

# Fluorescence Spectroscopic Studies of *in vitro* Interactions of Famotidine and Tapentadol Hydrochloride with Bovine Serum Albumin

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**ABSTRACT:** The *in vitro* interactions of Famotidine (FT) and Tapentadol hydrochloride (TAP) with bovine serum albumin (BSA) have been studied by fluorescence emission spectroscopy under different conditions. Quenching constants were determined using the Stern-Volmer equation. Two moles FT bound with 1 mole of BSA at 298 K and 3 mole FT bound with 1 mole of BSA at 308 K in presence of TAP. BSA was used for the study as it shows approximately 76% sequence homology to human serum albumin (HSA).

**Key words:** Famotidine, Tapentadol Hydrochloride, *in vitro*, bovine serum albumin, fluorescence, spectroscopy

## INTRODUCTION

Serum albumin is the principal transporter of fatty acids that are otherwise insoluble in circulating plasma. The three dimensional structure of bovine serum albumin (BSA) and the crystal structure of human serum albumin (HSA) has 76% sequence homology.<sup>1</sup> Famotidine (FT) is a histamine H<sub>2</sub>-receptor antagonist. It is known to prevent NSAID-induced UGI ulceration by reducing gastric acid secretion. FT inhibits H<sub>2</sub> receptors, thus reducing basal, nocturnal, and stimulated gastric acid secretion. Through inhibition of H<sub>2</sub> receptors present on parietal cells in the stomach FT prevents ulceration by reducing gastric acid secretion.<sup>2</sup> Tapentadol (TAP) is a centrally acting analgesic. It has an improved side effect profile when compared to

opioids and nonsteroidal anti-inflammatory drugs. Its dual mechanism of action makes it a useful analgesic to treat acute, chronic, and neuropathic pain.<sup>3</sup> Frequently both of these drugs are used concomitantly. Therefore, it has a chance to interact with each other or influence the binding with serum protein by each other.

Spectral methods are powerful and effective tool for studying the reactivity of chemicals and biological systems because it allows nonintrusive measurements of substances in low concentration under physiological conditions. There are several studies of albumin induced by drugs or other bioactive small molecules using spectral methods.<sup>4-7</sup> The fluorescence quenching data are usually analyzed by Stern-Volmer equation<sup>8</sup>:

$$F_0/F = 1 + K_{SV} [Q]$$

Here, F<sub>0</sub> and F are the fluorescence intensities in the absence and presence of quencher, respectively. The quencher concentration is [Q]. K<sub>SV</sub> is the Stern-

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Volmer quenching constant, indicates the strength of interaction between the drug and BSA. It is the slope of the plot of  $F_0/F$  against concentration of drug based on the fluorescence data at different temperatures.  $K_{sv}$  decreases with increasing temperature for static quenching while for dynamic quenching the reverse effect is observed.<sup>8</sup> Thermodynamic parameters and nature of the binding forces:

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

Here,  $\Delta S$  is the entropy change; constants  $K_a$  are analogous to the Stern-Volmer quenching constants  $K_{SV}$  at the corresponding temperature,  $R$  is the gas constant. The enthalpy ( $\Delta H$ ) and entropy ( $\Delta S$ ) changes can be determined from the slope and intercept of the fitted curve of  $\ln K_{SV}$  against  $1/T$ , respectively. The free energy ( $\Delta G$ ) can be estimated from the following relationship:

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

For reversible drug-protein binding, binding forces like hydrogen bonds, vander Waals forces, hydrophobic forces and electrostatic forces are involved.

**Binding constant and binding points.** When small molecule binds independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecule is given by the following equation:

$$\log [(F_0 - F)/F] = \log K + n \log [Q]$$

Where,  $K$  and  $n$  are the binding constant to a site and the number of binding per albumin, respectively. The values of  $K$  and  $n$  are calculated from the values of intercept and slope of the plot of  $\log [(F_0 - F)/F]$  versus  $\log [drug]$ , respectively.

In the present work, we studied the *in vitro* interactions between Famotidine (FT) and Tapentadol hydrochloride (TAP) with BSA by using fluorescence emission spectroscopy.<sup>9-11</sup>

## MATERIALS AND METHODS

All chemicals and reagents were of analytical grade and double distilled water was used throughout the study. The solutions of drug and BSA were prepared in phosphate buffer of pH 7.4 containing 0.01 M  $Na_2HPO_4$  (Active Fine Chemicals Ltd., Bangladesh) and 0.02M  $KH_2PO_4$ . Phosphate buffer of pH 7.4 was prepared. All fluorescence spectra were recorded on F-7000 spectrophotometer (Hitachi, Japan) equipped with 1.0 cm quartz cell. For different temperatures a thermostatic water bath (Unitronic Orbital, P Spectra, Spain) was used.

**Sample preparation and spectroscopic measurement.** Five mL of 20  $\mu M$  BSA solution, previously prepared in phosphate buffer of pH 7.4 was taken in each of the eight test tubes. FT was added in different volumes to seven out of eight test tubes to have the following concentrations: 20  $\mu M$ , 40  $\mu M$ , 80  $\mu M$ , 120  $\mu M$ , 160  $\mu M$ , 240  $\mu M$  and 320  $\mu M$ . The ratio of FT and BSA was ( $[FT]/[BSA]$ ) = 1, 2, 4, 6, 8, 12, 16) in the seven test tubes. The eighth test tube contained BSA solution only at pH 7.4. Phosphate buffer was marked as "control". BSA and the BSA-FT systems were stirred for 2 min and kept for 25 min at 298 K and 308 K, respectively. In case of BSA-(FT+TAP), the concentration of TAP was remained constant. The parameters of the fluorescence spectrophotometer were set properly. Then estimation of the fluorescence intensity of the system at 298 K and 308 K and at the excitation wavelength of 280 nm and 293 nm were done, respectively. The width of both entrance and exit slit were set to 5 nm. These emission spectra for all systems were recorded in the range of 320-460 nm for BSA at same experimental conditions.

## RESULTS AND DISCUSSION

In order to determine whether both tryptophan and tyrosine residues of BSA are involved in interaction with FT, the fluorescence of BSA excited at 280 nm and 293 nm in the presence of FT were compared. When 280 nm excitation wave length was used, fluorescence of albumin comes from both

tryptophan and tyrosine residues, whereas 293 nm wavelength only excites tryptophan-residues.<sup>12</sup>

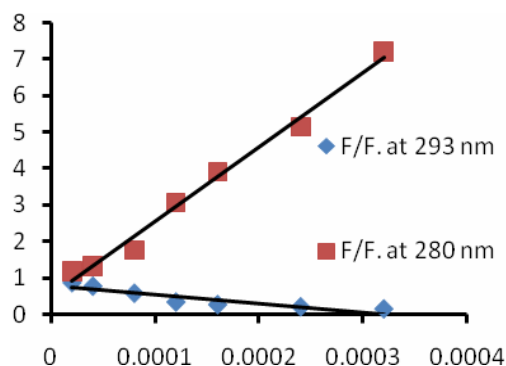


Figure 1. Fluorescence titration curve of BSA in the presence of FT at  $\lambda_{\text{max}} = 280$  and 293 nm.

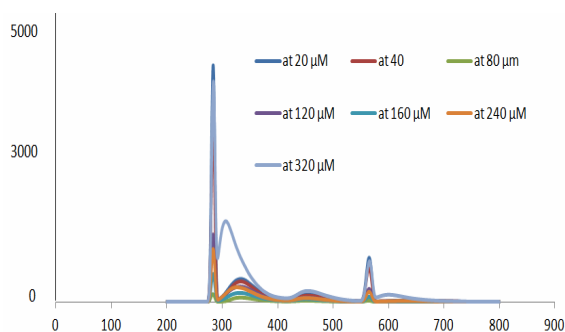


Figure 2. Fluorescence emission spectra of BSA in the presence of different concentration of FT ( $\lambda_{\text{max}} = 280$  nm,  $T = 298$  K).

The plots against  $[FT]/[BSA]$  (Figure 1) indicates that the fluorescence of BSA excited at 280 nm obviously differs from that excited at 293 nm in the presence of FT. This significant difference between quenching of serum albumin fluorescence shows that the both tyrosine and tryptophan residues participate in the molecular interactions between BSA and FT. Figure 2 shows the quenching of fluorescence of BSA. This indicates the strong interaction and energy transfer between FT and BSA. Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interaction with quencher molecule.

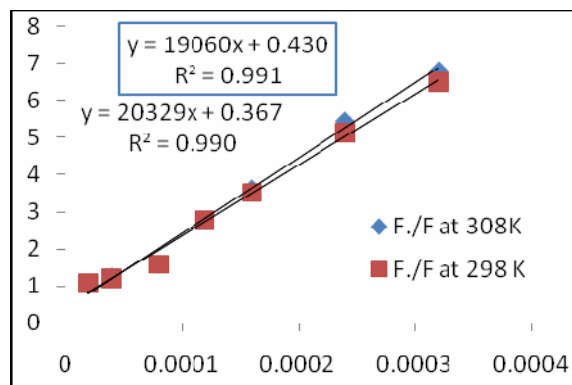


Figure 3. The Stern-Volmer plots for the quenching of BSA by FT at two different temperatures.

**Table 1.** Stern- Volmer quenching constant  $K_{SV}$  of the system of FT- BSA 280 nm ( $R =$  Correlation co-efficient).

T (K)	1/T	$K_{sv}$ ( $\times 10^3$ L mol <sup>-1</sup> )	$\ln K_{sv}$
298	0.00336	19.06	9.8553
308	0.00324	20.329	9.9198

From table 1 it is clear that the probable quenching mechanism of the FT-BSA binding reaction is due to dynamic quenching.

The curves for the interactions of FT on the fluorescence emission spectra of BSA in presence of TAP showed in below:

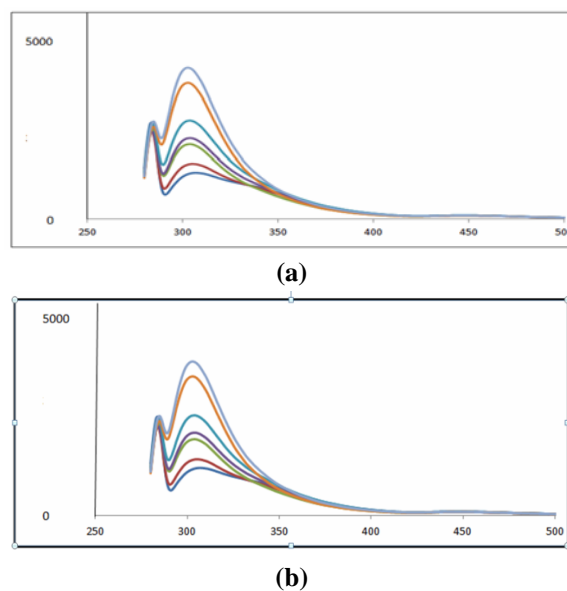


Figure 4. Fluorescence emission spectra of BSA-(FT+TAP) system at 280 nm at 298 K (a) and 308 K (b).

Figure 4 shows the fluorescence emission spectra of BSA with varying concentrations of FT in presence of TAP at 280 nm at 298K (Figure 4a) at 280 nm at 308 K (Figure 4b). It indicated that in presence of TAP there was a strong interaction and energy transfer between FT and BSA in both excitation wavelength ( $\lambda_{Ex_{max}}$  of BSA = 280 nm ) at two different temperatures. So it was found that the respective fluorescence intensity of BSA by increasing concentrations of FT in presence of TAP decreased in BSA-(FT+TAP) system.

**Determination of  $K_{sv}$  for BSA-(FT+TAP) system at 293 nm.** The following plots were drawn from the quenching of the interactions.

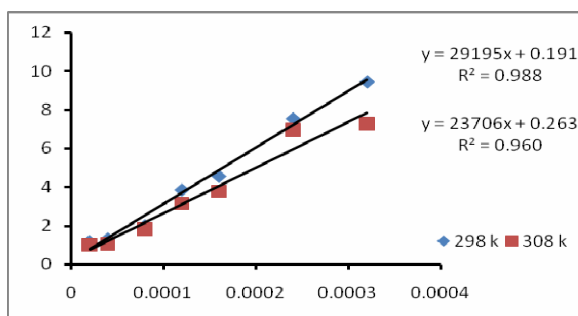


Figure 5. The Stern-Volmer plots for BSA-(FT+TAP) system at 293 nm at 298 and 308 K.

**Table 2. The Stern-Volmer quenching constant ( $K_{sv}$ ) for BSA-(FT+TAP) system at 293 nm at two different temperatures.**

T(K)	1/T	$K_{sv}(x 10^3 Lmol^{-1})$	$\ln K_{sv}$
298	0.00336	29.195	10.28175
308	0.00324	23.706	10.07348

It is evident from table 2 that the probable quenching mechanism for the FT-BSA binding in presence of TAP at 293 nm was due to static quenching. It has been seen that Stern-Volmer constant increased in BSA-(FT+TAP) system than BSA-FT system at 293 nm at both temperatures. It indicated that the quenching of BSA by FT in BSA-(FT+TAP) system was stronger than the quenching in BSA-FT system at 293 nm due to the presence of TAP. So, the quenching of BSA by FT was

increased in BSA-(FT+TAP) system at 293 nm due to presence of TAP.

**Table 3. Thermodynamic parameters for BSA-(FT+TAP) system at 293 nm at two different temperatures (298 and 308 K).**

T (K)	$\Delta H(KJ/mol)$	$\Delta S (J/mol)$	$\Delta G(KJ/mol)$
298	-15.8962	32.16	-25.4799
308	-15.8962	32.16	-25.8015

Table 3 indicates that the presence hydrophobic interaction and the possibility of hydrogen bonding was observed. The binding process was spontaneous. It was observed that the value of  $\Delta H$  and  $\Delta G$  for BSA-(FT+TAP) system increased and value of  $\Delta S$  decreased at 293 nm at both temperatures than BSA-FT system alone. It indicated that both stability and spontaneity of interaction of BSA with drugs in BSA-(FT+TAP) system at 293 nm increased with decreasing randomness (entropy) due to presence of TAP.

**Table 4. Binding constant and binding sites for BSA-(FT+TAP) system at 293 nm at two different temperatures.**

T (K)	K (Lmol-1)	log K	n
298	1.444X106	6.1598	1.4839
308	1.389X1011	11.143	2.8392

Table 4 indicates that the binding constant increased with the increase in temperature of BSA-(FT+TAP) system at 293 nm resulting in the increase of stability of the complex. The values of n were found to be  $\approx 2, 3$  respectively at temperature 298 K and 308K. The molar ratio of the BSA-(FT+TAP) system at 293 nm was 1:2 and 1:3, respectively at temperature 298 K and 308K indicated that 2 mole FT bound with 1 mole of BSA at 298 K and 3 mole FT bound with 1 mole of BSA at 308 K in presence of TAP. It was observed that the binding constant in BSA-(FT+TAP) system at 293 nm decreased (at 298 K) from BSA-FT system alone. It indicated that due to presence of TAP in BSA-(FT+TAP) system, the binding of BSA with drugs decreased at 293 nm. The binding constant in BSA-(FT+TAP) system at 293

nm increased (at 308 K) from BSA-FT system alone. It indicated that due to presence of TAP in BSA-(FT+TAP) system, the binding of BSA with drugs increased at 293 nm.

**Determination of K<sub>sv</sub> for BSA-(FT+TAP) system at 280 nm:** The Stern-Volmer constant (K<sub>sv</sub>) for BSA-(FT+TAP) system at 280 nm at two different temperatures were calculated from the slope of the plot of Fo/F versus concentration of FT in presence of TAP based on the fluorescence data at different temperatures.

**Table 5. The Stern-Volmer quenching constant (K<sub>sv</sub>) for BSA-(FT+TAP) system at 280 nm at two different temperatures.**

T(K)	1/T	K <sub>sv</sub> (X10 <sup>3</sup> Lmol <sup>-1</sup> )	lnK <sub>sv</sub>
298	0.00336	28.778	10.2674
308	0.00324	28.263	10.2493

From table 5 it is clear that the probable quenching mechanism was static. The quenching of BSA by FT was increased in BSA-(FT+TAP) system at 280 nm due to presence of TAP.

**Thermodynamic parameters and nature of the binding forces for BSA-(FT+TAP) system at 280 nm at two different temperatures.** The thermodynamic parameters and nature of the binding forces for BSA-(FT+TAP) system at 280 nm at two different temperatures are showed in the following table:

**Table 6. Thermodynamic parameters for BSA-(FT+TAP) system at 280 nm at two different temperatures (298 K and 308 K).**

T (K)	ΔH (KJ/mol)	ΔS (J/mol)	ΔG (KJ/mol)
298	-19.07234	23.31	-26.0187
308	-19.07234	23.31	-26.2518

Table 6 indicated that the hydrophobic interaction and hydrogen bonds were present. The binding process was spontaneous.

**Table 7. Binding constant and binding sites for BSA-(FT+TAP) system at 280 nm at two different temperatures (298 K and 308 K).**

T (K)	K (Lmol <sup>-1</sup> )	logK	n
298	1.2434X106	6.0946	1.4682
308	2.7555X106	6.4402	1.5569

From table 7, the value of n was found to be ≈2 at both temperature 298 K and 308 K. The molar ratio of the BSA-(FT+TAP) system at 280 nm was 1:2 at temperature 298 K and 308 K indicated that 2 mole FT bound with 1 mole of BSA at 298 K and 308 K in presence of TAP. It was observed that the binding constant in BSA-(FT+TAP) system at 280 nm increased (at 298 K and 308 K) from BSA-FT system alone. It indicated that due to presence of TAP in BSA-(FT+TAP) system, the binding of BSA with drugs increased at 280 nm.

## CONCLUSIONS

Both tryptophan and tyrosine residues of BSA participated in the interactions with FT at the excitation wavelength of 280 nm. FT-BSA binding reaction was due to dynamic quenching and this strong interaction was observed. The free energy change (ΔG) was negative, the enthalpy change (ΔH) was positive and the entropy (ΔS) was positive. Hydrophobic interaction was found, possibility of hydrogen bonding was rare and the binding process was spontaneous. The BSA-FT molar ratio of the system was 1: 2 at both 298 K and 308 K. That means 1 mole of BSA bound with 2 mol of FT. In case of FT with BSA at 293 nm, FT- BSA binding reaction was due to dynamic quenching. Strong interaction was observed. The free energy change (ΔG) and the enthalpy change (ΔH) were negative and the entropy (ΔS) was positive. Hydrophobic interaction was found, possibility of hydrogen bonding was found and the binding process was spontaneous. The BSA-FT molar ratio of the system was 1: 4 at both 298 K & 308 K. That means 1 mole of BSA bind with 4 mole of FT. In case of FT with BSA in the presence of TAP at 293 nm, FT-BSA binding in presence of TAP at 293 nm was due to

dynamic quenching. The free energy change ( $\Delta G$ ) and the enthalpy change ( $\Delta H$ ) were negative and the entropy ( $\Delta S$ ) was positive. Hydrophobic interaction was found, the possibility of hydrogen bonding was observed. The binding process was spontaneous. The stability and spontaneity of interaction of BSA with drugs in BSA-(FT+TAP) system at 293 nm increased with decreasing randomness (entropy) due to the presence of TAP.

The molar ratio of the BSA-(FT+TAP) system at 293 nm was 1:2 and 1:3, respectively at temperature 298 K and 308K. This indicated that 2 mole FT was bound with 1 mole of BSA at 298 K and 3 moles FT was bound with 1 mole of BSA at 308 K in presence of TAP. It was observed that the binding constant at 293 nm increased from BSA-FT system alone. It indicated that due to presence of TAP in BSA-(FT+TAP) system, the binding of BSA with drugs increased at 293 nm.<sup>13</sup>

### Competing interest

Authors have declared no competing interest.

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