

Advanced application of CD measurement for pharmaceutical research

Analyzing the interaction of human serum albumin and 3,5-diiodosalicylic acid

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 interaction with various compounds 1)-3).

Herein, the measurement of CD induced by the interaction of HSA titrated with 3,5-diiodosalicylic acid and subsequent analysis of the dissociation constant is presented.



JASCO J-1500 CD Spectrometer
at www.jascoinc.com

Keywords

Proteins, Induced CD, Pharmaceuticals

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Measurement Conditions

Sample:	HSA, 0.0228 mM, 100 mM acetate buffer (pH 6.3), 2 mL
Titration:	3,5-diiodosalicylic acid, (0.25 mM), 50 mL, 20 times
Column oven:	360 to 260 nm

Measurement System

J-1500 CD Spectrometer

PTC-517 Peltier thermostatted cell holder

ATS-522 Automatic titrator

Results

Chirality resulting from an achiral substance interacting with a chiral substance is called induced CD. Though, 3,5-diiodosalicylic acid is achiral, it exhibits chirality through interaction with HSA. The resulting CD spectra show a positive peak around 320 nm. (Fig. 1)

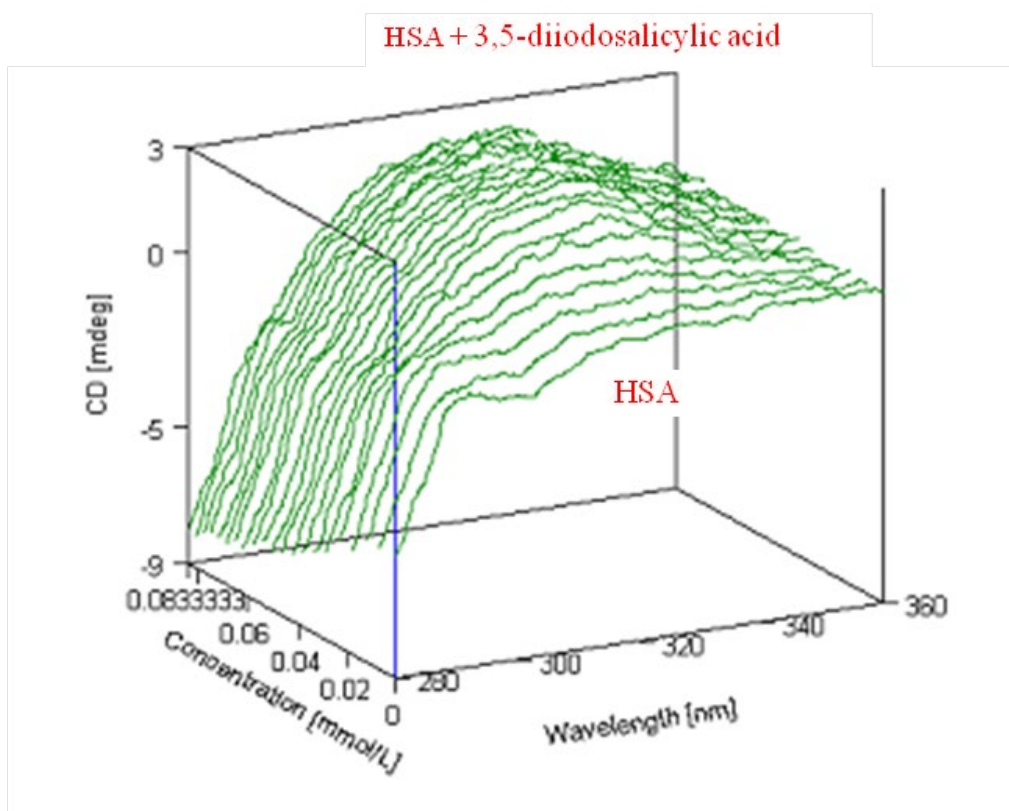


Fig. 1 3D-CD spectra of HSA titrated with 3,5-diiodosalicylic acid

HSA does not show a CD signal around 320 nm, but the induced CD from the interaction of HSA and 3,5-diiodosalicylic acid increases with additional titration of 3,5-diiodosalicylic acid. (Fig. 2)

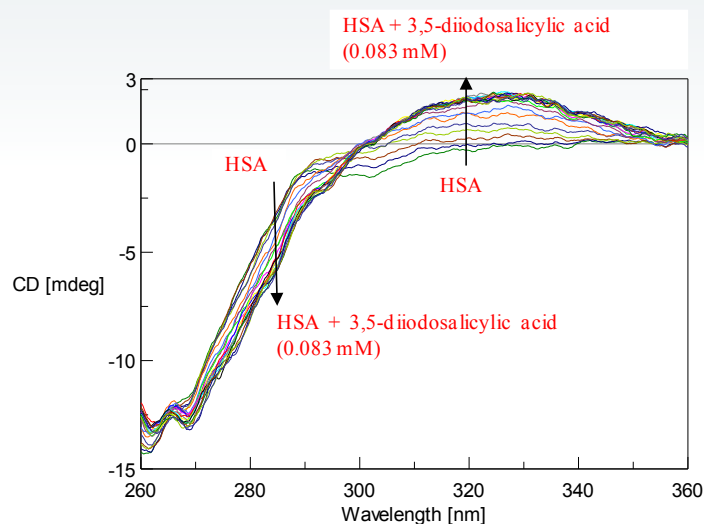


Fig. 2 2D-CD spectra of HSA titrated with 3,5-diiodosalicylic acid. The arrow represents increasing volume of 3,5-diiodosalicylic acid.

A plot of the increase of the induced CD signal (at 320 nm) versus the increase in 3,5-diiodosalicylic acid (Fig. 3) and the Hill plot (Fig. 4) are shown below.

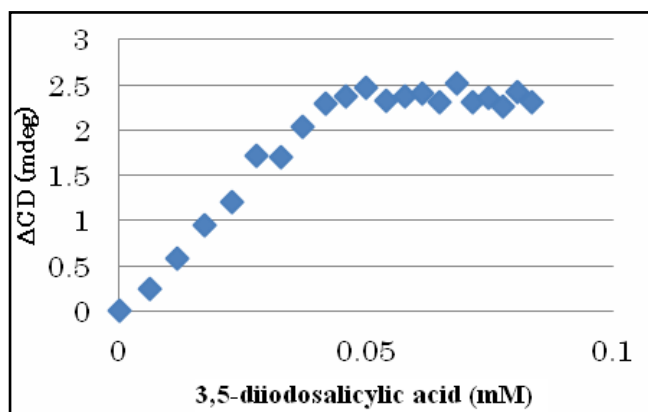


Fig. 3 Increase of induced CD (320 nm)

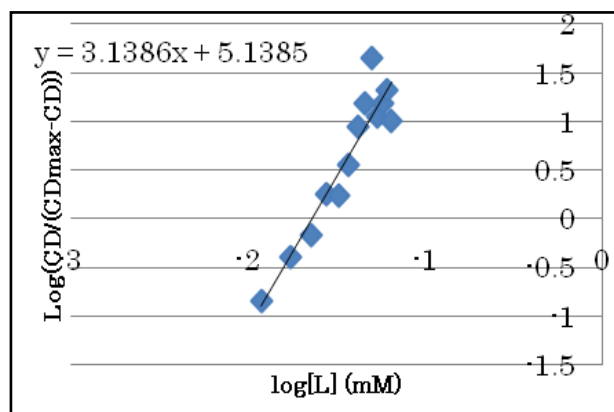


Fig. 4 Hill plot

The estimated dissociation constant (K_d), which is the concentration at which 50% of the 3,5-diiodosalicylic acid is bound with HSA is 0.023 mM. The Hill coefficient is approximately 3.1, indicating a positive cooperative reaction.

<References>

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