E. Gökoğlu and H. Bektaş / Hacettepe J. Biol. & Chem., 2026, 54(1), 57-64

Research Article

Hacettepe Journal of Biology and Chemistry

journal homepage: https://hjbc.hacettepe.edu.tr/
Dergipark Akademik: https://dergipark.org.tr/en/pub/hjbc

Studies on Biophysical Interactions Between a Sulfa Drug and Pepsin by Fluorescence Methods

Floresans Yöntemler ile Bir Sülfa İlacı ve Pepsin Arasındaki Biyofiziksel Etkileşimler Üzerine Çalışmalar

Elmas Gökoğlu* **and Hülya Bektaş***
Department of Chemistry, Hacettepe University, Ankara, Turkey.

ABSTRACT

The biophysical interaction of a sulfa drug, sulfonamide derivative sulfamerazine (Smz) with pepsin enzyme was examined using fluorescence and UV-vis absorption spectroscopies. Fluorimetric methods (steady-state/three dimentional fluorescence) were used to examine the binding properties of Smz to pepsin, and the results showed the formation of a complex with a static quenching. Thermodynamic parameters, binding and quenching constants were calculated at different temperatures. The results showed a spontaneous binding between pepsin and Smz through electrostatic/hydrophobic interactions. 3D spectra indicated the conformational/micro environmental changes in the structure of enzyme. Based on the FRET, the binding distance (r) for the drug-enzyme pair was obtained as 3.97 nm. To determine Smz, the limit of detection (LOD) and limit of quantification (LOQ) of Smz were calculated as $6.54x10^{-6}$ M and $2.18x10^{-5}$ M in the presence of pepsin.

Key Words
Pepsin, sulfa drug, sulfamerazine, binding mode, 3D fluorescence.

ÖZ

Bir sülfa ilacı olan sülfonamid türevi sülfamerazinin (Smz) pepsin enzimi ile biyofiziksel etkileşimi floresans ve UV absorpsiyon spektroskopileri ile incelenmiştir. Smz'nin pepsine bağlanma modunu araştırmak için kararlı hal ve üç boyutlu (3D) floresans yöntemleri kullanılmıştır ve sonuçlar statik sönümün varlığını ortaya çıkarmıştır. Bağlanma kısım sayıları, bağlanma sabitleri ve termodinamik parametreler farklı sıcaklıklarda hesaplanmıştır. Sonuçlar Smz'nin pepsine kendiliğinden bağlandığını ve hidrofobik/elektrostatik etkileşimlerin önemli rol oynadığını belirtmiştir. 3D spektrumlar, bağlanma modunda pepsin yapısındaki konformasyonel değişiklikleri göstermiştir. Işımasız enerji transferi teorisine dayanarak ilaç ile enzim arasındaki bağlanma mesafesi (r) 3,97 nm olarak hesaplanmıştır. Smz'nin belirlenmesi amacıyla, pepsin varlığında Smz için tespit sınırı (LOD) ve miktar tayin sınırı (LOQ) değerleri sırasıyla $6.54x10^{-6}$ M ve $2.18x10^{-5}$ M olarak hesaplanmıştır.

Anahtar Kelimeler
Pepsin, sülfa ilaç, sülfamerazin, bağlanma modu, 3D floresans.

Article History: Mar 3, 2025; Accepted: Oct 23, 2025; Available Online: Dec 30, 2025.
DOI: https://doi.org/10.15671/hjbc.1648847
Correspondence to: E. Gökoğlu. Department of Chemistry, Hacettepe University, Ankara, Turkey
E-Mail: gokoglu@hacettepe.edu.tr

INTRODUCTION

Sulfonamides, the active ingredient of sulfa group drugs, were among the first modern antibiotics and began to be used on humans in the 1930s. They have the structure of paraamino benzene sulfonylamide and their bacteriostatic effect is provided by the amino benzene ring, which is the active part [1]. As seen in Figure 1a, different sulfonamide derivatives are formed by adding different functional groups to the part given with the letter R in the sulfonamide structure. Thus, their chemical, physical, pharmacological and anti-infection properties also differ. Sulfonamide-containing compounds known to have antitumor effects are widely used in the pharmaceutical industry [2-5]. Some examples of sulfonamides currently used as drugs and their therapeutic effects can be given as follows: Trimethoprim-sulfamethoxazole for pneumonia; pyrimethamine-sulfadiazine to cerebral thoxoplasma; trimethoprim-sulfamethoxazole for urinary tract infections; silver sulfadiazine against bacterial infections in burn patients. Sulfamerazine (Smz, 4-amino-N-(4-methyl-2-pyrimidinyl) benzene sulfonamide) molecular structure is given in Figure 1b is one of the well-known and widely used sulfonamide antibacterial drugs in the treatment of bacterial diseases in humans and animals such as cattle, sheep and poultry, and has a broad spectrum in therapeutic applications [6]. Pepsin is a protease enzyme that catalyzes the hydrolytic cleavage of peptide bonds around hydrophobic or aromatic amino acids. It is produced by the main cells in the stomach that break down food proteins into peptides. Pepsin has a single polypeptide enzymatic chain consisting of 326 amino acids and its structure consists of two parts: N-terminal (1-172 amino acids) and C-terminal (173-326 amino acids). Intrinsic fluorescence of pepsin arises from five tryptophan residues (Trp39, Trp141, Trp181, Trp190, Trp300) and also contains thirteen tyrosine (Tyr) and thirteen phenylalanine (Phe) residues [7,8]. There are many reports in literature about on the interactions of pepsin with several ligands by fluorescence methods such as flavonoid analogs [9], fleroxacin [10], daidzein and genistein [11], trelagliptin [12]. In this study, fluorescence methods such as steady-state/3D fluorescence and also resonance energy transfer were used to examine the interaction of sulfamerazine (Smz, used here for chemical testing as a sulfa antibiotic drug) and pepsin enzyme at pH 2.0 (gastric juice acidity). The study also is important as it will provide healthy and useful information for other drugs containing sulfonamide structure, which are still widely used in physiological acidic environment.

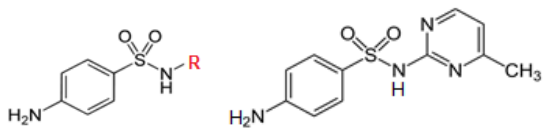

Figure 1. Molecular structures of (a) sulfonamide and (b) sulfamerazine.

MATERIALS and METHODS

Reagents

Sulfamerazine and pepsin (porcine gastric mucosa, EC 3.4.23.1, 35 kDa) were obtained from Sigma Aldrich Chemical Co. The chemicals were of analytical grade and were used without further purification. The stock solutions of pepsin ($3.45x10^{-4}$ M) and sulfamerazine ($5.05x10^{-4}$ M) were prepared in pH 2.0 citric acid-citrate buffer solution (0.02 M citric acid, 0.1 M NaOH). The solutions used in the experiments were prepared daily by diluting them with buffer from their stock solutions, and the solutions were stored at 4 ºC. Double distilled water was used in the studies.

Apparatus

The fluorescence measurements and the spectra were recorded on the Hitachi F-4500 spectrofluorimeter containing 150 W xenon lamp, band slits excitation/emission:2.5 nm/2.5 nm, 700 V PMT voltage using the FL Solutions 2.0 software computer program. The measurements were taken in 1.0 cm fluorescent quartz cells. UV-visible absorption studies were recorded with a UV-1700 PharmaSpec spectrophotometer (Shimadzu) with UV-Probe PC software. The pH of the buffer and the studied solutions was adjusted with a Mettler Toledo (FiveEasy Plus) digital pH meter.

Spectroscopic studies and procedures

In steady-state fluorescence spectral studies, the quenching of sulfamerazine on the intrinsic tryptophan fluorescence of pepsin was examined by fluorimetric titration method at 298, 303 and 310 K temperatures and pH 2.0 in citric acid buffer solution. Increasing concentrations of Smz solutions were added by microinjection into 2.5 mL of $2.0x10^{-6}$ M pepsin solution into the fluorescent cell. The solutions were excited at 280 nm and emission spectra, intensity measurements and Stokes shifts were recorded in the wavelength range of 290-350 nm. Titration data were then plotted according to the Stern Volmer equation.

Accordingly, binding and quenching constants and also thermodynamic and FRET parameters were calculated and explained in detail in the relevant sections. In all calculations, the intrinsic tryptophan fluorescence of pepsin was corrected in measurements for the inner filter effect [13]. 3D fluorescence spectra were recorded between emission wavelength 200 and 450 nm. Initial excitation wavelength was set at 200 nm and a scan speed of 1200 nm/min and band slits of 2.5 nm were recorded up to 450 nm with an increment of 10 nm.

RESULTS and DISCUSSION

Fluorescence quenching studies with steady-state

Fluorescence spectroscopy and related methods are used effectively in protein-drug interactions, providing descriptive information about the peptide structure and conformational changes of the protein. Figure 2 shows the fluorescence spectra obtained by adding increasing amounts of Smz to the pepsin solution. The fluorescence intensities occurring from the Trp residues of the enzyme decreased regularly with increasing drug concentration. It can be seen that emission of pepsin shifts from the 340 nm wavelength to the red by approximately 5-6 nm. This indicates that some changes occur in the microenvironment surrounding the Trp residues in the protein with the formation of a non-fluorescent enzyme-drug complex.

Fluorescence quenching occurs when the fluorescence quantum yield of a fluorescent substance such as a fluorophore, fluorescent probe, drug, dye etc, decreases which is described with the Stern-Volmer equation [13],

$$F_0 / F = 1 + K_{sv}[Q] = 1 + k_q \tau_0 [Q] \quad (1)$$

F_0 and F show the steady-state fluorescence intensities of pepsin without and with the quencher Smz. [Q] is quencher concentration. The quenching constants K_{sv} and k_q are called the Stern-Volmer quenching and quenching rate constant of the biomolecule, respectively. τ_0 is the average lifetime the biomolecule without quencher (for pepsin, $\tau_0 = 10^{-8}$ s [16]). Stern-Volmer graphs were drawn according to the experimentally obtained titration results at three temperatures (Figure3a). The obtained graphs show good linearity, indicating that quenching may occur through a static or dynamic mechanism. Stern Volmer equations, Ksv and kq constants obtained from the graphics are given in Table 1. According to these data, the decrease in Ksv values as the temperature increases indicates that static quenching dominates the enzyme-drug interaction. The maximum scattering collision quenching rate constant of various biopolymer quenchers is kq $1x10^{10}$ $M^{-1}s^{-1}$ [14]. Therefore, the rate constants of the quenching process of pepsin emission by Smz are larger than the kq of the scattering process. This confirms the existence of a static quenching with the formation of the ground state complex.

It has been evaluated that there are independent binding sites in the biomolecule, and the binding constant K_b and number of binding sites n can be obtained by using the double logarithmic equation [15];

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

Figure 3b shows the plots of log $(F_0-F)/F$ versus log [*Q*] for pepsin-Smz complex at different temperatures. The double logarithmic equations, calculated K_b and n values are given at these temperatures in Table 2. The value of n around one shows that there is only a single binding site on enzyme molecule for Smz. The fact that the obtained K_b values are around 10^5 shows that there is a strong interaction between the enzyme and the drug. In addition, the decrease in Kb values with increasing temperature shows that the unstable complex may partially decompose at higher temperatures. [17].

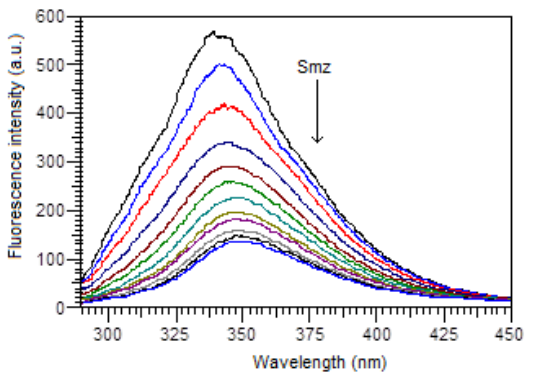

Figure 2. Steady-state fluorescence spectra for pepsin-Smz in condition at 298 K, λex= 280 nm, pH 2.0. [Pepsin]= $2.0x10^{-6}$ M and from high to low [Smz]= 0; 0.998, 1.99, 2.98, 3.96, 4.95, 5.93, 6.90, 7.87, 8.84, 9.80, 10.8 (x10-5) M.

Table 1. Stern-Volmer equations, quenching constants (K_{sv} and k_q) of pepsin-Smz system at three temperatures obtained from Fig.3a.

T (K)	Stern-Volmer Equation	K_{sv} (M^{-1})	$k_q x10^{12*}$	R^{2**}
298	$F_o/F = 0.5914 + 3.795x10^4$ [Q]	$3.795x10^4$	3.795	0.991
303	$F_o/F = 0.7870 + 2.627x10^4$ [Q]	$2.627x10^4$	2.627	0.973
310	$F_o/F = 0.8142 + 1.703x10^4$ [Q]	$1.703x10^4$	1.703	0.974

Table 2. Double logaritmic equations, calculated K_b and n of pepsin-Smz system obtained from Fig.3b at three temperatures.

T (K)	Double log Equation	K_b (M^{-1})	n	R^{2*}
298	$\log (F_0-F)/F = \log 5.834 + 1.324 \log$ [Q]	$6.819x10^5$	1.324	0.998
303	$\log (F_0-F)/F = \log 5.776 + 1.345 \log$ [Q]	$5.976x10^5$	1.345	0.995
310	$\log (F_0-F)/F = \log 5.727 + 1.383 \log$ [Q]	$5.332x10^5$	1.383	0.988

Binding mode and thermodynamic analysis

There are four types of non-covalent binding modes that may play role in ligand interaction to proteins. These are hydrogen bonds, van der Waals forces, electrostatic forces and hydrophobic interactions. Sign and magnitude of the parameters such as entropy change (ΔS) and enthalpy change (ΔH) allow the identification and evaluation of these binding modes involved in the binding process [18]. Here, van't Hoff graph drawn at three temperatures according to the equation given below, using the K_b values in Table 2.

$$\ln K_b = \Delta S/R - \Delta H/RT \qquad (3)$$

The values of ΔH and ΔS were obtained from the slope and intercept of the linear plot, respectively, and used the equation $\ln K_b = 7.1357 + 1873.5\ 1/T$ in Figure 4.

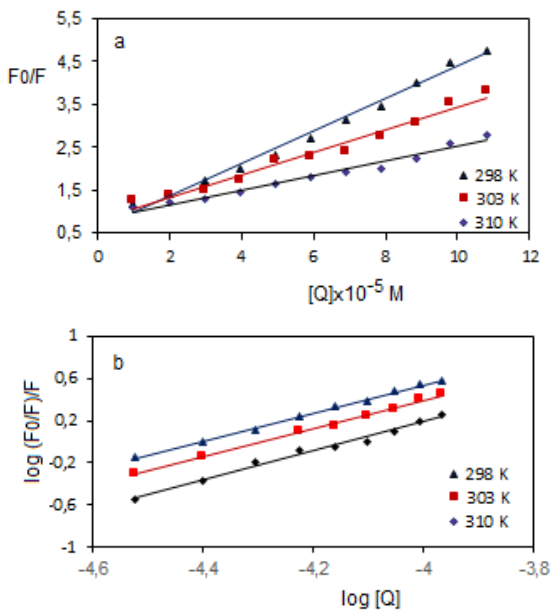

Figure 3. Stern-Volmer (a) and double logarithmic (b) plots of pepsin-Smz system at three temperatures, λex/λem= 280/340 nm and pH 2.0.

Gibbs free energy change (ΔG) is calculated from the below equation at different temperatures:

$$\Delta G = \Delta H - T\Delta S = -RT \ln K_b \qquad (4)$$

Thermodymanic parameters at three temperatures obtained are given in Table 3. The negative sign for DG means that the binding process is spontaneous and the formation of enzyme-drug complex is an exothermic process with the negative enthalpy change. Negative DH (-15.58 kJ/mol) and positive DS (59.33 J/molK) values show that the main forces between pepsin and Smz were electrostatic forces. However, positive values for ΔS are generally considered evidence of hydrophobic interaction because water molecules are regularly distributed around the ligand and the protein results in a random alignment as a result of the hydrophobic interaction. [19].

Three-dimensional (3D) fluorescence measurements

3D fluorescence spectroscopy has been used as a powerful and effective method to study the conformational and structural information of proteins in recent years. [20-22]. In 3D technique, the fluorescence spectrum is given by three axes: fluorescence intensity (I_F), excitation and emission wavelengths. Figures 5a and 5b show the 3D fluorescence spectra of free

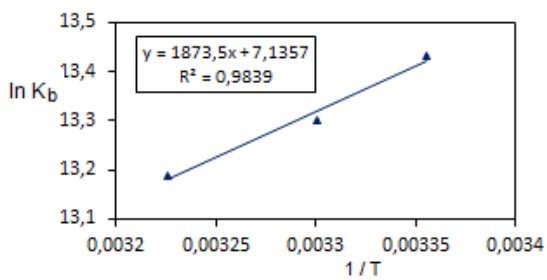

Figure 4. The van't Hoff graph of pepsin-Smz system.

pepsin and the pepsin-Smz binding system and their corresponding contour maps. These figures include two peaks: peak a (λex/λem=280/339 nm) that shows the spectral characteristics of the Trp and Tyr residues in protein molecules, such as the π-π* transition [23]; and the other b peak refers to the peaks corresponding to Rayleigh scattering at λex=λem. Table 4 shows the fluorescence properties of these spectra such as peak positions and Stokes shifts. It is stated that the addition of Smz to pepsin causes a decrease in the intensities of peak a and peak b, which indicates some conformational and microenvironmental changes in the peptide structure of the pepsin molecule.

UV-vis absorption spectral studies

UV-vis absorption spectroscopy is a simple but important technique used to explain protein-drug interaction studies. The formation of hypochromic

Table 3. Thermodynamic parameters of pepsin-Smz system.

T (K)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/molK)	Binding mode
298	-15.58	-33.26	59.33	Electrostatic/ hydrophobic interactions
303		-33.55		
310		-33.97		

Table 4. 3D fluorescence properties of free pepsin and pepsin-Smz system.

Peak	Pepsin			pepsin-Smz		
	Peak position $\lambda_{ex}/\lambda_{em}$ (nm/nm)	Stokes shift Δλ (nm)	Fluorescence intensity	Peak position $\lambda_{ex}/\lambda_{em}$ (nm/nm)	Stokes shift Δλ (nm)	Fluorescence intensity
Peak a	280/339	59	521.3	280/345	65	377.6

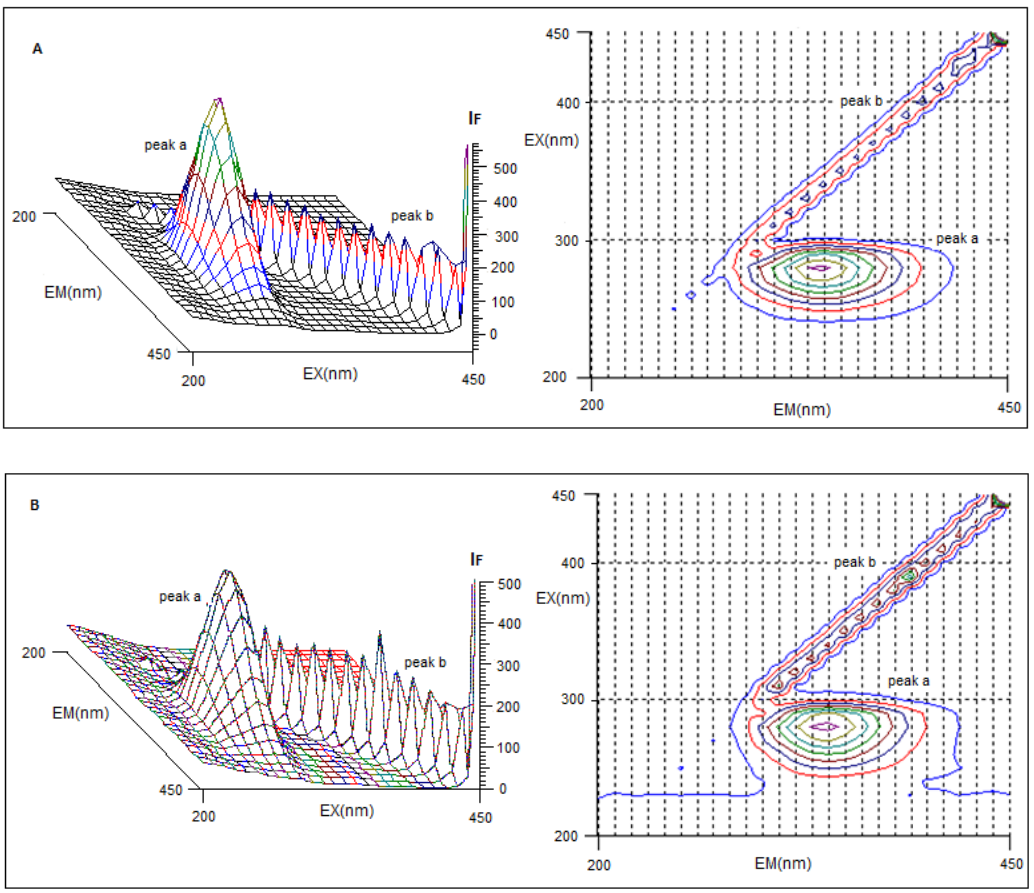

Figure 5. The 3D fluoresce spectra (the left) and their contour maps (the right) of (A) free pepsin ($2.0x10^{-6}$ M) and (B) pepsin-Smz ($2.0x10^{-6}$ M-$5.0x10^{-5}$ M) binding system.

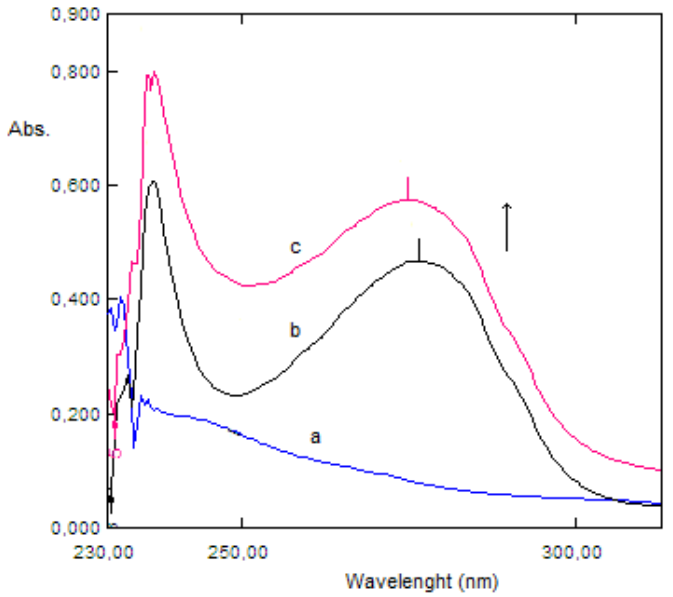

Figure 6. The absorption spectra of (a) 1.0x10^{-5} M Smz, (b) 1.0x10^{-5} M pepsin, (c) 1.0x10^{-5} M Smz-1.0x10^{-5} M pepsin.

and hyperchromic effects in the absorption spectrum affects the conformational change of the biomolecule and thus its spectral behavior [24] UV-vis absorption spectra of free pepsin (1.0x10^{-5} M) and 1:1 mol ratio pepsin-Smz (1.0x10^{-5} M-1.0x10^{-5} M) were recorded in Figure 6 at 298 K. It could be seen from the figure with the addition of drug, the absorbance intensity increased at 280 nm absorption peak of protein and a blue shift (3-4 nm) was observed. The results showed that there was a change in the conformation of protein and the microenvironment around the tryptophan residues [25].

Energy transfer parameters and binding distance

It is very important to collect information about a biomolecule's conformational states, transitions, dynamics, interactions and functional mechanisms in its environment. Fluorescence resonance energy transfer (FRET) is used to elucidate these parameters by using it as a molecular ruler at the biomolecular level. The efficiency of non radiative energy transfer from a donor to an acceptor fluorophore depends on the distance between both molecules. FRET can thus be used as an ideal tool to measure intra- and inter-molecular distances. Therefore, it allows finding intermolecular distances by transferring excitation energy from the electronically excited donor to the acceptor at ground level [13,26]. There are many studies using FRET to elucidate intermolecular interactions based on fluorescence quenching method [27-29]. Based o Förster's theory, the parameters are obtained from following equations:

$$E = 1 - F / F_0 \quad (5)$$

$$E = R_0^6 / (R_0^6 + r^6) \quad (6)$$

$$R_0^6 = 8.79 \times 10^{-25} (\kappa^2 n^{-4} Q_D J(\lambda)) \quad (7)$$

E is the energy transfer efficiency which can be obtained donor (pepsin) and acceptor (Smz) concentrations are equal (1:1 ratio), F and F_0 are the fluorescence intensities of donor in the absence and presence of acceptor. r is the acceptor-donor distance and R_0 is the critical distance (at 50% energy transfer efficiency). κ^2=2/3 is the spatial orientation factor of the donor-acceptor dipoles. n=1.336 is the refractive index of the medium, Q_D = 0.15 [10] is the fluorescence quantum yield of the donor in the absence of acceptor, $J(\lambda)$ is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor [13]. The overlap of these spectra is shown in Figure 7. The

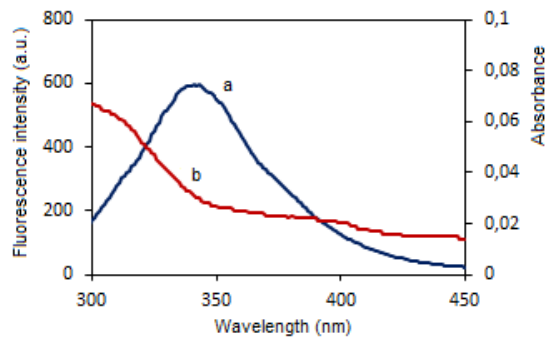

Figure 7. The overlap of emission spectrum of (a) 2.0x10^{-6} M pepsin and the absorption spectrum of (b) 2.0x10^{-6} M Smz.

Table 5. Energy transfer parameters of pepsin/Smz system.

Acceptor	J, Spectral overlap, $(M^{-1}cm^3)x10^{-14}$	R0 (nm)	Energy transfer efficiency, E (%)	r (nm)
Smz	2.36	2.95	14.4	3.97

parameter J(λ) was calculated by integrating the two spectra shown in the figure in the wavelength range of 300 and 450 nm. The parameters were listed in Table 5. According to the results, the fact that r is greater than R_0 may be another evidence of static quenching. Moreover, the distance r from donor to acceptor is less than 8 nm and $0.5R_0 < r < 1.5\ R_0$, indicating that non-radiative energy transfer occurs from enzyme to drug molecule.

Analytical parameters and determination of Smz

The analytical performance of the study was also examined and the determination of smz was tried to be found. The intrinsic emission of pepsin at fixed concentration as $2.0x10^{-6}$ M was quenched regularly with increasing the concentration of Smz and these were shown in Figure 2. The linear Stern-Volmer graph ($F_0/F = F_o/F = 0.5914 + 3.795x10^4$ [Q] with R^2=0.991 at 298 K) was used for the determination of Smz in the presence of pepsin at 0.998 $x10^{-5}$-10.8 $x10^{-5}$ M dynamic range of Smz under the experimental conditions described above. The limit of detection (LOD) and limit of quantification (LOQ) of Smz were obtained from the ratios 3*Sb/m* and 10*Sb/m*, respectively. *m* is the slope of the calibration graph and *Sb* is the standard deviation of the intercept of the graph [30,31]. The results are given in Table 6.

CONCLUSION

The interaction of a sulfa drug, sulfamerazine (Smz) with pepsin enzyme at pH 2.0 (gastric juice acidity) was studied using fluorescence methods such as resonans energy transfer, steady-state and 3D fluorescence as well as UV absorption spectroscopy. Experimental results showed that Smz is a quencher and interacts with pepsin through static quenching mechanism. The values of thermodynamic parameters reveal that the hydrophobic/electrostatic interactions play a major role in a complex formation of enzyme-drug. The distance (r) between the donor (pepsin) and the acceptor (Smz) was calculated as 3.97 nm using FRET. Conformational investigation results from 3D fluorescence spectra revealed that the binding of Smz to pepsin induced some micro-environmental and conformational changes in enzyme molecule. The detection (LOD) and quantification (LOQ) limits of Smz were calculated as $6.54x10^{-6}$ M and $2.18x10^{-5}$ M in the presence of pepsin, respectively. Furthermore, this study on drug-enzyme interactions is important as it guides the understanding of pharmacokinetics such as distribution in the body, side effects and excretion for sulfa drugs and analogues.

References

1. N. Rajendiran, J. Thulasidhasan, Interaction of sulfanilamide and sulfamethoxazole with bovine serum albumin and adenine: Spectroscopic and molecular docking investigations, Spectrochim. Acta Part A, 144 (2015) 183-191.
2. A. Casini, A. Scozzafava, A. Mastrolorenzo, T.C. Supuran, Sulfonamides and sulfonylated derivatives as anticancer agents, Curr. Cancer Drug Targets, 2 (2002) 55-75.
3. A.J. Bartlett, V.K. Balakrishnan, J. Toito, L.R. Brown, Toxicity of four sulfonamide antibiotics to the freshwater amphipod Hyalella Azteca, Envin. Toxicol. Chem., 32 (2013) 866-875.
4. A-C. Martel, S. Zeggane, HPLC determination of sulfathiazole in French honeys, J. Liq. Chromatogr. Relat. Technol., 26 (2003) 953-961.
5. S. Geng, G. Liu, W. Li, F. Cui, Molecular interaction of ctDNA and HSA with sulfadiazine sodium by multispectroscopic methods and molecular modeling, Luminescence, 28 (2013) 785-792.
6. X. Du, x. wang, J. Yao, H.Li, Y. Bao, J. Lan, Z. Zhao, W. Zhang, Study on the interaction between sulfamerazine and human serum albumin on molecular level using spectral analysis, Coll. Surf. A: Physicochem. Eng. Asp., 661 (2023) 130917.
7. M.D. Meti, Y. Xu, J. Xie, Y. Chen, Z. Wu, J. Liu, Q. Han, Z. He, Z. Hu, H. Xu, Multi-spectroscopic studies on the interaction between traditional Chinese herb, helicid with pepsin, Mol. Biol. Rep., 45 (2018) 1637-1646.

Table 6. The analytical results for determination of Smz in the presence pepsin.

Dynamic range of Smz (M)	$0.998\ x10^{-5}$ - $10.8\ x10^{-5}$
Standard deviation of the intercept (Sb)	0.0827
Limit of detection (LOD) (M)	$6.54x10^{-6}$
Limit of quantification (LOQ) (M)	$2.18x10^{-5}$
Slope of graph (m)	$3.79x10^4$
Replicate number (n)	5

8. W. Shi, Y. Wang, H. Zhang, Z. Liu, Z. Fei, Probing deep into the binding mechanisms of folic acid with a-amylase, pepsin and trypsin: An experimental and computational study, Food Chem., 226 (2017) 128-134.
9. J. Yu, X. Li, H. Liu, Y. Peng, X. Wang, Y. Xu, Interaction behavior between five flavonoids and pepsin: Spectroscopic analysis and molecular docking, J. Mol. Struct., 1223 (2021) 128978.
10. S. Lian, G. Wang, L. Zhou, D. Yanga, Fluorescence spectroscopic analysis on interaction of fleroxacin with pepsin, Luminescence, 28 (2013) 967-972.
11. G. Nan, P. Wang, J. Sun, J. Lv, M. Ding, L. Yang, Y. Li, G. Yang, Spectroscopy and molecular docking study on the interaction of daidzein and genistein with pepsin, Luminescence, 31 (2016) 1524-1531.
12. Z. Suo, X. Ma, Z. Meng, Q. Du, H. Li, Interaction between trelagliptin and pepsin through spectroscopy methods and molecular dynamics simulation, Spectrosc. Lett., 51 (2018) 332-339.
13. J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 3rd Edition, Springer, New York, USA, 2006.
14. J.R. Lakowicz, G. Weber, Quenching of fluorescence by oxygen. A probe for structural fluctuations in macromolecules, Biochemistry, 12 (1973) 4161-4170.
15. X.-Z. Feng, Z. Lin, L.-J. Yang, C. Whang, C.-l. Bai, Investigation of the interaction between acridine orange and bovine serum albumin, Talanta, 47 (1998) 1223-1229.
16. Y. Fang, H. Xu, L. Shen, F. Huang, S. Yibulayin, S. Huang, T. Shengli, Z. Hu, Z. He, F. Li, Y. Li, K. Zhou, K. Study on the mechanism of the interaction between acteoside and pepsin using spectroscopic techniques, Luminescence, 30 (2015) 859-866.
17. X. Li, M. Geng, Probing the binding of procyanidin B3 to trypsin and pepsin: A multi-technique approach, Int. J. Biol. Macromol., 85 (2016) 168-178.
18. P.D. Ross, S. Subramanian, Thermodynamics of protein association reactions: Forces contributing to stability, Biochemistry, 20 (1981) 3096-102.
19. H.J. Zeng, D. Yang, G.Z. Hua, R. Yang, L.B. Qu, Studies on the binding of pepsin with three pyrethroid insecticides by multi-spectroscopic approaches and molecular docking, J. Mol. Recognit., 29 (2016) 476-484.
20. L. Zhao, R. Guo, Q. Sun, J. Lan, H. Li, Interaction between azo dye Acid Red 14 and pepsin by multispectral methods and docking studies. Luminescence, 32 (2017) 1123-1130.
21. O.A. Chaves, B. Iglesias, C. Serpa, Biophysical characterization of the interaction between a transport human plasma protein and the 5,10,15,20-tetra(pyridine-4-yl) porphyrin, Molecules, 27 (2022) 5341.
22. X. Ma, J. He, Y. Huang, Y. Xiao, Q. Wang, H. Li, Investigation and comparison of the binding between tolvaptan and pepsin and trypsin: Multi-spectroscopic approaches and molecular docking, J. Mol. Recognit., 30 (2017) e2598.
23. J. Zhang, S. Zhuang, C. Tong, W. Liu, Probing the molecular interaction of triazole fungicides with human serum albumin by multispectroscopic techniques and molecular modeling, J. Agric. Food Chem., 61 (2013) 7203-7211.
24. X. Li, P. Li, Study on the interaction of β-carotene and astaxanthin with trypsin and pepsin by spectroscopic techniques, Luminescence, 31 (2016) 782-792.
25. M.A.G. Soares, P.A. de Aquino, T. Costa, C. Serpa, O.A. Chaves, Insights into the effect of glucose on the binding between human serum albumin and the nonsteroidal anti-inflammatory drug nimesulide, Int. J. Biol. Macromol., 265 (2024) 131148.
26. H. Sahoo, Förster resonance energy transfer-A spectroscopic nanoruler: Principle and applications (Review), J. Photochem. Photobiol. C: Photochem. Rev., 12 (2011) 20-30.
27. B.M. Aydın, M. Acar, M. Arık, Y. Onganer, The fluorescence resonance energy transfer between dye compounds in micellar media, Dyes Pigments, 81 (2009) 156-160.
28. J. Britton, E. Antunes, T. Nyokong, Fluorescence quenching and energy transfer in conjugates of quantum dots with zinc and indium tetraamino phthalocyanines, J. Photochem. Photobiol. A: Chem., 210 (2010) 1-7.
29. N. Janakiraman, A. Mohan, A. Kannan, G. Pennathur, Resonance energy transfer between protein and rhamnolipid capped ZnS quantum dots: Application in in-gel staining of proteins, Spectrochim. Acta Part A, 95 (2012) 478-482.
30. E. Gökoğlu, E. Yılmaz, Fluorescence interaction and determination of sulfathiazole with trypsin, J. Fluoresc., 24 (2014) 1439-1445.
31. C. Zhu, H. Zheng, D. Li, S. Li, J. Xu, Fluorescence quenching method for the determination of sodium dodecyl sulphate with near-infrared hydrophobic dye in the presence of Triton X-100, Spectrochim. Acta Part A, 60 (2004) 3173-3179.