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In situ SERS Detection of Emulsifiers at Lipid Interfaces using Label-free Amphiphilic Gold Nanoparticles†

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Here in, we fabricated amphiphilic gold nanoparticles (GNPs) that can self-assemble at oil-water interfaces. We applied those GNP s for in situ SERS detection of emulsifier molecules within the interfacial region of oil in water (O/W) emulsion systems.

Recent advances in the development of plasmonic metal nanoparticles have stimulated research into their applications as surface enhanced Raman scattering (SERS) probes in a variety of disciplines. SERS-active nanoparticles have been conjugated with various targets for sensor and imaging applications, including dyes, peptides, proteins, antibodies, and nucleic acids. In situ SERS detection is of particular interest due to its ability to monitor target molecules in their natural microenvironments. One of the biggest challenges for in situ SERS applications in multiphase heterogeneous samples is how to guide the nanoparticles to specific targets of interest, as the SERS-enhancement zone of the nanoparticles is in the range of several nanometers. Before choosing this technique for a particular application, one must consider the limitations imposed by the concentration of the target analyte, as well as the complexity, optical properties, and physical nature of the surrounding matrix. SERS-active nanoparticles that can self-assemble at the interface of multiphase systems, e.g. air-water, air-lipid, water-lipid, lipid-lipid have been fabricated via different methods. Cecchini et al utilized self-assembled nanoparticle arrays at liquid/liquid or liquid/air interfaces to detect multi-analytes from the aqueous, organic or air phases.

The objective of this study was to fabricate and apply amphiphilic gold nanoparticles (GNPs) that can self-assemble at oil-water interfaces for in situ SERS detection of emulsifier molecules within the interfacial region of oil in water (O/W) emulsion system. Many biological and non-biological materials are complex multiphase heterogeneous materials consisting of oil and water phases, such as pharmaceuticals, agrochemicals, petrochemicals, foods, personal care products, and biological fluids. Emulsions consisting of droplets of one phase dispersed within the other phase are good model systems for studying the behavior of more complex multiphase materials. The formation, stability, and functional performance of emulsions are largely determined by the thin layer of molecules that coats the droplets. For example, the interfacial characteristics influence such diverse properties as initial particle size distribution, stability to droplet aggregation, susceptibility to chemical degradation, and behavior in the gastrointestinal tract. To the best of our knowledge, it is the first report of in situ SERS detection of emulsifiers in O/W emulsions using amphiphilic GNPs.

GNPs (mean particle diameter, ~24 nm) were fabricated according to the Turkevich method, which used sodium citrate to reduce HAuCl₄. Particle size distributions and transmission electron microscopy images of these GNPs are presented in the ESI (Fig. S1 and S2). For surface modification, an aqueous colloid GNP suspension (~7.2×10¹¹ particles/mL) was incubated with an organic ligand solution (1% octanethiol in hexane solution). The ratio of GNP suspension to ligand solution was 1:1 (v/v). The thiol (SH) group in the ligand molecules is known to interact with GNP s to form strong gold-SH covalent bonds. Initially, two separate layers were formed (Fig. 1A) due to the immiscibility between hexane and water. The bottom layer (pink) was the aqueous phase containing GNPs, while the upper layer (clear) was the hexane phase containing octanethiol. After 12 h incubation with constant rotation (24 rpm, Fisher Scientific Ocelot rotator) at room temperature, the bottom layer lost its...
pink color, which indicated loss of gold nanoparticles from the bulk aqueous phase. Looking closely, we observed that the GNPs were concentrated in the interfacial layer between the two phases, which suggested that the modified GNPs had become amphiphilic. Using dark-field microscopy of the samples, we observed a color change from bright green to blue after ligand modification, which can be attributed to the change in surface plasmon resonance (SPR) of GNPs after conjugating with octanethiol (see ESI, Fig. S3).

To investigate the degree of ligand conjugation, we measured the Raman spectra of unmodified hydrophilic GNPs and modified amphiphilic GNPs (Fig. 1B). The details of the Raman instrumentation and data analysis are included in the supporting information. The Raman spectrum of hydrophilic GNPs is indicative of the molecular vibrations associated with anionic citrate molecules, indicating that they were present at the particle surfaces. The Raman spectrum of amphiphilic GNPs were fairly similar to the hydrophilic GNPs, which indicated that the citrate molecules were still bound to the nanoparticle surfaces. A more detailed analysis of the spectra showed that there were Raman peaks at 2883, 1465, 1305, and 1070 cm$^{-1}$, which were assigned as CH$_3$ stretch, CH$_2$ stretch and CC skeletal stretch, clearly increased after conjugating with octanethiol (Fig. 1B). It indicated that the octanethiol molecules partially covered the surface of the GNPs. The amphiphilic character of modified GNPs can therefore be attributed to the presence of hydrophilic groups (citrate) and lipophilic groups (octanethiol) at their surfaces. The principal component analysis (PCA) plot was shown in the supporting material (Fig. S4), indicating the SERS patterns of the GNPs before and after modification were statistically different.

The distribution of the polar and non-polar groups at the particle surfaces is currently unknown. A heterogeneous distribution may arise due to preferential accumulation of non-polar patches on the particle surfaces (Fig. 2). For example, unmodified GNPs may come into contact with the oil-water interface and a few octanethiol molecules bind to their surfaces, thereby increasing their local surface hydrophobicity. Subsequently, GNPs preferentially adsorb to the interface with an orientation whereby the non-polar patches point towards the hexane phase containing the ligand, thereby facilitating binding of more octanethiol molecules in the same vicinity. In this case, the GNPs would have a Janus-like structure, with some non-polar patches and some polar patches. Alternatively, there may have been an even distribution of polar and non-polar groups on the particle surfaces, and their ability to adsorb to the oil-water interface was simply a result of changes in their wetting behavior.

Further work is clearly needed to establish the surface chemistry of the amphiphilic GNPs developed in this study.

A number of other workers have functionalized GNPs using low molecular weight thiols, but these particles were primarily developed for aqueous-based applications, and few of them were used as probes for SERS analysis. Simard et al. fabricated amphiphilic GNPs through partial ligand exchange of alkanethiolate-protected gold nanoclusters with thio-carboxylic acids. Zhao et al. reported encapsulation of water-soluble vitamins using PEGylated GNPs for solubilization in a hydrophobic solvent. Here we used octanethiol to fabricate amphiphilic GNPs using a simple method, and then used them to develop SERS probes for providing intrinsic chemical information about molecules at oil-water interfaces in multiphase systems.

To prove that the amphiphilic GNPs preferentially accumulate at the oil-water interface in multiphase systems, we utilized a two-dimensional Raman mapping technique to scan water-in-oil (W/O) emulsions containing amphiphilic GNPs. W/O emulsions were chosen because their physical stability was sufficient to allow chemical mapping of individual droplets, i.e., no droplet movement occurred during the scan. W/O emulsions were prepared by blending 9% (w/w) water, 90% (w/w) canola oil, and 1% (v/v) amphiphilic GNP suspension together at 8000 rpm for 1 min (PowerGen Model 125 Homogenizer, Fisher Scientific). A W/O emulsion containing hydrophilic GNPs was prepared using the same method as a control. Raman images were taken by integrating signature peaks of oil (1656 cm$^{-1}$; double bond stretching C=C) and GNPs (Raman shift 1127 cm$^{-1}$) to determine the relative locations of the oil and GNPs in the emulsions. The oil-integrated images showed water droplets (blue color - low peak intensity) surrounded by oil (red color – high peak intensity) (Fig. 3A and 3B). The GNPs-integrated images showed amphiphilic GNPs (red color) at the oil-water interface, but the hydrophilic GNPs (blue color) presented inside the water droplets (Fig. 3C and 3D). The highest SERS signal enhancement takes place in the region between two nanoparticles (“hot spot”) due to an electromagnetic mechanism. The amphiphilic GNPs were concentrated at the
These amphiphilic GNP, adsorbed to the oil-water interface. Therefore, no significant signal was observed (Fig. 3F). The optical properties of the mixed systems also differed considerably when observed by dark field light scattering microscope (see ESI, Fig. S5), which again supported the proposed difference in location of amphiphilic and hydrophilic GNPs. These results confirmed that the unmodified GNPs were hydrophilic, while the modified GNPs were amphiphilic and adsorbed to the oil-water interface. The data indicate the amphiphilic GNPs can be applied for interfacial characterization of multiphase systems using SERS.

Amphiphilic GNPs were then used as probes for in situ SERS analysis of the molecular characteristics of two emulsifiers adsorbed to droplet surfaces in model oil-in-water (O/W) emulsions. β-lactoglobulin was used as an example of a model amphiphilic biopolymer, whereas Tween 20 was used as an example of a model nonionic surfactant. Emulsions were prepared by mixing 9% (w/w) canola oil, 88% (w/w) water, 2% (w/w) emulsifier, and 1% (v/v) modified GNPs suspensions together and stirring at 8000 rpm for 1 min (PowerGen Model 125 Homogenizer, Fisher Scientific). To inhibit the movement of the oil droplets during the SERS measurements, 1% xanthan gum (w/w) was added in the aqueous phase to increase its viscosity. Emulsions without modified GNPs (only oil, water, and emulsifier) were prepared as controls. No Raman signal (Tween 20 or β-lactoglobulin) was observed from the emulsifiers in the interfacial layers in the absence of GNPs (see ESI, Fig. S6), which was attributed to the fact that their intrinsic Raman signals were too weak to be detected. On the other hand, in the presence of amphiphilic GNPs, the Raman signals of both emulsifiers were enhanced and could be detected in the interfacial layer (using a laser power at 2 mW). In Figs. 4(a) and 4(b), the characteristic Raman bands of β-lactoglobulin and Tween 20 were clearly observed after extracting the background of oil signals. The enhanced signals were thought to be mainly from the molecules adsorbed onto the surface of GNPs. Compared with the references which were recorded using a pure solution (tween 20) or a solid powder (BLG) under the normal Raman parameters (without the use of GNPs, and a laser power at 24 mW), the extracted interfacial SERS spectra showed great enhancement and also spectral difference. The spectral difference was due to the conformational changes of the emulsifier molecules when they were adsorbed onto the GNPs surfaces. The physical stability of the emulsion containing GNPs was similar to that without GNPs. No stable emulsion was formed when only oil, water and GNPs were mixed together (data not shown). This information suggested that most of the emulsifier molecules need to be present at the oil-water interface in order to form a stable emulsion.

In summary, we developed a simple and straightforward method to fabricate amphiphilic GNPs. These amphiphilic GNPs can self-assemble at the O/W interface and enhanced the Raman signals of emulsifier molecules. Future studies are needed to elucidate more of the surface chemistry of the prepared amphiphilic GNPs and to study in depth about their interactions between emulsifiers in the emulsion systems, in order to better applying them for specific studies in multiphase systems.

Notes and references
