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

# Study the interaction of Human Serum Albumin with Aspirin Using Spectroscopic Techniques

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**M.Sc. Thesis**

**Jerusalem – Palestine**

**2018 /1440**

**Study the interaction of Human Serum Albumin with Aspirin Using Spectroscopic Techniques**

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A thesis Submitted in Partial fulfillment of requirement for the degree of Master of Science from the Department of physics, Faculty of Science and Technology, Al-Quds University.

**2018 /1440**

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

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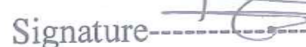

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2018/1440

## Dedication

To the of my husband, father, mother, brothers and sisters, my family with love and respect.

**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 study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

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Date: 12 /12 / 2018

**Acknowledgements**

At first and last my great commendation and thanks to Allah, who gave me the ability to accomplish this work.

I am very pleased to express my deep gratitude to my supervisor Dr. Husain Alsamamra for their supervision, guidance and insightful suggestions.

My sincere thanks go to my dear husband, lovely family and my friends for their unlimited support.

Kholoud Ali Awad Khalipha

## Abstract

Aspirin is an important for reduce the risk of heart attack and stroke. Human serum albumin is the major soluble protein constituent of the circulatory system and has many physiological functions including transport of a variety of compounds. In this work, interaction between Aspirin with human serum albumin was investigated by using fluorescence spectroscopy and UV absorption spectrum. From spectral analysis, Aspirin showed a strong ability to quench the intrinsic fluorescence of human serum albumin through a static quenching procedure. The binding constant (k) is estimated as $k=2.02\times10^4$ $M^{-1}$ for HSA-Aspirin. In addition The Stern-Volmer constant is calculated at room temperature for Aspirin.FT-IR spectroscopy was used to determine the protein secondary structure. The observed spectral changes indicates an increase of intensity for HSA-Aspirin interaction.. This variation of intensity is related indirectly to the formation of H-bonding in the complex molecules.

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# List of Symbols

| symbol | Description |
|---|---|
| N | Degree of freedom |
| R | the different part of 20 amino acids |
| $c$ | speed of light |
| $v$ | Frequency |
| λ | Wavelength |
| $E$ | Energy |
| $h$ | Planck's constant |
| $E_{total}$ | total energy |
| $E_{ele}$ | energy of the molecule's electrons |
| $E_{vib}$ | vibrational energy |
| $E_{rot}$ | rotational energy |
| $I$ | the intensity of light transmitted |
| $I_0$ | the intensity of light incident |
| ε | the molar absorption coefficient |
| $C$ | concentration of absorbing molecule in the sample |
| $l$ | length of the light path |
| $A$ | Absorbance |
| $F_x$ | restoring force |
| $f$ | the spring or force constant |
| $\Delta x$ | isplacement of the spheres along the x-axis from equilibrium position |
| $m_A$ | Mass of atom A |
| $m_B$ | Mass of atom B |
| $V$ | potential energy |
| $T$ | kinetic energy |
| $\mu$ | reduced mass |
| $\omega$ | circular frequency |
| $E_v$ | The potential energy for diatomic molecule for harmonic oscillator |

| $D_{eq}$ | dissociation energy |
|---|---|
| $r_{eq}$ | Equilibrium position |
| $r$ | Position |
| $\delta$ | constant for a particular molecule |
| $E_n$ | allowed vibration energy levels |
| $n$ | Interger |
| $W_e$ | oscillating frequency |
| $\dot{W}_e$ | oscillation frequency in wave number |
| $X_e$ | an-harmonicity constant |
| $a$ | absorptivity of the molecule |
| $b$ | distance that the light travels through the sample |
| $T$ | Transmittance |
| $mM$ | Mile molar |
| $n$ | nonbonding occupied molecular orbital |
| $\pi$ | Pi bonding occupied molecular orbital |
| $\sigma$ | Sigma bonding occupied molecular orbital |
| $\pi^*$ | Pi anti-bonding unoccupied molecular orbital |
| $\sigma^*$ | Sigma anti-bonding unoccupied molecular orbital |
| $A$ | recorded absorption at different concentrations |
| $A_\infty$ | the final absorption of the ligated protein |
| $A_0$ | the initial absorption of protein at 280 nm in the absence of ligand |
| $L$ | Concentration of ligand |
| $k_q$ | biomolecular quenching constant |
| $[l]$ | the quencher concentration |
| $k_{sv}$ | Stern-Volmer quenching constant |
| $\tau_0$ | unquenched lifetime |
| $F_0$ | the HSA fluorescence intensities in the absence of quencher |
| $F$ | the HSA fluorescence intensities in the presence of quencher |

# List of Abreviations

| abbreviation | Representation |
|---|---|
| G | Gram |
| IR | Infra-Red |
| VIS-UV | Visible Ultra Violet |
| ALB gene | Albumin gene |
| α-helix | alpha helix |
| β-pleated sheet | Beta pleated sheets |
| N-terminus | Nitrogen terminus |
| C-terminus | Carbon terminus |
| DNA | Deoxyribonucleic acid |
| FTIR | Fourier Transform Infrared |
| HSA | Human Serum Albumin |
| pH | potential of hydrogen |
| GLO | gulonolactone oxidase |

| PA | pernicious anemia |
|---|---|
| BSA | bovine serum albumin |
| DPPH | α, α-diphenyl-β-picrylhydrazyl |
| ITC | isothermal titration calorimetry |
| EMW | Electromagnetic waves |
| Far-IR | Far Infra-Red |
| Mid-IR | Middle Infra-Red |
| EM | Electromagnetic |
| $D_2O$ | Deuterium oxide |
| $H_2O$ | Water |
| HOMO | Highest Occupied Molecular Orbital |
| LOMO | Lowest Unoccupied Molecular Orbital |
| LED | light emitting diodes |
| CCD | charge-coupled device |
| PC | personal computer |
| OPUS | Optical User Software |
| Eq | Equation |

# Chapter One

# Introduction

## 1.1 Introduction

A technique used for the vibration of the atoms of a molecule is called Infrared (IR) spectroscopy. To obtain an infrared spectrum, determine what fraction of the incident radiation is absorbed at a particular energy when infrared radiation is passed through a sample. The appearance of any energy peak in an absorption spectrum corresponds to the frequency of a part of a sample molecule **[Banwell, 1972]**.

Infrared Spectroscopy is an absorption method pertaining to wavelengths in the region of 1 to 100 μm, extending the region of visible light to longer wavelengths and shorter frequencies (or energies). The IR light does not have sufficient energy to induce transitions of valence electrons, but can excite vibrational and rotational motions in molecules. Noted that the principle of IR spectroscopy are similar to ultra violet (VIS-UV) spectroscopy or other spectroscopic techniques except the differences in energy transfer from radiation to the molecules**[Wilson et al., 1955]**.

The length of a bond will vary in length when atoms move relatively to each other causing the bonds to stretch, or bend when atoms move out of plane relatively to one another. Reported linear frequencies to have a resonance frequency of 3N-5 and non-linear molecules have a frequency of 3N-6, where N is the degree of freedom, and some of these will interact with incident infrared radiation **[Hollowood and Miramontos, 2011]**.

Serum albumin, also known as blood albumin, is a type of globular protein found in vertebrate's blood. The albumin gene (ALB gene) is used to encode Human serum albumin and is similar to other mammalian forms such as bovine serum. They are all chemically similar **[Hawkins, 1982]**.

## 1.2 Proteins

Proteins are complex macromolecules. They are made up of successive amino acids, are covalently bonded together in a head-to-tail arrangement with substituted amide linkages called peptide bonds. The building blocks of proteins include 20 amino acids, which differ in the structure of their R-groups, and may be hydrophilic or hydrophobic, acidic, basic, or neutral. Proteins have the same basic structure, which is an amine group ($NH_2$), central carbon atom (alpha-carbon) and a carboxyl group (COOH), with the only difference on the side chain labeled R in the figure 1.1. **[Rosenberg, 2005].**

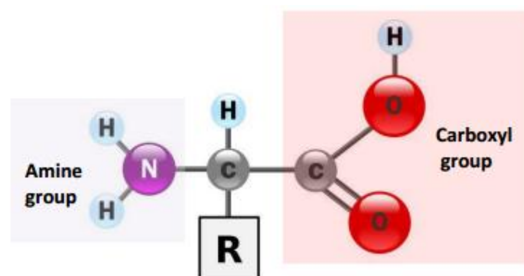


Figure 1.1: general structure of all amino acids **[Nelson, 2005]**.

Proteins are known as polypeptides because each protein molecule is made up of a long chain of amino acids, and each molecule is attached to it neighboring molecule through a covalent peptide bond. A large number of different proteins are known, with each type of protein showing a unique sequence of amino acids (see figure 1.2). This is exactly the same from one molecule to the next, with each with its own particular amino acid sequence **[Alberts et al., 2002]**.

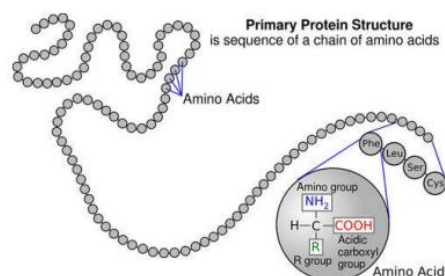


Figure 1.2: polypeptide (a chain made up of many linked amino acids).

**دراسة التفاعل بين الأسبرين مع مصل البيومين البشري باستخدام التقنيات المطيافية**

**اعداد : خلود علي عواد خليفة**

**اشراف : د.حسين السمامرة**

**ملخص:**

الأسبرين مهم في تقليل خطر الضربة القلبية والسكتة الدماغية ,البيومين المصل البشري هو البروتين الذائب الرئيسي وعنصر أساسي في الجهاز الدوراني وله الكثير من الوظائف الفسيولوجية تشمل نقل الكثير من المركبات الكيميائية. في هذا البحث تم دراسة التفاعل بين الأسبرين مع البيومين المصل البشري وذلك باستخدام تقنيات التحليل الطيفي الفلوري وايضا التحليل الطيفي لأشعة الفوق البنفسجية، ومن خلال التحليل الطيفي أظهرالأسبرين قدرة عالية على اخماد الطيف الفلوري لالبيومين المصل البشري من خلال اجراء الاخماد الاستاتيكي, تم حساب ثابت الربط للأسبرين والبيومين المصل البشري حيث كان ($2.02x10^4$ $M^{-1}$),وقد تم حساب ثابت شتيرن فولمر عند درجة حرارة الغرفة للأسبرين، أما التحليل الطيفي باستخدام تقنية تحويل فورييه للاشعة تحت الحمراء فتم استعمالها في تحديد بنية البروتين الثانوية، و ملاحظة التغيرات في الطيف وأشارت لزيادة التفاعل بين الأسبرين والبيومين المصل البشري، وهذا الاختلاف في الشدة يتعلق بطريقة غيرمباشرة لتكون روابط هيدروجينية في الجزيء المعقد.