existing instrumental methods or wet chemistry. The protocols and the methods may vary depend on the average size of AuNP, size distribution, and the methods that will be used. One the other hand, new techniques that can directly characterize the number of AuNP may be discovered. Either way, the quantitative AuNP analysis may further extend to new areas or may develop new physical or analytical concepts based on the colligative properties of the AuNP.

Acknowledgement

This work is supported by Rowan University Non-Salary Financial Support Grants.

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