

**Deanship of Graduate Studies  
Al-Quds University**



**Spectroscopic Study of the Interaction of Human Serum  
Albumin with Steroid Hormones:  
Progesterone and its parent compound Cholesterol.**

**Jafar Hamed Taha Ghithan**

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Prepared by:

Jafar Hamed Taha Ghithan

B.Sc. Physics, Birzeit University, Palestine

Supervisor: Dr. Musa Abu Teir.

Co-supervisor: Prof. Mahmoud Abu hadid.

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Spectroscopic Study of the Interaction of Human Serum Albumin with Steroid Hormones: Progesterone and its parent compound Cholesterol.

Prepared by: Jafar Hamed Taha Ghithan.  
Registration No.: 20714279

Supervisor: Dr. Musa Abu Teir  
Co-supervisor: Prof. Mahmoud Abu hadid

Master Thesis submitted and accepted, Date:     /     /2010  
The names and signatures of the examining committee members are as follows:

1-Head of Committee: ..... Signature .....

2-Internal Examiner: ..... Signature.....

3-External Examiner: ..... Signature .....

4-Committee member: ..... Signature.....

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## **Dedication:**

I dedicate this thesis to the brightness of hearts, watercress's and remedy of chests, mister prophet Mohammad "peace and blessings of Allah be upon him". To my parents, Hamed and Najla thank you for every prayer, and bean of perspiration ripple from your brows, fatigued for me. My wife Dalal my soul mate and confidant, for always being there for me. Thank you for your continual love, support, and patience as I went through this journey. I could not have made it through without you by my side. My brothers and sisters; Taha, Yousef, Belal, Asma', Assem, Yasser, and Maysa' for their love and support throughout the years, thank you for the laughing, the fighting, and everything in between. To my Future daughter ... "Baby Daddy desirous to enfold you".

*Jafar Hamed Taha Ghithan*

**Declaration:**

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed: \_\_\_\_\_

Jafar Hamed Taha Ghithan

Date: / / 2010

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## ABSTRACT

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It was found that the distribution and metabolism of many biologically active compounds in the body whether drugs or natural products are correlated with their affinities toward serum albumin. Thus, the study on the interaction of such molecules with albumin is of imperative and fundamental importance. Extensive studies on different aspects of drug-HSA interactions are still in progress because of the clinical significance of the processes.

In this study the interaction of steroid hormones (progesterone and its parent compound cholesterol) with human serum albumin at physiological pH have been studied using UV-VIS spectrophotometer, fluorescence spectrophotometer, and FT-IR spectroscopy.

The results showed that UV absorption intensity spectra were increased with the increase of progesterone or cholesterol molar ratios in fixed amount of HSA. From UV spectra the binding constants were obtained and equals ( $6.354 \times 10^2 \text{M}^{-1}$ ) for progesterone and ( $0.2641 \times 10^4 \text{M}^{-1}$ ) for cholesterol.

Beside that the results that have been obtained from analysis of fluorescence spectra indicated that progesterone and cholesterol have an ability to quench the intrinsic fluorescence of HSA through a static quenching procedure. The values of Stern-Volmer constant were determined to be ( $6.26 \times 10^2 \text{L mol}^{-1}$ ) for progesterone- HSA complexes and ( $6.21 \times 10^2 \text{L mol}^{-1}$ ) for cholesterol- HSA complexes. Also the quenching rate constant

values obtained were ( $6.20 \times 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$ ) for progesterone, and ( $6.21 \times 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$ ) for cholesterol.

The binding constants from fluorescence spectrum for progesterone- HSA complexes was found to be ( $6.56 \times 10^2 \text{ M}^{-1}$ ), and for cholesterol- HSA complexes was found to be ( $0.214 \times 10^4 \text{ M}^{-1}$ ). It was obviously noted that the obtained values agrees well with the values obtained using UV-VIS spectrophotometer, and that cholesterol binding constant is larger than progesterone binding constant, this refer to the structure of the two compounds which is consistent with that have been reported.

FT-IR spectroscopy with Fourier self-deconvolution and second derivative, as well as curve fitting procedures were used in the analysis of amide I, amide II, and amide III regions of HSA to determine protein secondary structure and hormone binding mechanism. It was observed that the intensity of absorption bands decreased as progesterone or cholesterol molar ratios increased. Also all peak positions of the three amide regions were assigned at different progesterone or cholesterol ratios.

In addition FT-IR spectra evidence showed that HSA secondary structure has been changed as progesterone or cholesterol molar ratios increased, which was observed in the reduction of  $\alpha$ -helices absorption band relative to  $\beta$ -sheets absorption band. The variation in the intensity is related indirectly to the formation of H-bonding in the complex molecules, which accorded for the different intrinsic propensities of  $\alpha$ -helix and  $\beta$ -sheets.

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