Application Note
CD Spectroscopy

High-throughput CD spectral measurement: biomedicines and pH dependency

Introduction

Biomedicine is a medicinal response to a physical or biological issue with the human body. Biomedicines use active ingredients derived from proteins and the research and development behind these methods is increasing daily. However, these biomedicines are more sensitive to environmental changes, such as a change in temperature, pH, and salt concentration, than traditional pharmaceuticals. This environmental sensitivity may be a potential cause of biomedicines' deactivation during production and storage processes.

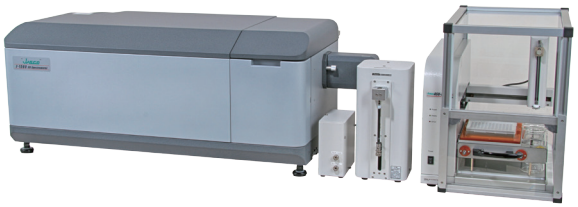
Circular dichroism (CD) measurements can provide information regarding changes in protein structure in small quantities of sample. Since protein structure and activity are closely related, CD measurements are now widely accepted in the quality control of protein, which includes biomedicines.

To meet the demand for increased sample throughput in the modern pharmaceutical laboratory, JASCO has developed a fully automated high-throughput CD spectral measurement system. This system is composed of a J-1500 CD spectrometer with an autosampler unit. The high-throughput CD system enables the automation of sample pretreatment, injection and washing.

In this application note, we will demonstrate the use of the ASU-800 automated system to evaluate the pH dependency on the structure of human serum albumin (HSA).

Keywords

Biomedicines, quality control, automated measurement, high-throughput screening, human serum albumin, circular dichroism, J-1500, ASU-800, biochemistry, pharmaceutical



JASCO J-1500 high-throughput CD system
View product information at www.jascoinc.com

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Experimental

The pH of human serum albumin (reagent 1) was adjusted by diluted sulfuric acid or sodium hydroxide (reagent 2) using a 1:4 ratio. The initial concentration of the 30 mg of HSA used was 0.05 mg/mL and the final concentration after mixing was 0.01 mg/mL. The mixed reagent was injected into a 10 mm rectangular cell in the sample compartment of the J-1500. The entire sampling procedure, including the mixing of reagents, CD spectral measurement, and the washing and drying of cells were pre-programmed so that a fully automated and unattended measurement could be performed.

Measurement conditions	
Data acquisition interval	0.5 nm
Path length	10 mm
Spectral bandwidth	1 nm
Scan speed	100 nm/min
Accumulations	2
Response time	1 sec
Reagent 1 concentration (HSA)	0.5 mg/mL
Reagent 1 used	30 mg
Mixed reagent concentration	0.01 mg/mL

Results

Figure 1 shows the CD spectra of human serum albumin for 10 different pH values (1.3, 2.2, 3.1, 4.1, 5.4, 6.7, 7.5, 8.4, 9.3, 10.7). The plot illustrates that the CD decreases as the pH increases, indicating structural changes to HSA.

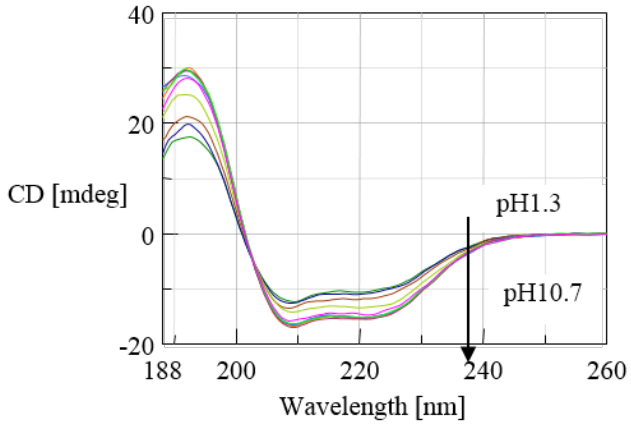

Figure 1. CD spectra of HSA for varying pHs. The arrow indicates the decrease in CD as the pH increases.

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Figure 2 shows these structural changes in more detail by plotting the CD values at 222 nm as a function of pH. 222 nm is a CD marker band for α-helices in proteins. By plotting this band, we can show the structural deviation of the protein's α-helical content. Between pH 5 to 10, the α-helical structure is conserved. However, in acidic conditions (<5) and basic conditions (>10), the decreased CD intensity suggests a slight denaturation of the HSA protein.

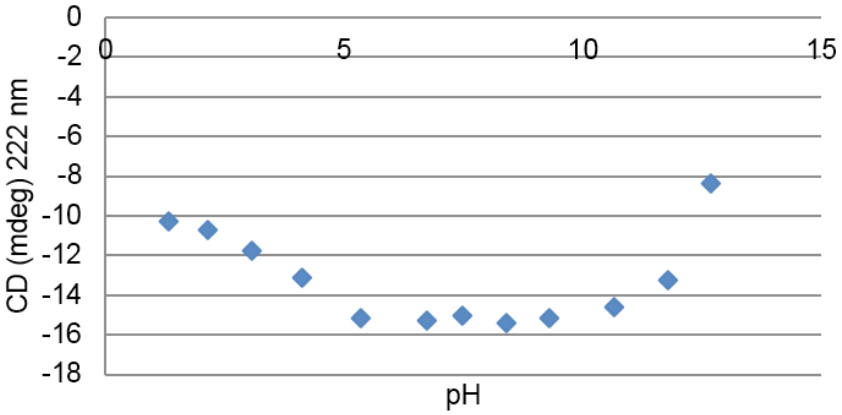

Figure 2. The pH dependency of the CD intensity of the 222 nm band.

Conclusion

By monitoring the pH dependency of human serum albumin using the J-1500 CD spectrometer and sample-handling ASU-800, numerous samples can be automatically measured to determine the structural integrity of the protein. This application note demonstrates that CD measurement is an effective tool for the quality control of biomedicines and that the JASCO J-1500 high-throughput CD system can assist pharmaceutical laboratories in the unattended screening of large numbers of samples.

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