Indian Journal of Advances in Chemical Science

Article

Interaction Study between Tiemonium Methylsulfate and Bovine Serum Albumin: Spectroscopic and Molecular Modeling Methods

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ABSTRACT

The interaction of tiemonium methylsulfate (TMS), an antispasmodic drug with bovine serum albumin (s), has been studied. The study has been investigated by fluorescence, ultraviolet–visible absorbance, viscometry, and molecular modeling techniques. The binding constants between TMS and bovine serum albumin (BSA) were calculated based on fluorescence quenching data at different temperatures. The negative ΔG° implied that the binding process was spontaneous and positive ΔH° and ΔS° suggested that hydrogen bonding force most likely played a major role in the binding of TMS to BSA. Moreover, the results obtained from molecular docking corroborate the experimental results obtained from spectroscopic investigations.

Key words: Tiemonium methylsulfate, Bovine serum albumin, Spectroscopic and molecular modeling studies.

1. INTRODUCTION

Tiemonium methylsulfate (TMS) (Figure 1) is chemically described as 4-(3-hydroxy-3-phenyl-3-(2-thienyl) propyl)-4-methyl morpholinium methylsulfate (salt). It is a quaternary ammonium antimuscarinic agent with peripheral effect similar to those of atropine and is used in the relief of visceral spasms [1]. It prevents the effects of acetylcholine by blocking its binding to muscarinic cholinergic receptors at neuroeffector sites on smooth muscle of gastrointestinal tract [2].

Serum albumin is the major protein constituent of blood plasma which facilitates the disposition and transportation of various exogenous and endogenous ligands to the specific targets [3]. Bovine serum albumin (BSA) is one of the serum albumins, and it has been widely investigated because of its structural homology with human serum albumin [4]. BSA has applied extensively in the fields of molecular biology and cell biology for its physicochemical properties was well characterized. Being the major binding protein for drugs and other physiological substances, BSA is considered as a model protein for studying protein-drug interaction *in vitro*. Thus, BSA has been studied extensively in the past years.

The study of interactions between proteins and drugs has become the hot spot in the field of life science, chemistry, and clinical medicine in recent years because most drugs undergo a greater or lesser extent of reversible binding to plasma proteins and blood cells [5-7]. The most important property of BSA is that it serves as a depot protein and transport protein for many drugs and other small bioactive molecules. Investigating on the interactions of TMS with BSA can elucidate whether TMS can transfer in the blood system and provide useful information for clinical medicine.

The literature survey reveals one ultraviolet (UV) spectrophotometric [8] and one high-performance liquid chromatography method [9] for the estimation of TMS in bulk and tablet dosage forms reported till to date but so far, the studies on the interactions between TMS and DNA or BSA have not been reported in the literature. Since, the drug is not included in any official pharmacopeia, that is, INN, till date. Thus, contribution to deeper insight into the study of interaction of TMS, antispasmodic with BSA is important for a better understanding of its therapeutic efficiency. In the present study, we investigated the interactions of TMS with BSA by UV–visible, fluorescence spectroscopy, viscometric and molecular modeling methods. Fluorescence and UV–visible spectroscopy employed to understand the quenching mechanism of TMS-BSA binding. The binding constants and number of binding sites are calculated. The results obtained from the viscosity experiments and molecular modeling validated those conclusions. We hope the results obtained in this work will provide some additional useful information for the evaluation of the safety performance of TMS through understanding their interaction with BSA.

2. EXPERIMENTAL

2.1. Chemicals and Reagents

Pharmaceutical grade of TMS INN was gifted by Global Calcium Ltd., Hosur, Tamil Nadu state, India, and certified to contain 99.9% w/w of TMS. It was used without further purification. BSA (>96%, essentially fatty acid free, lyophilized powder) was purchased from Sigma (India). All other chemicals were of analytical grade and used without further purification.

2.2. Apparatus

The UV absorption spectra were recorded on a double beam Elico UV–visible spectrophotometer equipped with a PC for data processing and using a 1.0 cm quartz cells with 0.2 nm path length. The range of wavelength was from 200 to 700 nm. Fluorescence spectroscopy was

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ISSN NO: 2320-0898 (p); 2320-0928 (e)
DOI: 10.22607/IJACS.2021.903004

Received: 26th March 2021;
Revised: 30th April 2021;
Accepted: 05th May 2021

executed using Hitachi F2700 spectrofluorimeter (JAPAN) equipped with a 1 cm quartz cell and a 150 W Xenon lamp. The widths of excitation and emission slits were set at 5.0 and 10 nm, respectively. A thermostatic bath was used to study the quenching mechanism at different temperatures.

The molecular docking studies of BSA with compounds were achieved using Autodock Vina, developed at the Scripps research institute (http://vina.scripps.edu) [10]. The input files for AutoDock Vina were prepared with AutoDock Tools (ADT), which is a Graphical User Interface for AutoDock and AutoDock Vina.

2.3. Protein Preparation

The crystal structure of BSA (3V03) was retrieved from Protein Data Bank and the ligand binding site location was analyzed by Q-Site Finder [11]. The cocrystallized ligand was removed. Using ADT, the water molecules were removed from the protein and polar hydrogen was added followed by adding Kollman charges. The Grid box has been set according to the binding site on protein and saved as pdbqt format, which was required by AutoDock Vina.

2.4. Ligand Preparation

The 2D structure of ligand were drawn by ChemSketch and converted to PDB format, required by ADT by Open Babel [12]. The rotatable bonds were selected within the ligand using Choose Torsions option in ADT and saved in pdbqt format. The Lamarckian Genetic Algorithm, which is a novel and robust automated docking method available in AutoDock [13], was employed.

3. RESULTS AND DISCUSSION

3.1. Fluorescence Studies

A strong fluorescence emission spectrum of TMS at 348 nm was observed in the range of 250–550 nm after excitation at 235 nm. The fluorescence emission spectra of TMS increased with increasing concentration of BSA (Figure 2).

The fluorescence intensity increases due to increase in the molecular planarity of the complex and decreases the collision frequency of solvent molecules with TMS. This is due to diffusion which occurs between adjacent base pairs of BSA. An enhancement in the fluorescence intensity was observed with the increasing of BSA concentration, but not altering the emission maximum and shape of the peaks. These results show that there is the binding between TMS and BSA.

The quenching nature of TMS in the presence of BSA was analyzed using Stern-Volmer equation [14],

$$F_0/F = 1 + k_q \tau_o [Q] = 1 + K_{sv} [Q] \quad (1)$$

In this equation, F_0 and F are the fluorescence intensities of TMS in the absence and presence of BSA, respectively, [Q] is the TMS concentration, k_q is the quenching rate constant, τ_o is the average excited-state lifetime of BSA in the absence of TMS (10^{-9}–10^{-7}s) [15], and K_{sv} is the Stern-Volmer quenching constant.

The Stern-Volmer plot at 25°C is shown in Figure 3a. The maximum, rate constant of collisional quenching of various quenchers with biopolymers is about 2.0×10^{10} L s^{-1} mol^{-1} [16]. From the slope of the line in Figure 3a, a greater value for k_q is obtained (K_{sv} and k_q values calculated were 5.611×10^5 L mol^{-1} and 5.682×10^{13} L mol^{-1} s^{-1} (R^2 = 0.9988), respectively, which suggest that the fluorescence quenching process may be mainly controlled by static quenching mechanism rather than dynamic).

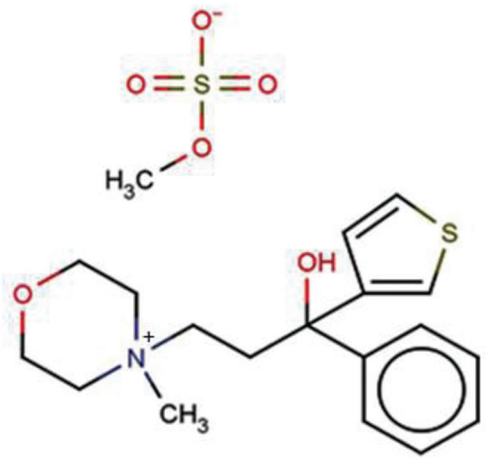

Figure 1: Chemical structure of tiemonium methylsulfate.

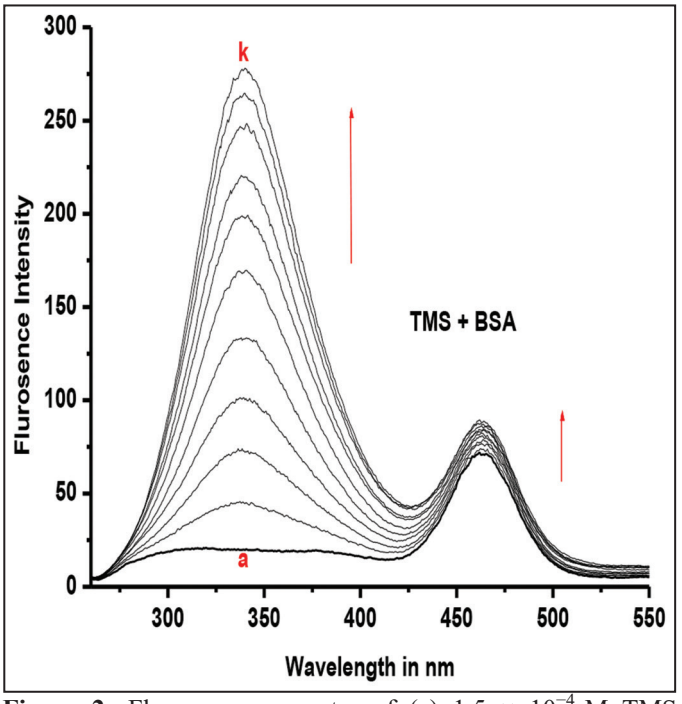

Figure 2: Fluorescence spectra of (a) 1.5×10^{-4} M TMS in presence of C_{BSA} = 0.0, 5.0, 10.0, 15.0, 20.0 25.0, 30.0, 35.0 μM L^{-1} (a to k).

The binding constant (K_b) and the number of binding sites (n) for TMS-BSA system can be determined by the following equation [17].

$$\log (F_0 - F)/F = \log K_b + n \log [Q] \quad (2)$$

Where, K_b and n are the binding constant and the number of binding sites in base pair unit, respectively. Thus, a plot of log (F_0 - F)/F] versus log [Q] (Figure 3b) yields the K_b and n values which are summarized in Table 1. An average value of one binding site on BSA for interaction with TMS was obtained from the slope of the regression line in Figure 3b.

3.2. Thermodynamic Parameters and the Nature of Binding Forces

There are several acting forces between small molecules and a biomolecules such as hydrophobic forces, hydrogen bonds, van der Waals, and electrostatic interactions. When the change in temperature is small, the enthalpy change (ΔH°) can be assumed as a constant, and

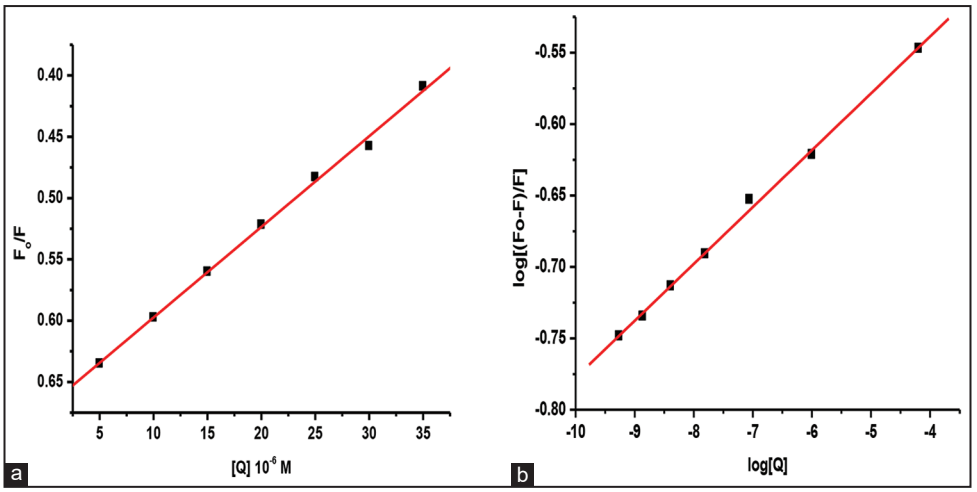

Figure 3: (a) Stern-Volmer plot of (F_0/F) versus [Q] for tiemonium methylsulfate-bovine serum albumin (TMS-BSA) system. (b) Plot of log [(F_0-F)/F] versus log [Q] for TMS-BSA system.

Table 1: Thermodynamic parameter (K, ΔG°, ΔH°, and ΔS°) and number of binding constant of BSA-TMS using spectrofluorimetric measurements

Temperature in K	Binding constant (K_b)	ΔH° (Jmol^{-1})	ΔG° (Jmol^{-1})	ΔS° (Jmol^{-1}K^{-1})	R^2
287	1.1025×10^6	21.077×10^3	-1.805×10^3	137.38	0.99873
297	3.5253×10^6		-10.922×10^3	193.32	0.99781
307	2.2528×10^6		-8.752×10^3	167.39	0.99793

then, its value and that of entropy change (ΔS°) can be determined from the van't Hoff equation.

$$\ln K_b = - (\Delta H°/RT) + (\Delta S°/R) \quad (3)$$

$$\Delta G° = \Delta H° - T\,\Delta S° = RT \ln K_b \quad (4)$$

Where, K_b is the binding constant at the corresponding temperature and R is the universal gas constant. The temperatures used were 278, 298, and 310 K. The values of ΔH° and ΔS° were obtained from the slope and intercept of the linear plot of ln K_b versus 1/T. The free energy change (ΔG°) was estimated from Equation (4). The values of ΔH°, ΔS°, and ΔG° are listed in Table 1.

From Table 1, the negative value of ΔG° reveals that the interaction process between TMS and BSA is spontaneous, while the positive sign of ΔH° and ΔS° associated with the interaction of TMS complex with BSA indicates that the binding is mainly entropy driven and the enthalpy is unfavorable and hydrophobic interaction plays a major role on the binding [18,19].

3.3. UV–Visible Spectroscopy

When a small molecule interacts with BSA and forms a new complex, a shift in the absorbance wavelength and/or a change in the molar absorptivity may occur [20]. UV–Vis absorption spectra of TMS with various amounts of BSA (Figure 4a) were recorded. It is clear that the absorption peak around 235 nm increased with the addition of the BSA, which indicates the interaction between two species.

The value of the binding constant (K_b) was obtained from the TMS absorption at 235 nm according to the following equation for weak binding affinities [21].

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \times \frac{1}{K[BSA]}$$

Where, A_0 and A are the absorbance of TMS at 235 nm in the absence and presence of BSA, ε_G and ε_{H-G} are the absorption coefficients of TMS and its complex with BSA, respectively. The plot of $A_0/(A-A_0)$ versus 1/[BSA] (Figure 4b) confirms the 1:1 stoichiometry (as was obtained in fluorescence experiments) and the binding constant (K_b) can be estimated from the ratio of intercept to slope [22]. The binding constant for TMS-BSA complex was calculated as 3.5253×10^6 M^{-1} at 25°C.

3.4. Viscosity Measurements

Spectrophotometric and spectrofluorometric methods can provide necessary but not sufficient clues to support binding modes, whereas hydrodynamic measurements which are sensitive to the length change are regarded as the most critical tests of a binding model in solution. Thus, to clarify the interaction between TMS and BSA, we carried out viscosity measurements. A classical intercalation mode is known to cause a significant increase in the viscosity of a BSA solution due to an increase in lengthening the BSA, while a non-classical intercalation or a groove mode would reduce the BSA viscosity [23]. The viscosity measurements were taken by varying the concentration ratio of BSA and each TMS. The values of relative specific viscosity $(\eta/\eta_0)^{1/3}$ versus (BSA)/(TMS) were plotted in the absence and presence of TMS (Figure 5). As it was observed from Figure 5, the relative specific viscosity of BSA exhibited a dependence on the concentration of TMS, which decreased with the value of (BSA)/(TMS), indicating non-classical intercalation mode of binding that may be realized through hydrophobic interaction between the TMS and BSA.

3.5. Molecular Modeling Study

Molecular modeling has been employed to study the interaction between TMS and BSA. The TMS was docked to BSA to determine the preferred binding site and binding mode. The best confirmation

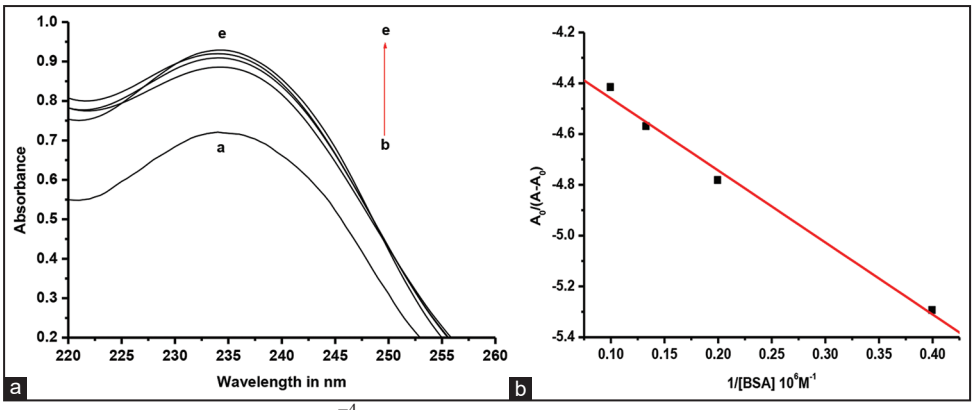

Figure 4: (a): Ultraviolet–visible spectra of 1×10^{-4} M tiemonium methylsulfate in presence of C_{BSA}= 0.0, 2.5, 5.0, 7.5, 10.0 µM L^{-1}. **(b):** Plot of $(A_0/(A–A_0)$ versus 1/(bovine serum albumin).

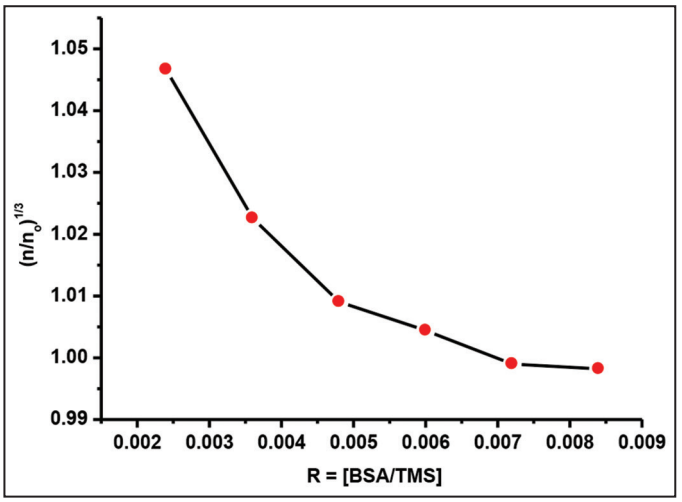

Figure 5: Effect of increasing concentration of bovine serum albumin on the relative viscosity of tiemonium methylsulfate.

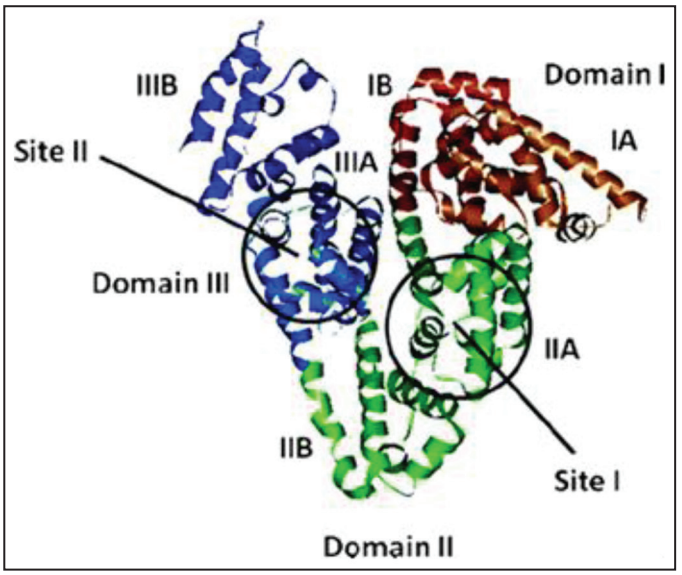

Figure 6: The crystallographic structure of bovine serum albumin.

was determined based on binding affinity and RMSD. In general, the first structure would have the lowest binding energy and RMSD (generally, should be <2A°) recommended by AutoDock Vina. The 3-D structure of crystalline albumin has revealed that BSA is made up of three homologous domains (I, II, and III): I (residues 1–183), II (184–376), and III (377–583), each containing two subdomains (A and B) that assemble to form heart-shaped molecule, Figure 6. The most important regions of ligand binding to BSA are located in hydrophobic cavity in subdomains IIA and IIIA, which is consistent with sites I and II, respectively [24-26].

The BSA was docked with TMS which shows nine best confirmations have the first lower binding energy, so this binding energy conformation was selected and analyzed as the best binding mode. The binding energy is located around −9.6–−10.5 kcal/mol and the best energy ranked results of the binding mode between TMS and BSA are shown in Figure 7.

The molecular docking of TMS and only amino acids residues surrounded TMS was clear. It is shown from Figure 7, TMS molecules in the subdomain IIA and IIIA cavity of BSA, in which there was a large hydrophobic region which can hold many drugs. The number of hydrogen bond represented in dotted lines. The presence of hydrogen bonds possibly enhanced the hydrophobicity of TMS-BSA system, making TMS-BSA system be a stable state. The whole molecule of TMS embedded into a hydrophobic cavity formed by TRP-212

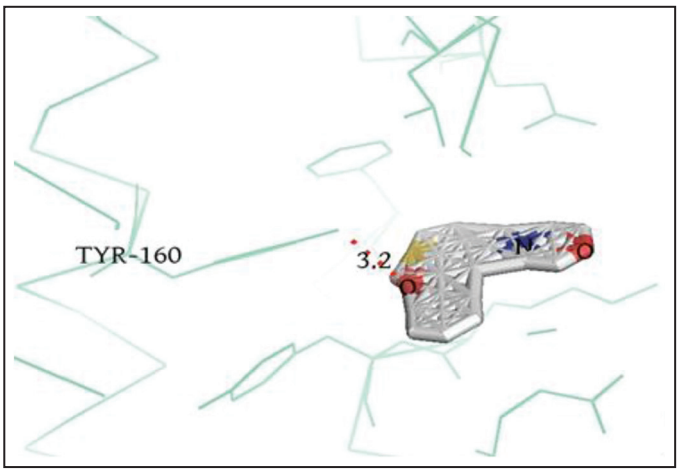

Figure 7: Molecular modeling of tiemonium methylsulfate and bovine serum albumin.

residue, LEU-196, LEU-345, LEU-451 residues, arginine (ARG-193, ARG-197, ARG-482, and ARG-483) residues, Tyrosine (THR-450), and other amino acid residues. The one hydrogen bond established between the TMS and the amino acid residues of BSA; they are –O-H

and TYR-160 with a bond length of 3.2°A. Thus, the binding forces to keep TMS-BSA system stable were conjectured predominantly hydrophobic interaction as well as hydrogen bonds. The energy of −10.922 kcal/mol was obtained for TMS-BSA complex.

4. CONCLUSIONS

In this paper, the interaction of TMS with BSA was studied by UV–Vis, fluorescence spectroscopy, viscometry, and molecular modeling techniques for the 1st time. Moreover, the large binding constant indicates that TMS has a high affinity with BSA. This work should be helpful for understanding the interaction of TMS with BSA and designing new antispasmodic drugs.

5. ACKNOWLEDGMENTS

The author thanks University Grants Commission, New Delhi, India, for financial support for this work (F. No. 42-308/2013 (SR) dated 28 March 2013). The author was grateful for Maharani's Science College for Women, Bengaluru-01 (*DST-FIST, STAR-DBT,* and *UGC-CPE* Facilities). Thanks are also due to Global Calcium Ltd., Hosur, Tamil Nadu state, India, for providing tiemonium methylsulfate as a gift sample.

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