HARRICK SCIENTIFIC PRODUCTS

No. 21169

ANALYSIS OF LOWER CONCENTRATION PROTEIN STANDARD SOLUTIONS BY ATR INFRARED SPECTROSCOPY

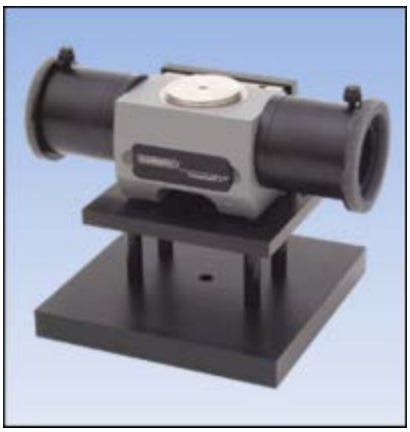

Figure 1. The Harrick ConcentratIR2™ multiple reflection ATR accessory.

INTRODUCTION

In a previous applications note, "Analysis of Protein Standard Solutions by ATR Infrared Spectroscopy," the ConcentratIR2™ with a silicon ATR crystal was used to analyze standard solutions of the protein bovine gamma globulin (BGG). It was shown that the ConcentratIR2™ is capable of qualitatively and quantitatively analyzing aqueous protein solutions. This is a follow up to that study to investigate the detection limits with lower concentrations of BGG using the extended silicon ATR crystal, which has more sensitivity over the standard silicon ATR crystal.

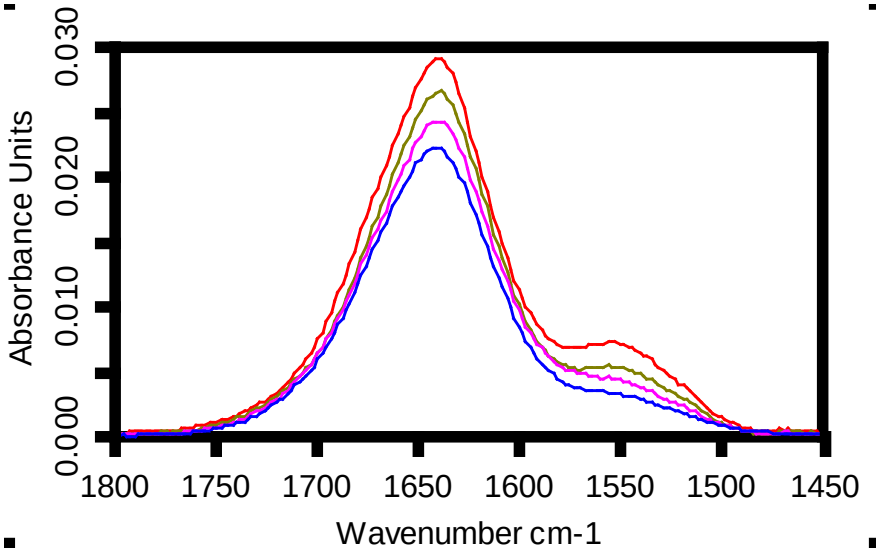

Figure 2. IR spectra of protein samples showing "Amide I" and "Amide II" bands. Red: 250 µg/mL, yellow: 125 µg/mL, pink: 62.5 µg/mL, blue: 31.25 µg/mL.

EXPERIMENTAL

Infrared spectra were collected on an FT-IR spectrometer equipped with a ConcentratIR2™ with the extended silicon ATR crystal optically contacted to a ZnSe crystal. An MCT detector cooled with liquid nitrogen was used. All spectra were a result of 64 averaged scans at a resolution of 4 cm^{-1}. The gain was set to 4, the aperture was set to 100 (fully open), and spectra were collected in the range 4000-650 cm^{-1}. Four BGG samples were analyzed, with concentrations: 31.25, 62.5, 125 and 250 µg/mL (Alpha Diagnostic International Inc.)

RESULTS AND DISCUSSION

The spectrum of pure water was subtracted from each protein spectrum to obtain the amide bands and the IR spectra of the amide bands were baseline corrected. Figure 2 shows the IR spectra of all protein samples in the spectral range of 1800-1450 cm^{-1}. The "Amide I" band is present at around 1650 cm^{-1}, and the "Amide II" band is present around 1550 cm^{-1}.

Resembling the results in the previous applications note, the

applications note

intensity of the peaks increases with increasing concentration. Note the band intensities are about 4X higher when using the extended Si ATR plate versus the standard Si ATR plate for 250 µg/mL and 125 µg/mL.

A calibration curve was produced using the peak area of the "Amide I" band and is shown in Figure 3. Peak area was used instead of intensity for more accuracy and reliability. The data indicates a linear relationship between peak area and concentration.

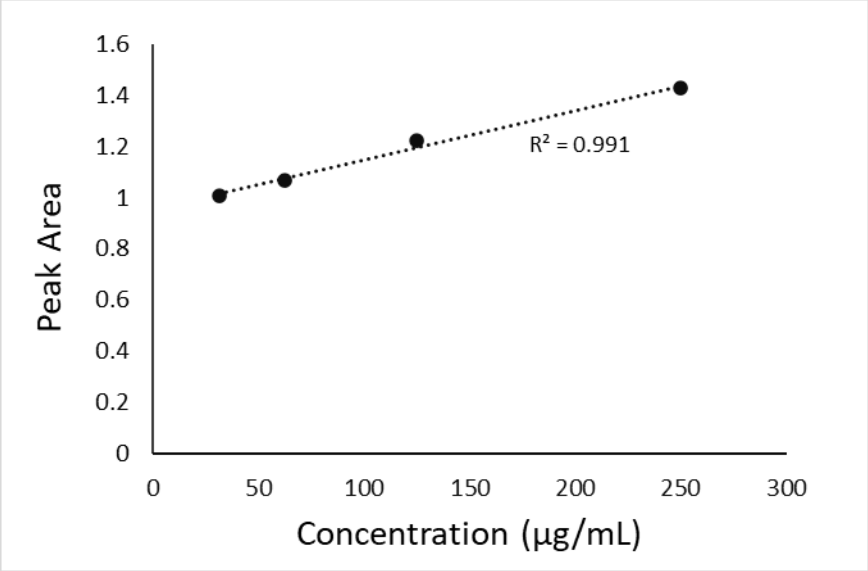

Figure 3. Calibration curve produced from the peak area of the "Amide I" bands in Figure 2.

CONCLUSION

The ATR spectra of the lower concentration standard solutions of BGG exemplifies the enhanced sensitivity of the ConcentratIR2™ configured with an extended silicon ATR sampling plate. A trend in the peak area of the "Amide I" band was used to show the possibility of obtaining a reliable concentration curve and demonstrates that it is capable of qualitatively and quantitatively analyzing lower concentration aqueous protein solutions.

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