Original Research Article

In-Vitro Fluorescence Spectroscopic Analysis of the Interaction of Glimepiride with Bovine Serum Albumin (BSA)

Abstract

Background: The significant study was made to investigate the interaction of an antidiabetic drug, glimepiride with bovine serum albumin (BSA) by fluorescence quenching method in two different temperatures (298K and 308K).

Methods: The overall study was carried out through fluorescence spectroscopy. Stern-Volmer equation determined the fluorescence quenching constant. The various thermodynamic parameters such as free energy (ΔG), enthalpy (ΔH), and entropy (ΔS) was found out by Van't Hoff equation.

Results: The data revealed that glimepiride interact with BSA and static quenching of fluorophore BSA occurred in presence of glimepiride. Both tryptophan and tyrosine residues of BSA is responsible for interactions with glimepiride. The hydrophobic forces participated in chief roles for BSA-glimepiride complexation and this was indicated by the values of thermodynamic parameters. The binding number (n) obtained was ≈1 pointed out that glimepiride and BSA has bound with 1:1 ratio.

Conclusions: Through fluorescence spectroscopic technique we revealed the nature of interaction of glimepiride with BSA, quenching mechanism for the interaction, and associated thermodynamic parameters.

Keywords: Glimepiride, Bovine serum albumin, Fluorescence spectroscopy, Drug-protein binding, Thermodynamic parameter, quenching

1. Introduction

Serum albumins are the most plenteous proteins in blood and it interacts with drugs to form drug-protein complexes [1]. The synergy of drug-protein interaction has incredible impacts on the pharmacokinetics and the pharmacodynamics of drugs that additionally has effects on bioavailability and toxicity and as a result it is a very crucial factor and can later contribute into drug therapy and the designing of the drugs [2–5]. We chose bovine serum albumin (BSA) as the

model protein since it bears approximately 76% similarity with human serum albumin (HSA) [6]. Moreover, BSA has 88% closeness in amino acid sequencing with HSA and so the 3D structure of BSA and HSA are of a close match. Also, the availability of BSA was gotten at remarkably pure form, was of great stability and lesser cost than HSA [2, 7, 8]. The reactivity of chemical and biological systems can be measured in low concentration under physiological conditions by spectral methods and as a result are regarded as the most dominant tools [9]. In our study, fluorescence spectroscopic method has been used due to its high sensitivity, relatively ease of use and reproducibility [10-13] to inspect the interaction of glimepiride with BSA molecule by calculating the participating amino acid residues, number of binding sites, thermodynamic parameters, fluorescence quenching rate constant and their binding constant. The drug glimepiride (Figure-1) used here, is an orally available antidiabetic drug which is a medium-to-long-acting potent sulfonylurea of third generation that helps in insulin production from the pancreas [14]. It is mostly used to control blood sugar in diabetic patients [15, 16]. In consideration of finding out the appropriate binding site of the drug when the attainable interactions occur with BSA, dose adjustments required or not were identified owing to the interactions held. With a view to upgrade the use of glimepiride as a preventive and personalized medicine the study is crucial.

2. Methods

2.1 Drugs and chemicals

BSA (product number: A 5611) was brought from Sigma-Aldrich. Glimepiride were gifts from Square Pharmaceuticals Ltd., Bangladesh. Every other reagents employed in the study were of analytical grade and bought from local supplier. Entire BSA solutions were prepared in fixed buffer solution that is in p^H 7.4. In the making of the buffer solution, a combination of disodium hydrogen phosphate (Na_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) have been applied.

2.2 Instruments

Fluorescence measurements were carried out utilizing a FL-7000 spectrofluorophotometer and a 1cm quartz cell (Hitachi, Japan). To maintain varied temperatures, a thermostat bath of Unitronic Orbital (P-Spectra, Spain) was utilized.

2.3 Sample preparation

5 ml of earlier made 20 µM BSA in phosphate buffer of pH 7.4 was taken in each of the 5 test tubes. Glimepiride was added in different volumes to 4 out of 5 test tubes to have the following concentrations: (0, 20, 40, 80, 160) µM, respectively. The ratios of glimepiride and BSA ([glimepiride]/[BSA]) in glimepiride BSA system of 4 test tubes were 1:1, 2:1, 4:1, and 8:1, respectively.

2.4 Spectroscopic measurement

At two individual temperatures (298 and 308K), two excitation wavelengths of BSA (280 and 293nm) noted the fluorescence emission spectra for glimepiride-BSA setup. The emission spectra were scripted for three times for each treatment in the range of 320–460 nm for BSA with the widths of both entrance and exit slits being set to 5 nm at the same conditions.

3. Results and discussion

3.1 The interaction of glimepiride with BSA

If by the usage of proper wavelengths of light, BSA is excited, then every of its fluorophores (tryptophan, tyrosine, and phenylalanine) are capable of emitting fluorescence. When an excitation wavelength of 280 nm is utilized, the fluorescence of BSA occurs in both tryptophan and tyrosine residues, whereas, for the 293 nm wavelength just the tryptophan residue is excited [17, 18]. The fluorescence of BSA being excited at 280 and 293 nm was compared in the presence of glimepiride that determines the interactions of residues of BSA with glimepiride. The plots F/F_0 against [glimepiride]/[BSA] at the two excitation wavelengths of 280 and 293 nm were compared at 298K, respectively. Here, F_0 being the fluorescence intensity of BSA, F is the fluorescence intensity of BSA in presence of glimepiride. Figure 2 shows that the fluorescence spectrum of BSA excited at 280 nm is different from that of when excited at 293 nm. This difference of quenching displays that both tyrosine and tryptophan residues participated in the molecular interactions between glimepiride and BSA.

3.2 Effect of glimepiride on the fluorescence emission spectra of BSA

For the determination of interaction of glimepiride with BSA, the fluorescence emission spectra were measured at two excitation wavelengths; at 280 nm and 293 nm at 298 K. Figure 3 illustrates that the fluorescence of BSA eventually goes down with the rising of concentration of glimepiride, stating that there is a powerful interaction and that energy transfers between glimepiride and BSA at both excitation wavelengths of BSA at the same temperature. As for

such reason, presence of quenching of intrinsic fluorescence of BSA occurred but it showed no significant shift of the emission maximum wavelength.

3.3 Fluorescence quenching analysis

Quenching is an important phenomenon where the fluorescence intensity of a substance declines in the presence of a quencher molecule [19]. The characteristics of the drug-protein interaction can be static or dynamic relying on the type of interaction involved. A different of processes can be resulting in quenching, such as, energy transfer, collisional quenching, complex formation, and excited state reactions. The composition of a complex between the quencher and the fluorophore was generally identified to be static quenching. However, during excitation, dynamic quenching occurs when there is a collision between the quencher and fluorophore [20]. The fluorescence quenching data are generally calculated by Stern-Volmer equation [21] which is:

$F_0/F = 1 + Ksv[Q]$

where F_0 and F are the fluorescence intensities in the non attendance and attendance of a quencher, [Q] is the concentration of the quencher, and Ksv is the Stern-Volmer quenching constant which displays the strength of interaction between albumin and a quencher molecule. The dependency on the temperature is what differentiates the static quenching from the dynamic quenching [21]. Dynamic quenching counts upon diffusion, and greater temperatures outcomes in greater diffusion coefficients. Thus, the Stern-Volmer quenching constants (Ksv) are expected to rise with rising temperature. In addition to this, an increased temperature is more likely to occur when the complexes decreases its stability and therefore a lesser value of static quenching constants occured [22]. The arrangement of quenching of BSA fluorescence by glimepiride was found by estimating the value of Stern-Volmer quenching constant (Ksv) at the excitation wavelength of 280 nm for BSA at two different temperatures (298 K and 308 K). The values were calculated from the slope of the plot of F/F_0 against the concentration of glimepiride that is relied on the fluorescence data (Figure 4) at the experimental conditions. The plots displayed that inside the experimental concentrations, the results were in good compliance with the Stern-Volmer equation. The plots were found to be linear, and Stern-Volmer quenching constants were got from the slopes at two varied temperatures as presented in Table 1. The Stern-Volmer quenching constant sloped down with the rising temperature for static quenching but for dynamic quenching, the opposite effect was noted [23]. It was observed that the static quenching happened for BSA in the presence of glimepiride by rising the temperatures from 298K to 308K.

3.4 Determination of thermodynamic parameters and nature of binding forces

Various types of forces like hydrogen bonds, electrostatic interactions, hydrophobic force, and Van-der Waals interactions help with the interaction of fluorescence active substance and the quencher. The thermodynamic criterions were estimated to explain the synergy between the drug and BSA, which has been calculated from the Van't Hoff equation [24]:

$\ln K_a = -(\Delta H/RT) + (\Delta S/R)$

where ΔH is the enthalpy change, R is the universal gas constant, Ka is the constant that is the analogous to the Stern-Volmer quenching constants, Ksv at the equivalent temperature and ΔS is the entropy change. The entropy change (ΔS) and the enthalpy change (ΔH) can be resolved from the slope and intercept of the curve of lnKsv versus 1/T, respectively (Figure 5). The free energy (ΔG) can be calculated from the subsequent relationship:

$\Delta G = \Delta H - T\Delta S$

and Table 2 shows that the enthalpy change (ΔH) and the entropy change (ΔS) are positive and the free energy change (ΔG) is negative. This negative ΔG value points out that the binding of glimepiride to BSA is spontaneous. According to the views of Ross and Subramanian [25], the model of synergy between a biomolecule and a drug is mostly considered as the evidence for a hydrophobic interaction [26] since the water molecules organized in a precisely manner around the drug and protein settles for a more random configuration. Therefore, it can be known that hydrophobic forces are presenting a dominant role in glimepiride-BSA interaction in the wavelengths of 280 nm and 298 and at 308 K temperature (Table 2).

3.5 Determination of binding constant and binding points

When glimepiride binds freely to a couple of equivalent sites on BSA, the equilibrium between free and bound glimepiride is shown by the corresponding equation [27]: Log $[(F_0/F)/F]$= log Ka+ n log [Q] where Ka is the binding constant and n is the number of binding sites per BSA molecule. The values of Ka and n were found from the values of the intercept and slope of the plot of Log $[(F_0/F)/F]$ against log [Q]. Table 3 portrays that the values of n were found to be ≈1 at both excitation wavelengths of BSA at two carried temperatures. The molar ratio of the glimepiride-BSA system at 280 nm was 1:1 which indicated that 1 mol of glimepiride ties with 1 mol of BSA.

4. Conclusions

As known earlier that the pharmacological activity of a drug is connected to protein binding. Due to variance in drug-protein interactions, the activity of a drug can be greater or lesser. This study indicates that both tryptophan and tyrosine engaged in the interaction of BSA and glimepiride. It was revealed that the fluorescence quenching of BSA took place due to static quenching. Fluorescence quenching constant values were calculated by using the Stern-Volmer equation and Van't Hoff equation that provided a measure of the thermodynamic parameters like ΔG, ΔH, and ΔS. The binding process for glimepiride has been observed to be spontaneous, exothermic, and entropy driven as identified by thermodynamic analysis, and hydrophobic forces played a major role in the binding of glimepiride-BSA complex.

Competing interests

The authors declare that they have no competing interests.

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Figures

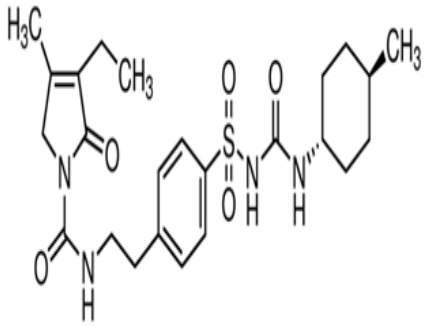


Figure 1: Chemical structure of Glimepiride

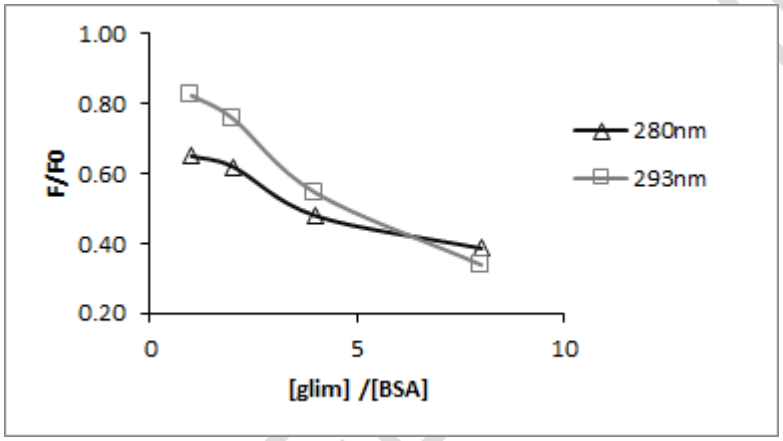
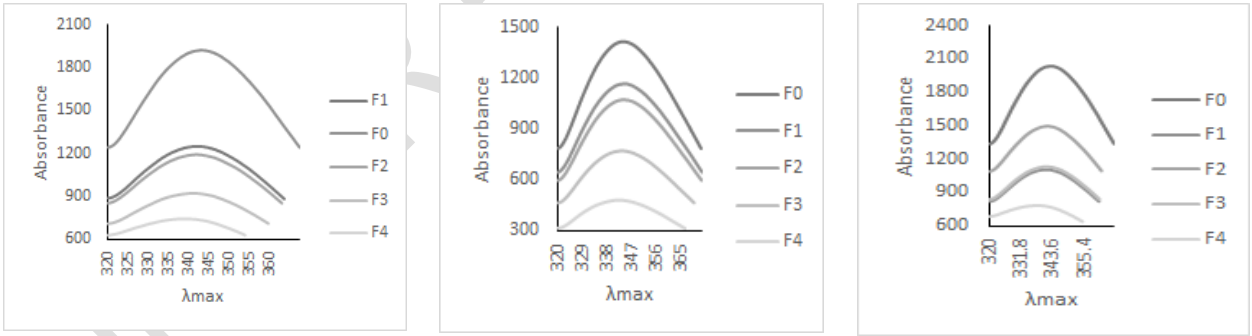


Figure 2: Fluorescence titration curve of BSA in the presence of glimepiride at the excitation wavelengths of 280 and 293 nm at 298 K



a b c

Figure 3: Fluorescence emission spectra of glimepiride-BSA system at the excitation of (a) 280 nm at 298 K, (b) 293 nm at 298 K, and (c) 280 nm at 308 K. [Concentration of BSA = 0 μM; concentrations of glimepiride 0, 20, 40, 80 and 160 μM]

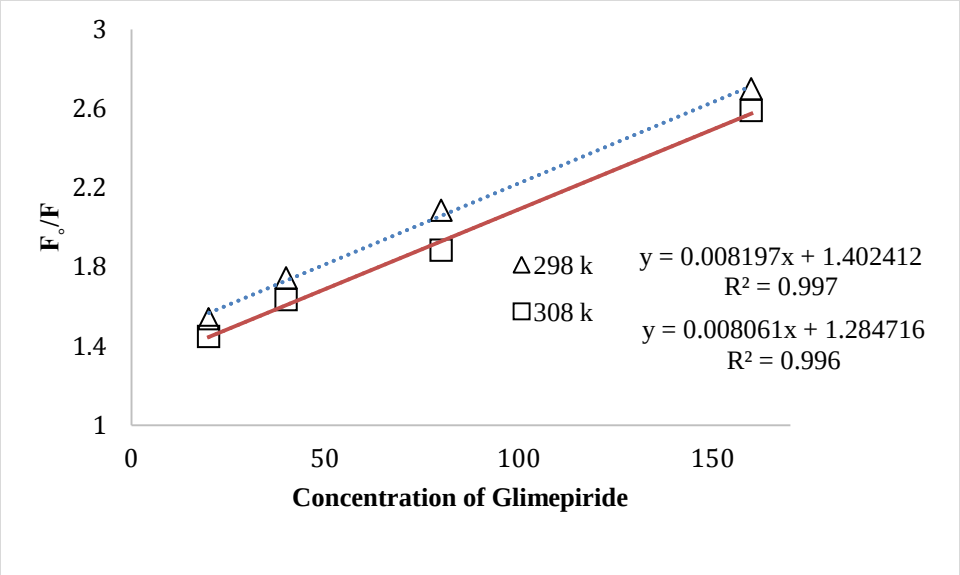


Figure 4: The Stern-Volmer plots for glimepiride-BSA system at the excitation wavelength of BSA at 280 nm at two different temperatures (298 and 308 K)

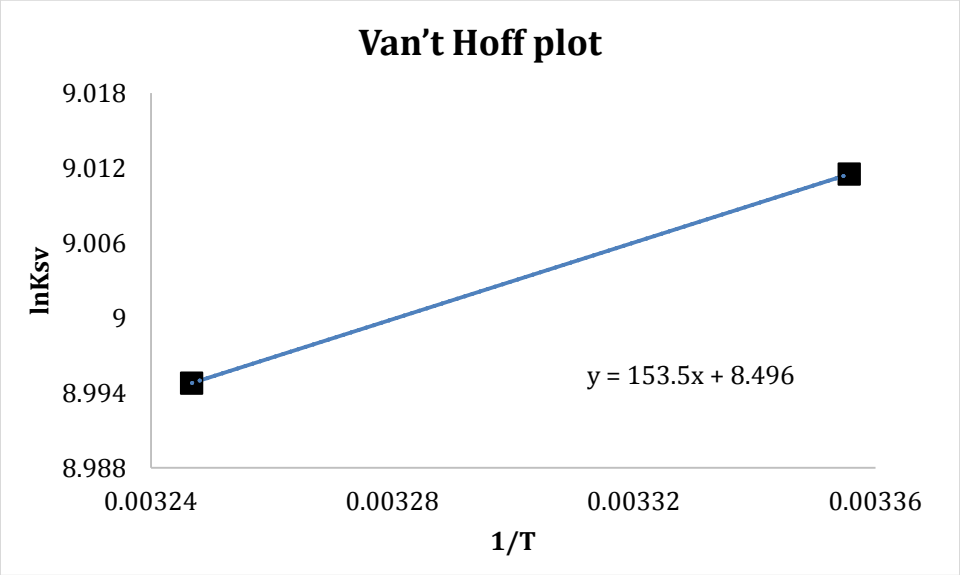


Figure 5 The Van't Hoff plot for glimepiride-BSA system at 280 nm at two different temperatures (298 and 308 K)

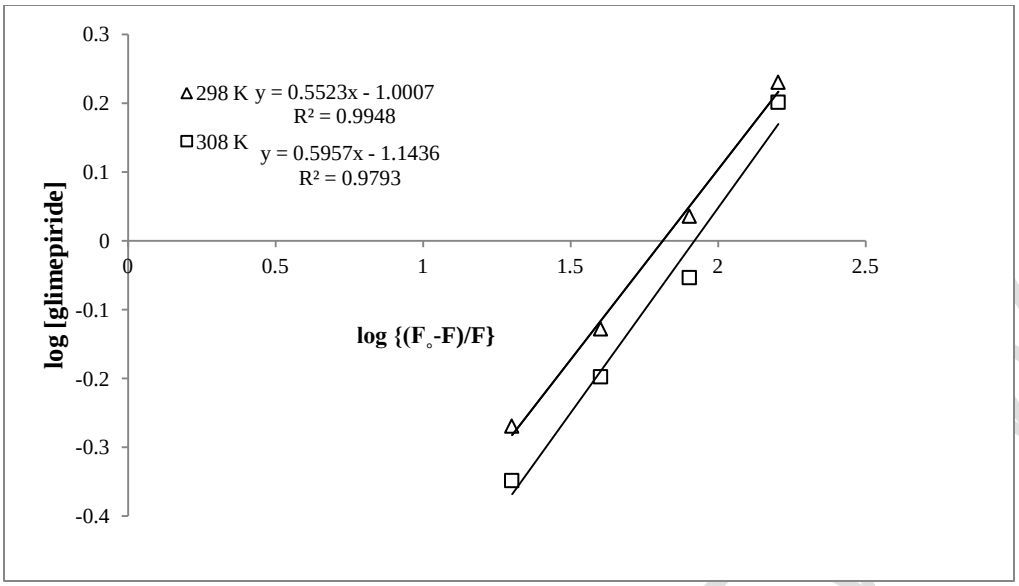


Fig. 6 Plot for binding constant and binding points for glimepiride-BSA at 280 nm at two different temperatures (298 and 308 K)

Tables

Table 1 : The Stern-Volmer quenching constant (Ksv) for glimepiride-BSA system at 280 nm at 298 and 308 K temperatures

Temperature (K)	Stern-Volmer quenching constant, K_{sv} (L mol^{-1})
298	8197
308	8061

Table 2: Thermodynamic parameters for glimepiride-BSA system at 280 nm at two different temperatures (298 and 308 K)

Temperature (K)	ΔH (KJ mol−1)	ΔS (J mol−1)	ΔG (KJ mol−1)
298	1276.70	70.59	-19757.89
308	1276.70	70.59	-20463.7

Table 3: Binding constant and binding points for BSA glimepiride system at 280 nm excitation wavelength of BSA at two different temperatures

Temperature (K)	Number
298	0.55
308	0.59