Application Note: 52082

Use of Antibody Modified SERS for Foreign Protein Detection in Milk

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Key Words

- DXR Raman Microscope
- Food Safety
- Milk
- Raman
- Surface-Enhanced Raman Scattering (SERS)

Introduction and Background

Ensuring food safety in our society continues to grow in importance. As a result the need for fast, sensitive, and specific tests of food products also continues to grow in importance. Testing for the accidental or intentional contamination of food during processing can be a time consuming and expensive process. Cross contamination of food products with foreign proteins can lead to serious problems involving allergic reactions and potentially expensive food recalls, whereas the intentional contamination of a food product with a bioterrorism agent would have profound effects.

A very large challenge when analyzing food is the complex nature of the food matrix. Testing for an analyte in pure water may be simple, but when there are other water soluble compounds, fat soluble compounds, and insoluble materials present in the sample, then the analysis becomes much more challenging. In addition to the complex nature of the matrix, the species of interest may be present in extremely low levels, so techniques need to be either sensitive or have a method of concentrating the species of interest. Current techniques used for food analysis include enzyme-linked immunosorbent assay (ELISA) or separation and chromatography methods. What is needed is a simple, fast, and economical means of testing food products.

This application note describes the use of Surface-Enhanced Raman Scattering (SERS) with antibody-modified silver dendrites for the detection of a foreign protein in milk, a complex food matrix. Milk was chosen as the sample matrix for a variety of reasons. The first is that milk is a suitably complex matrix with a variety of complicating factors – water and fat soluble compounds along with suspended solids. Other tests performed during the receipt of raw milk at a dairy plant take approximately 30 minutes, which imposes a time frame for the successful completion of the SERS analysis. And lastly, milk processing is a likely and vulnerable target for bioterrorism. In the experiments described below the egg white protein, ovalbumin (OVA), was the foreign protein introduced into milk.

Surface-enhanced Raman scattering (SERS) is a technique that can be used to improve upon traditional Raman analysis. SERS can be employed as a way to potentially lower sample fluorescence and enhance Raman scattering, both of these effects lead to a much improved limit of detection. A nanostructured metal substrate is used to enhance the signal from an analyte in contact with the substrate. The type of metal and the shape of the features of the nanostructure play an important role in the Raman signal enhancement. For more background and information on SERS please see our Technical Note #51874 “Practical Applications of Surface-Enhanced Raman Scattering (SERS)”. The Thermo Scientific DXR Raman microscope is the ideal instrument for this application, as it combines powerful performance capabilities, such as laser power control, alignment and calibration, with an impressive array of software for data collection and analysis, all together in an easy to use, accessible package.

The capability to sample for a specific protein, and minimize contributions from similar proteins, was achieved by using an antibody bound to the surface of a silver dendrite, which was used as the SERS substrate in these experiments.

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Experimental and Instrumentation

Preparation of the Antibody-Modified SERS Substrates

The silver dendrites used as SERS substrates were prepared through a simple replacement reaction. A piece of zinc metal was suspended in a solution of 0.20 molar silver nitrate for 1 minute, allowing the silver dendrites to form. Once the dendrites had formed they were mechanically removed from the zinc, rinsed with deionized water to remove unwanted ions, and then stored under water. When it was time to prepare the silver dendrites for binding of the antibody the pH of the water containing the dendrites was adjusted to 4.5 using 0.02 molar HCl. The pH is the isoelectric point of protein G (a protein that binds immunoglobulin), which is used to bind the antibody to the silver. Once the proper pH was reached, 500 µL of the silver dendrite suspension was mixed with 100 µL protein G solution (1 mg/mL) and mixed under constant rotation for 30 minutes at room temperature. The mixture was stabilized by the addition of bovine serum albumin (BSA), to a final concentration of 0.2% w/v (weight to volume), to block any unoccupied binding sites. The mixture was centrifuged at 2000 g for 1 minute and the resulting Ag-G pellet (the silver dendrites with protein G bound to them) was washed twice with phosphate buffered saline (PBS) at pH 7.4. Then the pellet was resuspended in 500 mL of 0.2% BSA, to reduce nonspecific binding. To bind the OVA antibody (antiOVA) to the Ag-G substrate 500 mL of 1 mg/mL antiOVA was incubated with the Ag-G substrates for 30 minutes under constant rotation at room temperature. Once the incubation was complete the mixture was centrifuged at 2000 g for 1 minute, and again the pellet was rinsed twice with PBS and resuspended in 500 mL of PBS.

Samples were prepared by adding OVA into whole milk and PBS. A concentration range of 0, 0.1, 0.5, 1, and 5 µg/mL of OVA was used to test the method. To prepare the samples for analysis 500 µL of the solution to be analyzed was incubated with 50 µL of the Ag-G-antiOVA suspension under constant rotation at room temperature for 15 minutes. A final centrifugation at 2000 g for 1 minute was performed and the resulting pellet was washed 3 times and then deposited onto a glass slide and allowed to dry at room temperature before analysis. Figure 1 illustrates the preparation of the modified silver dendrites and the Raman analysis.

Instrumentation

A DXR Raman microscope was used; it was equipped with a 780 nm laser, brightfield/darkfield illumination, 10× microscope objective, and a motorized microscope stage. The DXR microscope is particularly well suited as it offers research level Raman performance in a point and shoot design that sets up quickly and gives reliable results without expert tuning. With SERS, signal enhancement is such that fine laser power control is desirable to maximize signal without saturation of the CCD detector and to avoid sample damage. The DXR microscope features patented laser power control that provides precise control of power at the sample, making it an ideal choice for both SERS work and sensitive samples.

Samples were analyzed using 4 mW of laser power and four 15 second scans with a 25 micron slit aperture for each spectrum. Thermo Scientific OMNIC Array Automation software was used for the automated collection and processing of groups of samples. OMNIC™ Array Automation controls the movement of the motorized stage of a DXR Raman microscope and coordinates the stage movement with the spectral data collection of the samples. OMNIC Array Automation includes templates for many common multi-sample platforms, such as a 96 well-plate, and new templates can be easily created in the software. OMNIC Array Automation was used to collect 25 spectra per sample. The 780 nm laser was used to help minimize sample fluorescence, which was expected due to the complex organic nature of the milk matrix.

Data Analysis

Once collected, the data was analyzed using our Thermo Scientific TQ Analyst software, which is used for a range of chemometric calculations. TQ Analyst™ can be used for complex data analysis, particularly large sets of data. It can be used for quantitative analysis, qualitative analysis, and large calibration sets. The spectral data for the OVA samples was analyzed using principal component analysis (PCA) to determine the variance of the spectra. A qualitative predictive model was constructed based on the standards. Other data processing involved second derivatives and smoothing.

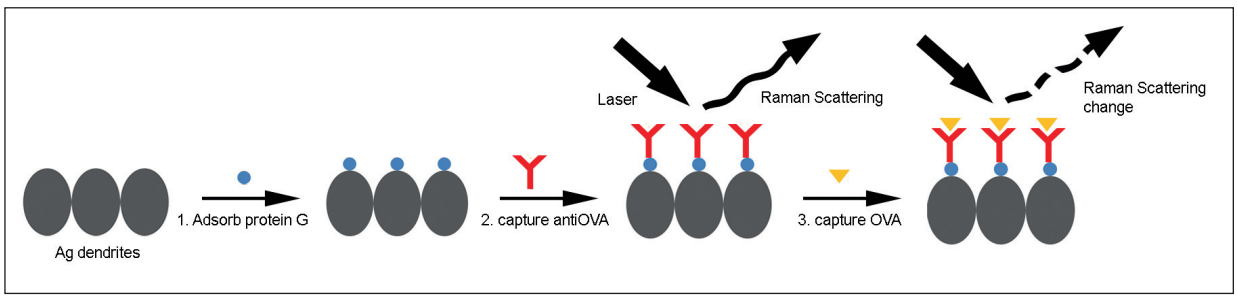

Figure 1: The preparation of the modified silver dendrites and Surface-Enhanced Raman Scattering (SERS) analysis of the bound protein

Results and Discussion

SERS spectra were collected at each step of the antibody binding process and also the final and most important step, the capture of the foreign protein, the OVA from milk samples. Figure 2(a) shows representative spectra of: the bare silver dendrite (Ag), the silver dendrite with protein G and antiOVA (Ag-G-antiOVA) attached, and the final step – the silver dendrite with protein G, antiOVA and the captured OVA (Ag-G-antiOVA-OVA). The broad peak seen at approximately 1070 cm^{-1} is due to residual nitrate, NO_3-, on the surface of the silver dendrite. The nitrate signal can be a useful tool to monitor the silver surface, because as protein G is bound to the surface the nitrate will be displaced and its signal will decrease. There were overall spectral changes with the binding of protein G onto the silver surface, and the sample spectrum changed again with the binding of the antiOVA to the protein G modified silver surface. The capture of OVA by the antiOVA bound silver substrate did not result in dramatic spectral changes. This in part can be attributed to the fact that the thickness of the electromagnetic layer responsible for the SERS enhancement is thin (approximately 10 nanometers). The binding event does cause small molecular structural changes to the antiOVA. By taking the second derivative of the spectra of the Ag-G-antiOVA and Ag-G-antiOVA-OVA the small structural changes can be visualized, shown in Figure 2(b).

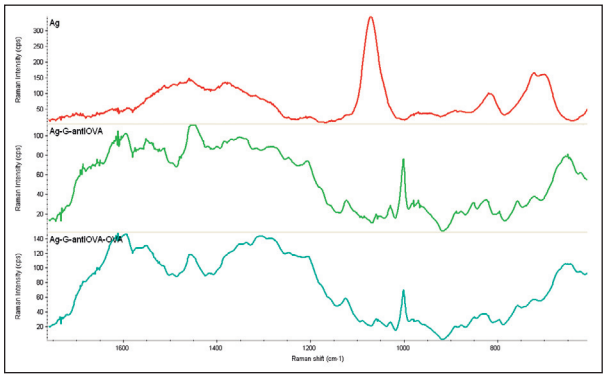

Figure 2a: Average spectra of the bare silver dendrite, silver dendrite with protein G and antiOVA bound to it (Ag-G-antiOVA), and silver dendrite with protein G and antiOVA with OVA captured (Ag-G-antiOVA-OVA)

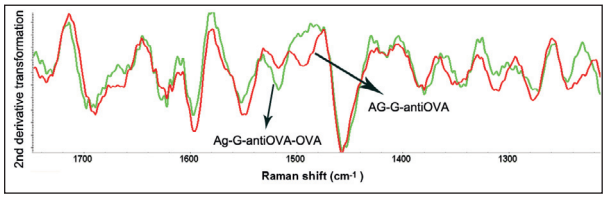

Figure 2b: Comparison of the second derivatives of the spectra for Ag-G-antiOVA and Ag-G-antiOVA-OVA

Principal Component Analysis (PCA) via TQ Analyst can be used to show how the spectra of the different stages of the binding and capture process cluster together. Figure 3 shows the PCA plot of the different spectral groups for the different substrate preparation stages. Clear grouping of the different stages occurs with the comparison of the first two principal components. Of greatest importance in the plot is the ability of the technique and analysis method to distinguish between the Ag-G-antiOVA and Ag-G-antiOVA-OVA bound groups. At the very least this means that the technique has potential as a qualitative test for the presence of a foreign protein.

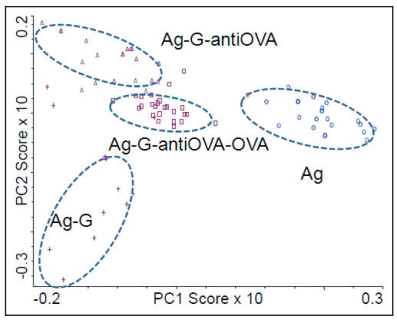

Figure 3: PCA plot of spectra from the different steps of the modified silver dendrite process

The next phase of data analysis focuses on the determination of the detection limit for the binding technique. Two sets of OVA samples were analyzed, those using OVA spiked milk and OVA spiked PBS solution. Again PCA was used for the data analysis, this time to determine which different concentrations could be distinguished. Figure 4 shows the resulting PCA plots for the limit of detection (LOD) of OVA in milk and PBS. The sample set with the best (lowest) limit of detection was the OVA spiked PBS samples. The PCA plot shows that it is possible to distinguish between the blank (zero OVA) samples and those samples containing OVA (0.1, 0.5 and 1.0 µg/mL), which means that the detection limit is 0.1 µg/mL, possibly lower. The clustering of all the spectra for the different concentrations means that there is not a way to distinguish the specific concentration of OVA, merely its presence or absence. The analysis of the milk samples spiked with OVA show a decrease in the sensitivity of the technique when a more complex matrix is encountered. The PCA results show that the limit of detection for the milk samples is between 1 and 5 µg/mL of OVA. The decrease in sensitivity can be most likely attributed to the presence of milk proteins that are involved in non-specific binding with the antiOVA or simple interference from other components of the milk.

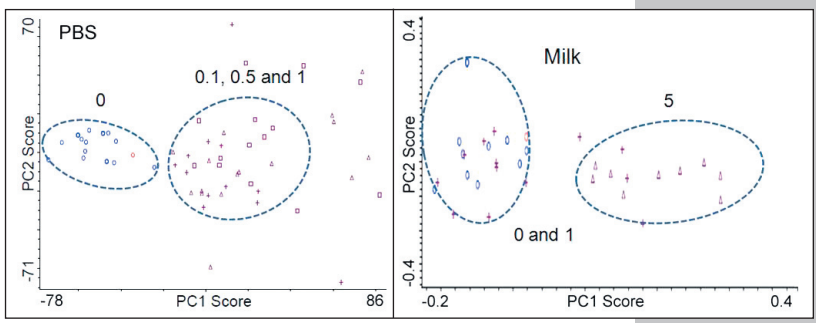

Figure 4: PCA plots for the limit of detection (LOD) of OVA in PBS solution, shown on left, and milk, shown on right

Conclusion

In conclusion this note shows how an antibody-modified silver surface can be used for the qualitative detection of a foreign protein that has been introduced accidentally or intentionally into a complex food matrix. Future work on the antibody-modified dendrite may lead to the lowering of the detection limit and the potential for quantitative results. By using previously prepared Ag-G-antiOVA complex on a silver surface the sample preparation and analysis takes less than 30 minutes. This time frame fits into the time constraints placed by other tests run at a dairy upon the arrival of a shipment of raw milk. A very important aspect of this work is that this technique can be modified for the analysis of other proteins by the choice of binding antibody, and this choice of other binding agents may allow the technique to be used on other types of molecules. The combination of the antibody bound SERS technique with the powerful capabilities of the DXR Raman microscope (laser power control, spatial resolution, system alignment and calibration) led to the success of this analysis. For more information about this application please see Reference 1.

References

1. "Detection of a Foreign Protein in Milk Using Surface-Enhanced Raman Spectroscopy Coupled with Antibody-Modified Silver Dendrites", Analytical Chemistry, 2011, 83, 1510-1513.

We have several application and technical notes about Surface-Enhanced Raman Scattering that may be of interest:

- Technical Note #51874 "Practical Applications of Surface-Enhanced Raman Scattering (SERS)"
- Application Note #52020 "Application of Automated Data Collection to Surface-Enhanced Raman Scattering (SERS)"

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