Protein Detection Using *Identity* Raman Plate Reader.

INTRODUCTION

Figure 1. *Digilab's Identity Raman Plate Reader.(Catalogue #RMI-78500-1 and #RMI-53200-1)*

Biopolymer (proteins, nucleic acids, polyamines etc.) at high concentrations can be easily detected by Raman spectroscopy [1-4]. Unfortunately, most of natural samples have these substances present at significantly lower levels, making application of the conventional Raman spectroscopy towards natural samples disputable. Sensitivity of the biopolymer's detection can be significantly enhanced by application of Surface-Enhanced Raman Scattering (SERS) technique. This Application Note describes how to use Identity Raman Plate Reader in both conventional Raman, and SERS mode to successfully detect some proteins in solution at different concentrations, particularly at the levels below 1%.

PROCEDURE

Materials and Methods: All measurements were performed with a Digilab Identity Raman plate reader (Figure 1). The system is configurable with either a 532 nm (Model #RMI-53200-1) or a 785 nm (Model #RMI-78500-1) laser. Raman scattering is collected in an 180° backscatter configuration by a spectrometer with a Peltier-cooled CCD array detector capable of <10 cm^{-1} spectral resolution. The Identity supports standard clear, flat bottom 96 and 384 well microtiter plates, as well as custom plate formats. The laser is focused through the bottom of the plate into the well for the analysis of liquids. Powders or coatings on the bottom of the well can be analyzed by placing the plate on a plate shim to raise the bottom of the well to the laser focal point. For SERS application, silver colloid was prepared according to Lee and Meisel [5] and was diluted 5-20 fold with corresponding buffer immediately before use. The colloid was mixed with the sample in 1:1 ratio inside the microplate well and measurement was taken within 1 min after the mixing and additionally 30 min later.

Results and Discussion: Conventional Raman spectroscopy has relatively low sensitivity (with lower detection limits being around 0.1%). Biopolymers in aqueous solutions can usually be detected only at the levels above 1% [1, 2]. Using SERS technique can significantly reduce the detection level for biopolymers [3, 4, 6]. By using a novel system for HTS Raman analysis, Identity plate reader, we studied two structurally different proteins: BSA, a globular protein with main structure represented by α-helix and gelatin, a randomly coiled protein, by both conventional Raman spectroscopy (Figure 2) and SERS technique (Figure 3). Several anions at different concentrations were used as coagulation agents.

The results show that:

1). Identity plate reader can detect as low as 5% gelatin (Figure 2), and 1% BSA (not shown) in aqueous solutions by using conventional Raman technique;

2). using SERS technique significantly enhance sensitivity of protein detection (BSA at levels below 0.1% is easily identifiable);

3). chloride at final concentration above of 50-100mM was sufficient for the observed enhancement effect. Using phosphate, or sulfate at 100mM was as effective as that of chloride, while carbonate anion at neutral pH was totally ineffective;

4). SERS signal linearly depends on the protein concentration in a buffer containing 100mM chloride when the protein is present at concentrations below 1%.

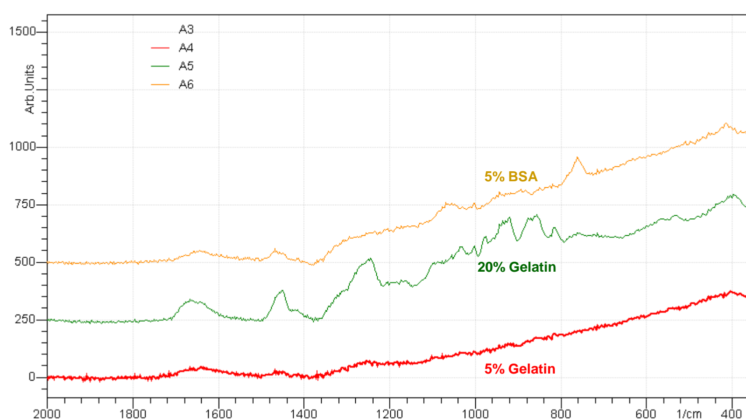

Figure 2. *Raman spectra of some proteins in water assayed by Identity equipped with 785nm Laser.*

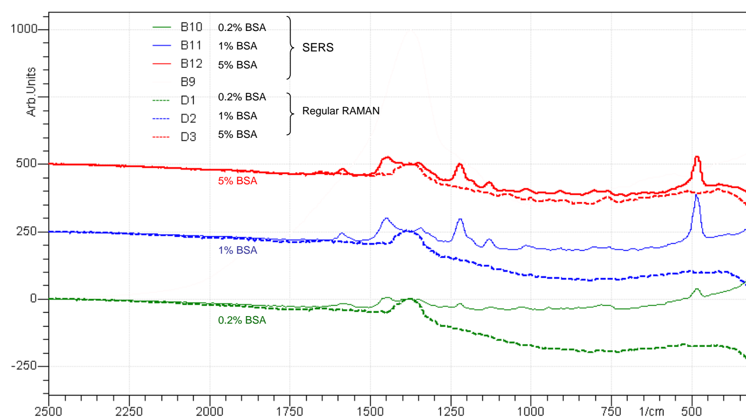

Figure 3. *SERS (solid lines) and conventional Raman (dashed lines) spectra of BSA at different concentrations (0.2-5%) in 0.1M NaCl, with 1/10 diluted silver colloid. (Background subtracted; Y values paired up.)*

Recommended Procedure: Switch Identity Raman plate reader on and start the Identity controlling software on the PC. Choose the "User Mode" for operation. For a system equipped with 785nm laser, we recommend using 100% power, while for the system equipped with 532nm laser, a 50% power is recommended for Raman data collection. Check inside the box for dark current correction. For detection of a protein in solution in the range of concentrations below 1%, add concentrated NaCl to the sample to make the final concentration of NaCl 100mM, dilute silver colloid with 100mM NaCl buffer 5-20 fold (the exact dilution factor needs to be determined experimentally for each batch of the colloid) and mix the sample with the diluted colloid in the well of the micro-plate immediately before the measurement (for a 96-well microtiter plate, use 0.1-0.15ml of sample and colloid solutions per well; for a 384-well plate, use 0.05ml of each solution per well). Perform Raman spectra analysis on Identity Raman plate reader using desired exposure time (from 0.01 to 60 sec per a single exposure) and necessary number of repeated exposures to be used for averaging of the final spectra. Collected spectral characteristics will be saved in files marked with the date (in the format year-month-day followed by the time (24hr based), the experiment has been started) and ready to be used by a third-party application software.

References:

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