RESEARCH ARTICLE

Binding interaction of sodium benzoate food additive with bovine serum albumin: multi-spectroscopy and molecular docking studies
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Abstract
Sodium benzoate (SB) is widely used as a preservative in food industry, and bovine serum albumin (BSA) is a major carrier protein similar to human serum albumin (HSA), the study of the binding between the two has great significance on human health. In this paper, we systematically investigated the binding of SB and BSA under the simulated physiological conditions combining with various common analytical methods, e.g., fluorescence, UV–vis absorption, synchronous fluorescence and circular dichroism (CD) spectra, as well as molecular docking method. The fluorescence quenching measurements were respectively carried out at 298 K, 303 K and 308 K using the Stern–Volmer method. The results reveal that ground state SB–BSA complex was formed within the binding constants from $2.02 \times 10^4$ to $7.9 \times 10^3$ M$^{-1}$. Meanwhile, the negative values of $\Delta H^0$ ($-43.92$ kJ mol$^{-1}$) and $\Delta S^0$ ($-111.6$ J mol$^{-1}$ K$^{-1}$) demonstrated that both the hydrogen binding interaction and van der Waals forces contributed to stabilizing the SB–BSA complex. The site marker competitive experiments show that the SB and BSA bound at site I. Furthermore, the experimental results of UV–vis absorption, synchronous fluorescence and CD spectra indicate that the binding of SB and BSA may change the conformation of BSA. In addition, the molecular docking experiment suggests that hydrogen bond was formed in the interaction between SB and BSA.

Keywords: Sodium benzoate, Bovine serum albumin, Multi-spectroscopy, Molecular docking modeling

Introduction
As a recognized food-grade preservative, sodium benzoate (SB), whose structure is shown in Fig. 1, is widely used in the food, cosmetic, and pharmaceutical industries [1–3]. For example, in the food industry, SB is used in a variety of foods and beverages, such as salads, kimchi, carbonated beverages, jams, juices, and soy sauce, due to its effective inhibition of fungal and bacterial growth during storage [2, 4]. In addition, SB is also applicable in clinically practice and can to treat various diseases, such as urea cycle disorders, liver disease, multiple sclerosis, early Alzheimer’s disease and Parkinson's disease [5, 6]. Although SB as a preservative is generally recognized as safe (GRAS), its concentration is limited to 0.1% by the US Food and Drug Administration (FDA) [7]. In recent years, studies have shown that the organic form of SB is nontoxic, but its synthetic form is toxic to organisms at chronic doses [8]. Furthermore, it has been reported that SB may damage mitochondrial DNA [9]. Nevertheless, the results of these investigations remain controversial. The interaction between SB and biomacromolecules requires a more in-depth research.

The effect of SB on health can be explored by studying the interaction between SB and Serum albumin (SA). SA, the most abundant translocator protein in blood circulation [10, 11], has several critical physiological functions, for example, the maintenance of the colloidal osmotic blood pressure, and the transportation of various...
endogenous and exogenous compounds [12, 13]. Bovine serum albumin (BSA) is not only widely used in biomedical and pharmaceutical applications, but also widely utilized as a ligand-biological model to study the interactions between small molecules and globular proteins, due to its high stability, low-cost, versatile ligand-binding properties, medical importance and high structural homology with human serum albumin (HSA) (approximately 76%) [14, 15]. BSA is a single-chain globular protein consisting of 583 amino acid residues, forming 17 disulfide bonds [16]. In addition, BSA contains two major specific ligand-binding sites located in the hydrophobic cavities in sub-domains II-A and III-A, which are also known as Sudlow’s site I and Sudlow’s site II, respectively [17]. Meanwhile, previous works have demonstrated that the durability and toxicity of chemicals have huge influence on the structure of BSA because of interaction effect [18]. Moreover, several studies have shown that secondary structure of BSA changes upon binding with small molecules [19–21]. Therefore, investigating the interaction between BSA and chemicals, especially small molecules is of great significance.

In this work, the interaction between SB and BSA was studied by employing multi-spectroscopic methods and molecular docking (MD) modeling. Fluorescence spectroscopy was first used for understand the binding mechanism, and quenching mechanism, binding constant, mode and site were analyzed. Then, UV–vis absorption, synchronous fluorescence and CD spectra were employed to determine the conformation and structure of BSA when SB was binding. Furthermore, to further interpret the experimental findings, molecular docking modeling was used to explore the molecular graph of the binding interaction.

**Experimental**

**Chemicals and reagents**

All chemicals used in this experiment, including BSA (lyophilized powder, Mw≈ 66.2 kDa, Sigma) and sodium benzoate (Sinopharm), are of high purity. Thus, no further purification is required after purchase. PBS buffer solution (20 mM, pH = 7.40) was prepared with the ultrapure water (ρ = 18.2 MΩ cm). Then, BSA solution was prepared using PBS as a solvent for the spectral experiments. Additionally, all solutions were stored in the dark environment with a low temperature of 4 °C before using.

**Measurements of the fluorescence spectra**

Fluorescence experiments were taken out with the RF-5301 (Shimadzu, Japan) fluorescence spectrophotometer. Firstly, BSA solution (1 × 10⁻⁶ M) was added to a quartz cell with size of 1.0 cm. An equal amount of BSA was added to the reference solution to eliminate the absorbance of BSA itself, and the absorbance of PBS buffer was subtracted through base line correction. Then, SB of different concentrations from 0 to 8 × 10⁻⁶ M was gradually dropped into the BSA using a microsyringe. The transportation of various molecules and materials in the blood circulatory system are regulated by albumin. Some of the small molecules entered into the blood are reversibly bound to plasma proteins, forming binding molecules. While those do not bind are free molecules. When the concentration of free molecules decreases, some of the binding molecules dissociate into free molecules, thus, they are always in dynamic equilibrium [22]. In our experiment, SB and BSA were let stand for 5 min to reach dynamic equilibrium [23]. During the fluorescence measurement, the slit width was set at 10 nm/10 nm, the excitation wavelength was 285 nm, and the scanning range of the fluorescence emission spectrum was 300–450 nm.

**Measurements of the synchronous fluorescence**

The synchronous fluorescence of SB–BSA was measured using the same concentrations of the mixture solutions as in the fluorescence quenching measurements, but at room temperature. Spectra were recorded at Δλ = 15 nm and 60 nm, which showed the tyrosine residue and tryptophan residue characteristics of the BSA.

**Measurements of UV–vis spectra**

The UV–vis absorption spectra were obtained by a UV-3600 (Shimadzu, Japan) spectrophotometer. The concentration of BSA was kept at 1 × 10⁻⁶ M, while the concentration of the added SB ranged from 0 to 8 × 10⁻⁶ M, each time with an increase of 2 × 10⁻⁶ M. The absorption spectra of the BSA between 230 and 330 nm were recorded in a 1 cm quartz absorption cell.

**Measurements of CD spectra**

CD spectra were recorded using the spectropolarimeter of JASCO J-810 and a 1.0 cm quartz absorption cell. Note that the spectrum of a cell only with PBS buffer solution
was firstly measured as background signal to remove the influence of PBS buffer solution.

**Molecular docking**

Molecular docking simulation was employed to study the molecular interaction between BSA and SB using Auto Dock Vina, an open-source software with significantly fast dock running speed and high molecular docking accuracy [24]. To prepare the protein and ligand molecules for docking study, BSA crystal structure was first retrieved from the Protein Data Bank (PDB ID: 3V03) (http://www.rcsb.org/structure/3V03), and then loaded on AutoDock Tools to remove additional molecules, e.g., all the water molecules. Next, Polar hydrogens and Gasteiger charges were added, respectively. The structure of SB was prepared by drawing the 2D chemical structure using ChemOffice and further optimized based on MM2 force field implemented using Chem3D. After that, the structurally optimized BSA and SB were employed to conduct molecular docking simulation. During the simulation, the size of the grid box along x-, y- and z-directions were all set at 18 Å and the grid spacing 1 Å. The grid box center was set at (88.537, 24.797, 13.111). The energy range and values were set at 4 kcal/mol and 100, respectively.

**Results and discussion**

**Fluorescence spectral analysis of the interactions involving SB with BSA**

**Fluorescence quenching of BSA**

Tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) residues are three main amino acids that can make protein generate endogenous fluorescence [25]. The major contribution to the changed fluorescence of BSA is from the environmentally-sensitive tryptophan (Trp) moiety [26]. The fluorescence emission spectra of BSA with various concentration of SB are shown in Fig. 2a. When excited at 285 nm, BSA had a characteristic band at around 344 nm. Furthermore, when the concentration of the added SB increased from 0 to $8 \times 10^{-6}$ M, the fluorescence intensity of BSA decreased significantly, indicating that the environment around the Trp residues of BSA varied with the addition of SB. Therefore, it can be inferred that there is a binding interaction between SB and BSA, and the binding site is located near the Trp residue [27].

The reaction temperatures for SB–BSA system were maintained at 298 K, 303 K, and 308 K, respectively. The fluorescence quenching data are analyzed by the Stern–Volmer equation [28]:

$$\frac{F}{F_0} = 1 + K_{SV}[Q] = 1 + k_q \tau_0 [Q]$$

where $F$ and $F_0$ are the fluorescence intensity of BSA with and without the quencher, i.e., SB, respectively. $K_{SV}$ is the Stern–Volmer quenching constant with the unit being M$^{-1}$, and $[Q]$ is the concentration of the quencher. $k_q$ is the quenching rate constant of BSA. $\tau_0$ is the average fluorescence lifetime of BSA in the excited state without the quencher (the order of magnitude is $10^{-8}$) [28]. $K_{SV}$ and $k_q$ value of BSA triggered by SB at different temperatures can be determined by calculating the slope of the curve, as shown in Fig. 2b. The values of the parameters $K_{SV}$, $k_q$ and R at different temperatures are listed in Table 1. It can be seen from the results that $K_{SV}$ decreases from $1.87 \times 10^4$ to $1.34 \times 10^4$ M$^{-1}$ as the temperature increases from 298 to 308 K. Moreover, the values of $k_q$ at various temperatures are all in the order of $10^{12}$ M$^{-1}$ s$^{-1}$, which
are much larger than the maximum diffusion collision quenching rate constant \(2.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}\), indicating that the SB-trigger BSA quenching process is static rather than dynamic.

**Interaction parameters and binding model for SB–BSA complex**

The binding constants \((K_b)\) and binding site \((n)\) of SB–BSA complex is calculated using the following formula [29]:

\[
\log \left( \frac{F_0 - F}{F} \right) = \log K_b + n \log [Q]
\]

According to the Eq. (2), \(K_b\) and \(n\) can be calculated from the curve of \(\log [(F_0 - F)/F]\) versus \(\log [Q]\), as shown in Fig. 3a. The calculated results are summarized in Table 2. These results show that within the temperature range studied, the value \(n\) of SB–BSA complex is close to 1, indicating that BSA has a single high affinity binding site for SB. \(K_b\) is calculated to be approximately \(10^4\), indicating strong binding interactions between SB and BSA.

It is also found that as temperature increases, \(K_b\) value decreases, suggesting that the stability of SB–BSA complex decreases with the increasing of temperature.

To better understand the binding between BSA and SB, the van’t Hoff Eq. (3) was used to calculate the thermodynamic enthalpy \((H_0)\) and entropy \((S_0)\) of BSA and SB complexation.

\[
\ln K_b = -\frac{\Delta H_0}{RT} + \frac{\Delta S_0}{R}
\]

As shown in Fig. 3b, the curves of \(\ln K_b\) and \(1/T\) were used to determine the thermodynamic parameters of SB–BSA complex at three different temperatures, i.e. 298 K, 303 K, and 308 K. Once the \(H_0\) and \(S_0\) values are determined, the variation in Gibbs free energy \((G_0)\) can be calculated by the following standard Eq. (4).

\[
\Delta G_0 = \Delta H_0 - T \Delta S_0
\]

Here, the binding constant values at three different temperatures, i.e. \(\Delta G_0\), \(\Delta H_0\) and \(\Delta S_0\), are listed in Table 2.

| **Table 1** Stern–Volmer Constants of Stern-Volmer quenching \((K_{SV})\) and bimolecular quenching rate \((K_q)\) at tested temperatures |
|---|---|---|---|
| \(T\) (K) | \(K_{SV} (10^4 \text{ M}^{-1})\) | \(K_q (10^{12} \text{ M}^{-1} \text{ s}^{-1})\) | \(R\) |
| 298 | 1.87 | 1.87 | 0.99327 |
| 303 | 1.58 | 1.58 | 0.99302 |
| 308 | 1.34 | 1.34 | 0.99579 |

\(R\) represents correlation coefficient.

| **Table 2** Calculated parameters of SB–BSA complex at different testing temperatures at pH 7.4 |
|---|---|---|---|---|---|---|
| \(T\) (K) | \(K_b (10^4 \text{ M}^{-1})\) | \(n\) | \(R\) | \(\Delta G_0\) (KJ/mol) | \(\Delta S_0\) (J mol\(^{-1}\) K\(^{-1}\)) | \(\Delta H_0\) (KJ/mol) |
| 298 | 2.02 | 1.00 | 0.9973 | -9.35 | -111.6 | -43.92 |
| 303 | 1.03 | 0.96 | 0.9933 | -8.78 | -111.6 | -43.92 |
| 308 | 0.79 | 0.95 | 0.9971 | -8.12 | -111.6 | -43.92 |

\(K_b\), \(n\), \(\Delta G\), \(\Delta H\) and \(\Delta S\) are binding constant, binding site, Gibbs free energy, thermodynamic enthalpy and entropy, respectively. \(R\) represents correlation coefficient.
The negative value of $\Delta G_0$ indicates that the interaction process between SB and BSA is spontaneous. And the positive $H_0$ and $S_0$ values indicate that hydrogen bonding and van der Waals interactions play a major role in the binding of the chemical to the protein [30, 31].

**Combination of fluorescent probes**

To determine the displacement percentage of the fluorescent combination probe, according to the method introduced by Sudlow et al., the fluorescence markers for distinct binding sites were chosen: ketoprofen for site I and ibuprofen for site II [32]. And the following equation is also adopted.

\[
\text{Probe displacement (\%)} = \frac{F_2}{F_1} \times 100
\]

where $F_1$ and $F_2$ represent the fluorescence intensity of the SB–BSA system in the absence and presence of the probe, respectively. As shown in Fig. 4. The increase in ketoprofen concentration results in significant decrease in the fluorescence intensity of the SB–BSA system. However, the increase in ibuprofen concentration has little impact on the fluorescence intensity of BSA. Therefore, SB and BSA are supposed to bind at site I [33].

**Conformational change of BSA**

**Analysis of the UV–visible absorption spectra**

UV absorption measurement, a very simple and effective method, is often used to observe the formation process and the change in the conformation of protein during the its binding interaction between with small molecule. The UV spectra of SB–BSA complex are shown in Fig. 5. All SB–BSA complex have absorption bands. The absorption band around 280 nm is the result of the $\pi-\pi^*$ transition of aromatic amino acids (Trp, Tyr, and Phe) [34]. Adding SB (0–8.0 × 10$^{-6}$ M) to the BSA solution enhances the absorption intensity (0.044–0.0556), and the maximum wavelength shows a slight blue shift at around 280 nm. The UV absorption intensity increases with the increase of SB concentration, indicating that complex is formed by SB and the amino acid residues of BSA.

**Synchronous fluorescence spectroscopy**

The synchronous fluorescence spectroscopy is a useful tool for obtaining information around chromophore microenvironment. Usually, the shift of the $\lambda_{em}$ represents the alteration of the polarity of the amino acid environment (particularly Tyr or Trp residues) [35]. The synchronous fluorescence spectra were carried out for investigating structural change in BSA with SB addition (Fig. 6). The fluorescence spectra characteristic of tyrosine and tryptophan residues are shown in Fig. 6a ($\Delta \lambda = 15$ nm) and Fig. 6b ($\Delta \lambda = 60$ nm), respectively [36]. It is obvious that the fluorescence intensity of the tryptophan residues are much stronger than that of tyrosine residues in Fig. 6. And no significant change is found in the fluorescent emission peak position of both tyrosine and tryptophan residues. Thus, the addition of SB did not rearrange the microenvironment of the tyrosine and tryptophan residues in BSA.

**Analysis of CD spectra**

To further explore whether SB can induce conformational change in BSA, CD spectroscopy experiments were performed. The two negative bands at 208 nm ($\pi-\pi^*$
transition) and 222 nm (n-π* transition) in CD spectrum of free BSA are characteristic of the protein α-helical structure [37], whose content can be estimated by [38]:

\[
a\text{-}\text{helix} \% = \left( \frac{-\text{MRE}_{208} - 4000}{33000 - 4000} \right) \times 100
\]

where MRE\textsubscript{208} is the MRE value observed at 208 nm, 4000 is the MRE value of the β shape and random coil conformation at 208 nm, and 33000 is the MRE value of the pure α-helix at 208 nm. The MRE\textsubscript{208} value used to indicate the change in secondary structure of BSA determined by [38]:

\[
\text{MRE}_{208} = \frac{\text{CD (m deg)}_{208}}{(10 \times n \times l \times C_p)}
\]

where \( n \) is the number of amino acid residues (583 for BSA), \( l \) is the cell path length, and \( C_p \) is the molar concentration of BSA. When SB:BSA ratio increases from 0 to 2:1, the band intensities of the CD spectra have a slight decrease, whereas the peaks positions remain unchanged, as shown in Fig. 7. The results demonstrate that the secondary structure of BSA has a partial change from α-helical content. The α-helical content decreased from 62.7% of free BSA to 61.88% (SB:BSA = 1:1) and 60.24% (SB:BSA = 2:1). Based on these analyses, we conclude that the addition of SB alters the secondary structure of BSA, resulting in a decrease in α-helical content, which is nonetheless still dominant in the secondary structure.

**Molecular docking**

To further elucidate SB–BSA binding interaction, molecular docking was used to simulate the molecular interaction between BSA and SB. The simulated result of the predominate configuration of SB–BSA complex is plotted in Fig. 8, where the binding energy is the lowest. Moreover, as shown in Fig. 9, the binding results indicate that SB is very close to the amino acid residues Tyr149, Leu237, Arg256, Leu259, Ala260, Ile263, Ser286, Ile289, and Ala290 at site I in subdomain IIA. The hydrogen bonding between SB and BSA (Tyr149, ARG256 with a bond length of 3.0 Å, 2.2 Å and 2.0 Å) is also responsible for maintaining the stability of the complex. Here, note that the calculated combined Gibbs free energy is \(-5.7\) kcal/mol \((-23.8\) kJ/mol) and binding constant (K\textsubscript{b}) is about \(1.45 \times 10^4\) mol/L, which is quite different with our experimental result (\(\Delta G = -935\) kJ/mol and K\textsubscript{b} = \(2.2 \times 10^4\) mol/L). This may be caused by the difference between X-ray crystal structure of BSA and its solution state in the aqueous system.

**Conclusion**

In this study, we combined the multi-spectroscopic methods and molecular docking modeling to systematically investigate the interaction between SB and BSA.
The multi-spectroscopic data show that the quenching of BSA binding with SB was caused by the formation of SB–BSA complex. The negative values of $\Delta H^0$ and $\Delta S^0$ demonstrated that hydrogen bonding and van der Waals forces contribute to make the SB–BSA complex stable. The negative values of $\Delta G$ indicate that the interaction process was spontaneous. Site marker competitive experiments show that SB and BSA bound at site I. In addition, the amino acid microenvironments and the secondary structure of BSA were altered by the addition of SB, as shown in UV–vis absorption, synchronous fluorescence spectroscopy and CD spectra data. Furthermore, molecular docking studies provided some valuable information on the interaction between SB and BSA and structural stability of their complex.

Abbreviations
SB: sodium benzoate; BSA: bovine serum albumin; HSA: human serum albumin; CD: circular dichroism; GRAS: generally recognized as safe; FDA: Food and Drug Administration; SA: serum albumin; MD: molecular docking.

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Authors' contributions
Experiments was accomplished by JY and WX. The molecular docking study was accomplished by JL. JY and XZ accomplished the writing of the article. XZ and YZ were the study designers and corresponding authors. All authors read and approved the final manuscript.

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Availability of data and materials
All data and material analyzed or generated during this investigation are included in this published article. The raw data can be requested from email of JY: 95634941@qq.com.

Competing interests
The authors declare that they have no competing interests.

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References
1. Baranowska I, Wojciechowska I, Solarz N et al (2013) Determination of preservatives in cosmetics, cleaning agents and pharmaceuticals using fast liquid chromatography. J Chromatogr Sci 52(1):88–94
2. Lennierz B-S, Vafai S-B, Delaney N-F, Clish A-B, Deik A-A, Pierce K-A, Ludwig D-S, Mootha V-K (2015) Effect of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans. Mol Genet Metab 114(1):73–79
3. Ikarashi Y, Uchino T, Nishimura T (2010) Analysis of preservatives used in cosmetic products: salicylic acid, sodium benzoate, sodium dehydroacetate, potassium sorbate, phenoxethanol, and parabens. Bull Natl Inst Health Sci 128:85–90
4. Nettis E, Colanardi M-C, Ferrannini A et al (2004) Sodium benzoate-induced repeated episodes of acute urticaria/angio-oedema: randomized controlled trial. Br J Dermatol 151(4):898–902
5. Yavav A, Kumar A, Das M, Tripathi A (2016) Sodium benzoate, a food preservative, affects the functional and activation status of splenocytes at non cytotoxic dose. Food Chem Toxicol 88:40–47
6. Häberle J, Boddaert N, Burlina A, Chakrapsani A, Dixon M, Huemer M, Karall D, Martinelli D, Crespo P-S, Santer R, Servais A, Valyannopoulos V, Lindner M, Rubin V, Dionisi-Vici C (2012) Suggested guidelines for the diagnosis and management of urea cycle disorders. Orphanet J Rare Dis 7(1):32
7. Misel M-L, Gish R-G, Patton H, Mendler M, Pahan K (2011) Immunomodulation of experimental allergic encephalomyelitis by cinnamon metabolite sodium benzoate. Immunopharmacol Immunotoxicol 33(4):586–593
8. Kehinde O-S, Christianah O-I, Oyetunji O-A (2018) Ascorbic acid and sodium benzoate synergistically aggravates testicular dysfunction in adult Wistar rats. Int J Physiol Pathophysiol Pharmacol 10(1):39
9. Piper J-D, Piper P-W (2017) Benzoate and sorbate salts: a systematic review of the potential hazards of these invaluable preservatives and the expanding spectrum of clinical uses for sodium benzoate. Compr Rev Food Sci F 16(5):868–880
10. Zhang Q, Ni Y, Koker S (2016) Competitive interactions between glucose and lactose with BSA: which sugar is better for children? Analyst 141(7):2218–2227
11. Leblanc A, Shiao T-C, Roy R, Sleno L (2014) Absolute quantitation of NAPQI-modified rat serum albumin by LC-MS/MS: monitoring acetylation, deacetylation, and binding in vivo. Chem Res Toxicol 27(9):1632–1639
12. Sharifi M, Dolatabadi JEN, Fathi F et al (2017) Kinetic and thermodynamic study of bovine serum albumin interaction with rifampicin using surface plasmon resonance and molecular docking methods. J Biomed Opt 22(3):037002
13. Janek T, Czyznikowska Z, Lucyżyński J et al (2017) Physicochemical study of biomolecular interactions between lysosomotropic surfactants and bovine serum albumin. Colloids Surf B 159:750–758
14. Mote U-S, Bhattar S-L, Patil S-R (2010) Interaction between felodipine and bovine serum albumin: fluorescence quenching study. J Lumin 129(1):1–8
15. Shama Y, Ryazudddeen (2017) Exploring thermodynamic parameters and the binding energetic of berberine chloride to bovine serum albumin (BSA): spectroscopy, isothermal titration calorimetry and molecular docking techniques. Thermochim Acta 655:76–78
16. Chaturvedi S-K, Ahmad E, Khan J-M, Alam P, Ishhtikhar M, Khan R-H (2015) Elucidating the interaction of limonene with bovine serum albumin: a multi-technique approach. Mol Bio Syst 11(1):307–316
17. Guan J, Yan X, Zhao Y-J, Sun Y-H, Peng X (2018) Binding studies of triclocarban with bovine serum albumin: insights from multi-spectroscopy and molecular modeling methods. Spectrochim Acta A 202:1–12
18. Shi JH, Zhou KL, Lou YY, Pan DQ (2016) Multi-spectroscopic and molecular modeling approaches to elucidate the binding interaction between bovine serum albumin and darunavir, a HIV protease inhibitor. Spectrochim Acta A 188:362–371
19. Skrt M, Benedik E, Podlipnik C, Poklar Ulrih N (2012) Interactions of different polyphenols with bovine serum albumin using fluorescence quenching and molecular docking. Food Chem 135(4):2418–2424
20. Tian J, Liu J, He W, Hu Z, Yao X, Chen X (2004) Probing the binding of scutellarin to human serum albumin by circular dichroism, fluorescence spectroscopy, FTIR, and molecular modeling method. Biomacromol 5(5):1956–1961
21. Makarska-Bialokoz M (2017) Investigation of the binding affinity of vitamin B12-Bovine serum albumin system using various spectroscopic methods. Spectrochim Acta A 184:262–269
22. Agarwal R-P, Phillips M, McPherson R-A et al (1986) Serum albumin and the metabolism of disulfiram. Biochem Pharmacol 35(19):3341–3347
23. Zhang J, Li WX, Ao BY et al (2014) Fluorescence enhancement of europium (III) perchlorate by benzoic acid on bis (benzylsulfinyl) methane complex and its binding characteristics with the bovine serum albumin (BSA). Spectrochim Acta A 118:972–980
24. Trott O, Olson A-J (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31(2):455–461
25. Shi J-H, Zhou K-L, Pan D-Q et al (2017) Spectroscopic and molecular docking approaches for investigating conformation and binding characteristics of clonazepam with bovine serum albumin (BSA). J Photochem Photobiol B 167:158–167
26. Sandhya B, Hegde A-H, Kalanur S-S et al (2011) Interaction of triprolidine hydrochloride with serum albumins: thermodynamic and binding characteristics, and influence of site probes. J Pharm Biomed Anal 54(5):1180–1186
27. Shen G-F, Liu T-T, Wang Q, Jiang M, Shi J-H (2015) Spectroscopic and molecular docking studies of binding interaction of gefitinib, lapatinib and sunitinib with bovine serum albumin (BSA). J Photochem Photobiol B 153:380–390
28. Zhao L-Z, Liu R-T, Zhao X-C, Yang B-J, Gao C-Z, Hao X-P et al (2009) New strategy for the evaluation of CdTe quantum dot toxicity targeted to bovine serum albumin. Sci Total Environ 407(18):5019–5023
29. Safarnejad A, Shaghaghi M, Dehgahan S, Soltani S (2016) Binding of carvedilol to serum albumins investigated by multi-spectroscopic and molecular modeling methods. J Lumin 176:149–158
30. Ross P-D, Subramanian S (1981) Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry 20(11):3096–3102
31. Xu Y-Y, Ou Z-Y, Wu W-H, Wang Y-F, Zhang B, Mao X-Y, Jiang L, Yang J, Hou S-F (2017) Synthesis of triangular silver nanoparticles and spectroscopic analysis on the interaction with bovine serum albumin. Anal Bioanal Chem 409(22):5327–5336
32. Yao D, Yu J, Pan Y-M, Huang F-P, Bian H-D, Yu Q, Liang H, Chen Z-F (2012) Spectroscopic studies on the binding of kaempferol-3,7-dihydroxy-8′-rhamnosylpyranoside to bovine serum albumin. Chin J Chem 30(2):439–444
33. Zhang G-W, Ma Y-D (2013) Mechanistic and conformational studies on the interaction of food dye amaranth with human serum albumin by multispectroscopic methods. Food Chem 136(2):442–449
34. Chi Z-X, Liu T-Y, Feng Y, Yang X-Y, Gao C-Z (2010) Binding of oxytetracycline to bovine serum albumin: spectroscopic and molecular modeling investigations. J Agric Food Chem 58(18):10262–10269
35. Ghosh K, Rathi S, Anora D (2016) Fluorescence spectral studies on interaction of fluorescent probes with Bovine Serum Albumin (BSA). J Lumin 175:135–140
36. Abert W-C, Gregory W-M, Allan G-S (1993) The binding interaction of Coomassie blue with proteins. Anal Chem 213(2):407–413
37. Yasmeen S, Riyazudddeen (2017) Biophysical insight into the binding of triprolidine hydrochloride to human serum albumin: calorimetric, spectroscopic and molecular docking approaches. J Mol Liq 233:55–63
38. Shi J-H, Chen J, Wang J, Zhu Y-Y, Wang Q (2015) Binding interaction of sorafenib with bovine serum albumin: spectroscopic methodologies and molecular docking. Spectrochim Acta A 149:630–637

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