Introduction

Human serum albumin (HSA) is the most abundant protein in blood plasma. HSA binds with pharmaceutical compounds and other in-vivo substances and plays an important role in the transport of these substances to target organs. Several studies have reported on the binding affinity of HSA and its interactions with a variety of compounds.\(^1\)\(^3\)

This application note demonstrates the use of the J-1500 CD spectrometer and ATS-530 automatic titrator to monitor the interaction of HSA titrated with 3,5-diiodosalicylic acid. While 3,5-diiodosalicylic acid is achiral, its interaction with chiral HSA induces circular dichroism.

Keywords

J-1500, circular dichroism, HSA, pharmaceuticals, ATS-530 automatic titrator, biochemistry, pharmaceutical

Experimental

<table>
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<th>Measurement conditions</th>
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<tbody>
<tr>
<td>Data acquisition interval</td>
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<tr>
<td>Scan speed</td>
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<tr>
<td>Spectral bandwidth</td>
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<td>Data pitch</td>
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<tr>
<td>Path length</td>
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<td>Accumulations</td>
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Analyzing the interaction of human serum albumin and 3,5-diiodosalicylic acid

Results

Chirality can be induced in an achiral substance interacting with a chiral substance. This interaction will exhibit circular dichroism. The resulting CD spectrum from the interaction of achiral 3,5-diiodosalicylic acid with chiral HSA shows a positive peak at 320 nm in Figure 1. While HSA does not show a CD signal at 320 nm, the induced CD from the interaction of HSA and 3,5-diiodosalicylic acid depicts this signal at 320 nm which increases with the increasing additions of 3,5-diiodosalicylic acid and is shown in Figure 2.

![Figure 1](image1.png)

**Figure 1.** 3-D graphical representation of the CD spectrum of HSA titrated with 3,5-diiodosalicylic acid.

![Figure 2](image2.png)

**Figure 2.** CD spectra of HSA titrated with 3,5-diiodosalicylic acid. The arrows represent the increasing volume of 3,5-diiodosalicylic acid.

The increase in the induced CD signal at 320 nm was plotted in Figure 3 as a function of the 3,5-diiodosalicylic acid concentration. Figure 3 is also known as a Hill plot which describes the cooperativity of the interaction between HSA and 3,5-diiodosalicylic acid. A dissociation constant, which is the concentration at which 50% of a substance is bound, can also be estimated from a Hill plot. Using Figure 3, the dissociation constant of 3,5-diiodosalicylic acid binding to HSA was determined to be 0.023 mM. The Hill coefficient is approximately 3.1 which indicates a positive cooperative reaction.
Conclusion

This application note demonstrates the use of the ATS-530 automatic titrator and J-1500 CD spectrometer to obtain CD spectra of the titration of 3,5-diiodosalicylic to HSA in order to determine a dissociation constant.

References


Figure 3. CD signal monitored at 320 nm for the titration of 3,5-diiodosalicylic acid to HSA.