Application note

Use of antibody-modified SERS for foreign protein detection in milk

Authors

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Figure 1. Thermo Scientific DXR3 Raman Microscope.

Introduction

It is increasingly important to ensure food safety in our society, which requires fast, sensitive, and specific tests of food products. Cross contamination of food with foreign proteins can lead to serious problems such as allergic reactions and potentially expensive food recalls, whereas the intentional contamination of food with bioterrorism agents would result in even more profound consequences. The difficulty, however, is that testing for the accidental or intentional contamination of food during processing can be time-consuming and expensive.

A major challenge of food analysis is the complex nature of the food matrix. Testing for an analyte in pure water may be simple, but analysis becomes much more challenging when there are other water- and fat-soluble compounds, as well as insoluble materials, present in the sample. In addition to the complex nature of the matrix, the species of interest may be present at extremely low levels, so techniques need to either be highly sensitive or have a method of concentrating the species of interest. Current techniques used for food analysis include enzyme-linked immunosorbent assays (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, which serves as a model complex food matrix. Milk was chosen as the sample matrix for a variety of reasons. First, milk is a suitably complex matrix with a variety of complicating factors: water- and fat-soluble compounds along with suspended solids. Second, other tests performed during the receipt of raw milk at a dairy plant take approximately 30 minutes, which imposes a timeframe for the successful completion of the SERS analysis. Lastly, milk processing is a likely and vulnerable target for bioterrorism. In the experiments described in this application note, egg white protein (ovalbumin or OVA) was used as the added foreign protein.

SERS 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, which, together, 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 nanostructure play an important role in the Raman signal enhancement. For more background and information on SERS, see our Technical Note #51874 “Practical Applications of Surface-Enhanced Raman Scattering (SERS)”. The Thermo Scientific™ DXR3 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, 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 a SERS substrate consisting of an antibody bound to the surface of a silver dendrite.

Experiment

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 M 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 antibody binding, the pH of the water containing the dendrites was adjusted to 4.5 using 0.02 M HCl. This 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 combined 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. The pellet was then 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 were 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; the resulting 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 sample solution were 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; the resulting pellet was washed 3 times, deposited onto a glass slide, and allowed to dry at room temperature before analysis. Figure 2 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 for this application 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 in order to maximize signal without saturation of the CCD detector and to avoid sample damage. The DXR Microscope provides precise, patented laser power control at the sample, making it an ideal choice for both SERS work and sensitive samples. (Note that a newer model, the Thermo Scientific DXR3 Raman Microscope, is now available, and offers superior speed and performance over this predecessor model.)

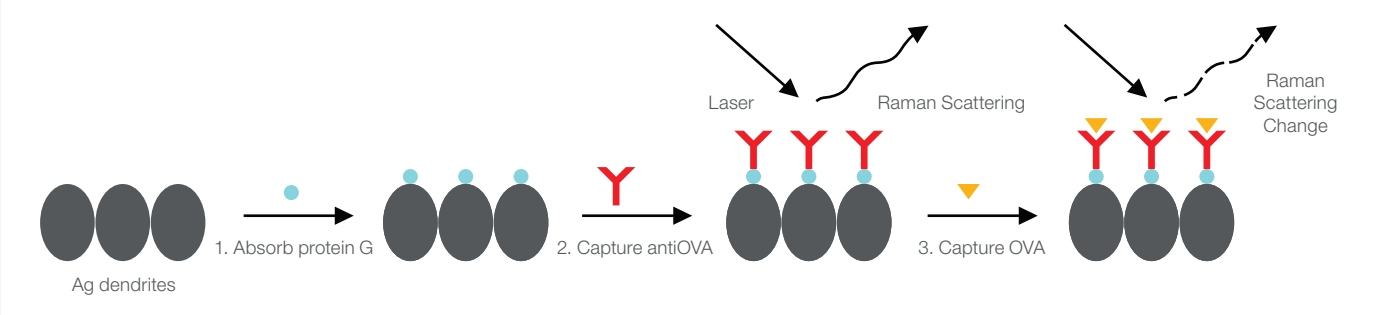

Figure 2. The preparation of the modified silver dendrites and SERS analysis of the bound protein.

Samples were analyzed using 4 mW of laser power and four 15-second scans with a 25 µm slit aperture for each spectrum. Thermo Scientific OMNIC™ Array Automation Software was used for the automated collection and processing of sample groups. OMNIC Software controls the movement of the motorized stage of the DXR Raman Microscope and coordinates the stage movement with the spectral data collection of the samples. OMNIC Software includes templates for many common multi-sample platforms (e.g., 96 well-plate) and new templates can be easily created in the software. OMNIC Software was used to collect 25 spectra per sample. A 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 Thermo Scientific TQ Analyst™ Software, which is used for a range of chemometric calculations. TQ Analyst Software is designed for complex data analysis, particularly large datasets, and can be used for quantitative and qualitative analysis, as well as 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.

Results and discussion

SERS spectra were collected at each step of the antibody binding process and also at the critical final step, when OVA was captured from the milk sample. Figure 3A shows representative spectra of the bare silver dendrite (Ag), the silver dendrite with protein G and antiOVA (Ag-G-antiOVA) attached, and 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; as protein G is bound to the surface the nitrate will be displaced and its signal will decrease. There were overall spectral changes resulting from 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 can be attributed to the thinness of the electromagnetic layer responsible for the SERS enhancement, which was approximately 10 nm. The binding event did cause small, structural changes to the antiOVA. By taking the second derivative of the spectra of the Ag-G-antiOVA and Ag-G-antiOVA-OVA, these changes can be visualized, as shown in Figure 3B.

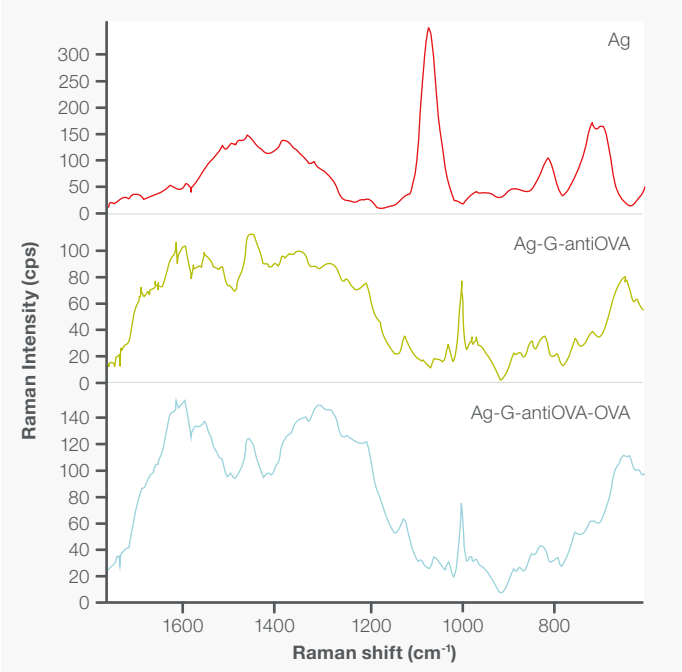

Figure 3A. Average spectra of the bare silver dendrite, silver dendrite with bound protein G and antiOVA (Ag-G-antiOVA), and silver dendrite with protein G, antiOVA, and captured OVA (Ag-G-antiOVA-OVA).

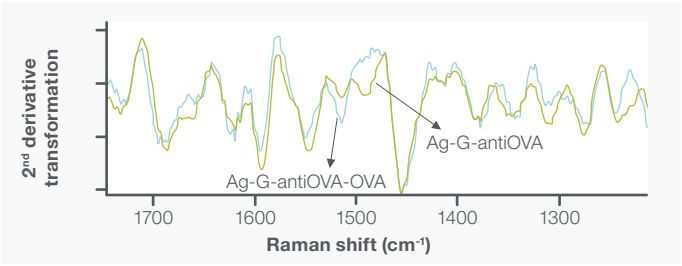

Figure 3B. Comparison of the second derivatives for the Ag-G-antiOVA and Ag-G-antiOVA-OVA spectra.

PCA via TQ Analyst Software was used to cluster together the spectra of the different binding and capture stages. Figure 4 shows the PCA plot for the different substrate preparation stages. Clear grouping of the different stages occurred when comparing the first two principal components. Notably, this method distinguished between the Ag-G-antiOVA and Ag-G-antiOVA-OVA bound groups, meaning that the technique can at least serve as a qualitative test for the presence of a foreign protein.

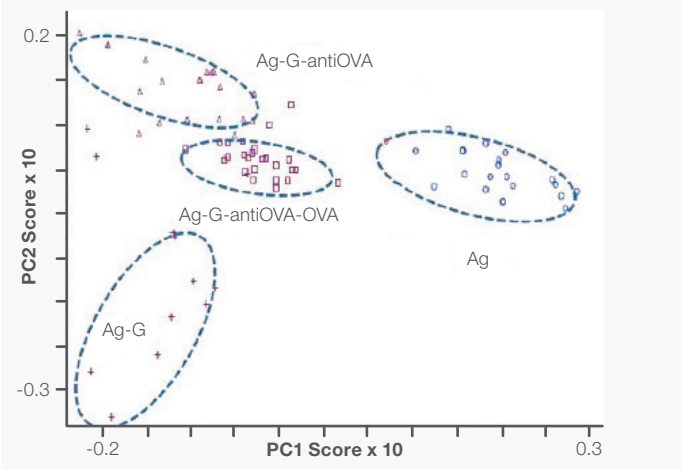

Figure 4. PCA plot of spectra from the different steps of the modified silver dendrite process.

Data analysis was also used to find the detection limit for the binding technique. Two sets of OVA samples were analyzed: OVA-spiked milk and OVA-spiked PBS solution. PCA was used for the data analysis to determine if different concentrations could be distinguished. Figure 5 shows the resulting PCA limit of detection (LOD) plots. The sample set with the best (lowest) LOD 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, or 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 shows 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 likely be attributed to the presence of milk proteins that are involved in non-specific binding with the antiOVA or simply to interference from other milk components.

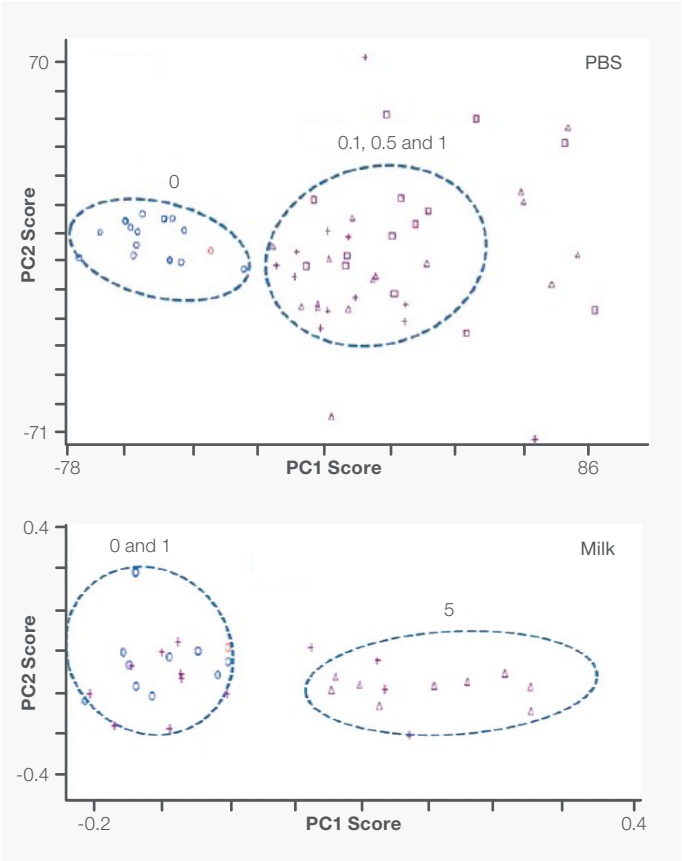

Figure 5. PCA limit of detection (LOD) plots for OVA in PBS (top) and OVA in milk (bottom).

Conclusion

In this application note, we have shown how an antibody-modified silver surface can be used for the qualitative detection of a foreign protein that has been introduced into a complex food matrix. Future work on the antibody-modified dendrite may lead to a lower detection limit and the potential for quantitative results. By using a previously prepared Ag-G-antiOVA complex on a silver surface, the sample preparation and analysis took less than 30 minutes. This fits within the time constraints placed by other tests run at a dairy on shipments of raw milk. Notably, this technique can be modified for the analysis of other proteins by changing the binding antibody; this may even allow the technique to be expanded to other types of molecules. Successful analysis is possible through the combination of antibody-bound SERS and the unique capabilities of the DXR3 Raman Microscope (laser power control, spatial resolution, system alignment, and calibration). For more information about this application, please see Reference 1.

Note: The data in this application note was collected using an older model DXR Raman Microscope. Currently, Thermo Fisher Scientific offers an improved model, the Thermo Scientific DXR3 Raman Microscope, which offers superior speed and performance over its predecessor model.

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.

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