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The Determination of Ligand Shell Composition of Bifunctional Alkanethiol-capped Gold Nanoparticles using GC/MS/MS

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Abstract

A methodology for the determination of two-component alkylthiol ligand shell composition of 2 nm gold nanoparticles by GC/MS/MS is presented. Nanoparticles were prepared with ligand shells composed of 1-dodecanethiol and 11-mercapto-1-undecanol with reaction solution ratios of the OH-terminated component of 25, 50 and 75%. The nanoparticles were decomposed in the presence of iodine and the resulting disulphide mixtures were analysed by GC/MS/MS. An efficient calibration strategy is shown to be effective in providing results comparable to an established ^{1}H-NMR analysis method, while minimizing thiol and nanoparticle consumption. The presented method provides an accessible and highly general option for the characterisation mixed monolayer-capped gold nanoparticles containing specialized ligands.

Introduction

Mixed-monolayer protected gold nanoparticles (AuNPs) are choice candidates for a variety of biological sensing, imaging, and cell-targeting applications by virtue of the ability to tune chemical recognition, biocompatibility, solubility, and other required properties[1-4]. For similar reasons, they have much to offer in many applications in materials science where precise control over nanoparticle interactions is required[5-7]. In many ways, it is the versatility of the mixed-monolayer coatings themselves that are central to the appeal of using AuNPs in both biological and materials applications[4, 8, 9]. The evaluation of the efficacy of such AuNP platforms often requires an accurate characterisation of the nanoparticle surface chemistry. Previous studies have indicated, however, that the ratio of different functional ligands used in the synthesis of the nanoparticles does not necessarily reflect the final ratios obtained in the ligand shell[10, 11], which necessitates methods for the accurate characterization of ligand shell composition. Quantifying the ratio of ligands on multifunctional nanoparticles has proven a challenge; where many techniques are capable of determining the presence of functional groups at the nanoparticle surface, accurate quantitation is not always possible[12]. Zhou et al. have used FTIR to estimate the relative amounts of different ligands within the ligand shell, however, this required comparison with another technique for calibration[10]. Similarly, nuclear magnetic resonance (NMR) spectroscopy has been used confirm the presence of different functionalities on the gold surface[13], yet the significant line broadening observed in 1H-NMR of nanoparticle ligand shells, and potential for interference from other functional groups and from the solvent limits the accuracy in quantifying ligand ratios. This problem has been circumvented somewhat through oxidative cleavage of the thiol ligands from the gold surface by exposure to I_2, which results in the formation and detection of disulphides[10, 11, 14, 15]. Assuming that all ligands are completely removed from the gold surface and that no ligands/functionalities are destroyed by side reactions, peak integration is proportional to the relative concentrations of the ligands on the gold nanoparticle. Although the peaks measured from disulphide are very sharp, they are often split into multiple peaks and are thus prone to interferences from overlapping resonances either in the ligand shell or the solvent. Iodine decomposition of gold nanoparticles has also been paired with other spectroscopic techniques (e.g. GC/FID[16]) in order to characterize the nanoparticle ligand shells. Zhou *et al.* have reported that by pairing the iodine decomposition of thioctic acid ligands on gold nanoparticles with high performance liquid

chromatography/mass spectrometry/ultraviolet/chemiluminescent nitrogen detection (HPLC/MS/UV/CLND) they were able to not only determine the relative concentrations of the different ligands, but to quantify the absolute amount of each ligand within a nanoparticle sample[10]. The thioctic acid ligand provides a particular advantage in the absolute quantitation of multifunctionalized ligand shells via I_2 decomposition, however, as the disulphide product that forms is equivalent in structure to the original ligand. In contrast, the product of thiol-protected gold nanoparticle decomposition ultimately yields a mixture of disulphides (Figure 1). While this complicates the quantitation of multi-functional thiol-protected nanoparticle shells, the prevalence and cost of thiol-capped nanoparticles today necessitates the development of additional accessible methods with minimal sample destruction. Furthermore, traditional methods of quantitation require calibration curves of each product of decomposition, and given that many applications of thiol-protected gold nanoparticles require the use of specialized thiols, methods that minimize thiol destruction during calibration offer a significant advantage.

In this paper we report the analysis of iodine-decomposed gold nanoparticles capped with mixed ligand shells containing both methyl- and hydroxyl-terminated alkanethiols by gas chromatography/mass spectrometry (GC/MS/MS). A one-pot calibration strategy is employed, and its merits and limitations are demonstrated. The methodology is demonstrated to provide an accessible and highly general alternative to NMR and other spectroscopic techniques.

Results and Discussion

Gold nanoparticles were synthesized by adaptation of the Brust-Schiffrin method, where predetermined ratios (25, 50, and 75%) of 11-mercapto-1-undecanol to 1-undecanethiol were added in order to vary the final ligand shell composition (herein we refer to these nanoparticles as 25, 50, or 75%-OH AuNPs). Transmission electron microscopy was used to assess the nanoparticle core size and shape (Figure 2). Iodine-decomposition of the nanoparticle samples were analysed by GC/MS/MS of the resulting disulphide mixtures (Figure 1). The peaks corresponding to compounds **I-III** were identified by GC/MS and there were no peaks found to be indicative of any remaining, unreacted thiol. The most prominent ion of each disulphide (*m/z* 374 for **I**, *m/z* 462 for **II** and *m/z* 185 for **III**) was selected for collection and analysis by GC/MS/MS (Figure 3). By comparing the peak heights (S) of the three disulphide products, the ratio of hydroxyl-terminated thiol (OH-thiol) was calculated according to equation 1.

$$\%OH\ detected = \frac{S_I + \frac{1}{2}S_{II}}{S_I + S_{II} + S_{III}} \times 100\% \qquad \textbf{(1)}$$

Figure 1: Products of the iodine-decomposition of methyl- and hydroxyl-terminated alkanethiol-capped AuNPs, post trimethyl silyl (TMS) derivatization.

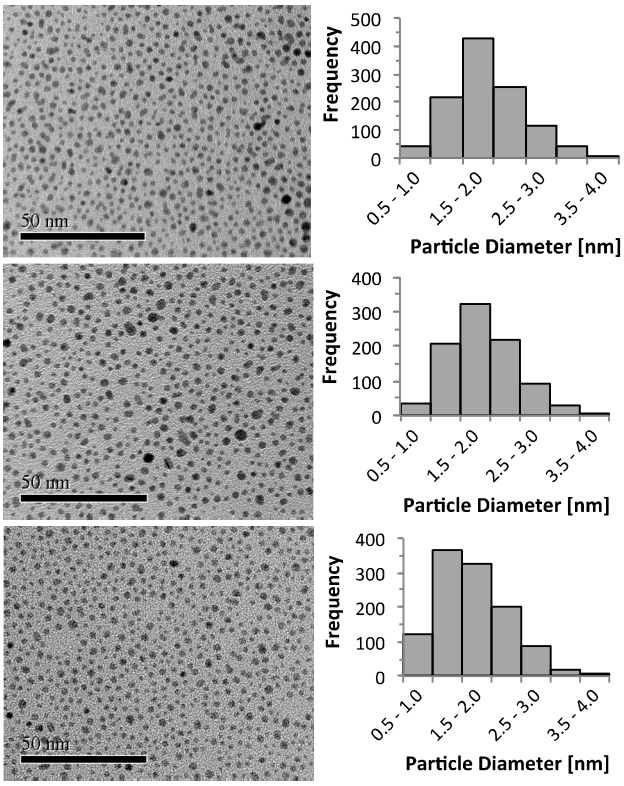

Figure 2: TEM images (left) and size distributions (right) of 25%-OH (top), 50%-OH (middle), and 75%-OH AuNPs (bottom).

The distinct peaks obtained suggest that products I, II and III sufficiently account for each disulphide and their expected relative retention in the column. We performed a straightforward and efficient calibration of the %OH-thiol detected by GC/MS/MS for disulphide mixtures prepared from a one-pot reduction of defined mole ratios of thiol precursors (Figure from these disulphide mixtures was generated and used to predict the actual %OH-thiol in the AuNP ligand shells (Table 1). We speculate that the sigmoidal shape arises from the change in distribution of disulphide products I-III with changing composition of the reaction mixture.

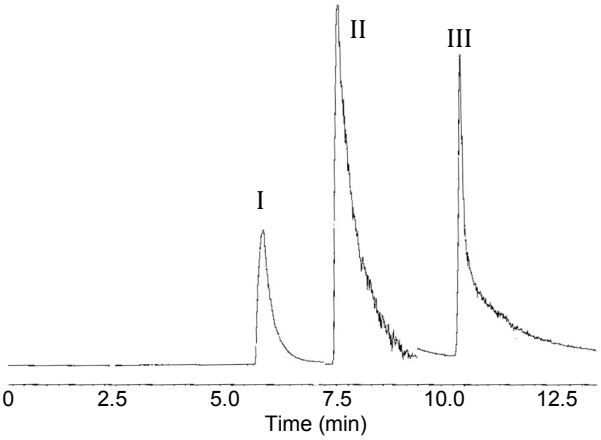

Figure 3. GC/MS/MS spectra of disulphide products from the decomposition of AuNPs synthesized from a 75% OH-terminated thiol composition.

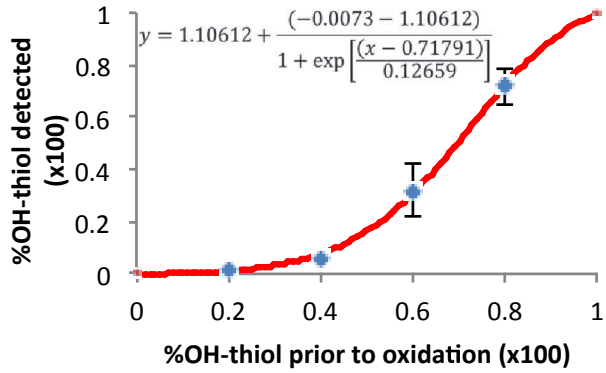

Figure 4: Sigmoidal fit of the GC/MS/MS peak ratios obtained from the dimerization reactions of known ratios of thiols. The adjusted R-squared value for this fit is 0.99856. The error bars indicate the standard deviation of independent samples run in triplicate.

Table 1: Core diameters (with standard deviation) of the bifunctional gold nanoparticles used in this study as well as ligand shell composition as determined by ^{1}H-NMR analysis of the undecomposed AuNPs, ^{1}H-NMR and GC/MS/MS analysis of the disulphide product of iodine-decomposed AuNPs. Uncertainties are presented at the 95% confidence interval.

% OH-thiol used in AuNP synthesis	AuNP core diameter (nm) [s.d]	% OH-thiol by ^{1}H-NMR of AuNPs	% OH-thiol by ^{1}H-NMR of disulphide	% OH-thiol by GC/MS/MS of disulphide	% OH-thiol by GC/MS/MS of disulphide using calibration curve
25	2.0 [0.6]	22	24	3 ± 3	28 ± 10
50	1.7 [0.5]	79	68	53 ± 1	71 ± 4
75	1.9 [0.7]	74	70 ± 4	60 ± 2	74 ± 1

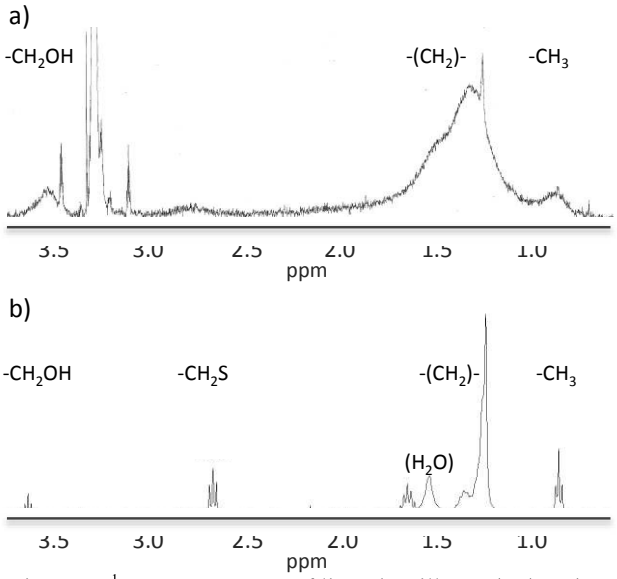

Figure 5: ^{1}H-NMR spectra of ligands still attached to the nanoparticle core (a), and after I_2 decomposition to disulphide (b) corresponding to 50%-OH AuNPs in d-chloroform and 25%-OH AuNPs in d-methanol, respectively.

For comparision, the gold nanoparticle samples were analyzed by ^{1}H-NMR spectroscopy before and after I_2 decomposition (Figure 5) by following established methods[17, 18]. Comparison of integrated peak areas for the peak at 0.8-0.9 ppm (corresponding to the ω-CH_3) and the peak between 3.5-3.6 ppm (corresponding to the α-CH_2 unit), led to an estimation of the ligand shell composition shown in Table 1. The %OH-thiol in the nanoparticle ligand shell obtained post-decomposition by GC/MS/MS agrees well with the values obtained by NMR analysis. There is less agreement with the values obtained by NMR of the nanoparticle-bound ligands (Figure 5a), primarily due to peak broadening and solvent interference. The one-pot method used to generate the standard solutions allows for a more efficient generation of a calibration curve, with less thiol consumption than if compounds I-III were synthesized and purified to generate three respective curves. The latter method would likely improve the uncertainty of the results and allow for absolute quantitation, however, the method presented is preferable where the thiols of interest are highly specialized and minimal thiol consumption is desired. We note that the sigmoidal calibration curve generated from these standard solutions generates greater uncertainty in thiol compositions less than 40% and greater than 90% OH-terminated thiol by virtue of low signal dependence (slope) upon %OH-thiol in those regions. However, at moderate compositions, we obtained comparable or improved precision than the results obtained by NMR after decomposition.

The final compositions of the ligand shells obtained are in good agreement with previous studies which have demonstrated how differential solvation of the ligands, controlled with choice of solvent, can lead to final ligand shell compositions that are biased toward one ligand over the other[10, 11]. In particular, OH-terminated thiols have been shown to be preferentially adsorbed over CH_3-terminated thiol during the synthesis of gold nanoparticles is performed in toluene.

As this is a destructive method, we sought to evaluate its accuracy when smaller samples of gold nanoparticles are used in the decomposition reaction. Care was taken to maintain the solvent environment when scaling down the amount of I_2 in the reaction, by maintaining the total volume of dichloromethane (800 µL) and ethanol (200 µL) present in each reaction vessel. Furthermore, the dried disulphide samples were dissolved in 200 µL dichoromethane prior to TMS-functionalization to pre-concentrate the solutions for

GC/MS/MS analysis. Table 2 lists the results of 0.1 mg to 1 mg samples of the 75% OH AuNPs after I_2 decomposition and analysis by both GC/MS/MS and ^{1}H-NMR. These results indicate that the GC/MS/MS method yields comparable accuracy as ^{1}H-NMR after the destruction of smaller quantities of AuNPs and there is less agreement between them. These results reflect the fact that such small samples are more prone to handling errors as these values as both methods are likely approaching their limit of detection for the total disulphide content in the case of NMR, and for the separated products I-III in the case of GC/MS/MS.

Table 2: Comparision of GC/MS/MS and ^{1}H-NMR analysis of decomposed 75% OH-AuNPs.

Amount of AuNPs decomposed (mg)	% OH-thiol by ^{1}H-NMR	% OH-thiol by GC/MS/MS
1	70 ± 4	74 ± 1
0.5	71	77
0.1	58	67

Experimental

Materials and methods

Gold(III) chloride trihydrate (>99.5%), tetraoctylammonium bromide (98%), sodium borohydride (≥ 98.5%), 1-undecanethiol (98%), 11-mercapto-1-undecanol (97%), acetonitrile (HPLC grade), chloroform (HPLC grade), and dichloromethane (HPLC grade) were obtained from Sigma-Aldrich Canada. Toluene (99.5%) was obtained from Caledon Laboratory Chemicals. Ethanol (anhydrous) was obtained from Commercial Alcohols Canada. Iodine crystals (99.5%) were obtained from Anachemia Science. *d*-chloroform and *d*-methanol were obtained from Cambridge Isotope Laboratories. Helium (99.999%) was obtained from Praxair Canada. Formvar and carbon-coated 400 mesh Cu transmission electron microscopy (TEM) grids were obtained from Ladd Industries, Inc. All chemicals were used as received. Deionized water with a resistivity of 18.2 MΩ/cm was prepared using an Elga Purelab UHQ filtration system. Derivatization grade N,O-Bis(trimethylsilyl)trifluoroacetamide (Supelco) was obtained from Sigma-Aldrich Canada.

Gold Nanoparticle Synthesis. A modified Brust-Schiffrin synthesis[19] was used to prepare 2 nm gold nanoparticles. Briefly, 30 mL of a 3 mM aqueous solution of hydrogen tetrachloroaurate was added to 80 mL of a 50 mM solution of tetraoctylammonium bromide in toluene. This mixture was stirred until the aqueous phase appeared colourless. A total of 0.84 mmol of 11-mercapto-1-undecanol and 1-undecanethiol with mole ratios of 10:90, 25:75 and 50:50 were added to the reaction mixture and stirred for 3 hours. Next, 25 mL of a freshly prepared 0.4 M aqueous solution of sodium borohydride was added dropwise to the reaction mixture which was then stirred for an additional 3 hours. The organic layer was then collected, washed three times with deionized water and then concentrated to a volume of approximately 2-3 mL using a rotary evaporator. For the 25%- and 50%-OH AuNPs, this concentrated solution was then diluted with 100 mL of anhydrous ethanol and 15 mL of deionized water and stored at 4°C overnight, yielding a black precipitate. The concentrated solution of 75%-OH AuNPs were instead dispersed in 400 mL of acetonitrile and stored at 4°C until precipitation occurred.

Gold Nanoparticle Purification. In order to remove residual thiol, and TOAB the 75%-OH AuNPs were repeatedly dispersed into acetonitrile using a vortexer and then centrifuged, with the supernatant removed between cycles. This process was repeated until no evidence of unbound thiol remained upon ^{1}H-NMR analysis of the nanoparticles. ^{1}H-NMR was performed in $CDCl_3$ on a JEOL JNM-GSX 270 MHZ spectrometer. For the 25%- and 50%-OH AuNPs, residual reactants were removed by size-exclusion chromatography using Sephadex LH-20 with ethanol as the eluent.

Gold Nanoparticle Size Characterisation. Dilute solutions (~ 1 mg/mL) of the nanoparticles in chloroform were spread dropwise at an air/water interface and the solvent left to evaporate for 10 min. Sections of the films were collected on TEM grids and allowed to air-dry. The films were analyzed by TEM on a JEOL 2011 scanning transmission electron microscope operating at 200 keV, equipped with a Gatan 4k x 4k Ultrascan digital camera. The average diameters of the nanoparticle samples were determined from area-

filling measurements using *ImagePro Plus 6.3* (Media Cybernetics Inc.) assuming a spherical core shape, with sample sizes of over 3000 nanoparticles.

Iodine Decomposition of Gold Nanoparticles. Nanoparticle samples were decomposed by adapting established procedures[11,14]. Briefly, approximately 1 mg of nanoparticles was dissolved in 800 μL dichloromethane. 200 μL of a 2.5 mg/mL solution of iodine dissolved in ethanol was added and the mixture was stirred for one hour in a closed vial. After stirring, the clear orange supernatant containing the disulphides was separated and placed under vacuum until dry and colourless (approximately 2-3 hours). The brown precipitate was discarded. The dried disulphide sample was dissolved in $CDCl_3$ and analyzed on a JEOL ESX 400 MHz NMR spectrometer.

Preparation of Standard Solutions for GC/MS/MS Calibration. Fresh stock solutions of the thiols (0.5 mg/mL) were prepared in anhydrous ethanol. Aliquots of these stock solutions were mixed in the predefined mole ratios to yield a total of 200 μg of thiol in ethanol. These solutions were heated to 50°C and 200 μL aliquot of 2.5 mg/mL ethanolic iodine solution was added. The solutions were then stirred at 50°C for 1 hour. Once cool, the disulphide mixtures were placed under vacuum until dry and colourless (2-3 hours), and then analyzed by GC/MS/MS as described below.

GC/MS/MS Analysis of Disulphide Mixtures. Disulphide samples were dissolved in 1 mL dichloromethane and then dosed with N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) to silylate the labile alcohol functional groups (10 μL BSTFA for every 100 μL disulphide solution). The samples were allowed to digest for one hour, and then analyzed on a Varian Saturn 2000 GC/MS/MS coupled with a Varian CP-3800 Gas Chromatograph equipped with a 30 m x 0.25 mm x 0.25 μm, 5% phenyl, 95% dimethyl polysiloxane column. Helium (purity 99.999%) was used as a carrier gas at a flow rate of 1.0 mL/min. Injections (1 μL) were performed at 275°C. The initial GC oven temperature of 240°C was held for 3 minutes and then ramped at a rate of $20°C min^{-1}$ to a temperature of 300°C. This temperature was held until the end of the data collection, which was a total of 13 minutes. The mass/charge ratio (*m/z*) of the selected ions for the monitoring of the disulphides correspond to **I** *m/z* 374; **II** *m/z* 462; and **III** *m/z* 185.

Conclusion

GC/MS/MS applied to the disulphide products of I_2-decomposed thiol-capped gold nanoparticles is a useful alternative for the characterization of multifunctional nanoparticles. The calibration strategy employed requires the use of minimal thiol and the methodology as a whole requires the destruction of few nanoparticles and yields comparable results to those obtained by NMR spectroscopy. In this case, the calibration strategy yielded a sigmoidal plot, which limits the dynamic range to moderate compositions. Nonetheless, by employing a general-purpose column and standard sample preparation techniques, the method is highly general and accessible, while requiring the use of minimal thiol for calibration. The method thus proves an attractive option for the quantitative characterisation of the ligand shell composition for nanoparticle with specialized ligand shells for biological or materials applications.

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