**THE CONCENTRATIR2™ MULTIPLE REFLECTION ATR** **NO. 21156**

# Analysis of Deuterated Proteins Using the ConcentratIR2™ ATR Accessory

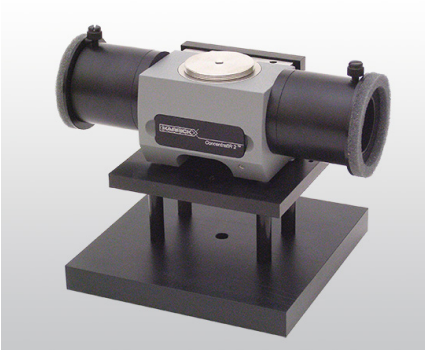

Figure 1. The ConcentratIR2™ multiple reflection ATR accessory.

## INTRODUCTION

Bovine serum albumin (BSA) is an often used protein concentration standard in laboratory experiments. BSA has various biochemical applications due to its small and relatively stable nature. Examples include use in ELISA's, immunoblots and immunohistochemistry. BSA is also widely used due to its ability to increase assay signals, and its low reactivity in many biochemical pathways. It can be purchased inexpensively since it can be obtained by purifying bovine blood which is a byproduct of the cattle industry. BSA and other proteins can be quickly examined as is using ATR.

BSA is frequently examined in water, where there is some overlap between the OH bend and the amide bands. In research, $D_2O$ is also used as a solvent for protein studies since its water band is shifted resulting in a less intense band in the amide region compared to water. This results in a better s/n ratio after pure deuterated water is subtracted from the raw data collected.

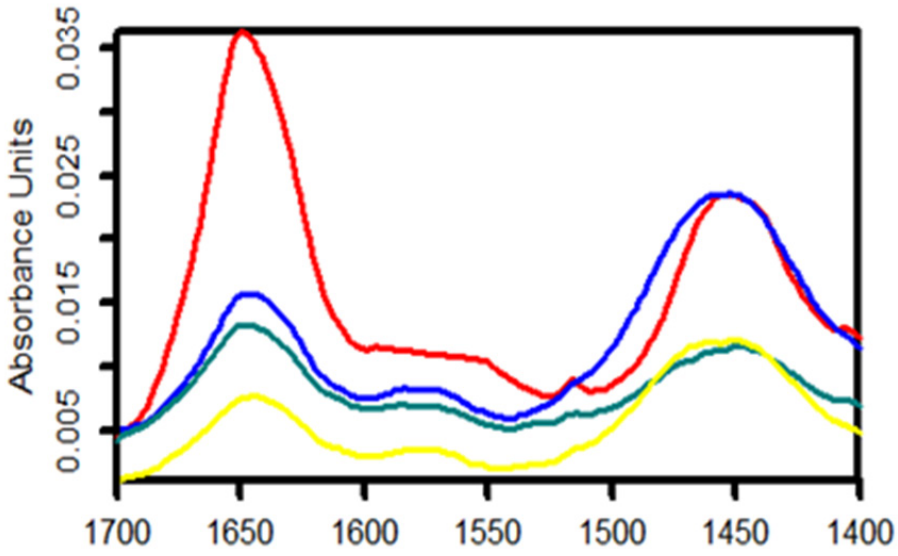


Figure 2. The ATR spectra of bovine serum albumin in $D_2O$ in concentrations of 5.0 mg/ml (red), 1.0 mg/ml (blue), 0.5 mg/ml (green) and 0.1 mg/ml (yellow).

This note shows the analysis of bovine serum albumin and its amide 1 and amide 2 bands in various concentrations in $D_2O$ solution.

## EXPERIMENTAL

Spectra were obtained on a FTIR spectrometer equipped with a liquid nitrogen cooled MCT detector using the ConcentratIR2™ accessory with its Si ATR crystal. Samples of BSA in $D_2O$ were used with concentrations of 0 mg/ml, 0.1 mg/ml, 0.5 mg/ml, 1 mg/ml, and 5 mg/ml. The clean ATR crystal was used for the background. Samples were introduced onto the crystal via a pipette and spectra were collected from 4000 to 650 $cm^{-1}$ using 64 scans at a resolution of 4 $cm^{-1}$.

## RESULTS AND DISCUSSION

Each spectrum of BSA had pure $D_2O$ subtracted from it to show the amide 1 and amide 2 bands present. Figure 2 clearly shows the amide 1 band at 1650 $cm^{-1}$ and the amide two band at 1455 $cm^{-1}$. These two peaks can be analyzed to help determine the secondary structure of the protein.

Figure 2 also shows the intensity of each band increasing as the samples become more concentrated. In Figure 3 the relationship is linear between concentration and peak intensity for the amide 1 bands as shown by the calibration curve, while the amide 2 bands also show a linear relationship in the calibration curve shown in Figure 4. It has been shown that when using $D_2O$ as a solvent the hydrogen deuterium exchange that results can have effects on the secondary structure of the protein being studied. These exchanges can affect amide band frequencies and could account for why the amide 2 band in Figure 2 is shifted to around 1455 $cm^{-1}$.

## CONCLUSION

In this note, we demonstrate that the ConcentratIR2 multiple reflection ATR accessory is suitable for analyzing protein solutions. The spectra of BSA in $D_2O$ contained amide 1 and amide 2 bands and calibration curves were generated for each band to show the linear relationship between peak intensity and concentration. The amide 1 bands displayed a linear relationship between peak intensity and concentration, as did the amide 2 bands. The possible effects of deuterium-hydrogen exchanges were able to be shown by the apparent shift of the amide 2 band to 1455 $cm^{-1}$.

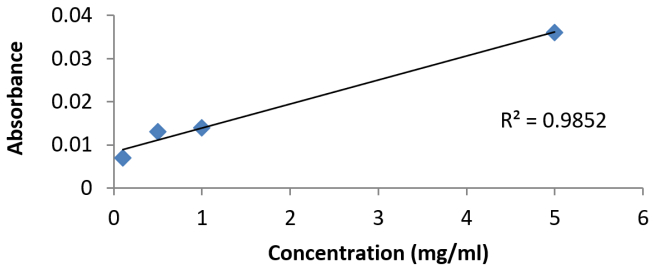


Figure 3. Calibration curve for the amide 1 band.

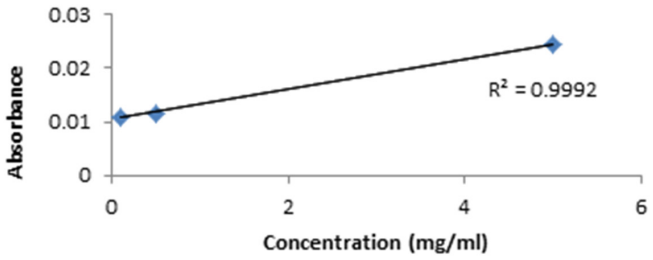


Figure 4. Calibration curve for the amide 2 band.

## REFERENCES

1. J. Baenziger and N. Méthot. "Fourier Transform Infrared and Hydrogen/Deuterium Exchange Reveal an Exchange-resistant Core of α -Helical Peptide Hydrogens in the Nicotinic Acetyl-choline Receptor ",*The Journal of Biological Chemistry,* 270, 29129-29137 (1995).
2. J. Kong and S. Yu, "Fourier Transform Infrared Spectro-scopic Analysis of Protein Secondary Structures", *Acta Biochimica et Biophysica Sinica*, 39(8), 549-559 (2007).