THE CHEMISTRY OF GOLD CLUSTERS IN PLASMA GENERATED WITH MALDI, LASER DESORPTION IONISATION AND LASER ABLATION FROM VARIOUS PRECURSORS

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1. Introduction

Nanotechnology is a rapidly growing science of producing and utilizing among others nano-sized particles. Their unique size-dependent properties make them superior and indispensable as they show unusual physical, chemical and biological properties. Nanomaterials are already having a huge impact on nanotechnology and nanomedicine. Nano-materials are present in toothpaste, sunscreens, sanitary ware coatings, medicaments, and even in food products but they can also represent health risks, like for example silver.

Generally, the behavior of metal particles with well-defined nanostructures has become one of the most active research areas. The inorganic nanoparticles exhibit significantly distinct physical, chemical and biological properties from their bulk counterparts. Discoveries in the past decade have demonstrated that the electromagnetic, optical and catalytic properties of noble-metal nanoparticles are strongly influenced by shape and size. Nanogold represents a tiny particle from a few gold atoms up to hundreds/thousands of atoms. This has motivated an upsurge on research on the synthesis routes that allow better control of shape and size for various nano-biotechnological applications.

Gold nanoparticles (GNP) are quite important in electronics, bio-analytics and in nano-medicine. They are produced by various technologies, mostly by chemical methods but also by laser ablation.

1.1. Short historical overview

GNP have been known for a long time but they were called colloidal gold. The history of GNP goes back to ancient times, for example, the so-called “Elixir of Life”, searched by alchemists, was a potion made of gold. Colloidal gold was used since Roman times to colour glass in yellow or red and famous bohemian ruby glass is also based on nano-gold. Paracelsus in the 16th century created a potion called Åurum Potabile, Herschel in 1842 used colloidal gold to record images on paper, etc. but Faraday in 1857 was perhaps the first one to understand that the colour of gold solutions was due to the small size of gold particles.

1.2. Applications of GNP in medicine

As nano gold is quite reactive, the gold particles are usually covered with a suitable layer of inorganic and organic material (Fig. 1), e.g. with thiols, sugars, etc. Such functionalized GNP are stable and are used in bioanalytics and biomedicine.

Most recently gold colloids have gained significant attention in the field of biomedicine. Due to their several exclusive properties, metallic gold acquired an excellent profile for use in these biomedical applications. Gold nanoparticles have a great impact in the medical society and they were used in cancer diagnosis, treatment and as a drug delivery vector for biological or pharmacological agents.

GNP have been applied as a drug carrier in diagnostic and medical fields as they comprise some explicit properties such as i) they are easily fabricated ii) they have a very good capacity to bind to target cancer cells, carbohydrates, antibodies, proteins, pharmacological agents rather than other nanoparticles.

It was recently reported that GNP can be used to detect some toxic metals like mercury in human body and in environmental bodies such as rivers, streams, lakes and oceans. When mercury contaminated fish and shell fish are ingested by children and women, it is dangerous to their health. Even if there are several conventional methods, a sensitive and accurate colorimetric method was developed which can detect mercury at nano-molar level by using DNA-functionalized gold nanoparticles.

During the last decade, fluorescence based assays have gained a prior position in immunochemistry-based in vitro diagnosis and in vivo imaging assays. In this field, radio chemical labels and chromophores have several advantages such as high sensitivity and potential for easy multiplexing. But these traditional radio chemical labels and organometallic chromophores were replaced by Au-QDs, where QDs are quantum dots, because of their exclusive characteristics including size dependent controllable emission spectra, narrow band width, broad excitation, high extinction coefficient and potential stability against photobleaching.
The combination of GNP and poly-amidoamine (PAMAM) dendrimers as capping ligands to biomolecules has recently been reported\textsuperscript{22}. So these AuQDs are suitable for biological applications. These dendrimers can be covalently or electrostatically conjugated to DNA\textsuperscript{23−24}, RNA\textsuperscript{25}, carbohydrates\textsuperscript{26} and proteins\textsuperscript{27−28}. PAMAM dendrimers have been proven as excellent cell transfection agents as they contain hydroxyl or amino groups at their end groups. So with the combination of a biologically inert material such as gold, along with good biological compatibility, the dendrimers penetrate into the cell membranes easily. PAMAM could efficiently conjugate with various biomolecules like DNA, proteins, RNA and carbohydrates.

1.3. Mass spectrometry of GNP

Among various methods used to study and characterize GNP, mass spectrometry is quite important. However, the ionization of GNP represents the major problem. It was reported\textsuperscript{29} that GNP are mostly decomposed during the ionization process and to obtain correct information about the size and the molecular weight of GNP is quite difficult. Recently, it was shown in MALDI that using 2-(4-hydroxyphenylazo) benzoic acid (HABA) matrix\textsuperscript{30} the real size of even very high Au clusters can be detected. Another technique to be considered is electrospray ionization mass spectrometry\textsuperscript{31} or visible-MALDI with tunable laser\textsuperscript{2}.

Successful MALDI analysis of GNP with HABA concerning chemically covered GNP, such as in Fig. 1 has also been reported in the literature\textsuperscript{32,33}. Mass spectrometric analysis of gold nanoclusters has also been reported in the literature\textsuperscript{2}. In this paper we are studying "naked" GNP, not covered with any ligand, and trying to develop a reliable approach to analyze such particles. We are studying the possibilities of TOF to characterize GNP in plasma and, to understand their plasma chemistry. Gold clusters are generated either by the use of matrices (MALDI) or by laser desorption ionization or ablation.

2. Experimental

2.1. Chemicals

Gold (III) chloride trihydrate, gallic acid, 2-(4-hydroxyphenylazo) benzoic acid (HABA), citric acid and nanodiamond powder (< 10 nm, purity 95 %) were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium azide and sodium hydroxide were from Merck (Darmstadt, Germany). The matrices α-cyano-4-hydroxycinnamic acid (CHCA), 2,5-di-hydroxybenzoic acid (DHB), sinapinic acid (SPA) and peptide mixture (Peppmix) for calibration were purchased from Bruker Daltonics (Bremen, Germany). Calmix (mixture of bradikinine, angiotensine I, renin, and adrenocorticotropic hormone) from Sigma Aldrich (Steinheim, Germany) was used in an AXIMA for external calibration. All other reagents were of analytical grade purity.

Water used to prepare all solutions was double distilled from the quartz apparatus by Heraeus Quartzschmelze (Hanau, Germany).

2.2. Instrumentation

Mass spectra were obtained either using a MALDI-TOF Auto-flex mass spectrometer (Bruker Daltonics, Bremen, Germany) or an AXIMA Shimadzu of Kratos (Manchester, U.K.). Mass spectrometers were equipped with delayed extraction, 337 nm nitrogen laser. Bruker spectrometer was provided with a hydrophobic coating target (Anchor Chip 600/384, Bruker Daltonics, Bremen, Germany). The accelerating voltage was set to 20 kV and laser repetition rate to 16 Hz. Mass spectra were obtained using linear positive or negative ion modes with delayed extraction. The mass spectra were accumulated for approximately 50–100 shots per preparation in total.

2.3. Synthesis of GNP

We have produced GNP from HAuCl\textsubscript{4} salt using various reducing agents like hydrogen peroxide, azide, citric\textsuperscript{36,37}, gallic or humic acids. The synthesis of GNP from citric acid was done in the following way: an aqueous solution of citric acid (2 ml 1 % solution) was mixed with 2 ml of acetone. The resulting mixture (2 ml) was diluted up to 4 ml with water. A solution of 0.25 mM Au(III) chloride (40 ml) was transferred to a clean 60 ml baker and heated in a water bath. When the temperature reached 80 °C, 4 ml of the reducing mixture (0.25 % citric acid in 25 % acetone) was added (pH of the mixture was 3) and stirred vigorously. After 10 min of continuous heating and stirring, the color of solution turned to wine red. It was the clear indication of gold nanoparticles formation. GNP were also synthesized by the reduction of Au (III) by gallic acid and the details are given elsewhere\textsuperscript{38}.

2.4. Samples preparation

The matrix solutions were prepared in the following way: CHCA (0.3 mg ml\textsuperscript{−1} in acetone-ethanol 2:1); DHB (0.16 mg ml\textsuperscript{−1} in acetonitrile); SPA (1 mg ml\textsuperscript{−1} in 70 % aqueous acetonitrile); and HABA (1 mg ml\textsuperscript{−1} in 70 % aqueous acetonitrile).

On the MALDI target plate 0.5−1 µl of matrix solution followed by 0.5−1 µl of sample was deposited. In LDI and LA modes, no matrices were used.

3. Mass spectrometry of gold clusters

3.1. Analysis of gold clusters with laser desorption ionization and TOF MS

GNP produced in aqueous solution were then ionized using a nitrogen laser 337 nm either (i) with the help of a matrix (MALDI mode) or (ii) via laser desorption ionization. Under linear and reflectron modes and dependent on the laser energy, single charged gold clusters Au\textsubscript{n} (\textit{n} = 1−55) were observed both in positive and negative ion modes. In addition, depending on the precursors used, various gold clusters like Au\textsubscript{n}Na\textsuperscript{m} (\textit{m} = 1−6) adducts, Au\textsuperscript{2+}, Au\textsubscript{2}H\textsuperscript{2+}, Au\textsubscript{2}H\textsubscript{2}O\textsuperscript{+}, Au\textsubscript{3}H\textsuperscript{+}, AuK\textsuperscript{+}, AuCa\textsuperscript{+} etc. were identified in the plasma (Fig. 2).
Using common matrices, like dihydroxycinnamic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA) and sinapinic acid for MALDI, no high clusters known from the literature were observed. Most probably high Au clusters are broken down by the action of the laser.

An example of LDI mass spectrum is given in Fig. 3, where GNP are synthesized by the reduction of HAuCl₄ and citric acid. The results in negative ion mode were similar to those obtained in the positive one.

A part of the mass spectrum is given in Fig. 4, where GNP were synthesized by the reduction of HAuCl₄ by gallic acid. The highest cluster we have detected here was Au₀₆. Negative ion mode results were similar to the results of positive mode.

3.2. Examination of matrices to ionize GNP

Different matrices to ionize GNP were examined. When DHB matrix was applied and using a laser pulse intensity threshold approach, we have been able to suppress, to some extent, nanoparticles fragmentation. Good results were also obtained by using DHB matrix. Additionally, the use of ND as a matrix for analysis of GNP was examined. Only several low gold clusters (n = 1–5) were observed. However the formation of several mixed Au-C clusters such as C₃Au, C₅Au, C₆AuH₂, C₇Au, C₈Au, C₉AuNa, C₁₀AuNa₂, C₁₁AuNa₄, C₁₃AuNa₄ and C₃₁Au were detected.

A part of LDI mass spectrum measured in the positive ion mode is given in Fig. 5. The spectrum is showing the formation of carbon-gold mixed clusters with good resolution and intensity.

Good agreement between model and experimental spectra was found (Fig. 6).

3.3. The use of GNP as a matrix for MALDI analysis

The use of GNP as a matrix was tested near the limit of the MALDI TOF MS detection (1 fmol) for the ionization of the organic molecule Huperzine A³⁹ (Hup A), known as anti-Alzheimer drug. It was observed that the use of the GNP as a matrix increases the signal of Huperzine A 100 times. The formation of adducts like Auₖ(Hup A)ₙ in the plasma phase...
was also detected. GNP were also examined as a matrix for ionization of mycotoxins (deoxynivalenol). However, the sensitivity was low and the procedure needs optimization.

4. Structure of gold clusters

Gold clusters show different structures. Low clusters are planar (Fig. 3). Recently, both compact and hollow gold cages which are fullerene like structures were reported\(^{40,41}\). An example of compact and hollow cage structures is given in Fig. 7.

Gold cluster cations have planar structures for \(n = 3−7\) and starting from \(n = 8\), they form slightly distorted 3-dimensional structures\(^{40,41}\). On the basis of quantum-chemical calculations, it has been reported that medium-sized gold clusters, such as \(\text{Au}_n\) with \(n = 32−35\), 42 and 50 also have cage-like structures\(^{42}\). Gold, having special properties, is the only metal which can form hollow cages\(^{43}\). Even though it has some unusual structural characteristics, gold clusters have become particularly attractive due to their diverse catalytic properties. In the case of hollow gold cages, clusters with 16–18 atoms have been experimentally observed and larger clusters, such as icosahedral \(\text{Au}_{12}\) were theoretically predicted\(^{44}\).

5. Conclusions

Common MALDI TOF MS instrumentation can be used to analyze gold species ionized in plasma by laser from GNP synthesized by chemical procedures.

Gold is forming in plasma positively and negatively single charged clusters \(\text{Au}_n^+\) or \(\text{Au}_n^-\) which are also reacting with the other ions and mixed \(\text{Au}_m\text{Na}_n\), \(\text{AuCa}\), \(\text{AuK}\), etc. clusters were also observed. The formation of gold hydrides, such as \(\text{AuH}\), \(\text{AuH}_2\), and hydroxide \(\text{AuOH}\) and peroxides \(\text{AuO}_2\), were also observed here for the first time.

When GNP were used as a matrix for ionization of nanodiamonds, carbon-gold mixed clusters which includes \(\text{C}_n\text{Au}\), \(\text{C}_n\text{Au}_2\text{Na}\) and \(\text{C}_n\text{Au}_3\text{Na}\), and others were detected.

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**Fig. 7. Structure of compact magic gold cluster \(\text{Au}_{20}\) (A, adapted from\(^{46}\)) and hollow golden cage \(\text{Au}_{16}\) (B, adapted from\(^{33}\)).**

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**References**

Rev. 54, 631 (2002).


