

Mass Spectrometry

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Analysis of Surface Bound Organic Ligands on Gold Nanomaterials Using Direct Sample Analysis –Time of Flight Mass Spectrometry

Introduction

In recent years, nanomaterials modified with organic molecules, have been used in various technologies including nanomedicine, biosensing and smart nanomaterials. Nanoparticles can be modified with an organic monolayer which often contains functional groups that act

as hooks onto which, biomolecules or other organic molecules can covalently attach.

The surface coating of nanoparticles with these organic ligands can provide distinct properties such as improved wettability, stability, and solubility of the particles. For example, a polar surface coating gives high aqueous solubility and prevents nanoparticle aggregation. In biological applications, nanoparticles are commonly coated with polyethylene glycol polymer since it increases their circulation time in the body and they experience less non-specific interactions compared to the unmodified nanoparticles^{1,2}.

Characterization of modified nanomaterials is essential for improvement in quality control assessment and process efficiency. Various spectroscopy techniques including infrared spectroscopy, and X-ray photoelectron spectroscopy have been used for surface characterization of nanomaterials,^{3,4} however, these lack specificity in identification compared to analytical technologies using high resolution accurate mass spectrometry (MS). Techniques such as matrix assisted laser desorption (MALDI) Time-of-Flight (TOF) mass spectrometry technology, which provide high resolution and mass accuracy, have also been used⁵. However, the MALDI-TOF MS is limited by a suitable choice of matrix for analysis of organic capped nanomaterials, inhomogeneous distribution of sample in matrix and matrix interference at the low masses (<300 Da).

In this study, we explore the use of ambient ionization TOF mass spectrometry, a technique that ionizes analytes in ambient conditions, to characterize the surface of nanomaterials. We used the Direct Sample Analysis (DSA™) system, an ambient ionization source which uses a “field free” corona discharge electron Reagent Ion Generator (RIG). The modified nanoparticles are layered on a steel mesh and then exposed to the source set at 350 °C for a few seconds. The organic capped ligands are released from the nanoparticles due to thermo-lability of the covalent bonds. The temperature of the source is not sufficient to volatilize the metallic core of the particles themselves. The released organic molecules ionized by the DSA source then enter the mass spectrometer and are detected with a high-resolution time-of-flight mass spectrometer delivering a mass accuracy of ≤ 2 ppm measured at m/z 1000. Using the accurate mass and isotope profile information provided by the TOF along with powerful visualization software tools, we were able to confirm the presence of the different types of ligands attached to nanoparticles. The DSA-TOF-MS offers an alternative technology for rapid analysis of organically capped nanoparticles without any sample preparation.

Experimental

All nanomaterials were obtained from a single manufacturer (Nanocomposix, San Diego, CA). The PerkinElmer DSA source was used as the ambient source for this study. The DSA was mounted on a PerkinElmer AxION™ 2 Time of Flight (TOF) mass spectrometer. The DSA source was maintained at a temperature of 350 °C during analysis. Mass spectra were acquired using the DSA controller software™, and the AxION Solo™ software was used for data analysis. The samples were spotted on the mesh for analysis. The total amount of nanomaterial loaded on the mesh was between 2-5 ug per sample.

Results

We analyzed gold (Au) nanomaterials capped with commonly used organic ligands such as lipoic acid and Cetyl trimethylammonium bromide (CTAB) that can then serve as linkers for other molecules. The lipoic acid modified gold nanoparticles were manufactured by reducing the disulphide bond in lipoic acid with dithiothreitol prior to attachment with Au nanoparticles. The free thiol groups in the reduced lipoic acid bind to the gold surface resulting in a strong covalent bond. Analysis of the lipoic acid Au nanoparticles in negative mode showed the $[M-H]^-$ ion of lipoic acid suggesting the lipoic acid

was released from the particles due to thermo-lability of the thiol-Au linkage (Fig. 1). The $[M-H]^-$ ion showed mass accuracy within 1 ppm and isotope profile within 2% of the expected value of lipoic acid, thus confirming presence of the ligand. The released lipoic acid is in its oxidized form due to the oxidizing environment of the DSA source.

Analysis of CTAB modified gold particles in positive mode showed the $[M+H]^+$ ion and the ion resulting from loss of a methyl group $[M-CH_3+H]^+$ (Fig. 2).

The standard solution of branched polyethyleneimine (BPEI) that was used to modify the gold nanomaterials was analyzed by DSA-TOF. The spectrum of BPEI was characteristic of a polymer containing repeating units (Fig. 3). The repeating units differed by 43.042 Da corresponding to the elemental composition of $[CH_2CH_2NH]$ thereby confirming the presence of BPEI. The nanoparticles were modified with BPEI using lipoic acid as the linker. Gold was covalently bound to the lipoic acid, the free carboxyl group of lipoic acid was then coupled to the terminal amine group of BPEI resulting in an amide bond (Fig. 4). The Au modified BPEI analyzed by DSA-TOF resulted in a spectrum similar to the standard BPEI (Fig. 5). However, a detailed examination of the spectrum also showed ions corresponding to lipoic acid-BPEI conjugate ions. The extracted ion chromatograms (EICs) of some of the major BPEI ions conjugated to lipoic acid (resulting in loss of water molecules) were observed only in the modified gold nanomaterials but were absent in the control BPEI standard and the Au particles (Fig. 6). Amide bonds such as the one linking BPEI to lipoic acid are thermo-labile in nature which may explain the reason for BPEI being the predominant ions in the mass spectra instead of the BPEI-lipoic acid conjugates.

The DSA-TOF provides rapid analysis without involving any extensive sample prep which makes it a suitable technique for quick quality control assessment of the modified nanomaterials.

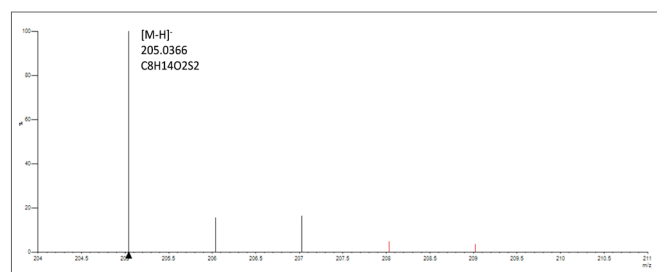


Figure 1. Spectrum of gold-lipoic acid nanoparticles analyzed in negative mode confirms presence of lipoic acid based on accurate mass (<2 ppm) of the monoisotopic mass and isotope profile (<6% of theoretical).

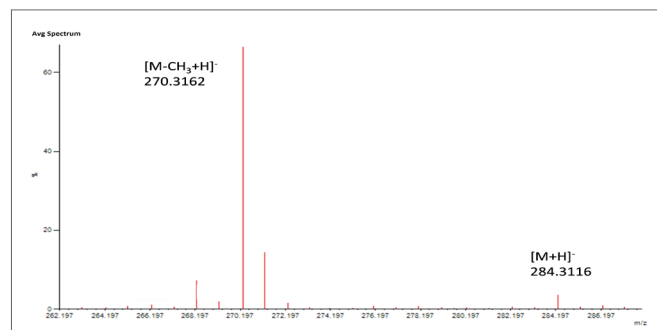


Figure 2. Spectrum of gold-CTAB acid nanoparticles analyzed in positive mode shows the $[M+H]^+$ ion corresponding to CTAB moiety and the ion showing loss of a methyl group from CTAB $[M+H-CH_3]^+$.

Fig. 7 shows the spectra obtained for BPEI conjugated gold nanoparticles from two different lots. The spectra from Lot 2 shows the classic BPEI polymer pattern unlike Lot 1 suggesting Lot 2 to be successfully modified by the ligand.

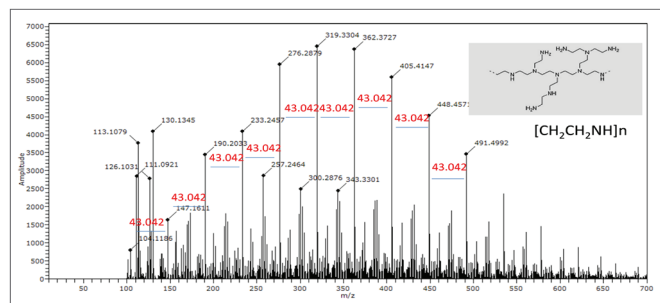


Figure 3. Spectrum of standard BPEI polymer solution shows repeating unit of 43.042 units corresponding to the elemental composition of $[\text{CH}_2\text{CH}_2\text{NH}]_n$.

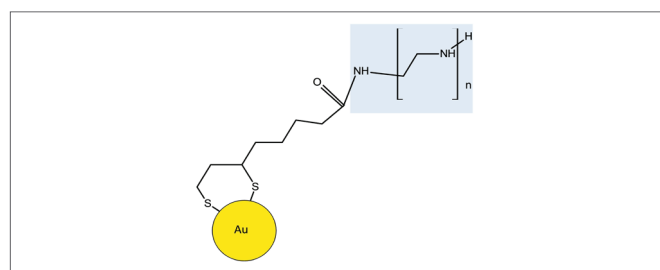


Figure 4. Schematic shows BPEI is attached to the lipioic acid-Au through an amide linkage. The BPEI moiety of the molecule is highlighted in blue.

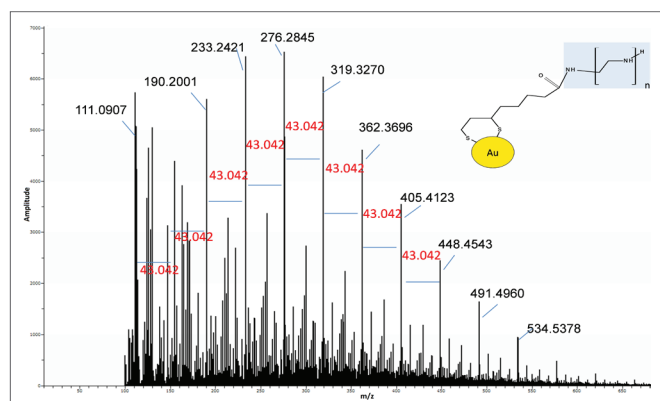


Figure 5. Spectrum of Au-lipioic acid-BPEI nanoparticles was similar to the BPEI standard suggesting BPEI was bound to the Au.

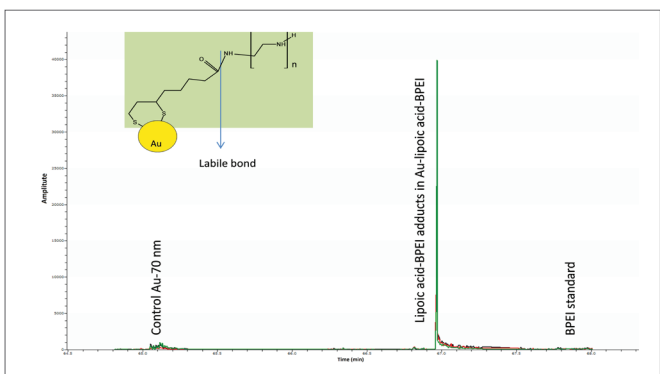


Figure 6. Overlay of EICs of m/z 421.2777, 464.3199, 507.3621 corresponding to the conjugates of the predominant ions of BPEI (m/z 233, 276, 319) with lipioic acid (minus water) were observed only in the Au-lipioic acid-BPEI sample and not in the negative controls (Au particles and BPEI standard). This suggests that BPEI is bound to AU via lipioic acid.

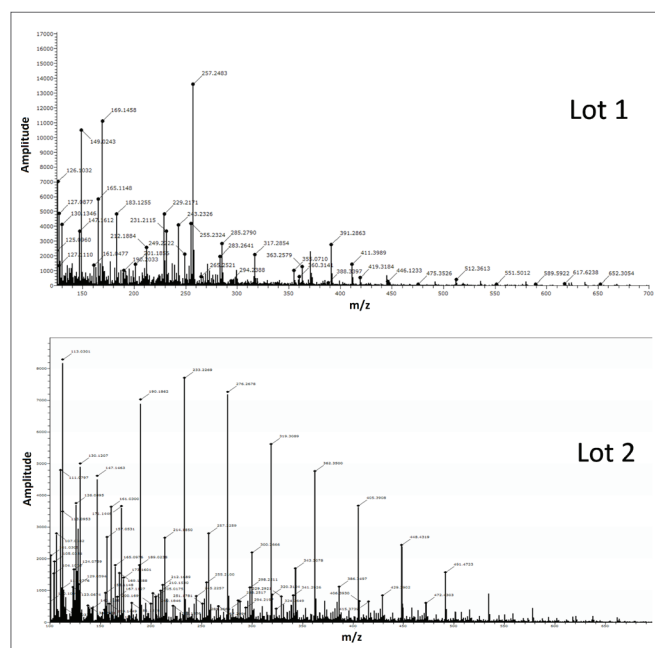


Figure 7. Two different lots of BPEI-lipoic acid-Au nanomaterials were analyzed by DSA-TOF (2 µg of each sample). Lot 2 showed the entire mass spectral profile of BPEI polymer, unlike Lot 1, suggesting it was successfully modified by BPEI.

Besides accurate mass and isotope profile information provided by the TOF mass spectrometer, powerful software visualization tools using the AxION Solo software can help to easily identify the target analytes in large batches of samples or in nanoparticles containing mixtures of capped ligands. For instance the green color coding depicted in Fig. 8 shows the presence of dodecanethiol dimer in the dodecanethiol capped gold nanoparticles and in the mixture of nanoparticles containing both dodecanethiol and CTAB conjugated to the gold surface but not in the negative controls color coded in grey (gold only nanoparticles and CTAB capped gold nanoparticles).

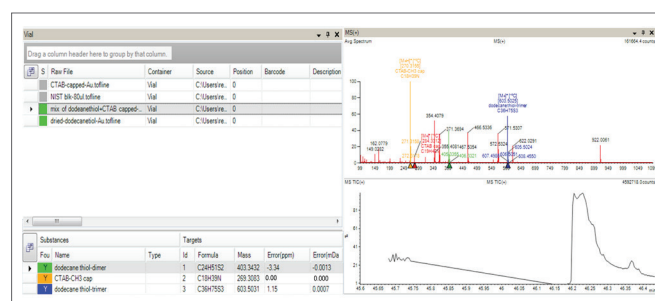


Figure 8. AxION Solo software shows presence of dodecanethiol (dimer and trimers) indicated in green color in the sample containing mixture of Au capped with dodecanethiol and Au capped with CTAB and also in the sample containing Au capped with dodecanethiol (top left hand corner of panel). The absence of the target in the negative controls (Au particles and Au capped with CTAB) is indicated in grey. The bottom left hand corner of the panel indicates all the target analytes along with the mass accuracy information for the selected sample which contained the mixture of capped ligands. The spectra for this mixed sample is shown in the top right hand corner.

Conclusion

The DSA-TOF provides a rapid analysis tool for quality assessment of the characterization of organic capped nanomaterials. Using accurate mass and isotope profile information provided by the TOF, along with powerful visualization software tools, we were able to confirm the presence of the different types of ligands attached to nanoparticles. Besides just identifying and confirming one type of organic monolayer covalently bound to the nanoparticle, we were also able to identify bilayers wherein, one organic monolayer is covalently modified with a second type of organic ligand.

References

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