International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Spectroscopic Studies of the Interaction of the Plant Alkaloid Capsaicin with Bovine Serum Albumin

Shabnam Kumari[1], Pritha Basu[2]

[1]Research Scholar, Department of Chemistry, T.M. Bhagalpur University, Bhagalpur Bihar-812007, India
Email: *shabnamsuman108[at]gmail.com*

Department of Chemistry, S.M. College Bhagalpur Bihar-812001, India
Corresponding Author Email: *titli.prithabasu1988[at]gmail.com*

Abstract: ***The binding study of capsaicin, the main component of chili pepper with the BSA was studied by the absorbance and fluorescence spectroscopic techniques. Capsaicin shows a high binding affinity with BSA in the order of 10^5 M^{-1}. The experimental data observed from the fluorometric study revealed that the quenching of fluorescence of BSA by capsaicin is due to the formation of a complex in the ground state and is of static nature. The synchronous fluorescence spectral study also reveals that binding of capsaicin also caused the structural changes in BSA. Overall, this study provides the insights on the interaction of the binding of the capsaicin to serum albumin and how they stabilize the protein which can be further used for drug development.***

Keywords: Alkaloids, Bovine serum albumin, anti-fungal, anti-cancer, anti-obesity, antioxidant

1. Introduction

Alkaloids are mainly a naturally occurring plant derivatives, different in respect of nitrogen base present in it, which works as hydrogen donor or receptor according to the functional groups [1]. These properties are significantly important for the interaction of alkaloids with the biological targets i.e., protein, enzymes, etc. The biological and pharmaceutical activities of alkaloids are of interest of recent research as they have impact on human health and have very low toxicity in comparison to other moieties. In recent decades our interest in natural drugs is increasing as the natural secondary metabolites have wide range of biological activities, less side effect and cost effective. There are lots of research is going on the development of new techniques to isolate and delivering the natural products as a new generation drug. Therefore, about 50% of pharmaceutical drugs are derived from natural products [2]. Alkaloids are mainly a wide group of heterogeneous nature compounds containing generally carbon, nitrogen, and hydrogen elements. In addition, the other element presents in capsaicin is oxygen in the. Capsaicin (figure 1), is a vanilloid, found mainly in the chili peppers of capsicum genus and is a colorless, odorless, crystalline alkaloid of molecular formula $C_{18}H_{27}NO_3$ and its molecular weight is 305.40 g/mol [3]. Capsaicin exhibits multiple biological effects and is known specially for its anticancer, antifungal, anti-inflammatory, ant obesity, antitumor, antimicrobial, pain relief properties [4-7]. Capsaicin (red chili peppers) are one of the main sources of vitamins like carotenoids, ascorbic acid and thiamine and the antioxidant property of capsaicin is due to ascorbic acid (vitamin C), capsaicin also has reducing nature due to presence of enediol structure [8]. Ascorbic acid concentration is dependent on the maturity of ripening of chili fruit, means ripe chili has more ascorbic acid content in compare of normal chili [9].

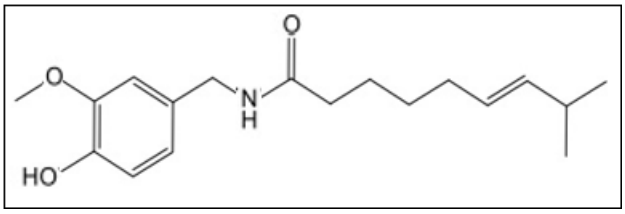

Figure 1: Chemical structure of capsaicin

Albumin is a well-known globular protein, and the most common albumin are serum albumin which are mainly found in blood plasma, and it plays an important role in transporting the endogenous and exogenous drugs in human body. Half of the plasma protein is made up of albumin (3.5 g/dL to 5 g/dL) [10]. Here our focus in this article on bovine serum albumin (BSA, hereafter used this symbol for bovine serum albumin), extracted from cows and it is a fifth fraction of the original so refer the other name is “Fraction V”. BSA in its mature state contains 583 amino acids after a respective cut off 18 residue single peptide and six amino acid further (the full length of BSA polypeptide residues are 607 amino acids) bound in a single chain cross linked with the 17 residues of cystine and the molecular weight is 66.5 kDa, present in three structurally different domains I, II and III each domain further divided into two sub- domains, A and B[11]. BSA and HSA (human serum albumin) has an excellent structural homology of about 80% and repeating pattern of disulphide bonds. The interestingly BSA binds other substances or drugs at ‘site 1’ in sub domain IIA, while other aromatic drugs bind to ‘site 2’ in sub domain IIIA [12] and it has the property to bind several types of hydrophobic ligands like warfarin, tryptophan, steroids, fatty acids, bilirubin and other dyes [13-15].

BSA is a globular protein with the non-glycosylated group breaks in nine loops arranged in heart-shaped molecule linked by disulfide bonds [15]. In many studies researchers have found that there are several activities like transportation and disposition of drugs in the context of drugs delivery in the blood stream as it forms a complex with serum albumins and they also optimize the blood osmotic pressure and blood pH [16,17]. Several spectral data taken by the researcher shows

that BSA specifically present in two tryptophan residues W^{131} and W^{214}, the other additional residues have been associated near the surface of the albumin molecule in the α-helix of the first domain [18]. In this regard the study of the interaction of BSA with small molecule is catching the attention [19-23]. In this paper we have shown how capsaicin reacts with BSA by spectroscopic and spectrofluorimetric techniques.

2. Experimental details

Bovine Serum Albumin (BSA) (M = 66.5 kDa, 0.97 mass fraction purity and globin free), and capsaicin (> 96% of purity, M = 305.40 g/mol) were purchased from CDH. The sample were prepared in 10 mM citrate-phosphate buffer of pH approx. 7.1. Citrate-phosphate buffer containing 5.2 mM Na_2HPO_4 and 0.453 mM citric acid. Concentration of the capsaicin and BSA were calculated by the absorbance measurement technique, by using the molar extinction coefficient of capsaicin 3410 M^{-1} cm^{-1} at 280 nm and for BSA 43,824 M^{-1} cm^{-1} at 279 nm. All the experiment was conducted in citrate-phosphate buffer of pH 7.1. All the reagents, above and the other one was used of analytical grade. Carefully, to remove any particulate matter, buffer solution was prepared from deionized water and buffer was passed through Millipore membrane filter of 0.22 µM pore size.

Absorption spectral studies were carried out on UV/Vis/Lambda 950 Perkin Elmer at room temperature (around 25°C) by using 1.0 cm path length quartz cells (Sterna cells, Inc.). For Steady state fluorescence measurement, Perkin Elmer Is 55 was used including for synchronous study. Temperature was maintained by using water bath during all experimental measurements. All the measurements were performed to set up the excitation and emission bandwidth at 5 nm and 10 nm. Fluorescence quenching of protein was studied by using excitation wavelength of 295 nm for the intrinsic Trp-moiety [24]. The fluorescence spectra of capsaicin were measured in the excitation range of 300-600 nm while maxima at 280 nm. The synchronous fluorescence spectra at two different wavelengths, i.e., Δλ at 15 and 60 nm were observed.

3. Results and Discussion

3.1 Absorption spectral Studies

Capsaicin shows absorption spectra in the range of 200-400 nm and this characteristic spectral pattern could be used to study the nature of binding to BSA with capsaicin. There are two peaks in this region, a sharp peak with maximum around 230 nm and a broad peak with maximum around 280 nm (Figure 2). The absorption spectral titration results of constant concentration of BSA (17 µM) with increasing concentration of capsaicin and constant concentration of capsaicin (5 µM) with increasing concentration of BSA are presented in Figures 2A & 2B, respectively. Binding of drugs with protein occurs complex formation and these results quenching of peaks. The spectral data were analyzed by Benesi-Hildebrand plot (Figure S1) to determine the equilibrium constant using the equation [25],

$$\frac{1}{\Delta A} = \frac{1}{A_{max}} + \frac{1}{K_{BH}(\Delta A_{\max})} \times \frac{1}{[Q]} \quad \ldots (1)$$

Where K_{BH} is the Benesi-Hildebrand binding constant and [Q] is the concentration of the free quencher (capsaicin). Applying to this equation form the linear plot gives K_{BH} value (see in Figure S1). The binding constant K_{BH} value is calculated is $3.49\times10^5 M^{-1}$

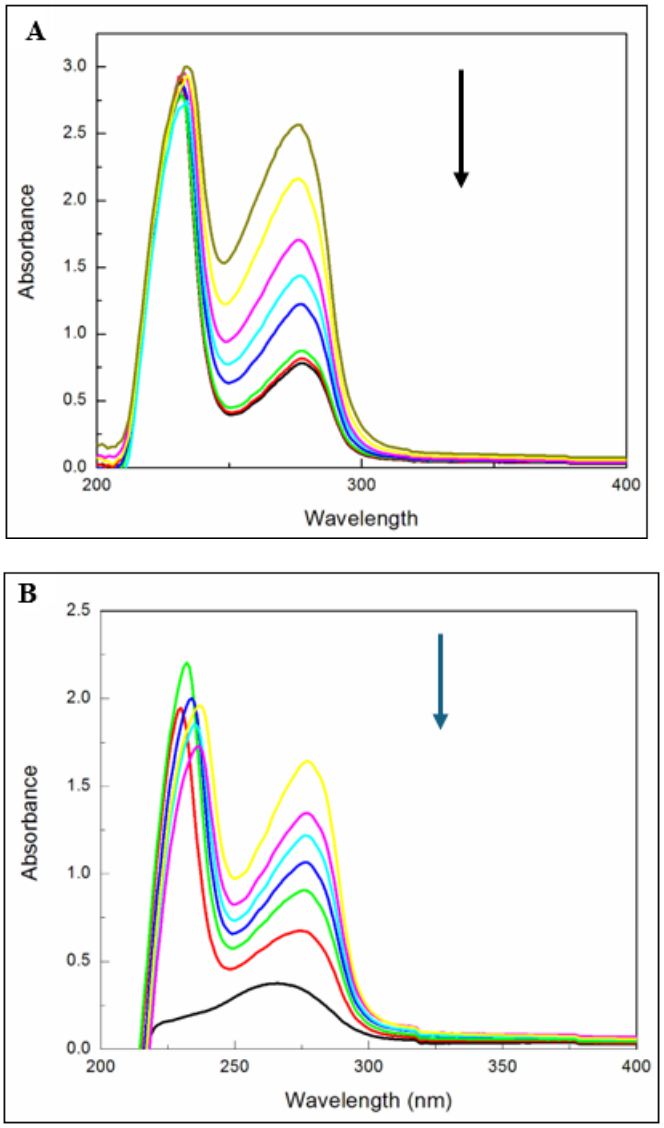

Figure 2: Absorption spectra of (A) BSA treated with increasing concentration of capsaicin, curve 1-8 respectively) and (B) Capsaicin with increasing concentration of BSA curves 1-6 respectively)

3.2 Fluorescence spectral studies.

BSA has two tryptophan moieties (Trp), one is Trp 134 and another is Trp 212 that possess intrinsic fluorescence property [26, 27]. The effect of capsaicin on the tryptophan residues of the protein was monitored by exciting at 295 nm in the fluorescence spectral technique. The first domain which is Trp 134 and the second domain which is Trp 212 are located on the surface of the hydrophilic and hydrophobic region of the protein, respectively [26]. BSA shows fluorescence emission maximum around 345 nm when excited at 295 nm of protein residues of Trp 212. The binding of capsaicin with `BSA causes the decrease in fluorescence intensity and the decreases in fluorescence intensity can be due to many reasons like molecular rearrangements, energy transfer, excited state reaction and collision quenching [28]. It has been monitored that proteins containing Trp residues have shown

a shorter wavelength emission maximum around 340 nm while a longer wavelength emission maximum is formed on or near the protein surface [24, 29]. The fluorescence quenching data of capsaicin with BSA are presented in Figures 3A & 3B. The quenching data were analyzed by Stern-Volmer equation,

$$\frac{F_0}{F} = 1 + K_q\tau_0[Q] = 1 + K_{sv}[Q]\ldots \quad (2)$$

Where F_o and F are the fluorescence intensities measured in the absence and presence of quencher, respectively. K_q is the rate constant, K_{SV} is dynamic quenching rate constant, τ_0 is the average lifetime of protein in absence of quencher, in the order of 10^{-8} sec [30], and [Q] is the quencher concentration (capsaicin). Static quenching refers to the complex formation of quencher-fluorophore in the ground state. On the other hand, dynamic quenching refers to the complex formation in the excited state. The plot of F_o/F versus [Q] is linear (a type of quenching), and K_{SV} values derived from the plot by using the Stern-Volmer equation, is 3.27×10^5 M^{-1} at 25°C.

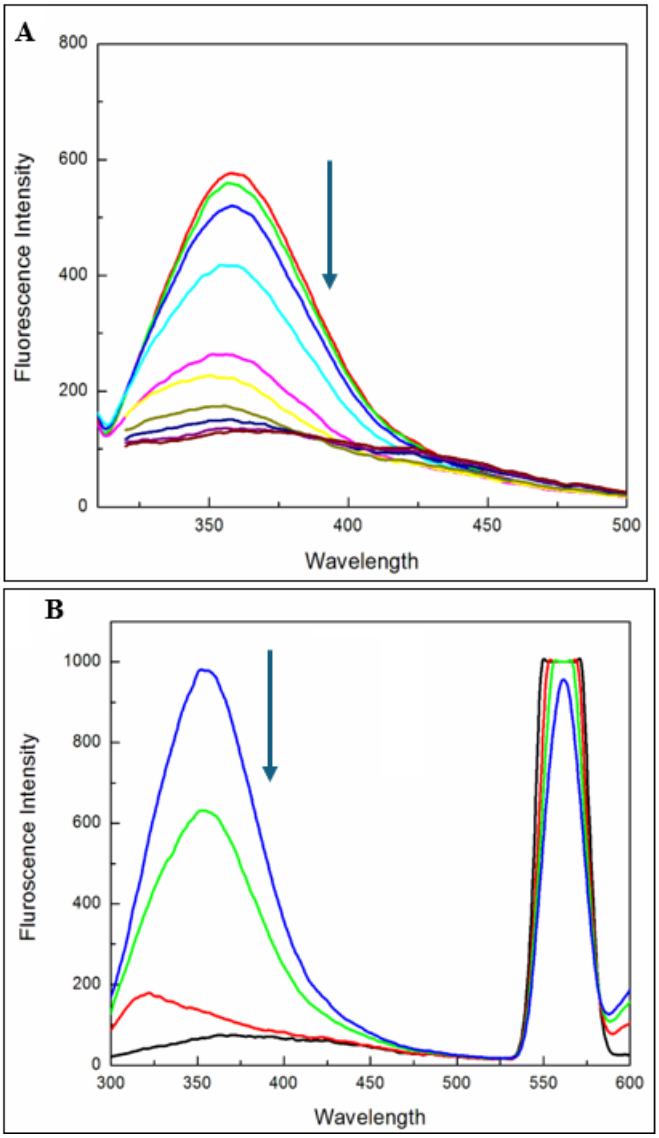

Figure 3: The fluorescence spectra of (A) BSA (10 µM) treated with 0.5, 1.5, 3.5, 6.5, 11.5, 16.5, 21.5, 26.5, 31.5 µM of capsaicin and (B) capsaicin (1µM) treated with 2, 4, 6, 8, 10 µM of BSA.

3.3 Spectral studies plot

Benesi-Hildbrand equation was used to calculate the value of K_{BH} (Fig S1) for absorbance spectral data and Stern-Volmer plot to calculate the value of K_{SV} (Fig S2) for fluorescence quenching studies. In both condition we plotted straight line graph to evaluate the constant values (plots of respective experiments are attached below in supplementary documents). The bindings constant observed from the both technique are in good relation are the values are respectively $3.49\times10^5 M^{-1}$ and 3.27×10^5 M^{-1}

3.4 Conformational Changes: Synchronous fluorescence

The conformational changes in protein associated with the binding of capsaicin was measured by using conformational studies of synchronous fluorescence technique proposed by Lloyd [31]. This is an effective technique to measure the fluorescence quenching of protein of possible shift of emission maximum in terms of the polarity around chromospheres. The excitation and emission wavelength are analyzed at 15 and 60 nm, this gives the alteration of the polarity around the protein residues to measure synchronous fluorescence of tryptophan (Try) and tyrosine (Tyr) [32]. The effect of capsaicin on the synchronous fluorescence of BSA with $\Delta\lambda$ are 15 and 60 nm are presented in the Figure 4 & 5, respectively. In this measurement it must be seen that the fluorescence intensity of BSA for $\Delta\lambda$= 60 nm of decreasing systematically with a small red shift. This indicates that, along with Trp is in polar environment and in the presence of capsaicin is more hydrophobic environment. On the other one, at $\Delta\lambda$ = 15 nm (Figure 5), there are almost no shift in maximum wavelength and reflecting graph shows some transformation around tyrosine.

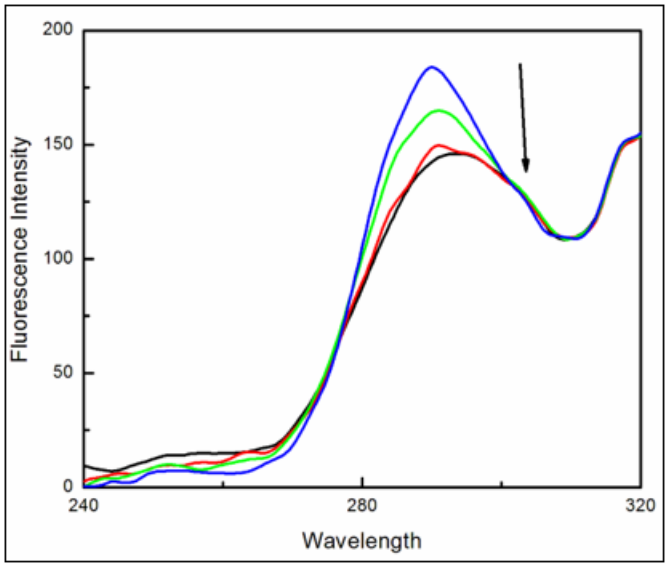

Figure 4: The synchronous spectra of BSA with increasing concentration of capsaicin at $\Delta\lambda$=15.

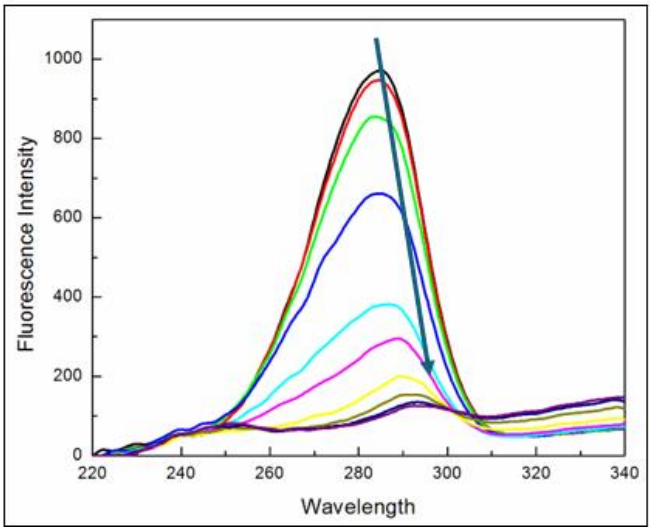

Figure 5: The synchronous spectra of BSA treated with capsaicin at Δλ=60

4. Conclusion

In recent years, there has been a growing interest in the medicinal potential of naturally occurring compounds found in plants. Among these, capsaicin, the pungent compound responsible for the heat in chili peppers, has drawn attention not only for its role in food but also for its broad pharmacological properties. Consumed globally in various diets, capsaicin has demonstrated diverse biological effects, including anti-inflammatory, analgesic, antioxidant, antifungal, and anticancer activities. As scientific focus shifts towards natural agents in drug development, it becomes essential to explore how such bioactive molecules function within physiological systems, particularly in relation to their interactions with plasma proteins that are vital for drug transport and metabolism.

To delve into these interactions, the current study investigated the binding behavior of capsaicin with bovine serum albumin (BSA), a commonly used model for human serum albumin (HSA) due to structural similarities [33]. However, differences in their spatial arrangements remain notable. Using absorption and fluorescence spectroscopy, the research aimed to analyze the nature and strength of capsaicin's association with BSA. Results revealed a high binding affinity, leading to the formation of a stable protein-ligand complex, which suggests that in the human bloodstream, capsaicin might circulate in a similar manner, bound to albumin. Such binding could significantly affect its distribution, bioavailability, and therapeutic potential. Further insights were obtained through fluorescence spectroscopy, which indicated that the presence of capsaicin led to a quenching of BSA's natural fluorescence. This effect, primarily attributed to interactions with tryptophan residues, suggests close contact between capsaicin and key aromatic amino acids. The observed quenching followed a static mechanism, indicative of ground-state complex formation. Interestingly, an increase in temperature led to a rise in the quenching constant, hinting that thermal energy may promote conformational changes in the protein that facilitate stronger binding. Such temperature-sensitive behavior could prove useful in designing controlled-release drug systems [34].

Additional analysis using synchronous fluorescence spectroscopy (at $\Delta\lambda$ = 15 and 60 nm) showed structural alterations in BSA upon capsaicin binding. Notably, changes were observed in regions near Trp37, located at the $\alpha 1\beta 2$ interface of BSA. These conformational shifts point to a deeper, more integrated interaction, rather than surface-level binding. This suggests that capsaicin could influence the functional dynamics of serum albumin, potentially modifying its affinity for other compounds, a characteristic that might be leveraged in drug design to regulate protein-ligand interactions [35]. Given the structural resemblance between BSA and human serum albumin, it is reasonable to anticipate that capsaicin would behave similarly in human systems. Understanding this interaction helps in predicting capsaicin's distribution, metabolism, and elimination in the human body. The ability of capsaicin to bind effectively with albumin may offer a strategy to improve its bioavailability, regulate its release profile, and increase therapeutic efficacy in clinical applications. From a pharmaceutical standpoint, capsaicin's albumin-binding capacity makes it a strong candidate for extended-release drug formulations. Drugs that bind to albumin tend to exhibit a longer half-life and enhanced systemic stability, which is particularly advantageous in managing conditions such as chronic pain, inflammation, obesity, and even cancer, where sustained drug activity is crucial.

However, capsaicin's interaction with serum albumin also raises important considerations. Since many drugs depend on albumin for transport, capsaicin could compete with or displace co-administered pharmaceuticals, potentially altering their efficacy. This underscores the need for further studies to assess the drug interaction potential of capsaicin, especially as it becomes more widely used in the form of supplements and functional foods. These findings lay the groundwork for incorporating capsaicin into the realm of nutraceuticals and food-based therapeutics. As healthcare moves towards more personalized and preventive models, naturally occurring compounds like capsaicin could play a significant role. By designing capsaicin derivatives that utilize its protein-binding capabilities, future therapies may be developed to target metabolic pathways, improve drug delivery, and personalize nutrition strategies, especially in the context of lifestyle-related diseases. In conclusion, this study highlights a robust and thermodynamically favorable binding interaction between capsaicin and BSA, marked by ground-state complex formation and protein structural changes. These results provide valuable insights into capsaicin's behavior in biological systems and suggest potential applications in drug delivery, therapeutic modulation, and clinical nutrition. As research continues at the intersection of food science and pharmacology, capsaicin stands out as a promising natural compound with diverse biomedical applications.

Acknowledgement
Preliminary laboratory facility was provided by the department of chemistry S.M. college, Bhagalpur (T.M.B.U.) and the spectroscopic and fluorometric facilities were providing by the UGC DAE Centre Kolkata. We are grateful to the reviewers by the positive comment and fruitful suggestions that helped us to correct and improve the manuscript on the right path.

Authors have no conflict of interest.

References

[1] Michelot, D., Melendez-howell. L. M., (2003). Amanita muscaria: chemistry, biology, toxicology, and ethnomycology. Mycol. Res., **107**: 131-146.
[2] Clark. A.M., (1996). Natural products as a resource for new drugs. Pharmaceutical Research. **13**: 1133-1141.
[3] "Capsaicin", Chem. Spider., Retrieved 9 June 2018.
[4] Szallasi. A. (2022). Capsaicin and cancer: Guilty as charged or innocent until proven guilty? Temperature. 13:1–5.
[5] Hsu, C. L., Yen., G. C. (2007). Effects of capsaicin on induction of apoptosis and inhibition of adipogenesis in 3T3-L1 cells. Journal of Agricultural and Food Chemistry. 55: 1730–1736.
[6] Leung, F.W. (2008). Capsaicin-sensitive intestinal mucosal afferent mechanism and body fat distribution. Life Sciences. 83: 1–5.
[7] Malagarie-Cazenave, S., Olea-Herrero, N., Vara, D., Díaz-Laviada, I. (2009). Capsaicin, a component of red peppers, induces expression of androgen receptor via PI3K and MAPK pathways in prostate LNCaP cells. FEBS Letters. 583: 141–147.
[8] Lu, J.M., Lin., P.H., Yao, Q., Chen, C. (2010). Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. Journal of Cellular and Molecular Medicine. 14: 840–860.
[9] Slanc, P., Doljak, B., Kreft, S., Lunder, M., Janes, D., Štrukelj, B. (2009). Screening of selected food and medicinal plant extracts for pancreatic lipase inhibition. Phytotherapy Research 23, 874–877 (2009).
[10] Yuwen, P., Chen, W., Lv, H., Feng, C., Li, Y., Zhang, T. et al. (2017). Albumin and surgical site infection risk in orthopaedics: a meta-analysis. BMC Surgery. 16: 7.
[11] Majorek, K.A., Porebski, P.J., Dayal, A., Zimmerman, M.D., Jablonska, K., Stewart, A.J. et al. (2012). Structural and immunologic characterization of bovine, horse, and rabbit serum albumins. Molecular Immunology. 52 (3–4); 174–182.
[12] Peters, T. (1996). All about Albumin: biochemistry, genetics, and medical application. San Diego: Academic Press. 11: 432.
[13] Yuan, H., Antholine, W.E., Subczynski, W.K., Green, M.A. (1996). Release of CuPTSM from human serum albumin after addition of fatty acids. Journal of Inorganic Biochemistry. 61(4): 251-259.
[14] Parker, W., Song, P.S. (1992). Protein structures in SDS micelle-protein complexes. Biophysical Journal. 61(5): 1435-1439.
[15] Bhattacharya, A.A., Grüne, T., Curry, S. (2000). Crystallographic analysis reveals common modes of binding of medium and long-chain fatty acids to human serum albumin. Journal of Molecular Biology. 303: 721-732.
[16] Gharagozlou, M., Boghaei, D.M. (2008). Interaction of water-soluble amino acid Schiff base complexes with bovine serum albumin: fluorescence and circular dichroism studies. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 71(4): 1617–1622.
[17] Krishnamoorthy, P., Sathyadevi, P., Cowley, A.H., Butorac, R.R., Dharmaraj, N. (2011). Evaluation of DNA binding, DNA cleavage, protein binding and in vitro cytotoxic activities of bivalent transition metal hydrazone complexes. European Journal of Medicinal Chemistry. 46(8): 3376–3387.
[18] Chadborn, A., Bryant, J., Bain, A.J., O'Shea, P. (1999). Ligand-dependent conformational equilibria of serum albumin revealed by tryptophan fluorescence quenching. Biophysical Journal. 76(4): 2198-2207.
[19] Monti, S., Ottani, S., Manoli, F., Manet, I., Scagnolari, F., Zambelli, B. et al. (2009). Chiral recognition of 2-(3-benzoylphenyl)propionic acid (ketoprofen) by serum albumin: an investigation with microcalorimetry, circular dichroism and molecular modelling. Physical Chemistry Chemical Physics. 11: 9104–9113.
[20] Katrahalli, U., Jaldappagari, S., Kalanur, S.S. (2010). Study of the interaction between fluoxetine hydrochloride and bovine serum albumin in the imitated physiological conditions by multi-spectroscopic methods. Journal of Luminescence. 130: 211–216.
[21] Liu, B., Zhao, E., Xue, C., Wang, J., Lu, Y. (2010). Studies on the antagonistic action between chloramphenicol and quinolones with presence of bovine serum albumin by fluorescence spectroscopy. Journal of Luminescence. 130: 859–864.
[22] Bourassa, P., Kanakis, C.D., Tarantilis, P., Pollissiou, M.G., Tajmir-Riahi, H.A. (2010). Resveratrol, genistein, and curcumin bind bovine serum albumin. Journal of Physical Chemistry B. 114: 3348–3354.
[23] Mandeville, J.S., Tajmir-Riahi, H.A. (2010). Complexes of dendrimers with bovine serum albumin. Biomacromolecules, 11: 465–472.
[24] Burstein. E.A., Vedenkina, N.S., Ivkova, M.N. (1973). Fluorescence and the location of tryptophan residues in protein molecules. Photochemistry and Photobiology. 18: 263−279.
[25] Benesi, H.A., Hildebrand, J.H. (1949). A Spectrophotometric Investigation of the Interaction of Iodine with Aromatic Hydrocarbons. Journal of American Chemical Society, 71: 2703−2707.
[26] Tayeh, N., Rungassamy, T., Albani, J.R. (2009). Fluorescence spectral resolution of tryptophan residues in bovine and human serum albumins. Journal of Pharmaceutical and Biomedical Analysis. 50: 107–116.
[27] Peters, T. (1985). Serum albumin. Advances in Protein Chemistry. 37: 161–245.
[28] Lakowicz, J.R. (1999). Principles of Fluorescence Spectroscopy. New York: Kluwer Academic/Plenum Publishers.
[29] Tayeh, N., Rungassamy, T., Albani, J.R. (2009). Fluorescence spectral resolution of tryptophan residues in bovine and human serum albumins. Journal of Pharmaceutical and Biomedical Analysis. 50(2): 107-116.
[30] Lakowicz, J.R., Weber, G. (1973). Quenching of fluorescence by oxygen. A probe for structural fluctuations in macromolecules. Biochemistry. 12: 4161–4170.

[31] Lloyd, J.B.F. (1971). Synchronized Excitation of Fluorescence Emission Spectra. Nature Physical Science. 231: 64−65.
[32] Miller, J.N. (1979). Recent advances in molecular luminescence analysis. Proceedings of the Analytical Division of the Chemical Society. 16: 203−208.
[33] Chaves, O.A., Tavares, M.T., Cunha, M.R., Parise-Filho, R., R Sant'Anna , C.M., Netto-Ferreira, J.C. (2018). Multi-Spectroscopic and Theoretical Analysis on the Interaction between Human Serum Albumin and a Capsaicin Derivative-RPF101. 8(3): 78.
[34] Yu, X., Liao, Z., Jiang, B., Hu, X., Li, X. (2015). Spectroscopic analyses on interaction of bovine serum albumin with novel spiro[cyclopropane-pyrrolizin]. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 137:129-136.
[35] Seetharamappa, J., Kamat, B.P. (2004). Spectroscopic studies on the mode of interaction of an anticancer drug with bovine serum albumin. Chemical and Pharmaceutical Bulletin. 52(9): 1053-57.