Journal of Medicine, Physiology and Biophysics
ISSN 2422-8427 An International Peer-reviewed Journal
Vol.49, 2018

Analysis of Aspirin-Human Serum Albumin Complex Interaction Using Various Spectroscopic Methods

Husain Alsamamra* Khloud Khalifa Saqer Darwish Musa Abuteir
Department of physics, Al-Quds University, Palestine

Abstract
The binding mechanism of aspirin with human serum albumin (HSA) was investigated by various spectroscopic techniques namely UV absorption, fluorescence spectroscopy and FTIR. Fluorescence data indicated that aspirin quenched the intrinsic fluorescence of HSA by static mechanism and hydrophobic interaction play the main reason in the aspirin binding. By using fluorescence and UV absorption, it was found that the binding constant of aspirin-HSA complex is in the order of 10^4 M^{-1}. FTIR results confirmed that the analysis of the secondary structure of HSA was changed due to the interaction of aspirin and a significant shift of the wavenumber values through amid bands was obtained.
Keywords: aspirin; HSA; binding mode and FTIR spectroscopy.

1. Introduction

Aspirin is a class of drugsknown as Acetylsalicylic Acid (Fig. 1) (Marcos 1983). The prototypical analgesic used in the treatment of mild to moderate pain, its molecular formula C9H8O4. It has anti-inflammatory and antipyretic properties and acts as an inhibitor of cyclooxygenase which results in the inhibition of the biosynthesis of prostaglandins (Pourgonabadi *et al*. 2011). Aspirin is also prescribed to patients at high risk for heart attack (Roth *et al.* 1975). The drug may be effective in reduction of risk of various cancers, including those of the colon (Alsamamra *et al*. 2017) and lung (Li *et al.* 2013). Its preventive effect against effect against adenocarcinomas may be explained by its inhibition of PTGS2 (COX-2) enzymes expressed in them (Li *et al.* 2008). Many studies have been reported on aspirin-protein interaction (Darwish *et al*. 2010).

Protein is an essential bio-chemical substance in our life and plays an important main target of all medicines in organism. Due to its abundance in plasma, human serum albumin is the carrier protein in human body with a high affinity for a wide range of metabolites and drugs (Arrondo *et al.* 1993). HSA is the soluble protein constituent of the circulatory system, it contributes to colloid osmotic blood pressure. HSA serves to bind and carry drugs which are poorly soluble in water (Abu Teir *et al.* 2011; Alsamamra *et al.* 2017). Several methods were listed in the literature to study the physical interaction between HSA and drugs such as electrochemistry, chromatography and spectral analysis (Bai *et al.* 2008). Spectral analysis is one of the applied methods because of its easy operation, low cost, and abundant theoretical formulation (Livio *et al.* 1982).

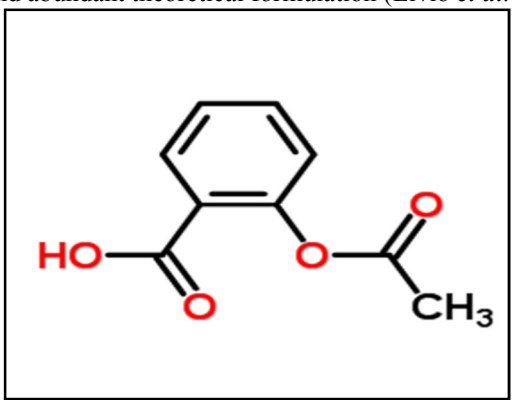

Fig.1. Chemical structure of aspirin

The goal of this work is to study aspirin-HSA complex interaction. It is highly important for pharmaceutical sciences to clarify the structure, function, and properties of aspirin-HSA complexes. Multiple spectroscopic methods; UV-Vis absorption spectroscopy, fluorescence spectroscopy and FTIR spectroscopy were employed to carry out detailed investigation of aspirin-HSA association.

2. Materials

HSA and aspirin were purchased from Sigma Aldrich chemical company. The HSA protein and the drug were used without further purifications. Thin films samples was used to take the data for FTIR measurements and for UV and fluorescence measurements; solution samples were used.

2.1 HSA stock solutions preparation
Phosphate buffer Saline (pH 7.4) was used to dissolve HSA to a concentration of (40mg/ml).

2.2 Aspirin stock solutions preparation
Aspirin has a molecular weight of (180.157 $g.mol^{-1}$), it was prepared to dissolve in phosphate buffer saline. Ultrasonic water path (SIBATA AU-3T) was used for one hour in order to ensure that all the amount of drug was completely dissolved.

2.3 HSA-aspirin solutions
HSA-Aspirin solutions with final concentrations were prepared by mixing equal volume from HSA protein to equal volume from different concentration of aspirin drug. HSA protein concentration in all samples kept at 40 $mg.ml^{-1}$. Final concentrations of aspirin drug in mM are (0.5 , 0.7, 0.9, 1.2 and 1.3).

3. Results and Discussions

3.1 UV-VIS
The absorption measurements of UV are very simple and applicable to illustrate the complex interaction (Bhattacharya *et al.* 2000). HSA UV spectra with and without aspirin were obtained. The binding constant represents the interaction strength between HSA protein and drugs, this constant can be obtained by using graphical analysis of the absorbance spectrum. The excitation (Fig. 2) has been done on 210 nm and the absorption is recorded at 235 nm for aspirin. According to the absorption spectra; an increase of the intensity as the aspirin concentration increases; the results obtained provide that an interaction between aspirin and HSA is happened and a protein–drug complex with certain new structure was formed (Alsamamra *et al*. 2018).

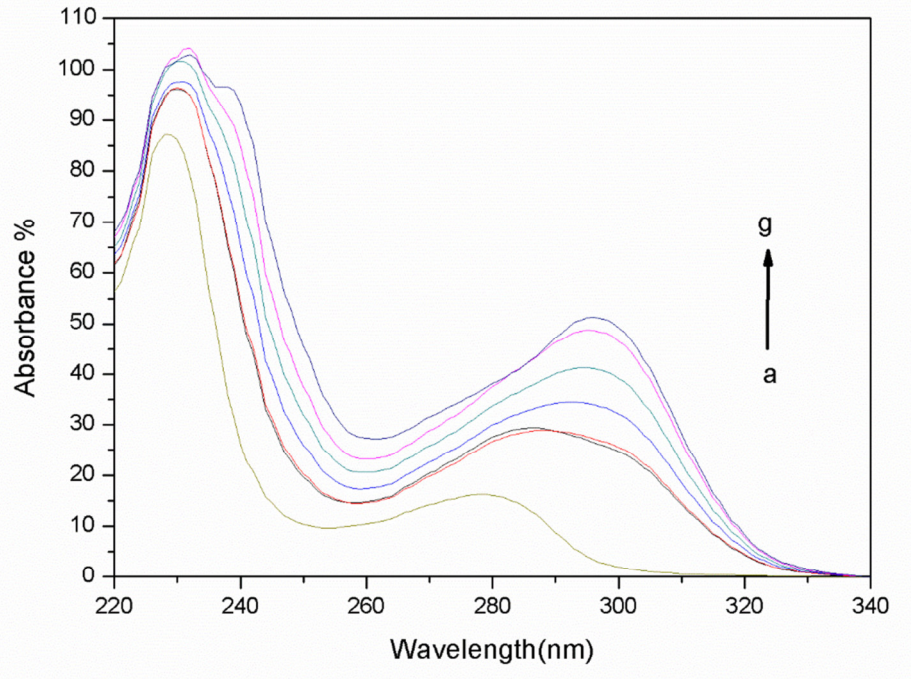

Fig. 2. UV-absorbance spectra of HSA with different concentrations of aspirin (a=freeAspirin,b=freeHSA,c=0.5mM,d=0.7mM,e=0.9mM,f=1.2mM,g=1.3mM)

The binding constant of HAS-aspirin complexes (Fig. 3) is determined by using UV-VIS spectrophotometer data according to published method (Stephanos 1996; Ouameur *et al.* 2004), it was assumed that there is only one type of interaction between aspirin and HSA in aqueous solution, which leads to establish the equation as follows:

$$K = [\text{ Aspirin:HSA }] / [\text{ Aspirin }][\text{ HSA }] \quad (1)$$

The absorption data were treated using linear reciprocal plots based on the following equation [Lakowicz 2006].

$$\frac{1}{A - A_0} = \frac{1}{A_\infty - A_0} + \frac{1}{K[A_\infty - A_0]} \cdot \frac{1}{L} \quad (2)$$

where A_0 corresponds initial absorption of protein at 280 nm in the absence of ligand , A_∞ is the final absorption of the ligated protein, and A is the recorded absorption at different concentrations (L).

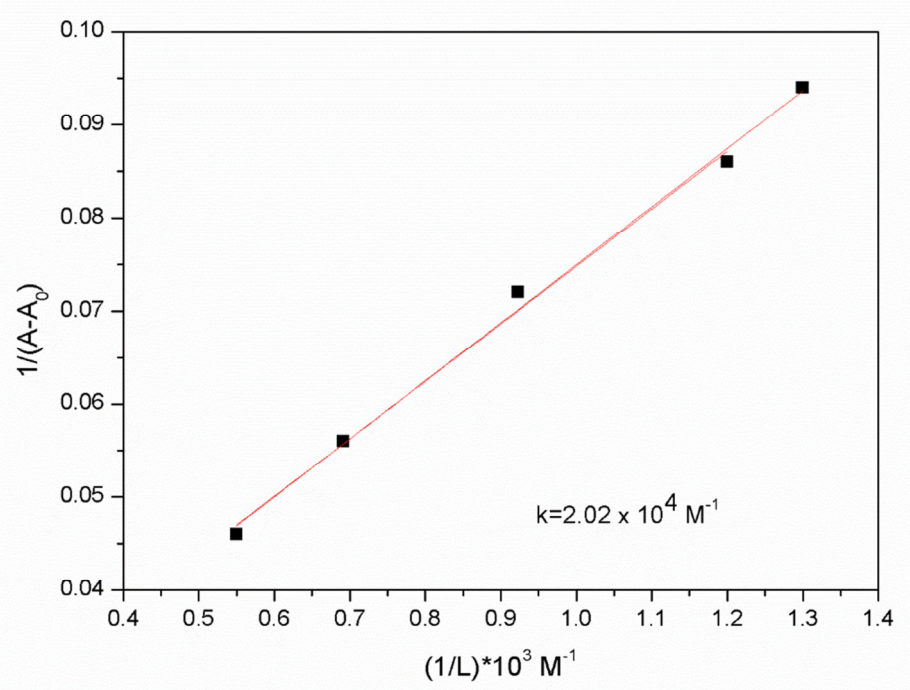

Fig. 3. The plot of $1/(A-A_0)$ vs. 1/L for HSA with different concentrations of aspirin.

Fig.3. illustrates the double reciprocal plot of $1/(A- A_0)$ vs. 1/L for HSA-aspirin complexes. The binding constant (K) can be estimated from the ratio of the intercept to the slope. The obtained values of the binding constants indicates a relatively weak interaction of aspirin ($K= 2.02x10^4\ M^{-1}$) when compared to other drug-HSA complexes with binding constants in the range of 10^5 and $10^6\ M^{-1}$ (Pourgonabadi *et al.* 2011). The presence of hydrogen-bonding interaction was the reason for the low stability (Boulkanz *et al*. 1995).

3.2 Fluorescence

A wide range of research problems in the biological and chemical sciences was studied using fluorescence spectroscopy. The data obtained can provide information of molecular processes, including the interaction of solvent molecules with fluorophores, complex binding interactions and conformational changes (Lakowicz 2006).

Various molecular interactions (such as molecular rearrangements, exited state reactions, energy transfer, ground state complex formation, and collisional quenching) can decrease the fluorescence intensity of a compound (Sheehan 2009).

HSA fluorescence results from the tryptophan, tyrosine, and phenylalanine residues. The contribution of intrinsic fluorescence of many proteins is mainly by tryptophan alone, since phenylalanine has a property of very low quantum yield and the fluorescence of tyrosine is almost totally quenched if it is ionized or near an amino group, a carboxyl group, or a tryptophan residue (Darwish *et al.* 2010).

Fig. 4 presents HSA emission exhibiting the peak maximum at 448 nm and was found to decrease progressively with increasing aspirin concentrations, illustrating that HAS and aspirin formed a complex due to the interaction.

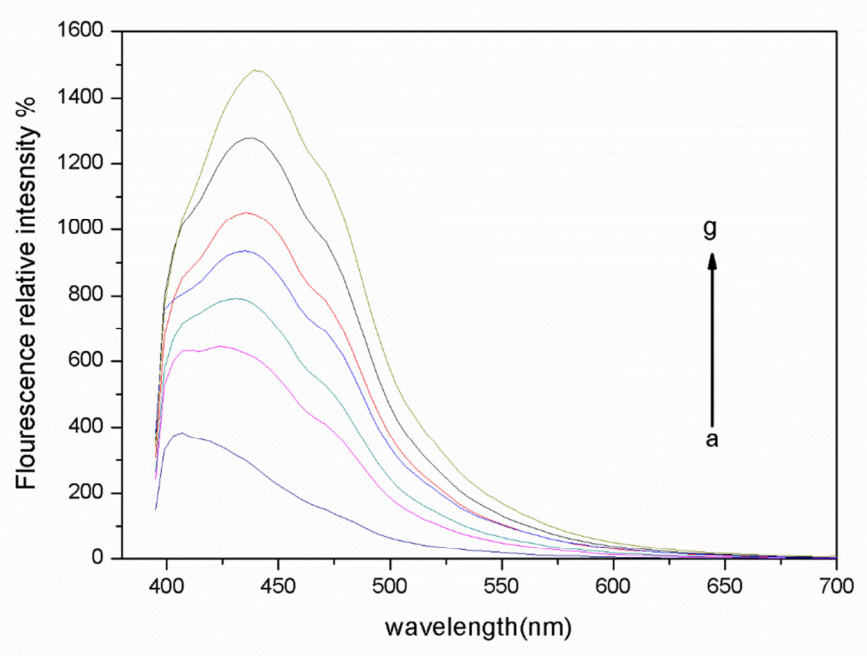

Fig. 4. Fluorescence emission spectra of HSA with and without aspirin in these concentrations (a=free aspirin, b=1.3mM, c=1.2mM, d=0.9mM, e=0.7mM,f=0.5mM,g=free HSA)

Stern-Volmer quenching constants (k_{sv}) and the quenching rate constant of the biomolecule (k_q)

A bimolecular process that decreases the fluorescence intensity without varying the emission spectrum of fluorescence is defined as fluorescence quenching; this is results from transient excited-state interactions or from formation of non-fluorescent ground-state species.

In order to examine the fluorescence quenching mechanism, the data of fluorescence quenching in steady state manner were used based on the classic Stern-Volmer equation (Krimm *et al.* 1986):

$$\frac{F_0}{F} = 1 + k_{sv}[L] = 1 + k_q\tau_0[L] \quad (3)$$

where F_0 and F are the HSA fluorescence intensity in the absence and presence of quencher (Aspirin); k_q is the HSA bimolecular quenching rate constant; τ_0 is the average fluorescence lifetime of the HSA molecule without quencher (of the order of 10^{-8} (Byler and Susi 1986); $[L]$ is the concentration of quencher, k_{sv} is Stern-Volmer fluorescence quenching constant. Linear curve was plotted according to the Stern-Volmer equation as shown in figure 5.

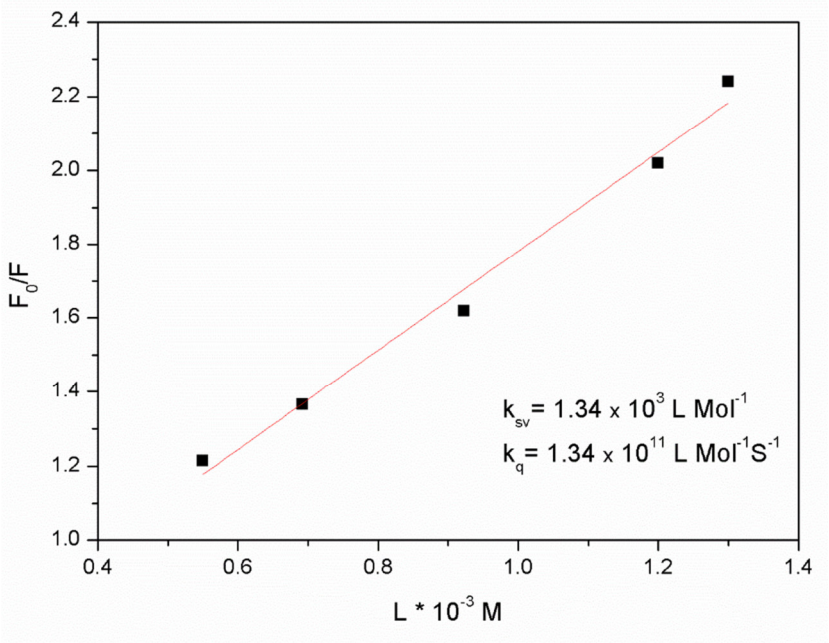

Fig. 5. The Stern-Volmer plot for Aspirin -HSA complex.

Fig. 5.represents the Stern-Volmer plot of HSA fluorescence intensity of aspirin respectively. The linear curve providing the existence of a single type of quenching (dynamic or static) and/or a single binding site for aspirin in the HSA neighborhood , The Stern-Volmer fluorescence quenching constant obtained by applying eq. (3) and is equal to (1.34×10^3) L mol^{-1} for HAS-aspirin complex, The Stern-Volmer quenching constant k_{sv} was obtained by the slope of the curve obtained in figure 5, The value is smaller than other k_{sv} values for the similar systems signalized earlier in literature (Bhattacharya *et al.* 2000 ; Bai *et al*. 2008). The value of k_q, which is equal to (1.34×10^{11}) L mol^{-1} s^{-1} for aspirin from equation (3) the value of $k_{sv} = k_q \tau_0$ from which we can calculate the value of k_q using the fluorescence life time of 10^{-8} s for HSA (Cheng et al). This value clearly confirms the existence of static mechanism of fluorescence quenching (Zsila *et al.* 2003, Alsamamra *et al.* 2018).

Determination of the binding constant using fluorescence spectrophotometer

The modified Stern-Volmer equation could be used when static quenching is dominant (Yang 1994)

$$\frac{1}{F_0 - F} = \frac{1}{F_0 KL} + \frac{1}{F_0} \quad (4)$$

Where K is the binding constant of aspirin-HSA complex, this constant can be calculated by plotting $\frac{1}{F_0 - F}$vs $\frac{1}{L}$, as seen in figure 6.

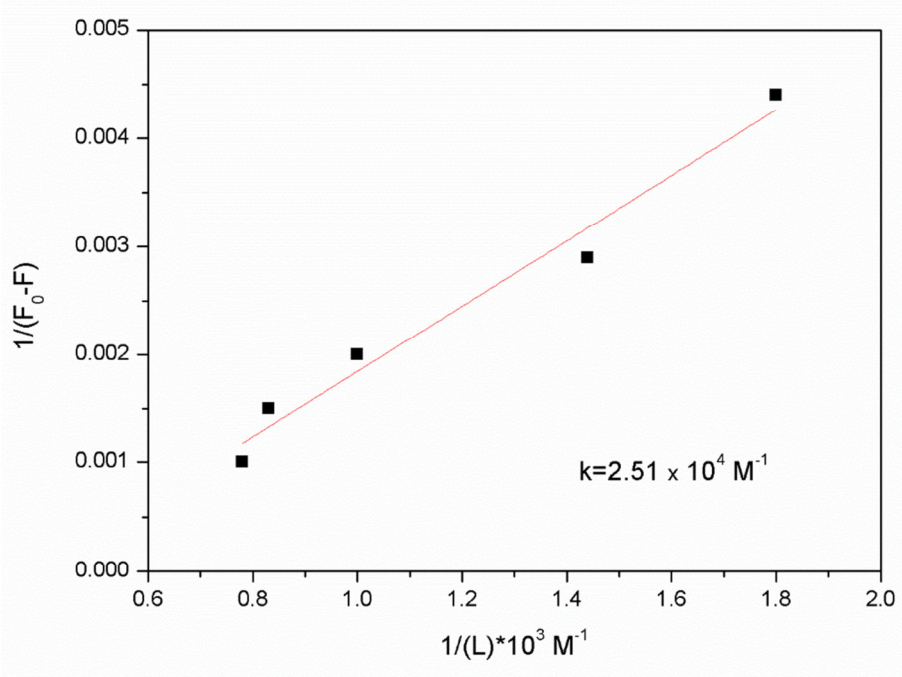

Fig. 6. The plot of $1/(F_0\text{-}F)$ vs $1/[L*10^3]$ for aspirin-HSA complex.

To determine the binding constant of HSA- aspirin complex, a plot of $\frac{1}{F_0 - F}$vs $\frac{1}{L}$ for different aspirin concentrations as shown in Fig.6. The plot is linear and has a slope of $\frac{1}{F_0 K}$ and intercept $\frac{1}{F_0}$according to eq. (4). The ratio of the intercept to the slope represents the value of K and was found to be (2.51×10^4 M^{-1}), this value agrees well with that obtained earlier by UV spectroscopy and confirms the idea of static quenching.

4.3 FT-IR spectroscopy

One of the useful technique for the investigation of hydrogen bonding is the FT-IR spectroscopy (Li et al., 2006), FT-IR has been found to be a powerful technique un the analysis of the secondary structure of HSA at different physiological systems (Arrondo *et al.* 1993). Infrared spectra provided the information about the proteins secondary structure. The different vibrational modes of the peptide moiety represents the number of proteins amid bands.

Amide I, amide II and amide III are the modes most widely used in protein structural studies. Amide I band ranging from 1600 to 1700 cm^{-1} and arises principally from the C=O stretching (Vandenbussche *et al.* 1992), has been widely accepted to be used (Workman and Kingston 1998). The amide II band is primarily N-H bending with a contribution from C-N stretching vibrations; amide II ranging from 1480 to 1600 cm^{-1} while amide III band ranging from 1220 to 1330 cm^{-1} which is due to the C-N stretching mode coupled to the in-plane N-H bending mode (Arrondo et al., 1993).

The second derivative of free HSA is shown in Fig.6, where the spectra is dominated by absorbance bands

of amide I and amide II at peak positions 1660 and 1540 cm^{-1}. Figure (7) illustrates the absorbance spectrum of HSA-aspirin complexes with different concentrations in the region from 1200 to 1800 cm^{-1}. It is clear that the absorbance increased as the aspirin concentration increases.

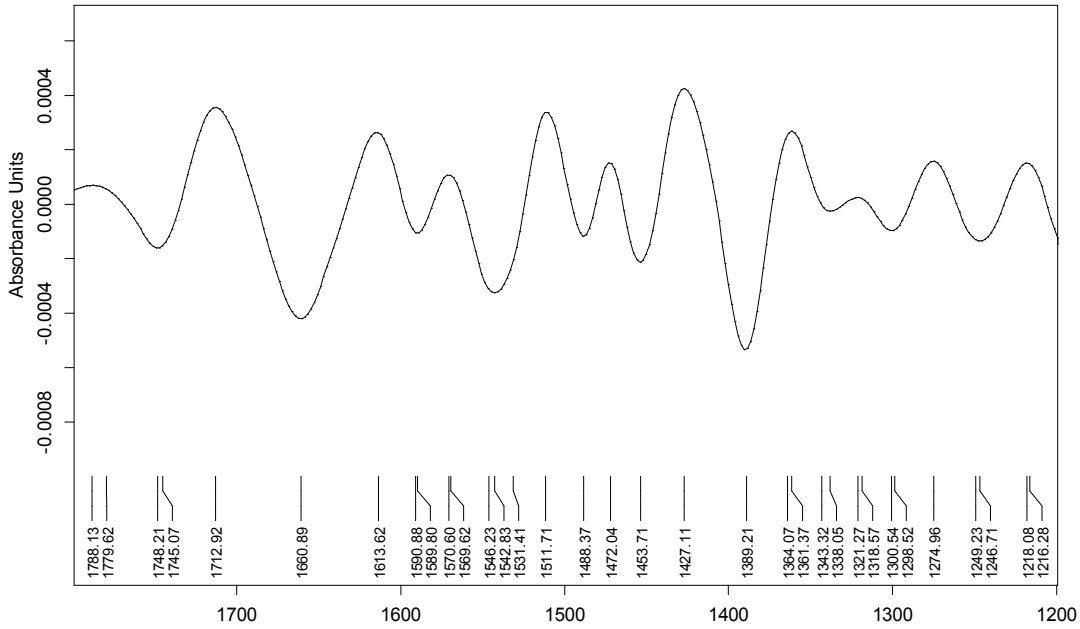

Figure 6: Second derivative of free HSA

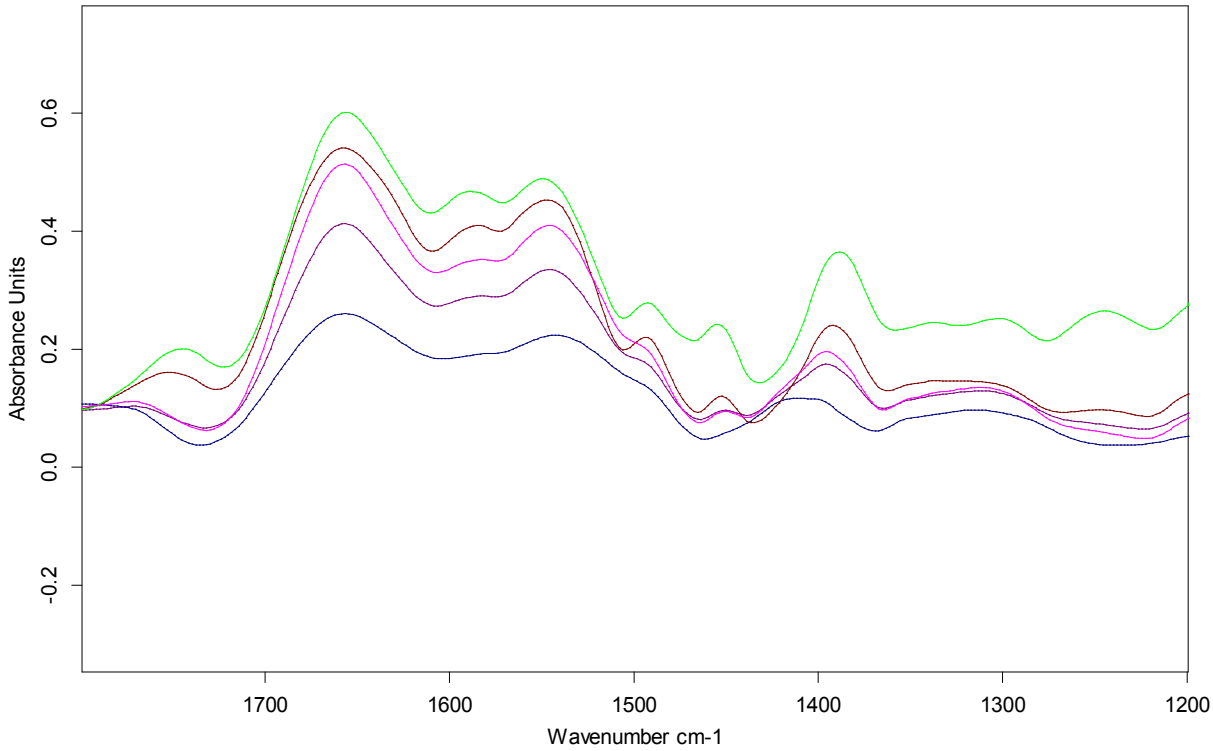

Figure 7: Different spectra of HSA and its complexes with different Aspirin concentrations in the region 1800-1200 cm^{-1}.

The peak position of HSA protein with different concentrations of aspirin is listed in table 4.1. As a comparison between free HSA and 1.3 mM concentration of aspirin; a significant shift of the wavenumber values in all amid bands ranges from 4 to 13 cm^{-1} which indicates that the drug interacts well with HSA and changes its secondary structure.

Table 4.1: Band assignment in the absorbance spectra of HSA with different Aspirin concentrations for Amid I-III regions

Band regions (cm^{-1})	Free HSA	0.5 mM	0.7 mM	0.9 mM	1.2 mM	1.3 mM
Amid I (1600-1700)	1661	1660	1658	1657	1655	1653
	1614	1610	1608	1607	1605	1604
Amid II (1480-1600)	1571	1574	1578	1581	1582	1584
	1543	1540	1538	1534	1534	1533
	1512	1514	1517	1517	1518	1519
	1472	1471	1471	1469	1468	1468
Amid III (1220-1330)	1343	1345	1347	1348	1350	1354
	1299	1293	1291	1290	1289	1288
	1247	1246	1245	1244	1242	1240

An intensity increase in the difference spectra of the aspirin-HSA complex for amide I, amide II and amid III bands, this happen due to drug binding to protein C=O, C-N and N-H groups.

In conclusion, our major goal in this investigative research was to distinguish the nature of the interaction between aspirin drug and human serum albumin by using spectroscopic techniques. The experimental results confirmed that aspirin quenched the intrinsic fluorescence of HSA through static mechanism. Furthermore, FTIR spectroscopy elucidate the changes in the secondary structure of the protein due the interaction with aspirin.

References

Abu teir, M. Ghithan, J. Darwish, S. Abu hadid, M. (2011), "Study of Progesterone Interaction with Human Serum Albumin: Spectroscopic Approach", *Applied Biological Sciences* **5**, 79-92.

Alsamamra, H. Khalid, I. Alfaqeh, R. Farroun, M. Abuteir, M. Darwish, S. (2018),"Spectroscopic Investigation of Procaine Interaction with Human Serum Albumin", *Journal of biomedical sciences* **7**, 1-10.

Alsamamra, H. Abusharkh, S. Abuteir, M. (2018),"A comparative study of the interaction of vitamin B12 with Human and Bovine Serum Albumins", *Romanian journal of biophysics* **28**, 125-136.

Alsamamra, H. Abusharkh, S. Darwish, S. Abu Teir, M. (2017) "Interaction of the Testosterone with Bovine Serum Albumin (BSA): UV-Visible Absorption Spectroscopy" *Journal of Chemical, Biological and Physical Sciences* **7**, 157-167.

Arrondo, L. Muga, A. Castresana, J. Goñi, M. (1993) "Quantitative studies of the structure of proteins in solution by Fourier-transform infrared spectroscopy", *Progress in Biophysics and Molecular Biology* **59**, 23-56.

Bai, S. Jain, M. Berg, O. (2008), "Contiguous binding of disulfide on the interface binding surface of pancreatic phospholipase A2", *Biochemistry* **8**, 2899-2907.

Byler, D. Susi, H. (1986) "Examination of the secondary structure of proteins by deconvolved FTIR spectra", *Biopolymers* **4**, 469-487.

Bhattacharya, A. Curry, S. Franks, N. (2000), "Binding of the general anesthetics propofol and halothane to human serum albumin", *Journal of Biological chemistry* **9**, 38731-38738.

Boulkanz, L. Balcar, N. Baron, M. (1995), "FT-IR analysis for structural characterization of albumin adsorbed on the reversed-phase support RP-C6", Spectroscopy, 1737-1746.

Darwish, S. Abu sharkh, S. Abu teir, M. Makharza, S. Abu hadid M. (2010) "Spectroscopic Investigations Of Pentobarbital Interaction With Human Serum Albumin", *Molecular Structure* **9**, 122–129.

Krimm, S. Bandekar, J. (1986) "Vibrational spectroscopy and conformation of peptides, polypeptides, and proteins", *Protein Chemistry* **5**, 181-364.

Lakowicz, J. (2006), "Principles Of Fluorescence Spectroscopy.3rd ", Springer Science & Business Media.

Li, X. Chen, D. Wang, G. Lu, Y. (2013) "Study of Interaction Between Human Serum Albumin And Three Antioxidants: Ascorbic Acid, A-Tocopherol, And Proanthocyanidins", *European Journal of Medicinal Chemistry* **70**, 22-36.

Li, J. Ren, C. Zhang, Y. Liu, X. Yao, X. Hu, Z. (2008), "Human Serum Albumin Interaction With Honokiol Studied Using Optical Spectroscopy And Molecular Modeling Methods", *Journal of molecular structure* **7**, 90–96.

Livio, M. Del Maschio, A. Cerletti, C. de Gaetano, G. (1982), "Indomethacin prevents the long-lasting inhibitory effect of aspirin on human platelet cyclooxygenase activity", *Prostaglandins* **23**, 787-796.

Marcus, A.J. (1983), " Aspirin as an antithrombotic medication", *New England Journal of Medicine* **309**, 1515-1523.

Ouameur, A. Mangier, E. Diamantoglou, S. Rouillon, R. Carpentier, R. Tajmir-Riahi, A. (2004), "Effects of organic and inorganic polyamine cations on the structure of human serum albumin" ,*Journal of Biopolymers*

33, 503-509

Pourgonabadi, S., Saberi, R, Chamani, K.(2011), "Investigating the antagonistic action between aspirin and tamoxifen with HSA: identification of binding sites in binary and ternary Drug-protein systems by spectroscopic and molecular modeling approaches", *Protein and Peptide Letters* **4,** 305-317.

Roth, G.J, Stanford. N, Majerus, P.W, " Acetylation of prostaglandin synthase by aspirin", *Proceeding of national academy of USA* **72**, 3073-3087.

Sheehan, D. Physical Biochemistry: Principles and Applications.2nd.John Wiley & Sons Ltd.2009.

Stephanos, J. (1996), "Drug-protein interactions: two-site binding of heterocyclic ligands to a monomeric hemoglobin", *Journal of Inorganic Biochemistry* **12**, 155-69.

Vandenbussche, G. Clercx, A. Curstedt, T. Johansson, J. Jornvall, H. Ruysschaert, J. (1992), "Structure and orientation of the surfactant-associated protein C in a lipid bilayer", *FEBS journal* **3**, 201-209.

Workman, J. Kingston, R. (1998), "Alteration of nucleosome structure as a mechanism of transcriptional regulation", *Annual review of Biochemistry* **6**, 545-579.

Yang, Z. (1994) "Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods", *Journal of Molecular Evolution* **7**,306-14.

Zsila, F. Bikdi, Z. Simonyi, M. (2003) "Probing the binding of the flavonoid to human serum albumin by circular dichroism, electronic absorption spectroscopy and molecular modelling methods", *Biochemical Pharmacology* **3**, 447-456.