Effect of Temperature and Additives on the Interaction of Ciprofloxacin Hydrochloride Drug with Polyvinylpyrrolidone and Bovine Serum Albumin: Spectroscopic and Molecular Docking Study

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Abstract: The fluoroquinolone antibiotic drug namely ciprofloxacin hydrochloride (CFH) is widely prescribed for the treatment of different bacterial infections. The interaction of CFH with a synthetic polymer, polyvinyl pyrrolidone (PVP), and biopolymer, bovine serum albumin (BSA) was studied by UV-visible and fluorescence spectroscopic methods at different temperatures. The binding constant (KB) for the CFH-PVP complex was determined from the Benesi-Hildebrand plot. PVP of different molecular weights (MW) (such as 24,000, 40,000, 360,000, and 700,000 gm. mole⁻¹) were used for the interaction between CFH and PVP. There was a gradual increase in KB value and the complexation reaction was found to be much enhanced with the augmentation of the MW of PVP. The values of KB were also found to be increased with increasing temperatures as well as with the increase of electrolyte/acetic acid concentration. The Gibbs free energy of binding (∆G₀) values of the interaction process was negative which indicates the complex formation is thermodynamically spontaneous. The positive values of enthalpy (∆H₀) and entropy (∆S₀) of binding connote that the binding force for CFH-PVP complexation is hydrophobic in nature and the complexation is entropy controlled. The negative intrinsic enthalpy (∆H*,₀) values indicate the high stability of CFH-PVP complexes. Molecular docking calculation discloses the existence of similar binding forces between CFH and PVP obtained by the analysis of experimental data from UV-visible spectroscopic method. The binding constant between CFH and BSA (KB), quenching constant (Ks), the number of binding sites (n), and the quenching rate constant (Kq) for the CFH-BSA system were also calculated. The values of Ks, Kq, and n for the CFH-BSA system are lower in 0.05 mol kg⁻¹ urea solution and higher in PVP solutions compared to those of aqueous medium.

Key words: antibiotic drug, polymer, bovine serum albumin, binding constant, thermodynamics

1 Introduction

Polymers are giant-like molecules having usually a carbon backbone and small repeating unit/units. The drug release in the bloodstream can occur through diffusion, degradation, or water penetration. The drug release through diffusion phenomenon is controlled by inert water-insoluble membrane barrier and it can happen either by reservoir diffusion systems or by matrix diffusion systems.

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The interactions between drug and polymer are significant as the majority of the administrative drugs bound with serum albumin of blood reversibly and transportation of these drugs happen through complexation with protein. The activity of the drug in the biological system gets influenced significantly by the type and degree of interaction of the drug with protein\textsuperscript{3–6}. To insight the pharmacological response of drugs and their doses form, the binding parameters can be utilized extensively\textsuperscript{3}. There are several advantages of using polymer in the drug formulation e.g. localized drug delivery, sustained drug delivery, lower dose frequency, reduced side effect, and biodegradable though high initial drug release is a disadvantage. In the case of formulation and development of less soluble drugs in aqueous vicinity, one of the greatest challenges is to increase the oral bioavailability. Different methods e.g. salt formation, solubilization, and component size reduction are the most commonly employed methods to enhance drug dissolution rate. But some practical limitations make these techniques less popular. Polymers can play significant performances in the biomedical as well as in the pharmaceutical sciences especially the plasma proteins which can act as a transporter in drug transportation\textsuperscript{7, 8}. In the pharmaceutical industries, the hot-melt extrusion method is now recognized where mixing of heated sample and subsequently shaping is carried out in a single continuous process\textsuperscript{9}. In the hot-melt extrusion method, PVP plays a significant role. Again, PVP can interact with some drugs and thus stabilize the molecular dispersion\textsuperscript{9}. PVP has the capability to inhibit/retard the recrystallization of active pharmaceutical ingredients by means of network formation or generating crystal surface surrounding the drug molecules\textsuperscript{10} and thereby limit the molecular mobility of active pharmaceutical ingredients\textsuperscript{11}. But the thermal degradation and hygroscopicity of PVP made it less applicable. However, the accurate exploration of composition as well as processing parameter by means of drug-polymer interaction, it can effectively be employed used in the drug formulation at moderate temperature\textsuperscript{9}. Serum albumin, the most plentiful protein among the plasma protein and play a significant role in the transportation of drug\textsuperscript{12–13} and thus drug-albumin complexes can be assumed as a model to explore the drug-protein interaction\textsuperscript{8}. Large numbers of amphiphilic substances having lower MW together with drugs interact with polymers. Therefore, the drug performance can significantly be affected by reversible interactions of biological polymers with drugs through the modification in transport and distribution phenomenon\textsuperscript{14}. BSA, mammalian albumin, has been extensively investigated due to its stability and neutrality in biological reactions as well as for low cost\textsuperscript{7, 8, 15}. It is found from such systems that the efficiency of absorption and release of drug is a function of ionic or electrostatic interactions amongst the drug and polymers\textsuperscript{16}. BSA (Scheme 1(A)) is a good carrier in blood for different substances and help to distribute throughout the body. The fate of less soluble substance depends on the interaction of that substance with BSA\textsuperscript{17–23}. Moreover, BSA is considered to be utilized in the protein-based nanoparticle synthesis as it is preferentially deposited at the tumor place\textsuperscript{17–23}. Abraxane, first, is used for the treat-
ment of breast cancer that proves the importance of albumin-based nanoparticles. For the treatment of different diseases caused by bacteria e.g. skin, joint and urinary tract infections CFH is widely used. Besides these, CFH is also applied for the treatment of infectious diarrhea and typhoid fever. The study of drug-polymer interaction is a key method to estimate the side effects of the drug. The binding of a drug with polymer plays a significant role in the case of distribution and long circulation of a drug. The drug-protein binding determines the ease of absorption, distribution, and excretion of the drug. Therefore, the study of drug-protein interaction has significant importance from an applied viewpoint. In addition, the molecular docking technique can be used to find out the location of the interaction of any solute on the polymer or protein molecules. So, in the current study, such initiative was considered to observe the location of the binding of CFH with PVP. Despite having ample articles on drug-polymer interaction, the interaction of PVP and BSA with CFH has not been reported yet. Keeping this in view, the study of the interaction of CFH with PVP was undertaken by UV-visible spectroscopic and molecular docking methods while the interaction of CFH with BSA was explored by fluorescence quenching technique. The values of binding constant (Kb), the number of binding sites (n), the quenching rate constant (Kq) and related thermodynamic parameters have been determined to characterize the interactions between CFH and polymers.

2 Experimental

2.1 Materials

The polymers PVP (98%) and BSA (99%) were obtained from BDH (London, England) and Merck (Darmstadt, Germany), respectively. CFH (99%) was provided by Novartis (Dhaka, Bangladesh) Ltd. Both CFH and PVP were utilized without any further refinement. CFH stock solution was prepared in water to avoid precipitation. Distilled and deionized water was used in all cases. The entire additives (KCl (99%), NaCl (99%), Na₂SO₄ (98%), NaOH (98%), HCl (98%), (NH₄)₂SO₄ (99%) and urea (99.5%)) were collected from Merck (Frankfurter Str, Darmstadt, Germany), and introduced with no purification.

2.2 UV-visible spectroscopic technique

The UV-visible spectroscopic technique was used to study the interaction between CFH and PVP. 5 mL PVP solutions having different concentrations of PVP were added to 5 mL 4 × 10⁻⁸ M CFH solutions taken in test tubes. The mixtures were then properly mixed and kept in an incubated electrical shaker (GFL, USA) for about one hour with constant stirring (80 rpm) at the desired temperature.

The absorbances of the mixture were observed through a 1601PC UV-visible spectrophotometer (Shimadzu, Japan) at λ_max of 277 nm. The baseline for each solvent was calibrated before each experiment. In each spectral run, the distilled deionized water was used as a reference. The experiments were carried out at different temperatures (303.15 to 318.15 K). An electrical temperature controller (Shimadzu-TCC-240A) having the precision of ±0.5 K and the capability of temperature controlling over 280.15-333.15 K was utilized to maintain the desired experimental temperature. The experimental pH was adjusted at the desired value with the addition of HCl and NaOH to the solution.

2.3 Fluorescence spectrophotometric measurements

Fluorescence emission spectra were recorded through a computerized spectrofluorophotometer (Shimadzu RF-6000 PC, Kyoto, Japan) furnished with a xenon lamp source and a 1.0 cm quartz cell. A microbalance (0.1mg accuracy) was employed for the accurate measurement of the sample. The initial concentration of BSA was kept fixed at 2.0 × 10⁻⁶ molkg⁻¹ prepared in water/urea solution while the CFH concentration was varied from 0 to 6.5 × 10⁻⁶ molkg⁻¹. A stock solution of CFH with a concentration of 1 × 10⁻⁴ molkg⁻¹ was prepared in water/urea solution and then the solution was diluted to prepare CFH solutions of different concentrations. The 2.00 mL solutions of drug and protein were mixed in a test tube and then the resultant mixture was shaken for a half-hour in a shaking incubator (GFL, USA) to mix properly and attain equilibrium condition. Fluorescence quenching spectra were recorded at 298.15 K in the range of 250-450 nm at an excitation wavelength of 280 nm. Both the excitation and emission bandwidth were kept constant at 5 nm. The experiments were repeated to study fluorescence quenching in a better way. To realize the influence of urea (0.05 mol kg⁻¹) and PVP solutions (0.5 and 1.40% (w/v)) on protein-drug binding a similar procedure was followed in urea and PVP solutions media where the concentration of protein was maintained constant.

2.4 Molecular Docking Methodology

The 3D coordinates of PVP and ciprofloxacin were downloaded from PubChem with CID6917 and CID2764 respectively. The geometries and energies of PVP and ciprofloxacin were optimized and minimized in Open Babel using the universal force field (UFF). Molecular docking between PVP and ciprofloxacin was performed on Hex 8.0.0 software (Dave Ritchie, Capsid research team at the LORIA/Inria, Nancy, France) with default settings. The interaction between PVP and ciprofloxacin was examined on the basis of shape complementarity as well as electrostatic potentials. After docking, post-processing was performed by OPLS (optimized potential for liquid simulation) minimization. The parameters such as grid dimension, receptor range, ligand range, twist range, distance range, translation

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step, and score threshold were set to 0.6, 180, 180, 360, 40, 0.8, and 0 respectively. A total of 2000 solutions were computed and the pose with the lowest energy in the top-ranked cluster was chosen as the best mode of interaction between PVP and ciprofloxacin. The results were analyzed, and figures were prepared in Discovery Studio 4.0 (Accelrys Software Inc., San Diego, USA).

3 Results and Discussion

3.1 Binding behavior between PVP and CFH

Figure 1 shows the UV absorption spectra obtained by the addition of increasing amounts of PVP solution to a $4 \times 10^{-5}$ M solution of CFH. Absorbances and the amplitude of the band of the drug-polymer system were increased with the increase of concentration of PVP which indicated the presence of interaction between CFH and PVP. In the structure of the PVP molecule, the effective chromophore is the lactam carbonyl group ($\geq N-C=O$) which is significant to explain interaction and it has the resonance hybrid of configurations I and II (Scheme 2). Thus, lactam carbonyl is an electrophilic substituent where nitrogen possesses a partially positive charge and oxygen possesses a partially negative charge. The drug CFH possesses both acid group and carbonyl functional groups in its chemical structure. In structure I, the carbonyl group of PVP may interact with the drug effectively by hydrogen bonding and in structure II, the positively charged nitrogen can electrically interact with the drug molecule.

However, the steric interference prevents the involvement of nitrogen atom in case of intermolecular interactions between PVP and CFH which makes the carbonyl group more feasible for hydrogen bonding (H-bonding) to the drug molecule, and thus drug-polymer complex might form. It was evident from the spectra (Fig. 1) that the interaction between CFH and PVP was not strong enough for a bathochromic or hypsochromic shifting of wavelengths with increasing concentration of polymer. In the present study, the only absorption was increased due to the addition of PVP. So, interactions between CFH and PVP might be weak interactions such as hydrogen-bonding or dipole-dipole interaction.

The $K_b$ values for the CFH-PVP system was determined by using the Benesi-Hildebrand equation:

$$\frac{[D]}{A} = \frac{1}{K_b[C]} + \frac{1}{\varepsilon}$$  \hspace{1cm} (1)

Where $[D]$ is the concentration of the drug, $A$ is the absorbance of the drug-polymer complex, and $[C]$ is the concentration of polymer. The $K_b$ value was obtained from the observed quotient of intercept and slope in the plot of $[D]/A$ versus $1/[C]$. The $K_b$ values for ionic surfactant with dyes was also estimated by others with the help of Benesi-Hildebrand equation.

Fig. 1 The UV-visible spectra of (A) pure CFH solution ($2 \times 10^{-5}$ M) and 0.45 % (w/v) PVP solution and (B) mixture of an equal volume of $4 \times 10^{-5}$ M CFH solution and PVP solution having diverse concentrations at 303.15 K.

Scheme 2 The resonance hybrid structures lactam carbonyl group.
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Table 1 The values of $K_b$ and different thermodynamic parameters of the CFH + PVP system in an aqueous medium having different molecular weights of PVP at different temperatures.

| $MW_{PVP}$ | $T$ | $K_b \times 10^{-4}$ | $\Delta G^o$ | $\Delta H^o$ | $\Delta S^o$ | $\Delta H_{s^o}$ | $T_c$ |
|------------|-----|---------------------|--------------|--------------|--------------|----------------|------|
| g.mol$^{-1}$ | K   | L.mol$^{-1}$ | kJ.mol$^{-1}$ | kJ.mol$^{-1}$ | J.K$^{-1}$mol$^{-1}$ | kJ.mol$^{-1}$ | K   |
| 24000      | 303.15 | 1.40  | -24.06  | 74.72        | 325.84       |                |      |
|            | 308.15 | 1.91  | -25.25  | 63.78        | 288.94       | -23.75         | 302.5 |
|            | 313.15 | 2.46  | -26.32  | 52.01        | 250.14       |                |      |
|            | 318.15 | 2.82  | -27.10  | 39.38        | 208.96       |                |      |
| 40000      | 303.15 | 2.52  | -25.54  | 49.57        | 247.78       |                |      |
|            | 308.15 | 3.26  | -26.62  | 32.27        | 191.13       | -27.40         | 311.3 |
|            | 313.15 | 3.90  | -27.52  | 13.76        | 131.84       |                |      |
|            | 318.15 | 3.97  | -28.01  | -5.99        | 69.20        |                |      |
| 360000     | 303.15 | 13.70 | -29.81  | -5.64        | 79.74        |                |      |
|            | 308.15 | 13.81 | -30.32  | 28.91        | 192.22       | -30.71         | 311.1 |
|            | 313.15 | 19.82 | -31.76  | 65.73        | 311.30       |                |      |
|            | 318.15 | 31.01 | -33.45  | 104.87       | 434.76       |                |      |
| 700000     | 303.15 | 536.12 | -39.05  | 5.26         | 146.19       |                |      |
|            | 308.15 | 543.32 | -39.73  | 5.68         | 147.35       | -41.89         | 322.6 |
|            | 313.15 | 573.11 | -40.51  | 6.11         | 148.88       |                |      |
|            | 318.15 | 582.43 | -41.20  | 6.55         | 150.11       |                |      |

The values of $K_b$ were found to be enhanced with the increase of the content of CH$_3$COOH medium having different MW of polymers. The $K_b$ values were found to be increased with the increase of the content of CH$_3$COOH medium. In the presence of an electrolyte, the water molecules around the PVP are removed from their vicinity by tying up them by electrolyte ions, and the solubility of PVP is reduced which makes PVP more available to CFH to interact, and thus binding is enhanced. In the CH$_3$COOH medium, the CFH molecules exist in the protonated form. The positive nitrogen atom ($N^+$) of CFH interacts electrostatically with the negative oxygen atom of the lactam carbonyl group and thus increases the drug-polymer binding. This binding phenomenon increases with the increase of the content of CH$_3$COOH and thus $K_b$ values are higher as compared to that in the aqueous medium. In basic condition, -COOH group of CFH dissociate and exist as anionic carboxylate ions ($\text{COO}^-\text{Na}^+$). This anionic carboxylate ion interacts electrostatically with the ($N^+$) ion of the lactam carbonyl group and thus the $K_b$ values are higher as compared to that in an aqueous medium.

### 3.2 Thermodynamics of binding between PVP and CFH

For a better insight into the interaction of PVP with CFH, the thermodynamic parameters of binding were as-

\[ \Delta G^o = -RT \ln K_b \]

\[ \Delta H^o = \Delta H_{s^o} + \Delta H_{s^o}^o \]

\[ \Delta S^o = \Delta S_{s^o} + \Delta S_{s^o}^o \]

The donating power of PVP experiences an increase with the increase of temperature which favors the formation of complexes.

The values of $K_b$ were found to be enhanced in the presence of NaCl/Na$_2$SO$_4$ but slightly reduced in (NH$_4$)$_2$SO$_4$ medium. In the presence of an electrolyte, the water molecules around the PVP are removed from their vicinity by tying up them by electrolyte ions, and the solubility of PVP is reduced which makes PVP more available to CFH to interact, and thus binding is enhanced. In the CH$_3$COOH medium, the CFH molecules exist in the protonated form. The positive nitrogen atom ($N^+$) of CFH interacts electrostatically with the negative oxygen atom of the lactam carbonyl group and thus increases the drug-polymer binding. This binding phenomenon increases with the increase of the content of CH$_3$COOH and thus $K_b$ values are higher as compared to that in the aqueous medium. In basic condition, -COOH group of CFH dissociate and exist as anionic carboxylate ions ($\text{COO}^-\text{Na}^+$). This anionic carboxylate ion interacts electrostatically with the ($N^+$) ion of the lactam carbonyl group and thus the $K_b$ values are higher as compared to that in an aqueous medium.

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\[ \Delta S^o = \Delta S_{s^o} + \Delta S_{s^o}^o \]

The donating power of PVP experiences an increase with the increase of temperature which favors the formation of complexes.
The assessed values of $\Delta G^0$ were found to be positive in almost all cases. The positive $\Delta H^0$ value indicates less-dominant hydrogen bond formation between the substrates, while largely positive $\Delta S^0$ value indicates the presence of predominant hydrophobic binding. Therefore the positive $\Delta H^0$ and $\Delta S^0$ values observed in all the CFH-PVP systems reveal that the interaction between CFH and PVP is principally hydrophobic in nature and the complexation process is entropy controlled. The negative $\Delta H^\circ$ values were also obtained in few cases which refer to the existence of weak electrostatic forces between CFH and PVP. The water molecules attached to the polymer and drugs are relatively structured. Upon complexation, drug and polymer molecules come close together releasing some of the ordered water molecules and thus entropy of the resultant complex is increased. The values of $\Delta H^\circ$ and $\Delta S^0$ for PVP with lower MW (24000 and 40000 g.mol$^{-1}$) decrease with the increase of temperature while following the opposite trend for PVP with higher MW (360000 and 700000 g.mol$^{-1}$).

The linear variation of enthalpy with the change of entropy is termed as enthalpy-entropy compensation and can be illustrated by the following equation:

$$\Delta H^\circ = T_s \Delta S^0 + \Delta H^*$$

In equation (8), the symbols $T_s$ and $\Delta H^*$ stand for compensation temperature and intrinsic enthalpy respectively. The values of $\Delta H^*$ were observed to be negative for all PVP having different MW and increased with the increase of MW of PVP (Table 1). The values of $T_s$ were found to be in the range of 302.5 to 322.6 K (Table 1). The $\Delta H^*$ values were observed to be lying in the range of $-23.75$ to $-41.89$ kJ mol$^{-1}$. The values of $T_s$ and $\Delta H^*$ can be utilized to describe the solute-solvent and solute-solute interaction respectively. The estimated values of $T_s$ in our current study can significantly be used to measure the water content in the protein solution.

### 3.3 Molecular docking results of PVP-CFH system

In literature, the binding study between BSA and CFH is already reported by molecular docking technique, therefore, here only PVP and CFH interaction was carried out by molecular docking. To observe the binding phenomenon between PVP and CFH in detail, a molecular docking technique was used. The interaction between PVP and CFH was determined using Hex 8.0.0 and the results are presented in Fig. 2 and Table 3. The interaction energy between PVP and CFH was estimated to be $-133.33$ kcal mol$^{-1}$. The CFH-PVP complex was stabilized by two electrostatic (Pi-Cation) interactions between the N-atom of PVP and two aromatic rings of CFH. Additionally, two carbon-hydrogen bonds were also formed between the O-atom of PVP and the H-atoms of CFH (Fig. 2).
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3.4 Binding behavior study between BSA and CFH

Fluorescence intensity of BSA solution was found to change with the addition of CFH in variable media. Figure 3 shows the fluorescence spectra of BSA solution and BSA + CFH mixture having different CFH concentrations in 1.4% (w/v) PVP solution. In the current study, the fluorescence quenching of BSA is persuaded by CFH. The fluorescence quenching data were scrutinized using the Stern-Volmer equation:

\[ \frac{F_0}{F} = 1 + K_{sv}[Q] \]  

In Eq. (9), \( F_0 \) and \( F \) elicit the fluorescence intensity in the absence and attendance of applied quencher, \( K_{sv} \) and \([Q]\) are Stern-Volmer quenching constant and equilibrium concentration of employed quencher respectively. The \( K_{sv} \) values were calculated from the plot of \( F_0/F \) against \([Q]\) (Fig. 4).

However, the quenching rate constant (\( K_q \)) was assessed via solving Eq. (10):

\[ \frac{F_0}{F} = 1 - \frac{K_q}{K_{sv}}[Q] \]  

Table 3 Interaction parameters between PVP and ciprofloxacin obtained from molecular docking.

| Interaction between donor-atom and acceptor-atom | Distance (Å) | Nature of interaction       | Energy (kcal mol\(^{-1}\)) |
|-----------------------------------------------|-------------|----------------------------|---------------------------|
| CFH:H - PVP:O                                 | 2.8769      | Carbon Hydrogen bond       |                           |
| CFH:H - PVP:O                                 | 2.8286      | Carbon Hydrogen bond       |                           |
| PVP:N - CFH                                   | 3.7635      | Electrostatic (Pi-Cation)  | -133.33                   |
| PVP:N - CFH                                   | 3.6563      | Electrostatic (Pi-Cation)  |                           |

Fig. 2 Interaction between PVP (golden color) and ciprofloxacin (teal color). Carbon hydrogen bonds and electrostatic (Pi-Cation) interactions are shown in green and blue dash lines. The N- and O-atoms are represented in blue and red sticks respectively.

Fig. 3 Fluorescence spectra of BSA solution (a) and BSA + CFH mixture having CFH concentrations of \(8.125 \times 10^{-7}\) (b); \(1.6 \times 10^{-6}\) (c); \(3.25 \times 10^{-6}\) (d) and \(6.50 \times 10^{-6}\) (e) mol kg\(^{-1}\) in 1.4% (w/v) PVP solution where \([\text{BSA}] = 2 \times 10^{-6}\) mol kg\(^{-1}\).

Fig. 4 Comparative Stern-Volmer plots describing BSA quenching by CFH at 298.15 K in an aqueous medium.
The values of $K_v$, $K_s$, $K_q$, and $n$ for the interaction between BSA and CFH in the aqueous solution and in presence of urea/PVP solutions were determined following the procedure as reported in the literature\cite{57, 60} and are included in Table 4. The fluorescence quenching phenomenon can be progressed by means of two mechanisms- (i) dynamic (diffusion-controlled) (ii) static (diffusion-independent)\cite{55, 59, 60}. The Stern-Volmer plots were found to be linear within the studied concentration range which enunciates either a single type of quenching or the presence of only a single binding site for a quencher\cite{55, 59, 60}. The Stern-Volmer plots for aqueous medium exhibit slightly upward curvature at higher quencher concentrations which reveals the presence of mixed quenching i.e., both static and dynamic\cite{54, 62}. The results display that the value of $K_v$ is two orders of magnitude for both systems (in water and urea solution) more than the maximum diffusion collision quenching rate constant $(2.0 \times 10^{10} \text{ L mol}^{-1} \text{s}^{-1})$ for different quenchers with biopolymer\cite{53}.

The values of $n$ represent that there exist greater binding sites in BSA for CFH in water medium compared to the urea medium whereas the values are increased in PVP solutions compared to the aqueous medium. Results also show that the $K_v$, $K_p$, and $K_s$ for the interaction between BSA and CFH in urea medium are lower compared to those in water (Table 4). This result affirms that urea has the denaturing ability of protein and thus reduces these values.

Since PVP is extensively utilized in different technological, industrial, and biological applications, the current study has extended to detect the effect of PVP on the binding between BSA and CFH. In the current study, the 0.55 and 1.40\% (w/v) PVP solutions were taken as media. The $K_v$, $K_p$, $K_s$, and $n$ values for the interaction between BSA and CFH in PVP solutions media are higher in magnitude compared to those in water/urea solutions and the values are enhanced with increasing PVP concentration (Table 4). The higher binding constants and number of binding sites (almost two times) of CFH + BSA system in PVP solution compared to the aqueous medium can be explained in the way that there might be the possibility of increasing resultant binding sites and hydrophobic regions in the mixture of PVP and BSA which facilitates the binding of CFH.

![Fig. 5](image-url) Representative plot of $\log \left( \frac{F_0 - F}{F} \right)$ vs. $\log [Q]$ for BSA + CFH mixed system in an aqueous medium.

$K_v = K_v/\tau_o$ \hspace{1cm} (10)

Where $\tau_o$ is the mean life span of the protein in absence of a quencher. The value of $\tau_o$ of the biopolymer is $1.0 \times 10^{-3} \text{ s}$\cite{55, 56} and thus $K_v$ values were of the order of $10^{-12} \text{ L mol}^{-1} \text{s}^{-1}$.

During the binding of small molecules independently to different equivalent sites of a macromolecule, the binding constant ($K_q$) and the numbers of binding sites ($n$) can be estimated solving Eq. (11)\cite{52, 53, 57, 58}:

$$\log \left( \frac{F_0 - F}{F} \right) = \log K_v + n \log [Q]$$ \hspace{1cm} (11)

The scheme of $\log \left( \frac{F_0 - F}{F} \right)$ vs. $\log [Q]$ (Fig. 5) was used for finding the value of $\log K_q$ and $n$ where $\log K_q$ is the intercept of the plot and $n$ is the slope of the plot. It is worth mentioning that all the calculations were done considering the fluorescence intensity at near maximum wavelength of 280 nm.

The $K_v$, $K_q$, $K_p$, and $n$ for the interaction between BSA and CFH in the aqueous solution and in presence of urea/PVP solutions were determined following the procedure as reported in the literature\cite{57, 60} and are included in Table 4. The fluorescence quenching phenomenon can be progressed by means of two mechanisms- (i) dynamic (diffusion-controlled) (ii) static (diffusion-independent)\cite{55, 59, 60}. The Stern-Volmer plots were found to be linear within the studied concentration range which enunciates either a single type of quenching or the presence of only a single binding site for a quencher\cite{55, 59, 60}. The Stern-Volmer plots for aqueous medium exhibit slightly upward curvature at higher quencher concentrations which reveals the presence of mixed quenching i.e., both static and dynamic\cite{54, 62}. The results display that the value of $K_v$ is two orders of magnitude for both systems (in water and urea solution) more than the maximum diffusion collision quenching rate constant $(2.0 \times 10^{10} \text{ L mol}^{-1} \text{s}^{-1})$ for different quenchers with biopolymer\cite{53}.

The values of $n$ represent that there exist greater binding sites in BSA for CFH in water medium compared to the urea medium whereas the values are increased in PVP solutions compared to the aqueous medium. Results also show that the $K_v$, $K_p$, and $K_s$ for the interaction between BSA and CFH in urea medium are lower compared to those in water (Table 4). This result affirms that urea has the denaturing ability of protein and thus reduces these values.

Since PVP is extensively utilized in different technological, industrial, and biological applications, the current study has extended to detect the effect of PVP on the binding between BSA and CFH. In the current study, the 0.55 and 1.40\% (w/v) PVP solutions were taken as media. The $K_v$, $K_p$, $K_s$, and $n$ values for the interaction between BSA and CFH in PVP solutions media are higher in magnitude compared to those in water/urea solutions and the values are enhanced with increasing PVP concentration (Table 4). The higher binding constants and number of binding sites (almost two times) of CFH + BSA system in PVP solution compared to the aqueous medium can be explained in the way that there might be the possibility of increasing resultant binding sites and hydrophobic regions in the mixture of PVP and BSA which facilitates the binding of CFH.

### Table 4

| Medium                | $K_v \times 10^{12}$ (Lmol$^{-1}$) | $K_q \times 10^{12}$ (Lmol$^{-1}$ s$^{-1}$) | $R^2$ | $\log K_v$ (Lmol$^{-1}$) | $n$ | $R^2$ |
|-----------------------|----------------------------------|-----------------------------------|-------|----------------------|-----|-------|
| Water                 | 8.75                             | 8.75                              | 0.974 | 5.32                 | 1.10| 0.974 |
| 0.05 mol kg$^{-1}$ urea solution | 7.45                             | 7.45                              | 0.999 | 4.75                 | 0.90| 0.996 |
| 0.55 (w/v) PVP solution | 164                             | 164                               | 0.889 | 11.07                | 1.96| 0.920 |
| 1.40 (w/v) PVP solution | 554                             | 554                               | 0.859 | 12.14                | 2.08| 0.964 |
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4 Conclusion
The binding of an antibiotic drug (CFH) with PVP and BSA has been carried out utilizing UV-visible and fluorescence spectroscopic techniques. The binding between CFH and PVP was found to be higher for PVP of higher MW. The values of $K_v$ for CFH-PVP were also observed to be enhanced with the increase of temperature. Salting out the effect of electrolytes increases the binding between CFH and PVP. The $\text{CH}_3\text{COOH}$ protonates the CFH molecules which might contribute to the binding by electrostatic interaction and increase the $K_v$ values. Binding of CFH and PVP is higher both in acidic and basic conditions as compared to that in aqueous vicinity. Different thermodynamic parameters connote the binding between CFH and PVP is spontaneous and interaction forces are mainly hydrophobic in nature while there is also the possibility of the presence of weak electrostatic force. Molecular docking results of the PVP-CFH system reveal the existence of both electrostatic and hydrogen bonding between PVP and CFH. So, there is a resemblance in the findings between experimental and molecular docking in terms of the binding forces present between the drug and PVP. The estimated $T_c$ values of the current study are in good agreement with the values for the water involvement in the protein solution. The values of $K_v$, $K_w$, and $n$ for the CFH-BSA system are reduced in 0.05 mol kg$^{-1}$ urea medium and increased in PVP solutions compared to those of aqueous medium. This study will be helpful to choose an appropriate polymer with definite MW for better formulation of the drug. Also, further study on the CFH-polymer system can be carried out using TEM/SEM to investigate the morphology of the systems.

Conflicts of Interest
There are no conflicts of interest to declare.

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