HARRICK SCIENTIFIC PRODUCTS

No. 21131

ANALYSIS OF PROTEIN STANDARD SOLUTIONS BY ATR INFRARED SPECTROSCOPY

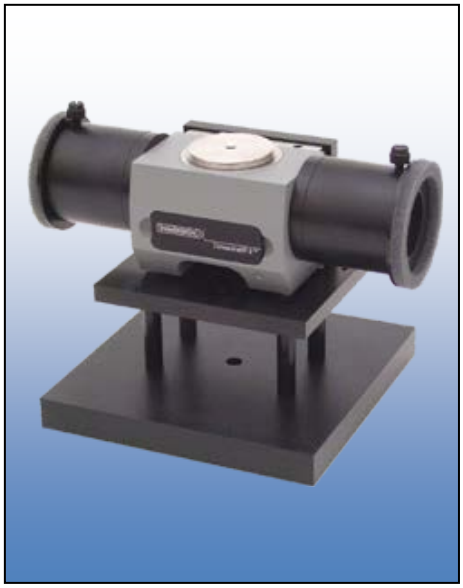

Figure 1. The ConcentratIR2™ multiple-reflection ATR accessory.

INTRODUCTION

One of the many applications of infrared (IR) spectroscopy is protein analysis. Proteins typically have a number of characteristic amide bands that can be used to elucidate secondary structure[1] as well as generate calibration curves for quantitative analysis. The ConcentratIR2™ is a multiple-reflection attenuated total reflection (ATR) FT-IR accessory (see Figure 1) that allows for analysis of strongly IR-absorbing samples, such as aqueous solutions, using a small sample size (as little as 10 µL). In this application, standard solutions of the protein bovine gamma globulin (BGG) were analyzed using the ConcentratIR2™ with a silicon ATR crystal. The "Amide I" and "Amide II" bands of BGG were observed and used to generate a calibration curve.

EXPERIMENTAL

IR spectra were collected on an FT-IR spectrometer equipped with a ConcentratIR2 with a silicon ATR crystal optically contacted with a ZnSe crystal. An MCT detector cooled to 77 K 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 8, and the aperture was set to 100 (fully open), and spectra were collected in the range 4000-650 cm^{-1}. The spectrometer and accessory were purged with filtered air (water and carbon dioxide removed) that was produced by a Parker Balston

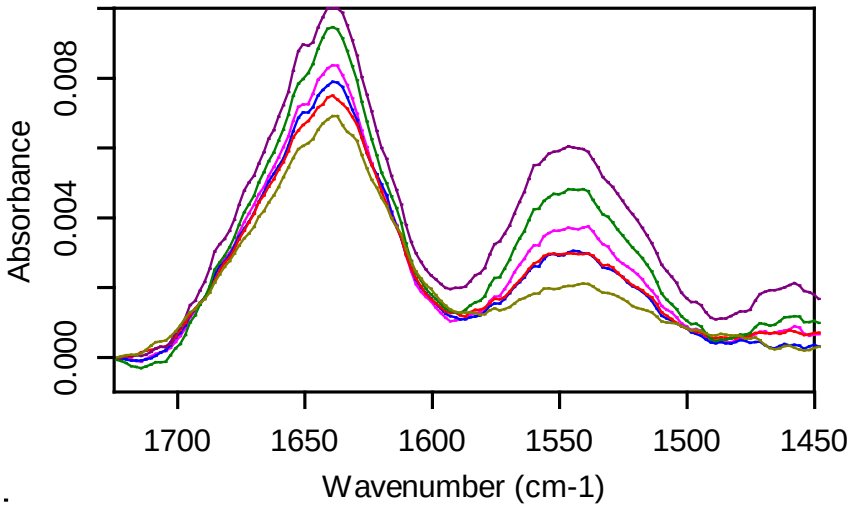

Figure 2. IR spectra of protein samples showing "Amide I" and "Amide II" bands. Table 1 gives the various sample concentrations and the color of their spectra on the graph.

Sample	Conc. (µg/mL)	Spectrum Color
1	2000	Purple
2	1500	Green
3	750	Pink
4	500	Blue
5	250	Red
6	125	Yellow

Table 1. Concentrations of standard protein samples and the color of their spectra on the graph in Figure 2.

applications note

Analysis of Protein Standard Solutions by ATR Infrared Spectroscopy

Model 75-62 FT-IR Purge Gas Generator at 40 SCFH. Each BGG protein sample was kept refrigerated right until analysis. One drop of sample was placed on the ATR crystal of the ConcentratIR2™ for analysis. Six BGG samples were analyzed, with concentrations ranging from 125-2000 µg/mL (Thermo Scientific Bovine Gamma Globulin Standard Pre-Diluted Set, Product #23213).

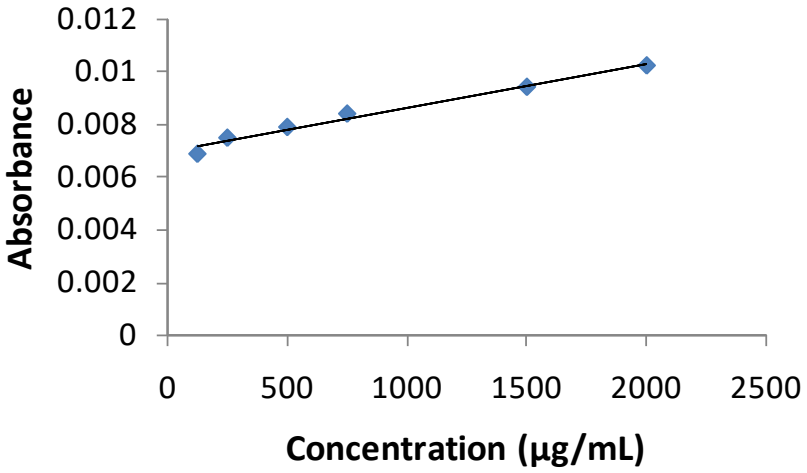

Figure 3. Calibration curve generated from the maximum absorbance of the "Amide I" bands.

Results and Discussion

The IR spectra of the BGG protein samples were baseline-corrected and the spectrum of pure water was subtracted from each protein spectrum to reveal the amide bands. Figure 2 shows the IR spectra of all protein samples in the spectral range of 1700-1450 cm^{-1}. The "Amide I" band is clearly present at around 1645 cm^{-1}, and the "Amide II" band is present around 1550 cm^{-1}. The "Amide I" and "Amide II" bands are a result of the addition of secondary structure bands, and calculations could be performed to estimate the secondary structure of the protein. The intensity of the peaks also clearly decreases with decreasing concentration.

A calibration curve was generated using the peak maximum intensity of the "Amide I" band and is shown in Figure 3. The data suggests a linear relationship between absorbance and concentration. Better calibration may be achieved using partial least squares analysis with an increased number of samples, as well as with better lab temperature and humidity control.

Conclusions

Multiple-reflection ATR accessories such as the ConcentratIR2™ are capable of qualitatively and quantitatively analyzing aqueous protein solutions. The IR spectra of standard solutions of BGG contained "Amide I" and "Amide II" bands that are useful in determining the secondary structure of the protein. A trend in the intensity of the "Amide I" band was used to demonstrate the possibility of obtaining a reliable concentration curve, particularly if more samples are used.

References

1. Barth, A. *Biochimica et Biophysica Acta* 1767 (2007) 1073-1101.

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