BSA-Testosterone Dec-2017

Article · October 2019

DOI: 10.24214/jcbps.B.8.1.15767.]

1 author:

Husain Alsamamra
Al-Quds University
25 PUBLICATIONS **286** CITATIONS

SEE PROFILE

JCBPS; Section B; November 2017 – January 2018, Vol. 8, No. 1 ; 157-167. E- ISSN: 2249 –1929

[DOI: 10.24214/jcbps.B.8.1.15767.]

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section B: Biological Sciences

CODEN (USA): JCBPAT Research Article

Interaction of the Testosterone with Bovine Serum Albumin (BSA): UV-Visible Absorption Spectroscopy

Husain Alsamamra, Sawsan Abu Sharkh, Saker Darwish, Musa Abu Teir*

Department of Physics, Biophysics Research Laboratory, Al-Quds University, Palestine.

Received: 11 November 2017**; Revised:** 22 December 2017; **Accepted:** 29 December 2017

Abstract: The molecular interactions between BSA and Testosterone have been successfully investigated. The absorption, distribution and metabolism of many molecules can be altered based on their affinity to BSA. BSA is often increases the apparent solubility of hydrophobic ligands in plasma and modulate their delivery to cells. In this study, the interaction between Testosterone and BSA has been investigated using UV- absorption spectrophotometry and fluorescence spectroscopy to determine the binding constant. From UV- absorption spectrophotometry which showed a decreasing in the absorption intensity with increasing of the molecular ratios of testosterone to BSA, it is found that the value of the binding constant of testosterone to BSA, K equals $0.415*10^3$ M^{-1} at 293 K. While from the Fluorescence spectroscopy there was a quenching in the intensity with increasing of the molecular ratios of testosterone to BSA and it gave the same value of the binding constant as uv-absorption spectroscopy.

Keywords: Testosterone; Bovine Serum Albumin, binding mode; binding constant; UV–spectroscopy, Fluorescence spectroscopy.

1. INTRODUCTION

Hormones are the most familiar to the general public, due probably to the widespread pharmacological use and abuse of steroid hormones for diverse purposes, such as contraception and body building[1]. Steroids hormones mainly can act as a chemical messenger in a wide range of species and target tissues to

produce both slow genomic responses and rapid non-genomic responses[2]. They have many physiological effects on human body; their incorrect concentration may cause abnormalities in human body[3]. Steroid hormones help control metabolism, inflammation, immune functions, salt and water balance, development of sexual characteristics and the ability to withstand illness and injury[4]. In human all steroid hormones are derived from cholesterol and differ only in the ring structure and side chains attached to it[5], so as cholesterol is a non-polar and hydrophobic molecule steroid hormones are insoluble in water but lipid soluble thus they have to be carried in the blood bound to specific carrier proteins such as sex hormone-binding globulin or corticosteroid-binding globulin. Sex steroid binding globulin carries testosterone and estradiol[4].

Testosterone is a steroid hormone from the androgen group which is found in males and in smaller amount in female. It is 7-8 times concentrated in human males' plasma than in human females. The metabolic consumption of testosterone in males is greater. Testosterone is classified as a strong androgen and secreted primarily from the testicles of males and the ovaries of females, while small amounts of testosterone and weak androgens such as anabolic steroids are secreted by the adrenal gland[6]. The chemical structure of testosterone is as shown[5] in **Figure (1)** ($C_{19}H_{28}O_2$). It is classified as Δ4 steroid as the double bond (un-saturation site) is located at 4-5 position. Testosterone chemical structure lacks the 2-carbon side-chain attached to the 17 position existed in the cholesterol structure, making it a 19-carbon steroid, also the side-chain has been replaced by a 17β-hydroxyl[5].

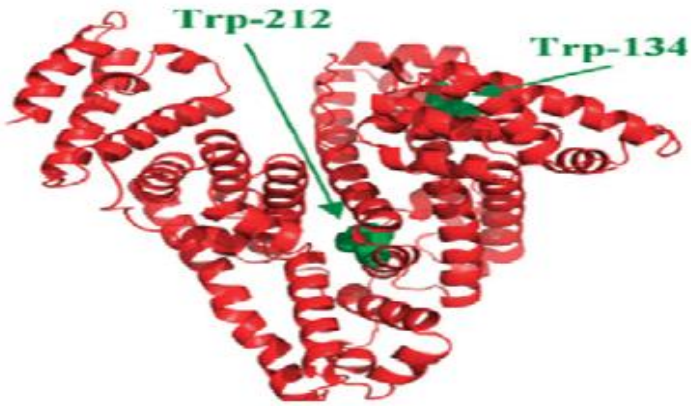
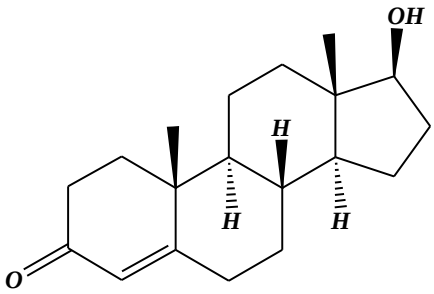


Figure 1: structure of the bovine serum albumin, with tryptophan residues shown in green color

Serum albumin is a major soluble protein constituent of the circulatory system and has many physiological functions such as acting as a plasma carrier by nonspecifically binding to several hydrophobic steroid hormones and as a transport protein for hemin and fatty acids[7]. Albumins are characterized by a low content of tryptophan and methionine and a high content of cystine and charged amino acids[8,9]. Bovine serum albumin (BSA),its molecular structure shown in **Figure (2**) which is an example of a mammalian albumin, has been studied extensively because of its stability, neutrality in many biochemical reactions, and low cost[10,11]. Brown elucidated the 607 amino acid residue, primary structure of BSA in 1975, twenty one of which are tyrosine (Tyr) residues and two of which are

tryptophan (Trp) residues located at positions 134 and 212, respectively[8,10]. These two Trp residues cause BSA to have intrinsic fluorescence.



$C_{19}H_{28}O_2$

Figure 2: Chemical structure of Testosterone.

Bovine serum albumin (BSA) is constituted by 582 amino acid residues and, on the basis of the distribution of the disulfide bridges and of the amino acid sequence, it can be regarded as composed of three homologous domains (I, II and III) linked together. Each domain can be subdivided into two subdomains, A and B. BSA has two tryptophans, as mentioned above which they are embedded in subdomains IB and IIA, respectively[12].During the last three decades, substituted indane-1,3-dione derivatives have shown wide applicability in the fields of medicine[13,14] and biology[15]. Many studies on the biological activity of these compounds report their anticoagulant[16], anti-inflammatory[17], analgesic[18], antibacterial[19] or bronchial dilating[20] action. However, literature data on their interaction with proteins is relatively scarce [21-23]. Because they are compounds of important biological activity, we considered that such studies could elucidate important aspects of drug pharmacokinetics.

The distribution and metabolism of many biologically active compounds in our body whether drugs or natural products are correlated with their affinities toward serum albumin which is the most abundant protein carrier in our plasma. So the study of the interaction of such molecules for example testosterone with albumin is of a fundamental importance. Some investigations have been applied on Testosterone-HSA interaction but none of them investigated the interaction of the Testosterone-BSA and none of them determined the binding constant (K) of Testosterone-BSA nor the effect of testosterone complexes on the protein structure. Some investigations only indicated that the interaction occurred and others used the equilibrium dialysis method to calculate the binding constant (k) [24, 25].Other spectroscopy techniques are usually used in studying the interaction of drugs and proteins, fluorescence and UV spectroscopy are commonly used because of their high sensitivity, rapidity and ease of implementation. Several reports have been published studying the interaction of proteins with drugs by uv spectroscopy and fluorescence technique[26-28].The binding of Testosterone to BSA was investigated by means of UV-absorption spectroscopy and Fluorescence spectroscopy. Spectroscopic evidence regarding the drug binding mode and drug binding constant are provided here.

2. MATERIALS AND METHODS

2.1. Materials: BSA (fatty acid free), testosterone in powder form were purchased from Sigma Aldrich chemical company and used without further purifications. The data were collected using samples in the liquid form for UV-VIS and fluorescence measurements.

2.2. Preparation of stock solutions: BSA was dissolved in phosphate buffer saline, at physiological pH 7.4, to a concentration of (80 mg/ml), to get a final concentration of (40 mg/ml) in the final hormone-BSA solution.

Testosterone with molecular weight (288.42 g/mol) was dissolved in phosphate buffer saline (0.7622 mg/ml), the phosphate buffer saline was at room temperature, the solution was placed on a shaker for one hour in order to dissolve the testosterone powder with buffer, then it was placed in ultrasonic water path (SIBATA AU-3T) for 8 hours to ensure that the entire amount of testosterone was completely dissolved. The solution was placed in a water path with a temperature range 37-40c° for one hour to let the solution completely dissolved and became homogenous.The final concentrations of BSA-Testosterone solutions were prepared by mixing equal volume of BSA and hormone. BSA concentration in all samples kept at 40 mg/ml. However, the concentration of hormone in the final protein hormone solutions was reduced such that the molecular ratios (BSA: Testosterone) are 10:18, 10:14, 10:10, 10:6 and 10:2. All solutions were prepared at the same time at room temperature.

2.3. UV/VIS spectrophotometer (NanoDrop ND-1000) : The absorption spectra were obtained by the use of a NanoDrop ND-1000 spectrophotometer. It is used to measure the spectrum of the samples in the range between 220-750nm, with high accuracy and reproducibility.

2.4 Fluorospectrometer (NanoDrop 3300): The fluorescence measurements were performed by a NanoDrop ND-3300 Fluorospectrometer at 25 ºC. The excitation source comes from one of three solid-state light emitting diodes (LED's). The excitation source options include: UV LED with maximum excitation 365 nm, Blue LED with excitation 470 nm, and white LED from 500 to 650nm excitation. A 2048-element CCD array detector covering 400–750 nm, is connected by an optical fiber to the optical measurement surface. The excitation is done at the wavelength of 360 nm and the maximum emission wavelength is at 439 nm.

3. RESULTS AND DISCUSSION

3.1 UV-absorption spectroscopy: The Testosterone-BSA Binding constants were determined using UV absorption spectroscopy as reported for several drug- protein complexes[29-31]. The absorption spectra for different concentration of testosterone in BSA are shown in **Figure (3)**. Assuming only one type of interaction between progesterone and BSA in aqueous solution, leads to establish Eqs. (1) and (2) as follows:

BSA + Testosterone ↔ Testosterone: BSA … (1)

K = [Testosterone: BSA]/ [Testosterone][BSA] …(2)

The absorption data were treated using linear reciprocal plots based on the following equation.

$$\frac{1}{A-A_0}=\frac{1}{A_\infty-A_0}+\frac{1}{K[A_\infty-A_0]}\cdot\frac{1}{L} \quad \dots (3)$$

where A_0 corresponds to the 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 Testosterone concentrations (L). The double reciprocal plot of $1/(A-A_0)$ vs. $1/L$ is linear as shown in **Figure (4)** and the binding constant (K) can be estimated from the ratio of the intercept to the slope to be 0.2779×10^3 M^{-1}.

The binding constant value shows a relatively Testosterone-BSA interaction in comparison to other drug-BSA complexes with binding constants[16] in the range of 10^5 and 10^6 M^{-1} .

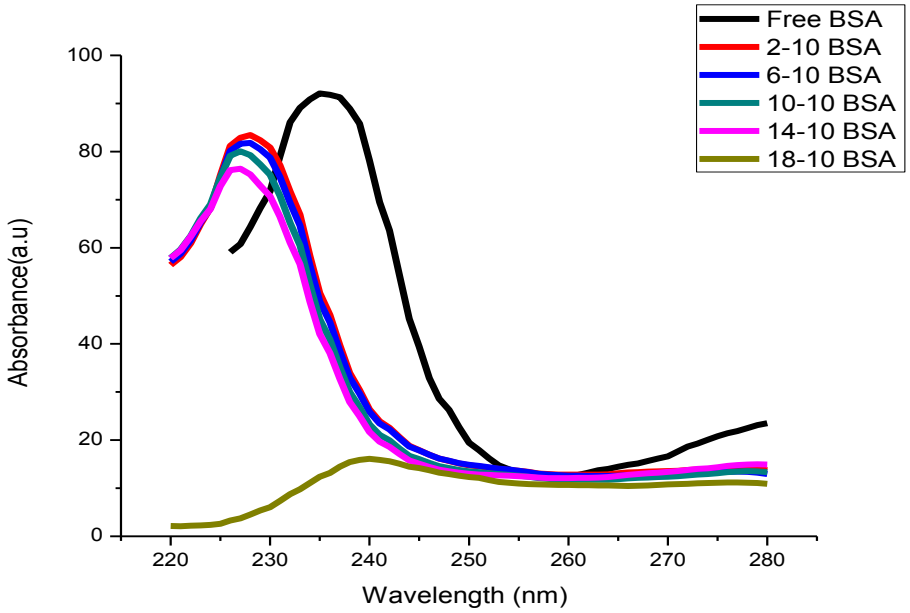


Figure 3: UV-absorbance spectra of BSA with different molar ratios of testosterone.

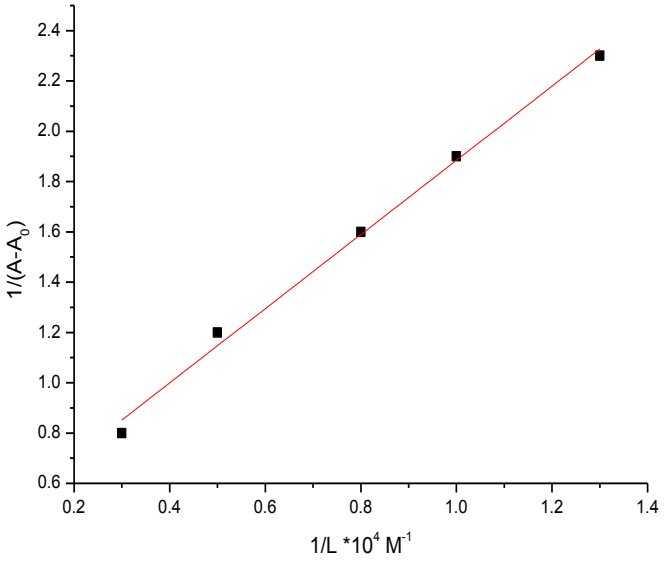


Figure 4: The plot of $1/(A-A_0)$ vs 1/L for BSA with different concentrations of testosterone.

3.2 Fluorescence spectroscopy: The fluorescence of BSA results from the tryptophan, tyrosine, and phenylalanine residues. The intrinsic fluorescence of many proteins is mainly contributed by tryptophan alone, because phenylalanine has 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[32].

The fluorescence spectra of BSA at various ratios of Testosterone (10:18, 10:14, 10:10, 10:6, and 10:2.)mol L^{-1} are shown in **Figure (5).** The fluorescence intensity of BSA decreased regularly with the increasing of Testosterone concentration. The peak position shows little or no change at all.

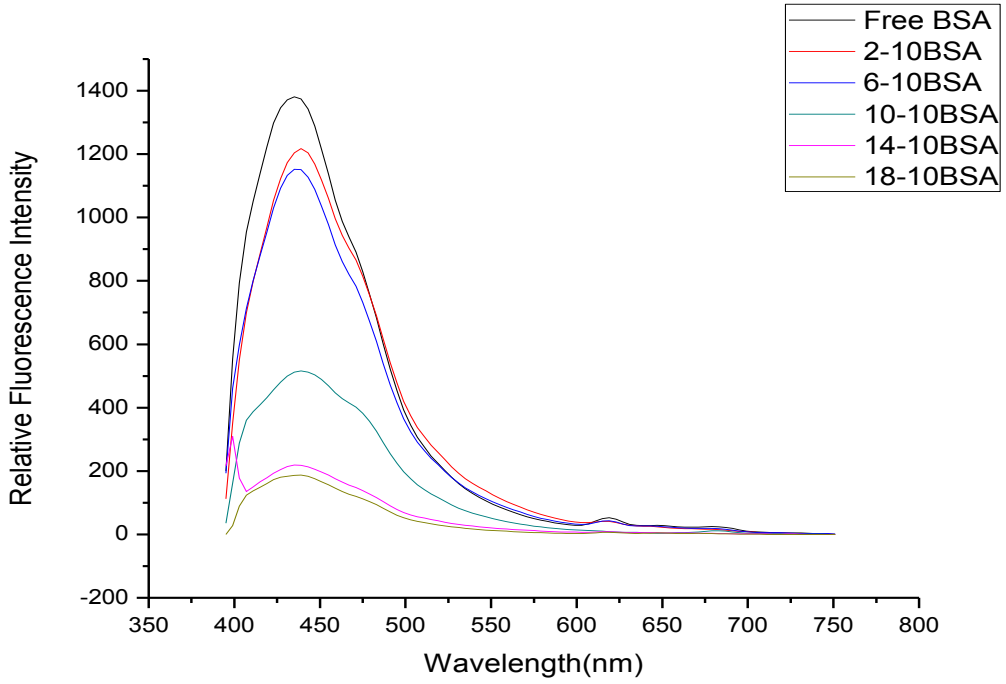


Figure 5: Fluorescence emission spectra of BSA in the absence and presence of testosterone.

The Dynamic quenching process can be described by the Stern-Volmer equation[33].

$$\frac{F_0}{F} = 1 + K_q \tau_0 (L) = 1 + K_{sv} (L) \qquad \ldots (4)$$

Where F and F_0 are the fluorescence intensity with and without quencher, K_q is the quenching rate constant of the biomolecule, K_{sv} is the Stern-Volmer quenching constant, τ_0 is the average lifetime of the biomolecule without quencher, and (L) is the concentration of Testosterone.

As can be seen from **Figure (6),** the Stern-Volmer plot is linear and the slope is equal to K_{sv} (28.8×10^2 L mol^{-1}). Fluorescence quenching can be induced by different mechanisms, which were usually classified

into dynamic quenching and static quenching. Dynamic quenching arises from collisional encounters between the fluorophore and quencher, and static quenching resulting from the formation of a ground state complex between the fluorophore and the quencher.

The quenching rate constant K_q, can be calculated using the fluorescence[34] life time of 10^{-8}s for BSA as it is for HSA.

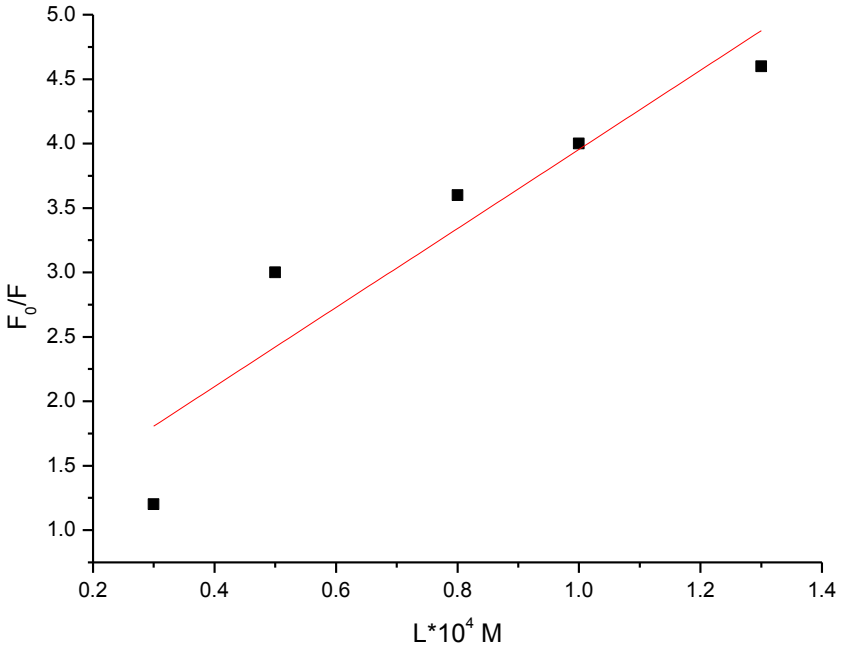


Figure 6: The Stern Volmer plot for Testosterone –BSA complexes.

The obtained value of the quenching constant K_q is 28.8×10^{10} L $mol^{-1}s^{-1}$ which is larger than the maximum dynamic quenching constant for various quencher[35]s with biopolymer ($2x10^{10}$ $L.mol^{-1}.s^{-1}$). This result confirms that a static quenching is dominant in the formed complexes [36]. When the static quenching equation is used[33]

$$\frac{1}{F_0 - F} = \frac{1}{F_0 K(L)} + \frac{1}{F_0} \qquad \ldots (5)$$

Where K is the binding constant of Testosterone with BSA, and can be calculated by plotting $1/(F_0\text{-}F)$ vs 1/L, **figure(7)**.

The value of K can be determined from the slope and the intercept shows that the value of K is 0.415×10^3 M^{-1}, which agrees well with the value obtained earlier by UV spectroscopy and supports the effective role of static quenching. The highly effective quenching constant in this case has lead to a lower value of binding constant between the drug and BSA due to an effective hydrogen bonding between Testosterone and BSA.

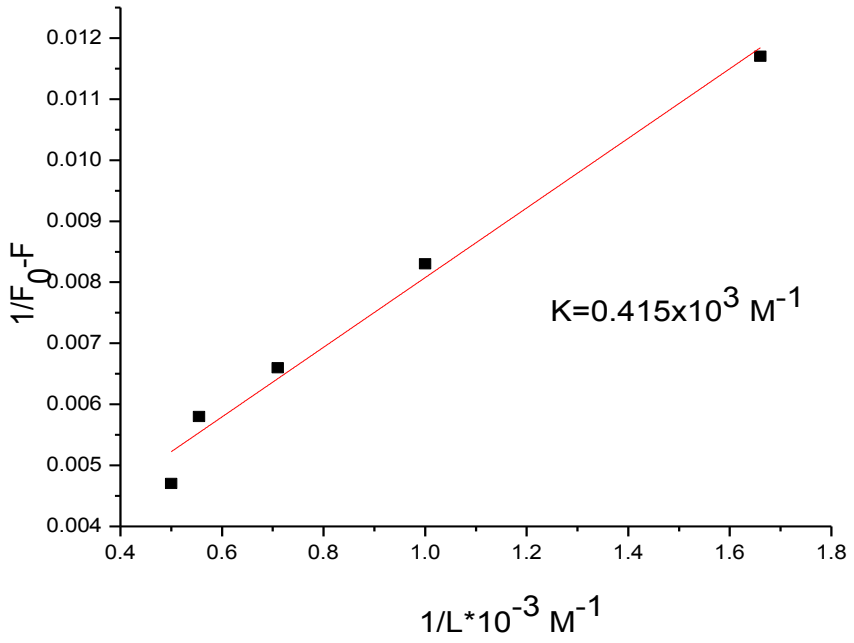


Figure 7: The plot of $1/(F_0\text{-}F)$ vs $(1/L) \times 10^4$ for Testosterone –BSA complexes.

4. CONCLUSION

The binding of Testosterone to BSA has been investigated by UV-absorption spectroscopy, and fluorescence spectroscopy. From the UV and Fluorescence Investigations we determined values for the binding constant and the quenching constant. The results indicate that the intrinsic fluorescence of BSA was quenched by Testosterone through static quenching mechanism.

ACKNOWLEDGEMENTS

This work is supported by the German Research Foundation DFG grant no. DR228/24-2

REFERENCES

1. D.G.Hardie, Biochemical Messengers, Hormones, Neurotransmitter and Growth Factors, 1st, University Press, Cambridge, 1991.
2. A.W. Norman, M.T. Mizwicki, D.P. Norman, Nature Reviews: Steroid-Hormones Rapid Actions, Membrane Receptors and a Conformational Ensemble model. Nature Reviews.2004, 3.
3. M.M. Abu Teir, J.H. Ghithan, S.M. Darwish, M.M. Abu-Hadid, Journal of Applied Biological Sciences, 2010, 4 (3)79-92.

4. C.A.Frye, "Steroids, reproductive endocrine function, and affect. A review". Minerva Ginecol, 2009, 61 (6): 541–562. PMID 19942840.
5. S.F. Brandt, Introduction to Steroid Hormones, 1999. http://www.rosehulman.edu/~brandt/Chem330/EndocrineNotes/Chapter_1_Steroids.pdf
6. W.L. Reed, M.E. Clark, P.G. Parker, S.A. Raouf, N. Arguedas, D.S. Monk, E. Snajdr, V. Nolan, E.D. Ketterson , "Physiological effects on demography: a long-term experimental study of testosterone's effects on fitness",2006.
7. J. R. Brown, "Structure of bovine serum albumin," *Federation Proceedings*, 1975, 34, 591.
8. J. E. Patterson and and D. M. Geller, "Bovinemicrosomal albumin: aminoterminalsequence of bovineproalbumin," *Biochemical and Biophysical Research Communications*, 1977, 74, 3, 1220–1226.
9. K. Hirayama, S. Akashi, M. Furuya, and K. Fukuhara, "Rapid confirmation and revision of the primary structure of bovine serum albumin by ESIMS and Frit-FAB LC/MS," *Biochemical and Biophysical Research Communications*, 1990, 173, 2, 639–646.
10. T. J. Peters, "Serum albumin," *Advances in Protein Chemistry*, 1995, 37, 161–245.
11. A. Tarushi, C. P. Raptopoulou, V. Psycharis, A. Terzis, G. Psomas, and D. P. Kessissoglou, "Zinc(II) complexes of the second-generation quinolone antibacterial drug enrofloxacin: structure and DNA or albumin interaction," *Bioorganic and Medicinal Chemistry*,2010,18, 7, 2678–2685.
12. Y. Moriyama, D. Ohta, K. Hachiya, Y. Mitsui, K. Takeda. Fluorescence Behavior of Tryptophan Residues of Bovine and Human Serum Albumins in Ionic Surfactant Solutions: A Comparative Study of the Two and One Tryptophan(s) of Bovine and Human Albumins, *J. Protein Chem*. 1996, *15*, 265-271.
13. I. Hall, O. Wong, L. Chi, S. Chen, Cytotoxicity and Mode of Action of Substituted Indan-1, 3- diones in Murine and Human Tissue Cultured Cells. *Anticancer Res*. 1994, *14*, 2053-2058.
14. G. Le Baut,; S, Loire, ;L. Sparfel,; M. Creuzet, ; C. Feniou,; H. Pontagnier, ; G. Prat, N-substituted 2-aminomethylene-1,3-indanediones for treating arterial hypertension and spasmodic conditions. *US Patent 4758561*, 1984; [*Chem. Abstr*. 1986,105.]
15. M.A. Salama, M.N. Yousif, A.G. Hammam, Syntesis and reactions of 4, 5-diaryl-2-mercaptoimidazoles. *Pol. J. Chem.* 1988**,** *35*, 83-88.
16. De Winter, M.L.; Nauta, W.T. Pharmacochemistry of 2-diarylmethyl-1, 3-indandiones. I. Synthesis. *Eur. J. Med. Chem*. 1977, *12*, 125-130.
17. Van Der Goot, H.; Eriks, J.C. Substituted benzamides as anti-inflammatory agents. *Eur. J. Med. Chem*. 1978, *13*, 425-428.
18. Kubovic, M.; Prazic, M.; Atanackovic D. Analgetic Property of Vitamin K. *Proc. Soc. Exp. Biol. Med.* 1955, *90*, 660-662.

19. E. Gori, Antibacterial activity of Coumarin and Indandione Compounds. The first international conference on Thrombosis and Embolism, Basel, Switzerland, 1954; 271-274.

20. H. Blumberg, H.B. Dayton, S.M. Gordon, Bronchodilator Action of the Anticoagulant Warfarin Sodium. *Science*, 1958, *127*, 188.

21. L.P. Zalukaev, O.V. Kozyreva, Allosteric Effects in the Mechanism of Action of Derivatives of Indane-1, 3-dione on Proteins. *Pharm. Chem. J*. 1979, *13*, 1126-1128.

22. M. Esmann, P.C. Sar, K. Hideg, D. Marsh, Maleimide, Iodoacetamide, Indanedione, and Chloromercuric Spin Label Reagents with Derivatized Nitroxide Rings as ESR Reporter Groups for Protein Conformation and Dynamics. *Analyt. Biochem*. 1993, *213*, 336-348.

23. G. Drochioiu, M. Murariu, I. Mangalagiu, I. Druta, Highly Selective Assay of Proteins in Dilute Solutions. *Talanta*, 2002, *56*, 425-433.

24. Pearlman, H. W, Crepy. O. 1967. The Journal of Biological Chemistry, 1967, 242, 2,182-189.

25. G. Litwack, Axelord. J. 1970. Binding of Hormones to Serum Proteins. Biochemical Actions of Hormones, Volume.1. New York: Academic Press, 1970, INC. 209-260.

26. T. Wybranowski, M. Cyrankiewicz, B. Ziomkowska, S. Kruszewski, 2008. The HSA affinity of warfarin and flurbiprofen determined by fluorescence anisotropy measurements of camptothecin. Bio Systems, 2008, 94, 258– 262.

27. J. Li, C. Ren, Y. Zhang, X. Liu, X. Yao, Z. Hu, Human serum albumin interaction with honokiol studied using optical spectroscopy and molecular modeling methods. Journal of Molecular Structure, 2008, 881, 90–96.

28. Y. Li, Ying, W. He, D. Ming, F. Shenga, D. Zhi, Human serum albumin interaction with formononetin studied using fluorescence anisotropy, FT-IR spectroscopy, and molecular modeling methods. Bioorganic and Medicinal Chemistry, 2006, 14, 1431–1436.

29. S. Darwish, S. Abu sharkh, M. Abu Teir, S. Makharza, M. Abu-hadid, Journal of Molecular Structure, 2010,963, 122.

30. H.A. Tajmir_Riahi, ScientiaIrania, 2007, 14 (2), 87-95.

31. I. Carrie, J. Portoukalian, R. Vicaretti, J. Rochford, Potvin S. and Ferland G. Menaquinone-4 concentration is correlated with sphingolipid concentrations in rat brain. J. Nutr. 2004; 134: 167-172.

32. A. Sulkowska, Interaction of drugs with bovine and human serum albumin. J. Mol. Struct., 2002, 614: 227-232.

33. F. Cui, J.Wang, Y. Cui, X. Yao, G. Qu , Y. Lu , Investigation of interaction between human serum albumin and N6-(2-hydroxyethyl) adenosine by fluorescence spectroscopy and molecular modelling. Luminescence, 2007, 22: 546-553.

34. F.Q. Cheng, Y.P. Wang, Z.P. Li, D. Chuan, Spectrochimica Acta Part A,2006, 65, p144.

35. J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 3 rded, Springer Science+Business Media, LLC, 2006.

36. M.M. Abu Teir, J. Ghithan, S. Darwich, M.M. Abu-hadid, Journal of Applied Biological Science,2012, 6 (3), P45-55.

Corresponding author: Musa Abu Teir,

Department of Physics, Biophysics Research Laboratory, Al-Quds University, Palestine

Online publication Date: 29.12.2017