



## Microsampling of Low-Concentration Aqueous Proteins Using a Novel Hydrophobic Sampling Device and the $\mu$ Max™ Sample Compartment Infrared Microscope

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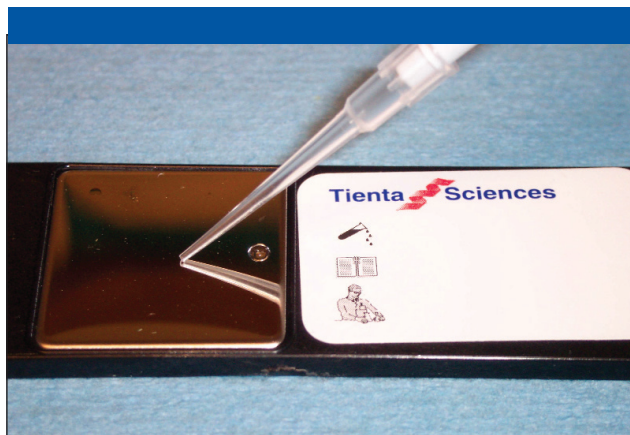
Proteins are the most important group of molecules for biopharmaceutical, medical and proteomics research. Infrared and Raman spectroscopy are the most powerful nondestructive techniques for characterizing protein secondary structure. Infrared detection is a sensitive tool to identify the secondary structure of the protein and has the additional advantage over Raman that it is not sensitive to fluorescence which is difficult to avoid with natural biological samples. Based on the infrared spectrum of protein solutions, the structure of the protein can be characterized in the 1400–1800  $\text{cm}^{-1}$  range. In biomedical or biopharmaceutical samples, however, some of the proteins are in very low concentration aqueous solutions which are difficult samples in the infrared due to the strong absorption of water.

One of the new techniques proposed here is a combination of infrared microscopic detection of concentrated proteins using a hydrophobic mirror surface for sample preparation. The specially treated surfaces allow a drop of protein solution to bead up and dry in a controlled small ring. The structure of the protein using this technique remains solvated after the evaporation of the water, leaving a concentrated ring of proteins with a very similar structure to what it had in solution. The size of the ring requires that the optical sampling be smaller than 50  $\mu\text{m}$ . Thus, an infrared microscope is a requirement for this application to achieve the necessary visual targeting and a good signal-to-noise.

### Experimental Conditions

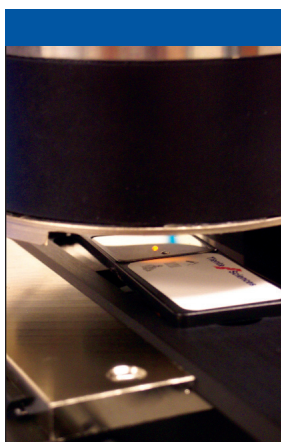
Protein concentration from polar dilute solutions such as aqueous media was recommended mainly for Raman spectroscopy by using a SpectRIM™ hydrophobic stainless steel substrate (1–3). The substrate surface is a highly polished stainless-steel plate with an ultra thin (< 50 nm) hydrophobic coating, mounted on a microscope slide. To compare the protein secondary structures in solution and on the hydrophobic surfaces, for the latter a 15- $\mu\text{m}$  path length liquid cell with a specially coated  $\text{CaF}_2$  transmission plate was used.

Sample preparation consisted of depositing a 2- $\mu\text{L}$  sample with a micropipette onto the surface of the SpectRIM plate (Figure 1) and allowing the solution to dry. Concentration of the protein solutions was around 2  $\mu\text{M}$ . The drying usually took only a few minutes, and after that the slide was placed on the positioning table of a  $\mu$ Max™ Infrared Microscope (Figure 2) which was installed in the sample compartment of an FTIR spectrometer. The beam was focused on a clear area of the plate for background measurement, and on the ring for sample measurement (Figure 3). The measurement time was typically less



**Figure 1:** Application of protein solution to a reflective SpectRIM hydrophobic stainless steel substrate.

than one minute with either 4 or 8  $\text{cm}^{-1}$  resolution. For reference, the aqueous solution of the proteins were measured in a 6- $\mu\text{m}$  path length BioCell™  $\text{CaF}_2$  liquid cell in transmission (BioTools, Inc.).



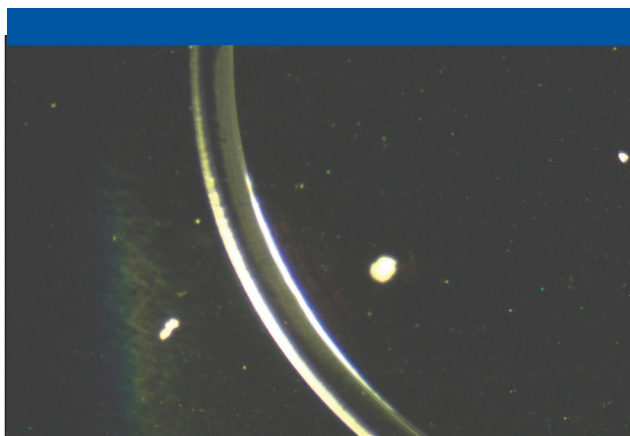
**Figure 2:** Protein sample plate placed in a  $\mu$ Max™ sample compartment microscope. Depending on the sample and the plate, transmission, reflectance or ATR microscopy can be used.

### Results

Ovalbumin and lysosyme proteins were used to test the new sampling technique. The 2- $\mu\text{L}$  samples produced approximately 2-mm diameter rings, 3–10  $\mu\text{m}$  wide depending on the protein concentration. When the concentration and the delivered volume were kept constant the rings were very well defined and sharp, and had a perfect circular shape. The usability of a new

protein sampling technique in the infrared depends on how strongly the drying changes the protein structure.

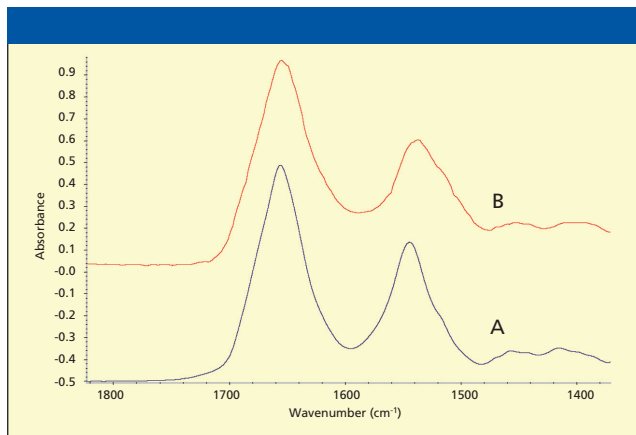
To test this, spectra of protein solutions were measured with water as background using 6- $\mu\text{m}$  path length cell. Figure 4 shows the Amide I and II regions of the spectrum of lysozyme in solution and on the hydrophobic slide. Spectra A and B are very similar, with only a small shift observed in the Amide II (1544  $\text{cm}^{-1}$ ) band due to changes in hydrogen bonding. There do not appear to be any conformational changes upon drying of protein solution on the hydrophobic surface.



**Figure 3:** Portion of a typical protein sample ring on SpectRIM slide (from a 1- $\mu\text{m}$  ovalbumin solution).

Spectra taken with the  $\mu\text{Max}^{\text{TM}}$  Infrared Microscope using different protein conformations were different according to the anticipated different secondary structure. Based on the spectra, the proteins can be identified and their secondary structure analyzed. Bacterial proteins were similarly analyzed and reported earlier (4).

Figure 5 shows the comparison of the normalized spectra of albumin and lysozyme.

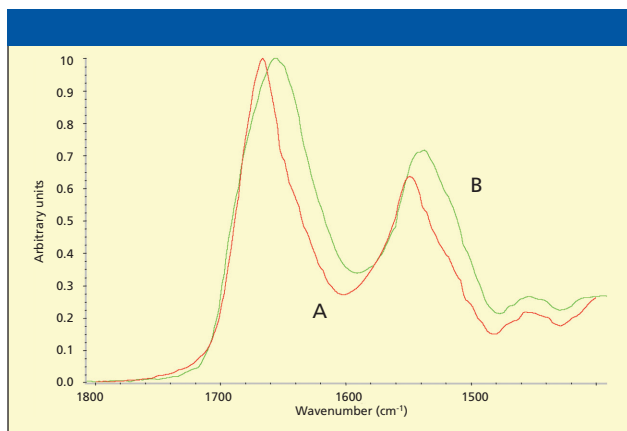


**Figure 4:** Infrared transmittance spectra of lysozyme in aqueous solution (A), lysozyme on SpectRIM steel reflectance slide (Spectra are normalized between 0 to 1 for comparison).

## Conclusions

Combination of the new SpectRIM hydrophobic stainless steel substrate with infrared microscopy is a powerful novel technique

to determine secondary structure. The protein type secondary structure, the  $\alpha$ -helix,  $\beta$ -sheet, bend, and turn content can be determined from the infrared spectra using specialized protein database and programs (5). Transmission plates with infrared transmitting substrates, such as  $\text{CaF}_2$ , were also available on an experimental basis and could provide even better signal-to-noise than the micro-reflectance measurements.



**Figure 5:** Infrared transmittance spectra ovalbumin (A) and lysozyme (B) on SpectRIM steel reflectance slide. Spectra are normalized between 0 to 1 for comparison.

## Acknowledgements

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## References

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- (3) More information on the SpectRIM<sup>TM</sup> slides can be obtained from Tienta Sciences, Indianapolis, Indiana or found at [www.tientasciences.com](http://www.tientasciences.com).
- (4) Emilia E. Gerischer, Daniell B. Kelly, Kathryn S. Kalasinski, April A. Shea, Gabor Kemeny, Dongmao Zhang, Laurence A. Nafie, and Rina K. Dukor. Paper # P1.39.A, ICAVS3, Delavan, WI (Aug.14-19, 2005).
- (5) BioTools, Inc Wauconda, IL., [www.btools.com](http://www.btools.com).

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