

Detection of Biological Species by Surface Enhanced Raman Scattering

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ABSTRACT

In recent years there has been a strong interest in the development of rapid detection techniques of biological species, especially pathogens that are involved in food poisoning, water contamination, and air-borne diseases. Conventional detection methods are time consuming and usually involve overnight culture of bacteria in a suitable growth medium. In this study we used surface-enhanced Raman scattering (SERS) to develop a rapid method of identifying bacteria. Molecules in close contact to the nanometallic surfaces show a huge enhancement in their Raman signature. The enhancement can be as great as 10^8 - 10^{14} . We also explored the application of dry nanostructured surfaces to characterize biomolecules by softly landing them on plasma-etched silver surfaces. By such landings, biomolecular gas phase ions were allowed to chemically bind to the surface so that the Raman effect was significantly enhanced and fluorescence was suppressed.

Keywords: bioaerosol, biomolecules, electrospray, Raman spectroscopy, reactive landing

1 EXPERIMENTS

In the studies of bacteria, bacterial cells were contacted with a colloidal suspension of silver nanoparticles to deposit silver on the wall surface. The samples were then illuminated with a 514.5 laser beam, and SERS spectra were acquired. Figure 1 shows the Raman system used to obtain SERS spectra of bacteria in solution. The inset shows the modified system used for bioaerosol collection. We have shown that an aerosolized stream of bacteria can be mixed with the nanocolloidal silver suspension to yield strong Raman signals. Additional details of the experiments were published by Sengupta *et al.* [1].

In another set of experiments we used confocal SERS to characterize trace amount of biomaterials deposited on roughened silver (Ag or AgO). The apparatus used to produce an electrospray of biomaterial and to deposit it by soft landing on a metallic surface has been previously reported by Volny *et al.* [2]. The purpose of the reactive landing experiments was to explore the possibility of further increasing the Raman enhancement by reducing the contact distance between the bio-analyte and the underlying surface.

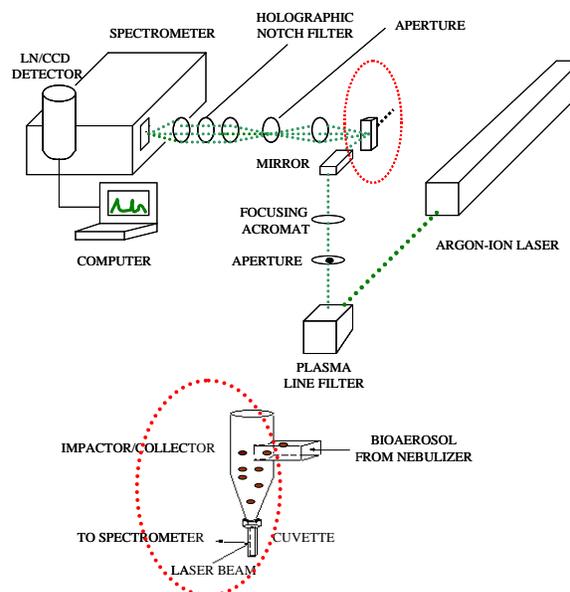


Figure 1: Raman apparatus and bioaerosol collection set-up.

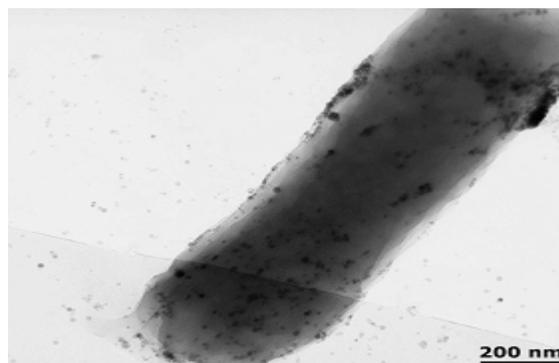


Figure 2: Bacterial cell coated with silver colloidal particles.

Furthermore, the intrinsic fluorescence of the bioanalytes is expected to be quenched because of the interactions of the excited states in fluorophores with electrons in the conduction band of the metal surface [3].

2 RESULTS AND DISCUSSION

SERS is a powerful tool to probe bacterial cell wall surfaces and provides rich structural information of the different chemical moieties present in the cell wall. Figure 2 shows a transmission electron microscope (TEM) image of a bacterial cell coated with silver nanoparticles. The size of the nanoparticles ranged between 10-20 nm and they were synthesized following a borohydride reduction protocol [4]. The image also suggests the formation of the localized silver clusters, which may play an important role in the signal enhancement. The aggregated sites provide a hot spot where the local electric field is significantly higher than that around the isolated particles. The aggregated cluster also affects the position of the plasmon absorption band, which is red-shifted with the increase in cluster size. The resonance between the metallic plasmon band and the incident wavelength promotes a large enhancement of the electric field

In this work, we report the studies that we have performed to maximize the Raman enhancement by optimizing several experimental parameters affecting size and the charge of the colloidal particles.

Previous work revealed the importance of the concentration ratio of analyte to colloidal particles to maximize the signal [5]. By optimizing the volume ratio of colloid to bacteria and pH of the colloidal suspensions, the signal was maximized and the fluorescence was quenched. The bacteria *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa* were studied using this technique. As shown in Figure 3, the spectra of different bacteria are very similar in the Raman shift region >1100, but there are some differences at lower Raman shifts. The spectral differences may be due to the subtle differences in the cell wall compositions of different bacterial species. The detailed characterization of the structural fingerprints obtained from the bacterial studies is discussed in Sengupta *et al.* [5]. We found that bacterial samples induce aggregation of the colloidal particles, which gradually leads to the development of a secondary extinction band. Figure 4 is a representative UV/VIS spectra of colloidal particles mixed with bacteria. The spectra demonstrate the change in the particle size distribution over time. The strong resonance coupling of the secondary plasmon band at around 500-550 nm and the laser line is expected to further enhance the Raman signal. We observed a time evolution of the SERS signal due to the changes in the particle size distribution, which suggests the necessity of having optimum size of the nanoparticles to maximize the enhancement.

Figure 5 illustrates the temporal evolution of the signal for different bacterial species in contact with the silver nanoparticles. The plot is constructed by taking the normalized intensity of the major band at 1355 cm^{-1} over time as the growth indicator.

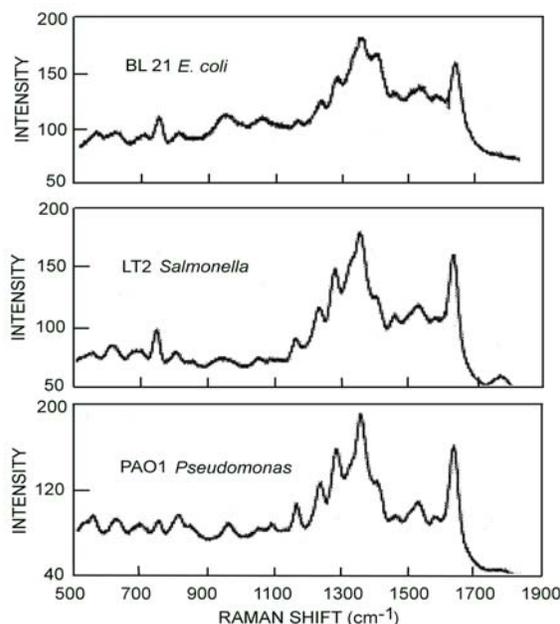


Figure 3: SERS spectra for three types of bacteria.

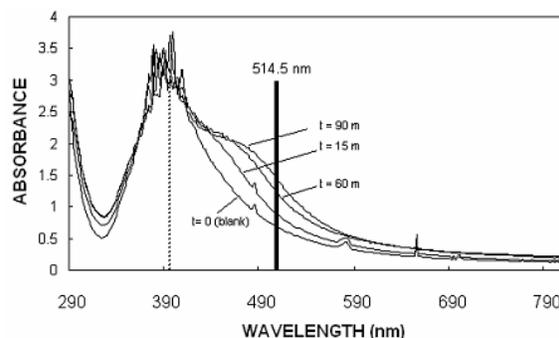


Figure 4: UV-VIS spectra of silver colloidal solution mixed with bacteria.

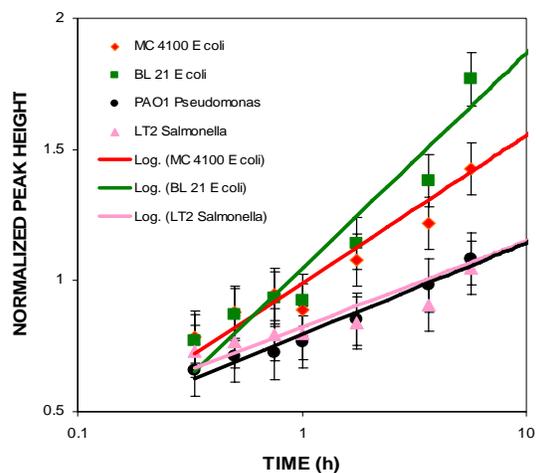


Figure 5: Time evolution of the major SERS peak.

Another important parameter that can influence the binding of the nanoparticles to a biological surface is the charge state of these particles. We investigated the binding process at different pH's. The binding is much stronger at acidic pH and the signal evolution is much faster. However, the colloidal stability deteriorates rapidly under acidic pH conditions [5]. At basic pH > 9, we found little or no binding. The optimum pH to work with the biological samples coated with nanocolloidal particles was found to be 7.8-8.1.

After optimizing few parameters mentioned above we explored the detection limit of our system. From our studies, we found that the detection limit of our system can be as low as 100 colony formation unit (cfu)/ml [6]. However, for very dilute bacterial samples, to recover SERS fingerprints masked by the large water libration background, it is necessary to subtract the water background. Details of this background subtraction process are discussed in Sengupta *et al.* [6]. We also demonstrated that a particular bacterium can be identified in a binary mixture with another species by their SERS fingerprints [6].

In experiments with lower molecular weight biomolecules, we investigated the SERS characterization by landing the biomolecular gas phase ions on plasma-etched metallic surfaces. By depositing them using mild eletrospray conditions the biomolecules are allowed to react with the surfaces thereby forming strong bonds. The non-destructive deposition technique is known as reactive landing and the biomolecules retain their activities in their bound state [7]. The binding allows the nanometallic surfaces to be in very close contact with the biomaterials and further enhances the Raman signal.

From our preliminary studies, we observed strong SERS signal from organic dyes such as crystal violet and Rhodamine-B. The surface roughnesses of the plasma-etched surfaces are in the range of 20-25 nm as estimated from the AFM image shown in Figures 7A and 7B. The contact layer of the landed molecules is retained even after strong washing treatment with water and methanol. Figure 8 shows the SERS spectrum of crystal violet dye after thorough washing. These results suggest that our technique is extremely sensitive to the surface morphology and can provide information of the various surface active phenomenon that are limited to the first few contact layers. The detection limit for crystal violet studies was found to be sub zeptomolar.

From our experiments with molecules such as proteins, we found that it is necessary to optimize the surface roughness to achieve Raman enhancement. Our initial studies with trypsin yielded little enhancement. This may be due to the size mismatch between a protein and the surface grooves. Trypsin is a globular protein and expected to sit on the top of the ridges and have little contact with the surface if the groove spacing is small. To overcome this problem we sandwiched the trypsin between a plasma-etched surface and an electrospayed layer of silver ions. We obtained a strong SERS signal by using this methodology. Figure 9 demonstrates the SERS spectra of trypsin molecules sandwiched between the two SERS active surfaces.

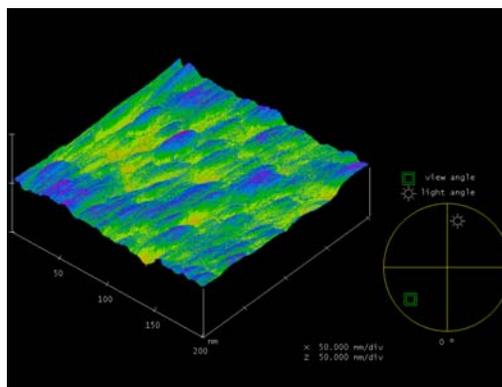


Figure 7A: Tapping mode AFM image of a roughened silver surface after the landing of crystal violet molecules and subsequent thorough washing.

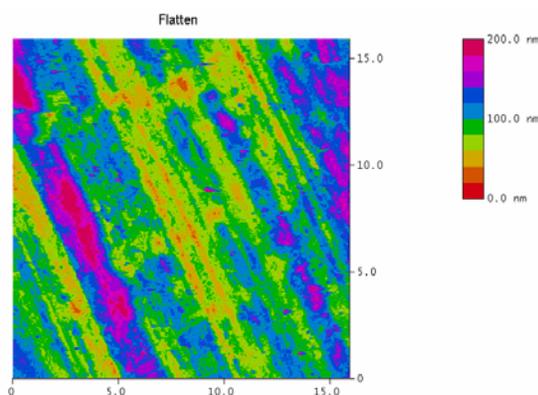


Figure 7B: Flattened view of the tapping mode AFM image of the same surface shown above.

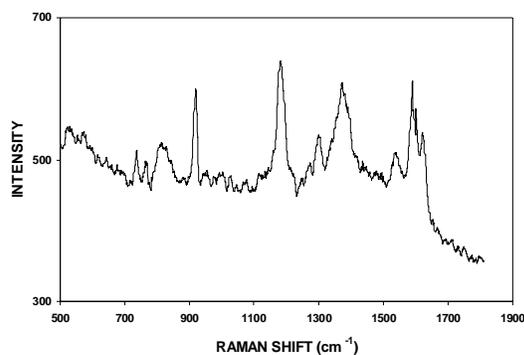


Figure 8: SERS spectra of approximately 10 picomoles of crystal violet molecules reactively landed on plasma etched silver surface.

Similar studies were performed by landing crystal violet on roughened Pt surfaces. The SERS signal on the Pt surface was found to be much weaker than that obtained from the silver surfaces.

However, we still obtained a picomolar detection limit using platinum as SERS active substrate.

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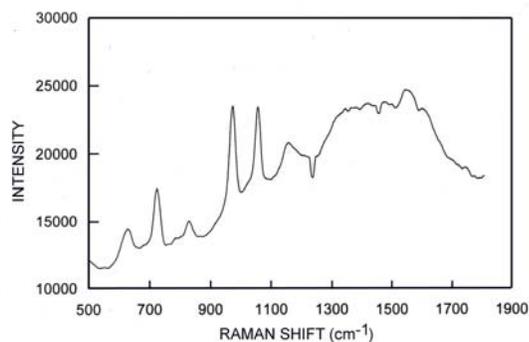


Figure 9: A SERS spectrum for trypsin molecules sandwiched between silver atomic clusters and plasma etched silver surface.

4 CONCLUSIONS

We have demonstrated that surface-enhanced Raman spectroscopy is an effective tool for the chemical characterization of biological material, capable to detect trace amount of biomaterials on metallic surfaces. The detection method for bacteria in silver colloidal solutions can be easily integrated to a bioaerosol sampling system to collect the airborne microorganisms in real time and transfer them to the SERS active silver nanocolloidal suspensions. By landing the biomaterials on a dry surface, SERS can be a sensitive tool to study the surface-active processes and to understand the binding behavior of biological molecules to the inorganic surfaces.

5 REFERENCES

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