

ANALYSIS OF DEUTERATED PROTEINS USING THE CONCENTRATIR2™ 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.

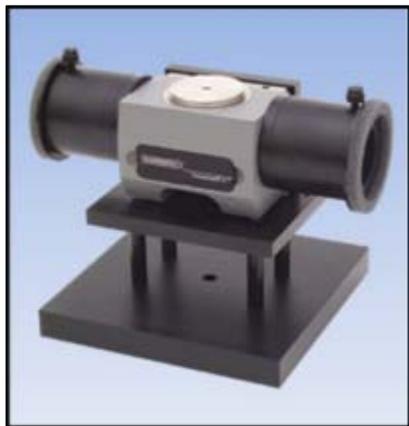


Figure 1. The ConcentratIR2™ multiple reflection ATR accessory.

BSA is frequently examined in water, where there is some overlap between the OH bend and the amide bands. In research, D₂O 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.

This note shows the analysis of bovine serum albumin and its amide 1 and amide 2 bands in various concentrations in D₂O 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 D₂O 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 D₂O subtracted from it to show the amide 1 and amide 2

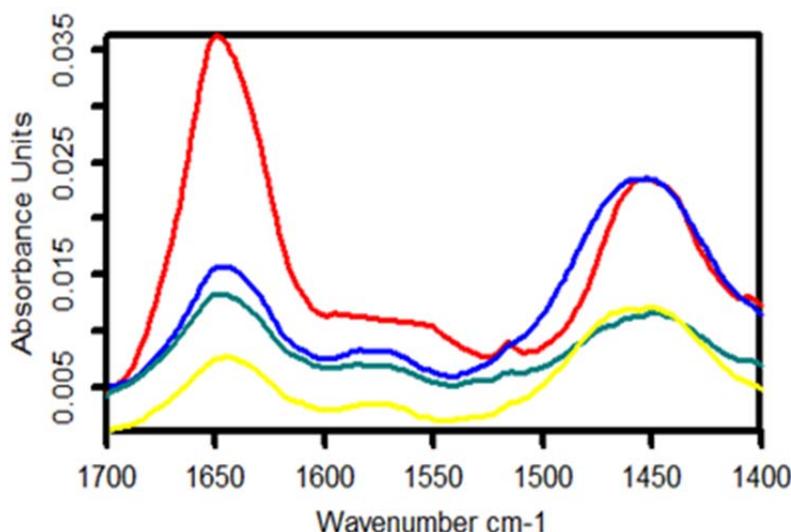


Figure 2. The ATR spectra of bovine serum albumin in D₂O in concentrations of 5.0 mg/ml (red), 1.0 mg/ml (blue), 0.5 mg/ml (green) and 0.1 mg/ml (yellow).

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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

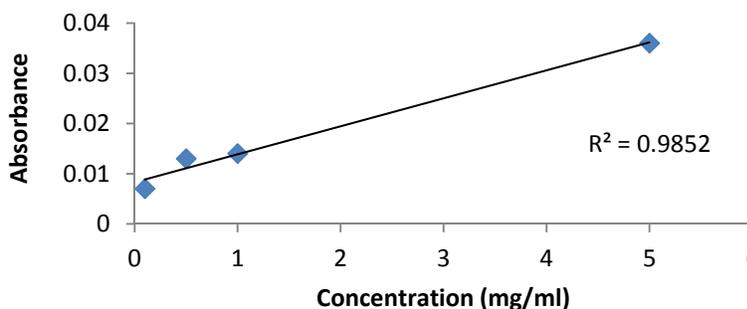


Figure 3. Calibration curve for the amide 1 band.

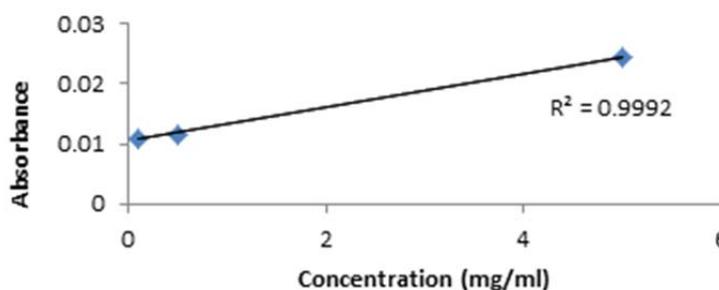


Figure 4. Calibration curve for the amide 2 band.

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} .

REFERENCES

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HARRICK SCIENTIFIC PRODUCTS
141 Tompkins Ave., 2nd floor • PO Box 277 • Pleasantville, NY 10570

www.harricksci.com • E-mail: info@harricksci.com

Phone (international): 914-747-7202 • Phone (in USA): 800-248-3847 • Fax: 914-747-7209