Rapid Detection of an Egg Allergen Ovalbumin from Milk and a Stainless Steel Surface using IMS-SERS

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ABSTRACT

Food allergies are adverse immune responses to food proteins that occur when the body's immune system mistakenly identifies a foreign protein as harmful. In the food industry, special care must be taken to prevent the unintentional addition of allergens to food products through contact with contaminated equipment. It is important to have a quick, easy and cost-effective detection method for allergenic proteins, such as the egg protein ovalbumin, in food products and on food processing equipment. Here we show the benefits of using Immunomagnetic Separation and Surface Enhanced Raman Spectroscopy for the detection of ovalbumin in milk and on a stainless steel surface. These results show superior detection levels to the currently used industry method, Enzyme Linked ImmunoSorbent Assay.

INTRODUCTION

- 11 million people have food allergies in the US
- 5% of children <5 yr old have allergic reactions to food [1]
- Over 230,000 hospital visits each year are due to acute immune attack [2].
- Undeclared allergen in food is the #1 cause (43%) of food recalls due to:
  - Cross contamination in the plant
  - In plant mistakes
  - Undeclared allergens in ingredients
- Even a small amount of allergenic protein can trigger an allergenic reaction.
- Prior reasons show need for rapid detection methods.
- One possibility is Surface Enhanced Raman Spectroscopy (SERS).

OBJECTIVE: This study was undertaken to explore the feasibility of SERS coupled with immunomagnetic separation to rapidly detect ovalbumin (OVA) – an allergenic egg white protein added into milk and onto a stainless steel surface.

METHODS

IMS-SERS procedure consisted of following major steps:

(1) Bind the antibody (antiOVA) to protein G conjugated Dynabeads
(2) Immunocapture antigen (OVA).
(3) Eluate antibody (antiOVA) and antigen (OVA) from the Dynabeads
(4) Mix with silver (Ag) dendrites and deposit on a glass slide. Dry for Raman measurement

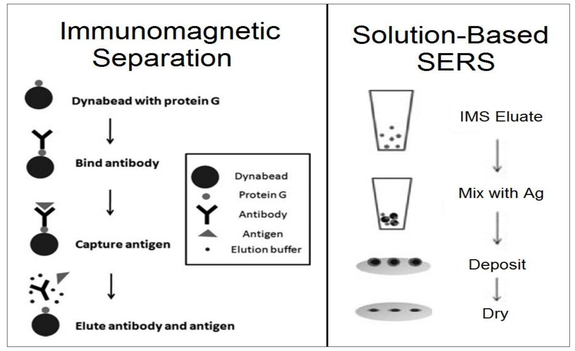

Figure 1 Schematic illustration of the IMS-SERS assay

Silver (Ag) dendrites were used as a nano-enhancer in this study. They were prepared through a simple replacement reaction involving both zinc and silver nitrate [3].

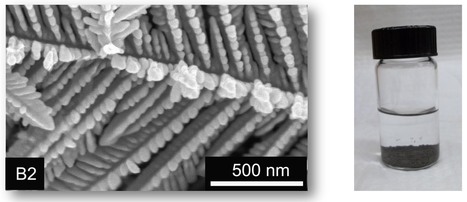

Figure 2 SEM figure of silver dendrite.

For milk samples:

(1) OVA was spiked in whole milk samples at levels of 0, 1, 2, 4, 5 µg/mL to act as test samples.
(2) Each sample (1000 µL) was then incubated with the prepared Dynabead-antiOVA complex (50 µL) for IMS and then measured using SERS.

For swab samples:

(1) OVA was spiked in water (500 µL) at the levels of 0, 5, 10, 25 µg/mL.
(2) OVA samples were pipetted onto a stainless steel surface and dried using a hot plate (~ 60° C).
(3) The surface was swabbed for 30 seconds with a cotton swab.
(4) The swab was then placed in water (1000 µL) and swirled for 2 minutes to dissociate the OVA from the swab.
(5) The OVA was captured from the solution using previously described IMS-SERS method.

An Almega Raman microscope (Thermo Fisher Scientific, Madison, WI) was used in this study. This instrument facilitates 785 nm excitation of SERS spectra through a 10x microscope objective. Quadruplicate SERS measurements were taken with a 25 µm slit aperture for 5s integration time. Spectra were collected using the Thermo Scientific OMNIC™ Software and analyzed using TQ Analyst™

RESULTS

The spectrum exhibited differences (Figure 3) between the negative control (0) and spiked samples (4 µg/mL), indicating the successful capture of OVA out of milk.

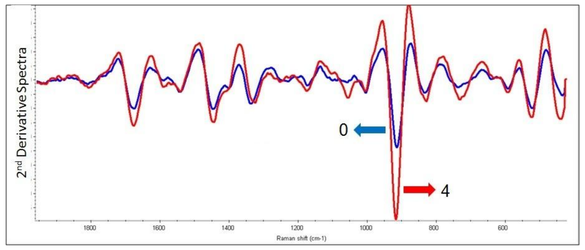

Figure 3 Second derivative spectra of antibody and Ag dendrites before and after capturing OVA from milk

The differences were further analyzed by principal component analysis (PCA), a common statistical tool that separates nearly identical spectral information and builds qualitative models. As shown in Figure 4, the discrimination was observed in milk between the negative control (0 µg/mL) and OVA-spiked samples at 4 µg/mL. Data becomes distinct between 1 and 4 µg/mL, indicating that the limit of detection (LOD) in milk is 4 µg/mL.

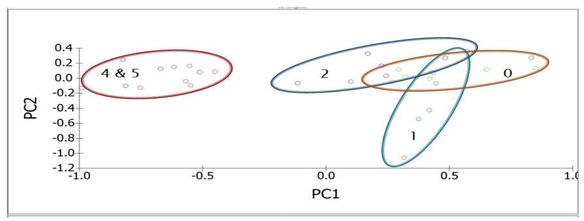

Figure 4 PCA plot of SERS analysis of OVA in milk.

The method was also validated in surface swabs. A PCA analysis of the swab SERS spectra shows a clear distinction between the negative control (0 µg/mL) and the sample spiked at 25 µg/mL (Figure 4), indicating that the limit of detection (LOD) on surfaces is 25 µg/mL.

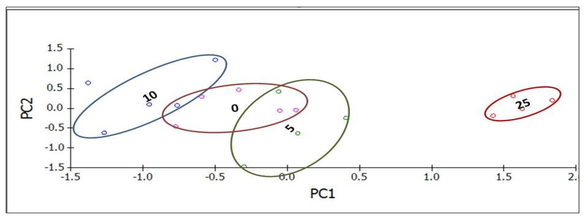

Figure 5 PCA plots of SERS measurements of OVA from surface swabs.

CONCLUSIONS

- This SERS method provides a rapid and simple "Yes/No" way to detect foreign proteins in a complex food matrix such as milk or on the surface of equipment.
- If the Dynabead-G-antibody complex is assembled prior to sensing, the total time for capture and detection of OVA is less than 30 min.
- The LOD for the OVA was 4 µg/mL in milk and 25 µg/mL from surface swabs using PCA.

DISCUSSION

- The detection limit could be further improved to 1 µg/mL in milk and 10 µg/mL from surface swabs using PCA with lower concentration data (data not shown)
- Further analysis of the surface swab procedure show approximately 80% OVA capture from the stainless steel surface with opportunity for improvement. This method has tremendous potential to be used in an industry setting.
- The 10 µg/mL level of detection limit from surface swab is 25 times more sensitive than the currently used protein surface detection method ELISA and 50 times more sensitive than ATPase swabs [4].
- The OVA level of detection in milk is similar to the ELISA level of detection in food matrices, but the SERS method is much more rapid. ELISA methods range from 1.5 to 3 hours, while the SERS method is less than 30 minutes.
- Future studies will include OVA detection using other food matrices, an aptamer-based SERS method instead of antibody-based SERS method, and a portable Raman instrument.

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