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Spectroscopic Investigations of Anesthetic Drugs (Pentobarbital and Propofol) Interaction with Human Serum Albumin

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M.Sc. Thesis

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Thesis Approval

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Dedication

I dedicate this thesis to all of my wonderful family members who have supported me throughout my life and allowed me to achieve my goals: to my father Eid, who helped in making my educational decisions and sent me on the path to my graduate career; to my mother Riad; who raised me to be the person I am today, she has been with me on every step of the way, through good times and bad. Thank you for all the unconditional love, guidance, and support that you have always given me, helping me to succeed and instilling in me the confidence that I am capable of doing anything I put my mind to, to my husband Sami for his encouragement and support; to my supporting brothers; to my nice sisters; to my daughter Rawand who gave me more reasons to succeed; and to any future child…I just don't know who you are yet. Thank you for everything. I love you!

Sawsan Eid Hammed Abu sharkh

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: _____

Sawsan Eid Hamed Abu sharkh

Date: / / 2010

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ABSTRACT

The interaction of anesthetic drugs pentobarbital and propofol with HSA has been investigated by using UV-absorption, fluorescence spectroscopy, and Fourier transform infrared (FTIR) spectroscopy. The binding constants of pentobarbital and Propofol have been determined by both UV- absorption, and fluorescence spectroscopy. The values of the binding constants calculated at 293k are 1.812×10^{4} M^{-1} for pentobarbital and $2.55x10^{3}$ M^{-1} for propofol. The Stern–Volmer quenching constant values were found to be 3.875×10^{7} $L.mol^{-1}$ pentobarbital and $9.686x10^{6}$ L mol^{-1} propofol. The UV-absorption intensity of HSA-drug complexes has increased with increasing of pentobarbital and propofol concentration.

The fluorescence data reveals a decrease in HSA-drug emission intensity with the increase of pentobarbital and propofol concentration. This decrease of intensity indicates that both of pentobarbital and propofol have a strong ability to quench the intrinsic fluorescence of HSA through a static quenching mechanism.

FTIR spectroscopy with Fourier self-deconvolution technique and second derivative resolution enhancement, as well as curve-fitting procedures were applied in the analysis of the amide I,II, and III regions to determine the effects on protein secondary structure and drug binding mechanisms. All peak positions in the three amide regions (amid I, amide II and amide III) have been assigned and any effects due to concentration changes have been investigated. The FTIR spectra measurements indicate a change in the intensity of absorption bands due to change in the drug concentrations. In addition, a larger intensity decrease in the absorption band of the α-helix relative to that of β-sheets has been observed. This variation in intensity is related indirectly to the formation of H-bonding in the drug HSA complexes, which accounts for the different intrinsic propensities of α-helix and β-sheets in HSA.

It was found that the (carbonyl groups and N-H groups) and the hydroxyl group which are substituted on aromatic ring of pentobarbital and propofol respectively play an important role in the protein's secondary structure changes. The analysis supports that propofol with the lower binding constant has a faster sedation time than pentobarbital.

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