

Fabrication of lipophilic gold nanoparticles for studying lipids by surface enhanced Raman spectroscopy (SERS)[†]

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A simple fabrication method for preparing lipophilic gold nanoparticles (AuNPs) suitable for use as substrates in surface-enhanced Raman scattering (SERS) applications of lipids was developed. The AuNPs were shown to be dispersible within lipid phases, where they greatly increased the SERS signals of lipids.

Conventional Raman spectroscopy has been widely used to characterize the chemical composition of bulk lipids.^{1–3} It has also been used to monitor lipid oxidation in bulk oils based on changes of chemical bond vibrations caused by molecular alterations associated with oxidation reactions.^{4–7} However, Raman signals are intrinsically weak and are thus restricted to bulk oil analysis. Recent advances in the fabrication of noble metal nanoparticles that can greatly enhance Raman signals has led to the development of powerful surface-enhanced Raman spectroscopy (SERS) methods that can sensitively characterize trace amounts of compounds. The weak Raman signatures of analytes adsorbed on or in close proximity (several nanometers) to noble metal nanoscale-roughened surfaces (typically silver or gold) are enhanced tremendously through electromagnetic and charge transfer mechanisms.⁸ Previously, we evaluated silver dendrites as a SERS substrate to enhance canola oil, however, the enhancement was limited.⁹

Gold nanoparticles (AuNPs) have been fabricated and further modified with a variety of functionalizing ligands such as dyes, proteins, peptides, nucleic acids, and drug molecules for different SERS based sensing and imaging applications.¹⁰ However, few studies have reported using AuNPs for SERS measurement of lipids. The major reason is that most AuNPs

are hydrophilic particles that are immiscible with lipid phases.^{11,12} This problem can be partly overcome by drying AuNPs onto solid surfaces and then applying the lipid molecules on top. However, the Raman signals produced are typically highly variable and rather weak. An alternative approach is to fabricate lipophilic AuNPs that are dispersible within lipid phases since this would allow for *in situ* SERS measurement. Lipophilic gold nanoparticles have already been used in studies involving biochemical transport^{13,14} and biochemical shielding¹⁵ due to cellular membrane compatibility. The most common strategy to modify the hydrophobicity of AuNPs is the two phase method, which requires the use of a phase transfer agent, tetraoctylammonium bromide (TOAB). TOAB is mixed with the gold until all of the gold nanoparticles go into the organic toluene layer. Dodecanethiol was then added followed by sodium borohydride which acts as the reducing agent. The organic layer with the suspended gold can then be extracted.^{16,17}

In this study, we report a much simpler method to fabricate lipophilic gold nanoparticles by modifying the AuNPs in citrate buffer through ligand exchange using octanethiol in isopropyl alcohol. We then demonstrate the efficacy of these lipophilic AuNPs by using them to provide molecular information about lipids in diluted oils, and by using them to monitor lipid oxidation. To the best of our knowledge, this is the first article reporting the utilizing of lipophilic AuNPs for SERS studies of lipids.

AuNPs were synthesized using the Turkevich method which involves the reduction of chloroauric acid (HAuCl₄) with sodium citrate.¹² One ml of 1% sodium citrate was added to 100 ml of boiling 0.01% chloroauric acid. The resulting AuNPs had a mean diameter of 24 nm calculated from the measured particle size distribution (in Fig. S1[†]). Hydrophobic modification of AuNPs was evaluated using different alkanethiols in different organic solvents. Alkanethiols have been widely used to modify gold nanoparticles by adsorbing the thiol group onto the gold surface.^{18–21} The thiol group readily attaches to the gold nanoparticle as a self-assembled monolayer (SAM). The most appropriate combination of components for functionalizing the

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AuNPs determined in our preliminary experiments was octanethiol in isopropyl alcohol (data not shown). Isopropyl alcohol has an intermediate polarity between water and octanethiol, therefore it can mix with both water and octanethiol and promote uniform contact between gold surfaces and octanethiol molecules under consistent low rotation.²² Functionalization was carried out by adding 20 ml of AuNP suspension, 20 ml of isopropyl alcohol, and 1 ml of octanethiol (5% v/v, 8.644×10^{-7} M) to a test tube. The resulting mixture was then gently agitated overnight at room temperature using a Thermo Fisher Rotator at the speed of 24 rpm. Twenty ml of hexane was then added to the test tube and a homogenizer (Fisher Scientific PowerGen 125) was used to agitate the sample for 3 minutes. This procedure led to the modified hydrophobic AuNPs moving into the hexane layer. If required, a more concentrated suspension of AuNPs can be prepared by using less volume of hexane (*e.g.* for a $4\times$ concentration, 5 ml of hexane was used). After extraction, a sonicator (Fisher Scientific Model 50) was used to further disperse the hydrophobic AuNPs in hexane.

There was a distinct difference in the visual appearance of the gold nanoparticle suspensions after surface modification. The modified (lipophilic) AuNPs in hexane suspension had a dark blue appearance, whereas the unmodified (hydrophilic) AuNPs in aqueous solution had a red appearance (Fig. 1a). Visible absorbance spectra (Fig. 1b) also indicated that there were significant changes in the optical characteristics of the suspensions after surface modification. The change of absorbance indicates two things: the successful modification of the octanethiol and the aggregation of the modified AuNPs. For SERS analysis, aggregation of AuNPs generates stronger signals due to the "hot-spot" effect.^{23,24} Before adding the modified AuNPs in the oil samples, we used sonication to disperse them well.

Raman spectroscopy (DXR, Thermo Scientific) was used to provide information about the surface composition of the unmodified AuNPs in aqueous solution and of the modified AuNPs in hexane. As shown in Fig. 1c, the band at 2150 cm^{-1} (C–O stretching) in the hydrophilic AuNPs spectrum disappeared in the lipophilic AuNPs spectrum. In addition, the bands ($1125, 1050, 900, 850\text{ cm}^{-1}$) which are assigned to various vibration modes of C–C and CH_2 were clearly enhanced,

indicating the conjugation of the octanethiol molecules. Principal component analysis (PCA, by TQ analyst software, Thermo Fisher) was used to statistically analyze the data variance. Clear separation was found between these two data clusters in the PCA plot (Fig. 1d), indicating the conjugation of the octanethiol on the AuNP surfaces was fairly homogenous.

The ability of the lipophilic AuNPs to greatly increase the Raman signal from lipids was demonstrated by dispersing them within a common food-grade oil, *i.e.* canola oil purchased from a local grocery store. Canola oil was diluted to a final concentration of 3% using hexane for comparing between conventional Raman and SERS techniques on diluted oil. Diluted oil samples ($10\ \mu\text{l}$) were deposited on a gold coated slide. After the hexane was evaporated, the samples were measured under the Raman microscope for analysis. As shown in Fig. 2a, the normal Raman spectrum of 3% canola oil (without AuNPs) only showed weak signals using a 5 mW laser power. However, when the lipophilic AuNPs was added, a clear and enhanced lipid spectrum was obtained. The lipid spectrum contained similar peaks as the normal Raman spectrum of 100% oil, although some small peaks from the ligand molecules on the AuNPs were also observed. The ligand peaks can be extracted from the spectra if needed. This data demonstrated the improved sensitivity of the lipid measurement using the modified lipophilic AuNPs. These spectral data were further analyzed using PCA to check the consistency of the enhancement (Fig. 2b) and demonstrated the enhancement produced by the lipophilic AuNPs was fairly consistent, though some variance still existed, probably due to the size variance of the aqueous AuNPs. The enhancement may be further improved by using more monodisperse AuNPs.

The efficacy of the lipophilic AuNPs as SERS substrates were further demonstrated in a preliminary lipid oxidation study. One ml of canola oil in a test tube was put in a dark incubator at $55\text{ }^\circ\text{C}$ for one week. After one week, the oil was noticeably oxidized with a distinct rancid odor. The oxidation was also confirmed by measuring the hydroperoxide value (Fig. S2†).

The oil was then analyzed using normal Raman and SERS at day 0 and day 7. For the SERS measurement the oil was diluted to 3% using hexane containing lipophilic AuNPs. As shown in Fig. 3a, no significant difference was observed between the

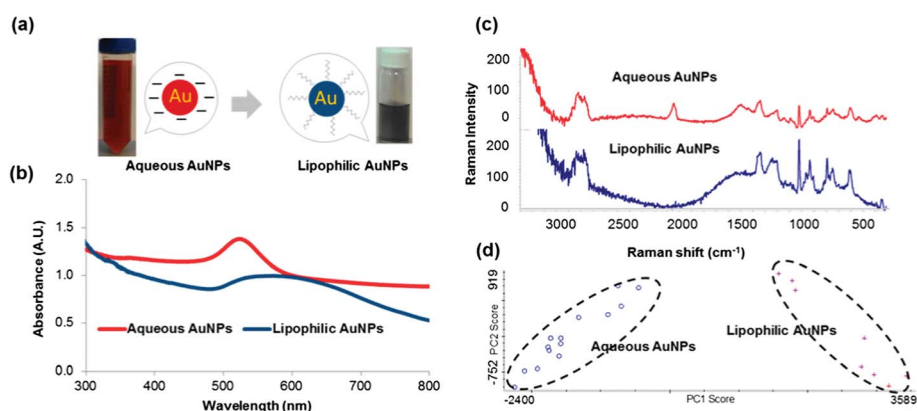


Fig. 1 (a) Optical colors, (b) absorbance spectra, (c) Raman spectra and their PCA plot of AuNPs in aqueous solution and AuNPs in hexane. PC1 score revealed 94.9% of the variance, and PC2 score revealed 3.7% of the variance.

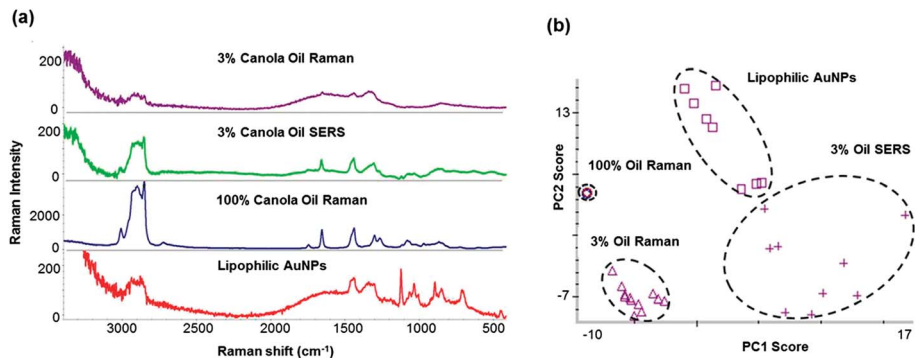


Fig. 2 (a) Raman spectra of 3% canola oil diluted with hexane, 3% canola oil diluted with hydrophobic AuNPs contained hexane, 100% canola oil and the hydrophobic AuNPs in hexane as a control, and (b) their PCA plot. PC1 score revealed 40.8% of the variance, and PC2 score revealed 27.8% of the variance.

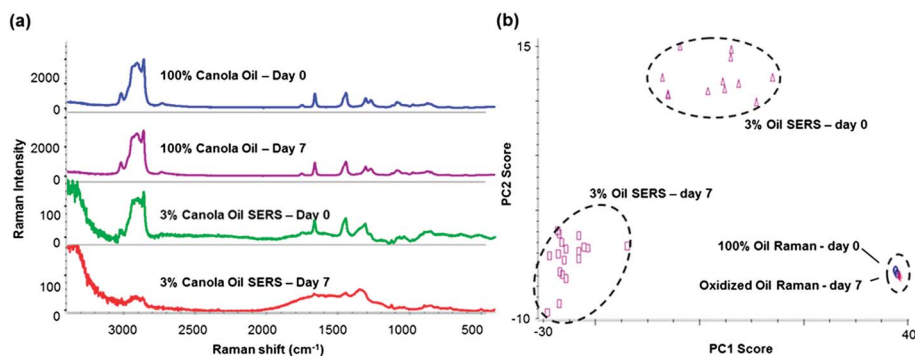


Fig. 3 (a) Normal Raman spectra of commercial canola oil (100%) on day 0 and after 7 days of incubation, SERS spectra of 3% canola oil on day 0 after 7 days of incubation inoculated with lipophilic AuNPs and (b) their PCA plot. PC1 score revealed 75.4% of the variance, and PC2 score revealed 6.9% of the variance.

normal Raman spectra at days 0 and 7, while a clear difference was found between the SERS spectra. Bulk lipid oxidation using normal FT-Raman has been studied and reported before. The changes of chemical bond vibration caused by molecular alterations associated with the oxidation reaction were minor and mainly resulted from formation of primary oxidation products.⁴⁻⁷ The changes in the SERS spectra were noticeably larger even though the total oil concentration was much lower (3% oil for SERS compared to 100% oil for conventional Raman). The statistical variance was analyzed using PCA to check the difference observed in the spectra was significant or not. As shown in Fig. 3b, the two clusters of the normal Raman data nearly overlapped with each other while the two clusters for the SERS data were clearly separated, indicating the large variance caused by oxidation was captured using the SERS method and the difference was statistically significant.

To further investigate the change of the spectra after oxidation, we carefully compared the SERS spectra of oil on day 0, day 7 and the AuNPs control. Three spectra were normalized based on the AuNPs at 1127 cm⁻¹. As shown in Fig. 4, it is clear to see that the major lipid peaks from 2950 to 2850, 1665, and 1450 cm⁻¹ decreased greatly in the day 7 sample compared to the day 1 sample. Looking closely, we observed three increased/new peaks (*i.e.* 1348, 816, 503 cm⁻¹)

in the day 7 sample, compared to the day 1 and the control sample. This change was due to the loss of the reactant and production of the oxidation products after 7 day incubation. More importantly, we speculated there was competition between the lipids and oxidation products on the AuNP surfaces for signal enhancement. Compared between the

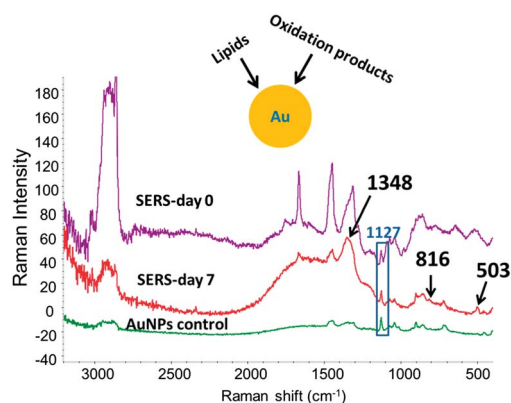


Fig. 4 SERS spectra of (a) 3% canola oil on day 0, (b) 3% canola oil on day 7, (c) AuNPs control. Spectral intensity was normalized based on the AuNPs peak at 1127 cm⁻¹.

spectra, despite the concentration of the lipids were much higher than that of the oxidation products, the drop of the lipid intensity was large. This indicates that the lipids were not able to compete with the oxidation products onto the AuNP surfaces. Certain oxidation products may have more affinity to the AuNP surfaces thus being selectively enhanced. However, there are various oxidation products that can be produced from vegetable oils, such as hexane, heptane, hexanal, *cis*-2-hexenal, heptanal, *cis*-2-heptenal, 2-octanone, *cis*-*trans*-2,4-heptadienal, *cis*-*cis*-2,4-heptadienal, nonanal, and *cis*-*cis*-2,4-decadienal.²⁵ It is difficult to assign the peaks to one or two oxidation products at this moment. Further experiments are needed to investigate the origination of the three increased/new peaks.

The observed spectral change of canola oil after oxidation was found very different than that from silver dendrites in the previous study.⁹ Using silver dendrites, we observed the overall peak intensity decrease in the oxidized samples which was due to the saturation of the analytes on the substrate surface. After dilution, some new peaks (*i.e.* 1777 and 1633 cm⁻¹) were noticed. Using lipophilic AuNPs, we found both decreased and increased of peaks, and the new peaks that emerged were quite different. The difference between two substrates was thought mainly due to the different sample preparation method. For silver dendrites, we directly deposited the oil samples on the surface. All molecules in the samples would get on the surface and be enhanced. Therefore, the enhancement selectivity was limited. While for AuNPs, we were able to mix and incubate the oil sample with the AuNPs due to the lipophilic modification. During the time of the incubation, molecules that were more affinitive to the AuNPs would be captured and selectively enhanced.

In summary, we reported a simple fabrication method to prepare lipophilic and SERS-active AuNPs and proved that they can enhance lipid signals compared with normal Raman measurements. The potential application of these lipophilic AuNPs was also demonstrated in the lipid oxidation study. One advantage of using lipophilic AuNPs in SERS study of lipids is by inoculating lipophilic AuNPs in lipids, we may be able to sensitively and selectively measure the lipids in a complex multicomponent environment (such as foods and biological systems) *in situ*. Future studies will further investigate the molecular mechanism of the spectral changes due to oxidation, as well as optimize of the lipophilic AuNPs (size and ligand) for SERS measurement of lipids and applying them in various lipid studies.

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