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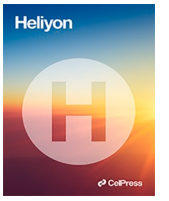

Research article

Interaction of the lysozyme with anticoagulant drug warfarin: Spectroscopic and computational analyses

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ABSTRACT

Warfarin is a cardiovascular drug, used to treat or inhibit the coagulation of the blood. In this paper, we have studied the interaction of lysozyme with warfarin using several experimental (fluorescence, UV–visible and circular dichroism spectroscopies) and computational (molecular docking, molecular dynamics and DFT) approaches. Experimental studies have suggested that there was a strong interaction between lysozyme and warfarin. Inner filter effect played important role in fluorescence experimental data which show that the emission intensity of lysozyme decreased on the addition of warfarin, however, after inner filter effect correction the actual outcome turned out be the fluorescence enhancement. The extent of binding, increased with temperature rise. The interaction was primarily taken place via the dominance of hydrophobic forces. Small amount of warfarin didn't influence the secondary structure of lysozyme; however, the higher concentration of warfarin caused a decrease in the helicity of the protein and a consequent partial unfolding. Molecular docking studies were also performed which revealed that warfarin binds with lysozyme mainly with hydrophobic forces along with a significant contribution of hydrogen bonding. The flexibility of warfarin played important role in fitting the molecule into the binding pocket of lysozyme. Frontier molecular orbitals of warfarin, using DFT, in free as well as complexed form have also been calculated and discussed. Molecular dynamics simulations of unbound and warfarin bound lysozyme reveal a stable complex with slightly higher RMSD values in the presence of warfarin. Despite slightly increased RMSF values, the overall compactness and folding properties remain consistent, emphasizing strong binding towards lysozyme through the results obtained from intermolecular hydrogen bonding analysis. Essential dynamics analysis suggests warfarin induces slight structural changes without significantly altering the conformation, additionally supported by SASA patterns. Aside from the examination of global and essential motion, the MM/PBSA-based analysis of binding free energy elucidates the significant binding of warfarin to lysozyme, indicating a binding free energy of −13.3471 kcal/mol.

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1. Introduction

Therapeutic agents or drugs are widely used to treat numerous diseases or illnesses. Most of the drugs have side effects and might show some toxicity ranging from extremely low to very high. The toxic or unfavorable effects are generally dose dependent [1]. To overcome these adverse effects the drug-delivery systems are designed to improve the therapeutic efficacy, release at the action site and reduce toxicity [2]. The role of a drug-delivery system is to give the stability of the therapeutic molecule, to transport the drug throughout the body and to release it at the target so that the maximum efficiency with least toxicity could be obtained [3].

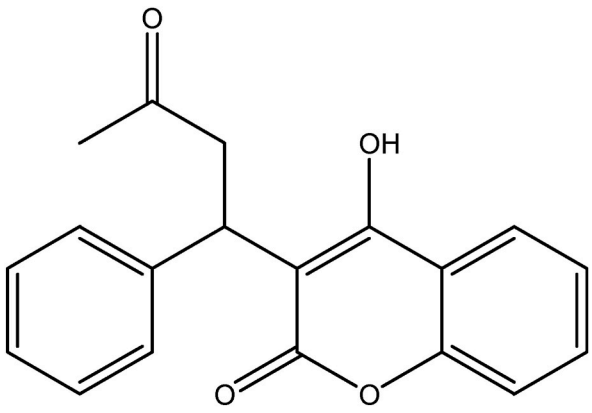
A diverse class of substances exist which can be used in fabricating the drug-delivery systems, for instance, polymers, dendrimers, liposomes, surfactant micelles, nanomaterials, proteins, etc. [3]. The use of proteins in the drug delivery systems is very common due to the extensive biological occurrence of these substances. Although a lot of proteins are also toxic and harmful [4–6] but only the protein that are compatible with biological systems can be used for such purposes. Proteins are large biomolecules which may have several binding pockets to accommodate the small molecules inside them due to which these can serve as the good carrier agents [7]. There are a large number of proteins, like, β-lactoglobin and α-lactoglobin, lysozyme, etc., which are being used in designing the drug delivery systems.

Understanding the interaction of these proteins with the drug is an important aspect of pharmaceutical chemistry as well as biochemistry. A stronger interaction of the drug and protein will help in slow release of the drug although it will increase the half-life of the drug in the body, in contrary, a weak interaction may result in the fast release in which the drug may not react to its site of action.

Lysozyme is a very important protein which is naturally finds in many secretions like, saliva, mucus, milk, etc. Small amount of lysozyme is also found in human blood. Lysozyme has been found to be a convenient substance and has shown many promising properties like antibacterial, anticancer, immunomodulatory, antifungal and antiviral activities [8]. There are many drug-delivery formulations which contain lysozyme as an important component [9,10].Lysozyme was used as a renal-selective drug delivery agent for transporting naproxen [11] and the angiotensin-converting enzyme inhibitor captopril [12]. Recently, the role of lysozyme as a potential nitric oxide cardiovascular drug carrier has been investigated [13]. The hydrogels obtained from the hen egg white lysozyme amyloids were also found to be the promising drug-delivery agents. The membrane disrupting function of lysozyme has been utilized to specifically target antimicrobial drug(s) to pathogen cells [14]. Along with the use of lysozyme alone, it was also used in combination of other substance(s) for the effective delivery of several therapeutic agents. Lysozyme microspheres incorporated with gold nanorods were also used for the encapsulation and release of an anticancer drug, 5-Fluorouracil [15]. Ultrasonically synthesized lysozyme microspheres have been found to be the prospective candidates to capture the drugs on their surfaces [16]. Nanogels obtained from the self-assembly of lysozyme and pectin were found to be the excellent carrier for the antitumor agent, methotrexate whose anticancer activity increased when encapsulated within the nanogels as compared to the free drug [17]. Likewise, the nanogels fabricated from the combinations of lysozyme and carboxymethylcellulose were very effective in releasing the drug methotrexate to improve its bioavailability. Biocompatible gold nanoparticle-loaded lysozyme–dextran nanogels have been synthesized and proposed to be a promising system for simultaneous drug delivery and biomedical imaging. Considering the exceptional properties of lysozyme in various applications, we have selected it as a model system to understand it binding with an important cardiovascular drug, warfarin.

Warfarin (Scheme 1; Drug Bank ID: DB00682) is a very common drug given to the patient suffering with blood clotting. Although, it is one of the most prescribed medicine to cure the blood clotting, it has several serious side effects which include severe bleeding [18], acute kidney injury [19], etc. Warfarin is either administrated orally or intravenously. To reduce its adverse effect of overdosing there is need to design optimal delivery systems that can improve the pharmacological action of warfarin [20]. The hydrophobic nature of warfarin is also an important aspect that is considered in designing the drug-delivery systems [21].

There are two well-known targets of warfarin which are vitamin K epoxide reductase complex subunit 1 and nuclear receptor subfamily 1 group I member 2 and a total of six cytochrome P450 enzymes, namely CYP2C9, CYP1A2, CYP2C19, CYP3A4, CYP2C8 and CYP2C18, are responsible for the warfarin metabolism [22–26]. Warfarin has been found to reversibly inhibits vitamin K epoxide



Scheme 1. Chemical structure of warfarin.

reductase by forming a T-shaped stacking interaction with residue Y139 of the proposed TYA warfarin-binding motif [27]. The interactions of warfarin with serum albumin have been studied a lot using several methods [28–33] which show that there was a strong binding between them. Warfarin is also very well-known site marker for the drug binding site 1 of human serum albumin [34] and this is the reason it has been used in numerous studies for competitive binding site analyses. Apart from the targets, metabolic enzymes (described above) and serum albumins, the interaction of warfarin was only seen with α1-acid glycoprotein [28,35] and to the best of our knowledge, there is no report on the binding of warfarin with proteins other than these two. Therefore, it would be interesting to see this much studied and important drug with other proteins to know that whether it interacts strongly with other proteins or not and what will be its effect on the structure of the proteins.

Therefore, we have selected lysozyme protein to see its interaction with warfarin and also to understand its potential as a carrier because it can bind a lot of molecules in its large cavity [36–42]. Additionally, the effect of warfarin on the structure of the protein which also occurs inside human body was also a topic of concern. For studying the interaction between lysozyme and warfarin, we

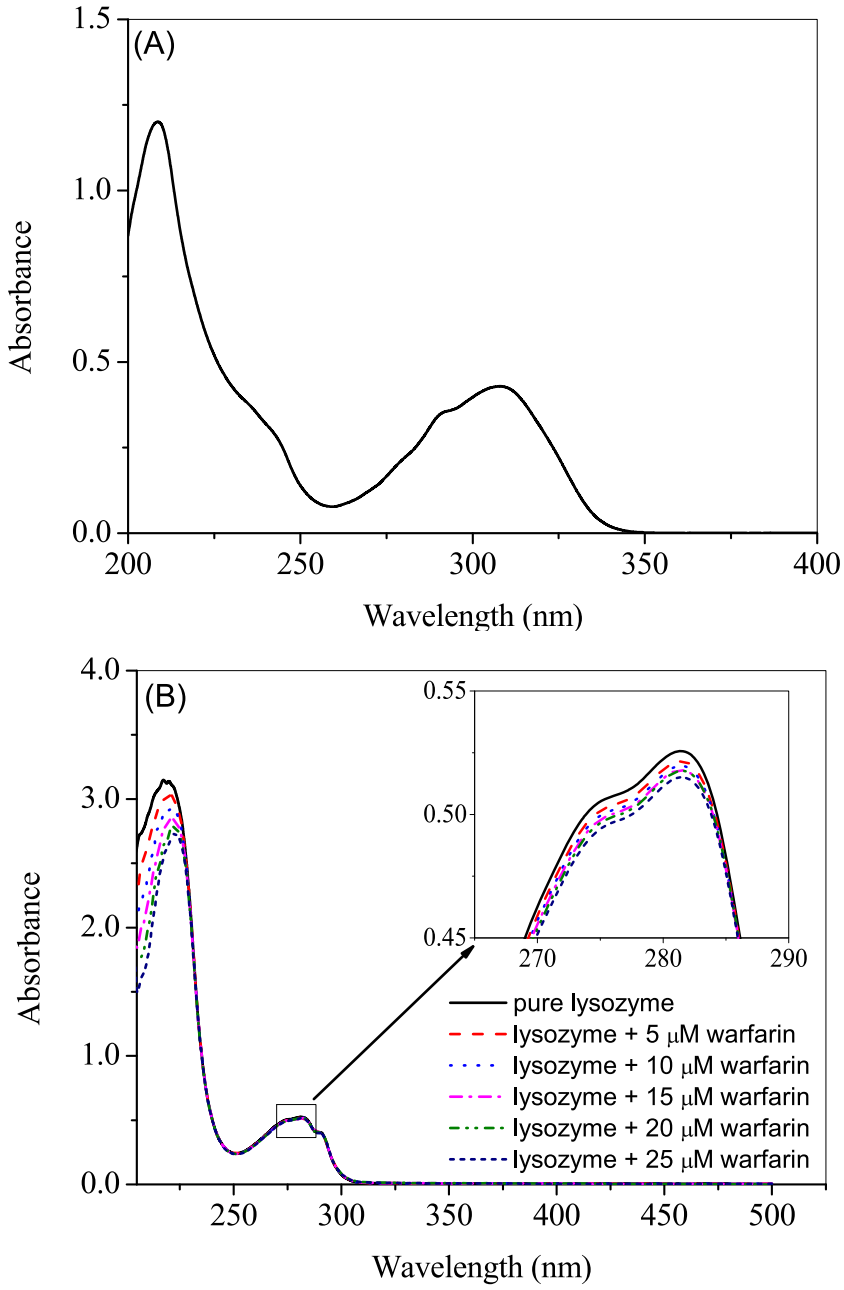


Fig. 1. (A) UV–visible spectra of 25 μM warfarin 25 °C. (B) Difference UV–visible spectra of lysozyme in presence of various concentrations of warfarin.

have employed several experimental as well as computational methods including molecular docking, Density Functional Theory (DFT) and molecular dynamics simulations.

2. Materials and methods

Lysozyme (≥98 %, L4919) and warfarin (≥98 %, A2250, CAS No.:81-81-2) were purchased from Sigma. UV–visible studies were performed on PerkinElmer Lambda 45 spectrophotometer within the range of 200 nm–500 nm as required. Intrinsic fluorescence measurements were performed on Hitachi F 7000 spectrofluorometer equipped with the programmable temperature controller. The excitation and emission slit widths were adjusted to 5 nm with a PMT voltage of 500 V. circular dichroism (CD) spectrophotometric recordings were obtained with a Jasco J-815 spectropolarimeter using a quartz cuvette of 0.1 nm. The studies were carried out in the 20 mM tris buffer of pH 7.4 and at 25 °C unless stated otherwise. Molecular docking simulations were performed using AutoDock Vina [43]. program and the DFT calculations were carried out using Orca program [44].

The energy gap between HOMO and LUMO was obtained using following equation:

$$\Delta E = E_{LUMO} - E_{HOMO} \tag{1}$$

The energy associated with HOMO and LUMO was then used to calculate the chemical potential (μ) and chemical hardness (η) of the system [45,46]:

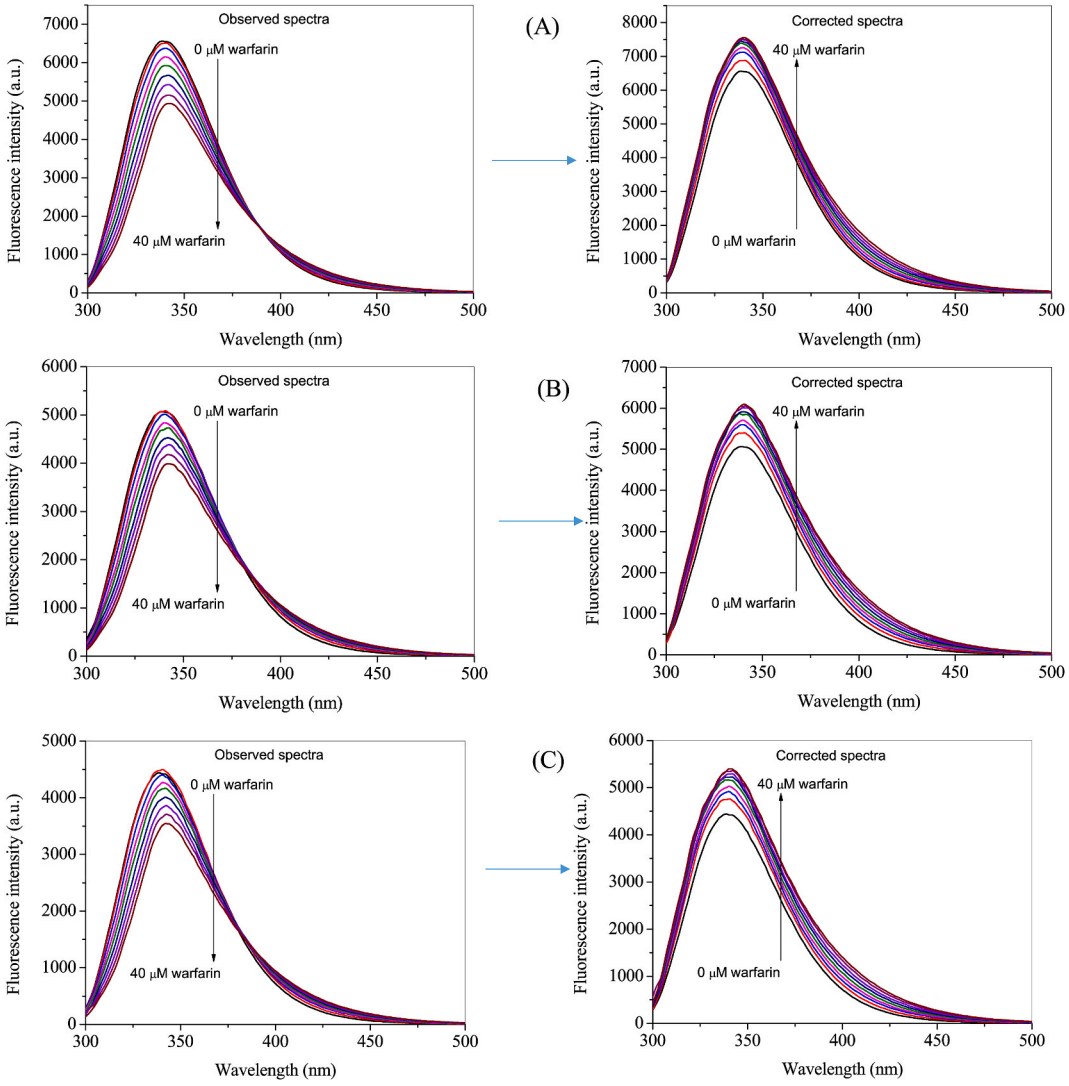


Fig. 2. Observed (left panel) and corrected (right panel) fluorescence emission spectra of lysozyme in presence of several concentrations of warfarin ranging from 0 to 40 μM with a constant increment of 5 μM at the excitation wavelength of 280 nm at 25 (A), 35 (B) and 45 °C (C).

$$\mu = \frac{E_{LUMO} + E_{HOMO}}{2} \tag{2}$$

$$\eta = \frac{E_{LUMO} - E_{HOMO}}{2} \tag{3}$$

Generally, the ionization potential (I) is defined as the $-E_{HOMO}$ value, while the electron affinity (A) is equal to $-E_{LUMO}$ [47]. Thus, the electronegativity (χ) and electrophilicity (ω) can be calculated according to the following equations [45,48,49]:

$$\chi = \frac{I + A}{2} \tag{4}$$

$$\omega = \frac{\mu^2}{2\eta} \tag{5}$$

Molecular dynamics simulations were performed using Gromacs Version 2021.3 program [50] and the detailed methodology is given in the supporting information. In addition to molecular dynamics simulations, the MM/PBSA based binding free energy analysis was performed between lysozyme and warfarin with the aid of g_mmpbsa package [51].

3. Results and discussions

3.1. UV–visible absorption spectroscopy

The UV–visible spectrum of pure warfarin is given in Fig. 1 (A). It shows two strong peaks at one at 208 nm and other 308 nm and two shoulders at around 235 nm and 290 nm. UV–visible spectroscopy is one of the basic techniques to study the binding between biomolecules with the ligands. The change in the spectrum of the biomolecule in presence of the ligand can give the primary idea of interaction between them. To get the clear picture of the change in the spectrum of complex, difference spectrum is calculated by subtracting the spectrum of same amount of ligand from the cumulative spectrum of the complex [52,53]. The difference spectra of lysozyme in presence of various concentrations of warfarin are shown in Fig. 1 (B). At 280 nm wavelength the absorption peak

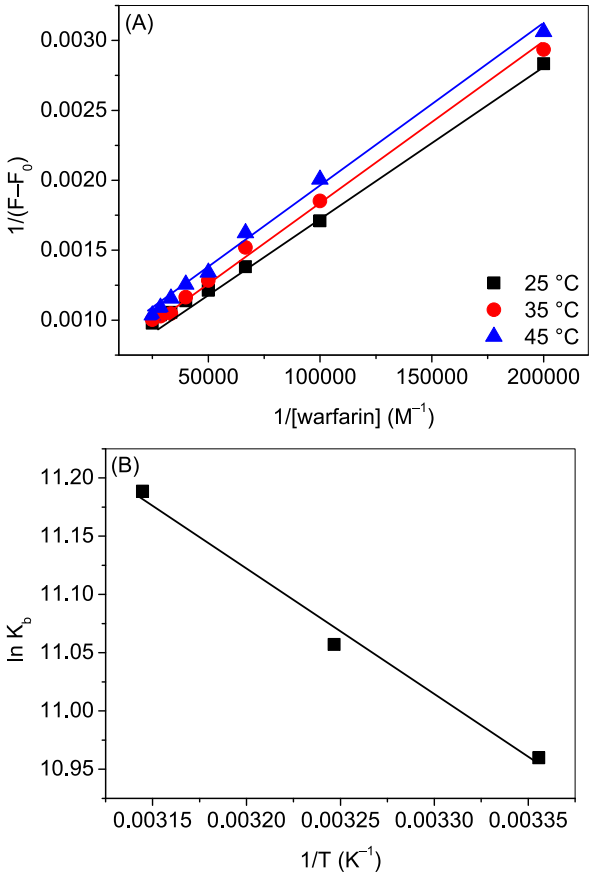


Fig. 3. (A) Plots of $1/(F–F_0)$ vs. $1/[warfarin]$. (B)Van 't Hoff plots for the interaction of lysozyme with warfarin.

appeared in the UV–visible spectrum protein is due to the absorption of aromatic amino acid residues (tryptophan, tyrosine and phenylalanine) and if the microenvironment of these amino acids changes after the incorporation of the ligand, the spectral property of these amino acids also changes. As can be seen in Fig. 1 (B) inset that there is a gradual decrease in the absorbance of lysozyme after the addition of the warfarin, which is a clear indication of the alterations in the microenvironment of the amino acids that suggest the complex formation between the drug and the protein [54].

3.2. Intrinsic fluorescence

All proteins that have the aromatic amino acids described above have the property to fluoresce intrinsically, therefore, the change in the microenvironment of these fluorophores could be studied with the help of fluorescence spectroscopy. Among these amino acids, tryptophan is the largest contributor of the fluorescence followed by tyrosine while phenylalanine has very weak fluorescence and can be regarded as negligible in comparison to the other two. When the protein is excited at 280 nm it gives a strong emission peak of tryptophan at 340 nm the small or negligible emission of tyrosine at 315 nm. It can be seen from Fig. 1 (A) that warfarin has a strong absorption at 280 nm and a small absorption at 340 nm, therefore, the fluorescence spectra should be corrected for inner filter effect. The observed and corrected (using Eq. S1) fluorescence spectra of lysozyme in presence of various concentrations of warfarin at 25 °C (A), 35 °C (B) and 45 °C (C) are given in Fig. 2. In the left panel observed spectra are given while corrected spectra are given in right panel. The gradual decrease in the fluorescence intensity of lysozyme on the addition of warfarin can be seen in the observed spectra at all temperatures while corrected spectra exhibited that the intensity of lysozyme increased on the addition of warfarin [55]. This type of behavior was also observed in other recent works on the interaction of lysozyme with some anti-inflammatory drugs [40,41].

3.3. Analysis of fluorescence data

The fluorescence enhancement data have been analyzed to obtain the binding constants (K_b) using the method developed by Bhattacharya et al. [56–58]:

$$\frac{1}{\Delta F}=\frac{1}{\Delta F_{max}}+\frac{1}{K_b[L]}\times\frac{1}{\Delta F_{max}} \tag{6}$$

where $\Delta F = F - F_0$, ΔF_{max} is the maximum change in the fluorescence; F_0 and F are the fluorescence intensities of lysozyme at 340 nm in absence and presence of warfarin; [L] represents the concentration of warfarin.The values of K_b have been obtained from the linear regression of Eq. (6) using the plots of $1/(F–F_0)$ vs. 1/[drug] (Fig. 3 (A)) given in Table 1. Warfarin show strong interaction with lysozyme which increases with rise of temperature. In comparison to the interactions of other drugs with lysozyme studied recently, like cefoperazone, ibuprofen, paracetamol, diclofenac, indomethacin, azithromycin and digitoxin [40–42,59], warfarin shows the stronger interactions than most of these drugs except digitoxin which has the association constants in same order of magnitude, whereas the association constants of antibiotic drug azithromycin [60] were 10 times more than the one of warfarin. Estimation of thermodynamic parameters.

Thermodynamic parameters like free energy change (ΔG), enthalpy change (ΔH) and entropy change (ΔS) give the idea about the feasibility of a process as well as the types of forces involved in that process. For instance, a negative value of ΔG corresponds to the spontaneity of the process and vice versa. Positive values of ΔH and ΔS are associated with the involvement or dominance of hydrophobic interactions whereas their negative values are connected to the presence of hydrogen bonding and van der Waals forces [61]. These thermodynamic parameters for an interaction could be evaluated using well-known Van 't Hoff equation (given in supporting info as Eq. S1 and Eq S2) by studying the interaction at several temperatures. The plot of ln $K_b$vs 1/T (Van 't Hoff plot) is given in Fig. 3 (B) and the values of thermodynamic parameters are given in Table 1. The negative values of ΔG shows that the interaction between warfarin and lysozyme is a spontaneous process. Table 1 shows that the values of both ΔH and ΔS are positive which is a clear indication of the involvement of hydrophobic interactions as major forces in the binding of warfarin and lysozyme. The dominance of the hydrophobic interactions could be justified on the basis of the structure of warfarin which is also hydrophobic in nature [21]. Further, warfarin also interacted with another important protein human serum albumin through the dominance of hydrophobic interactions [62]. Far-UV Circular dichroism spectroscopy.

3.4. Far-UV CD study

Secondary structure of a protein plays important role in determining the protein function. The secondary structure of proteins is

Table 1
Analyzed values of binding parameters and thermodynamic parameters for the interaction of lysozyme with warfarin at various temperatures.

Temperature (°C)	Binding parameters		Thermodynamic parameters		
	K_b (M^{-1})	R^2	ΔG^0 (kJ mol^{-1})	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})
25	5.7×10^4	0.9981	−27.1	9.0	121.2
35	6.3×10^4	0.9977	−28.4		
45	7.2×10^4	0.9956	−29.6		

determined by the pattern of hydrogen bonding between amide hydrogens and carbonyl oxygens of the neighboring amino acids. The secondary structure contains several structures like α-helices and β-sheets and turns.

Circular dichroism (CD) is an important technique to study the secondary as well as tertiary structural changes in a protein. The far UV CD spectrum of pure lysozyme, given in Fig. 4 (A), resembles the typical spectrum of α-helical protein which has two negative peaks at 208 nm and 222 nm. With the addition of 10 μM warfarin to the lysozyme no observable change in the helicity of the protein could be observed while increasing the concentration of warfarin caused a slight decrease in the α-helicity of lysozyme. The small decrease in the α-helicity of lysozyme in presence of warfarin could be regarded as the partial unfolding of the protein.

For getting clearer picture of the secondary structure of lysozyme in presence of warfarin, CD data have been converted to the mean residue ellipticity (MRE), which is the molar circular dichroism for individual protein residues, using the following equation:

$$\mathbf{MRE} = \frac{\theta_{\text{obs}}(m\ \text{deg})}{10 \times n \times C \times l} \tag{7}$$

where θ_{obs} is the observed ellipticity in millidegrees, C is the concentration of protein in molar, n is the number of amino acid residues and l is the length of the light path in centimeter. From the calculated MRE values at 222 nm, % α-helical contents could be calculated using the following equation by Chen et al. [63].

$$\%\alpha\ -\ \text{helix} = \left(\frac{\text{MRE 222 nm}\ \ -2,340}{30,300}\right) \times 100 \tag{8}$$

The calculated value of % α-helix of pure lysozyme was found to be 39.3 % which is very close to the reported value [64,65]. In presence of 10 μM warfarin, this value remains unchanged while 50 μM warfarin decreased the value slightly to 37.4 %. The values of % α-helix of lysozyme in absence and presence suggest that the lysozyme became partially unfolded in presence of higher concentration of warfarin.

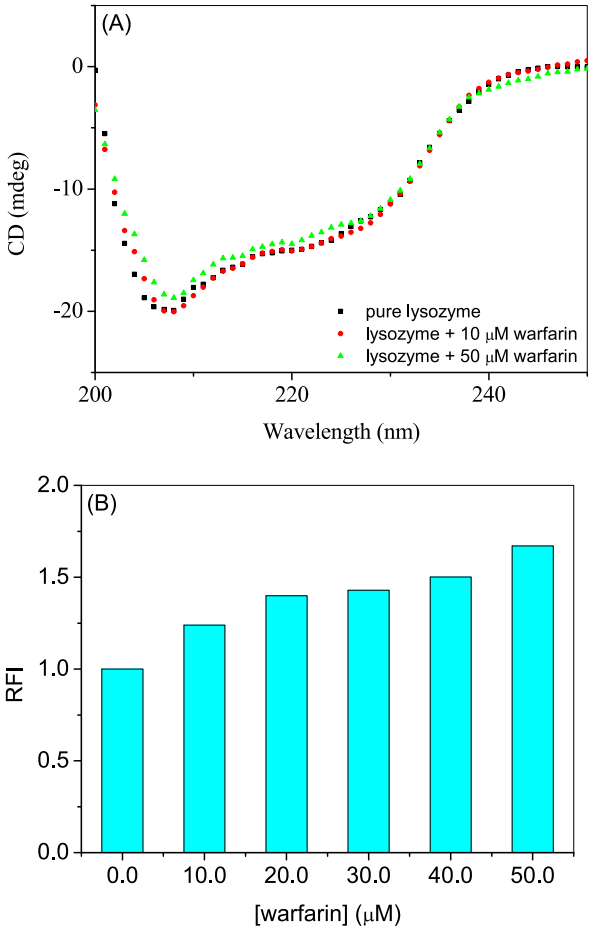


Fig. 4. Far-UV CD spectra of lysozyme in absence and presence of warfarin at 25 °C.

3.5. Resonance Rayleigh scattering (RRS) study

To understand the effect of warfarin on the size of lysozyme RRS studies have been performed. RRS is an important method to understand the relative size of the proteins during folding/unfolding due to their interaction [66,67]. In RRS the excitation and

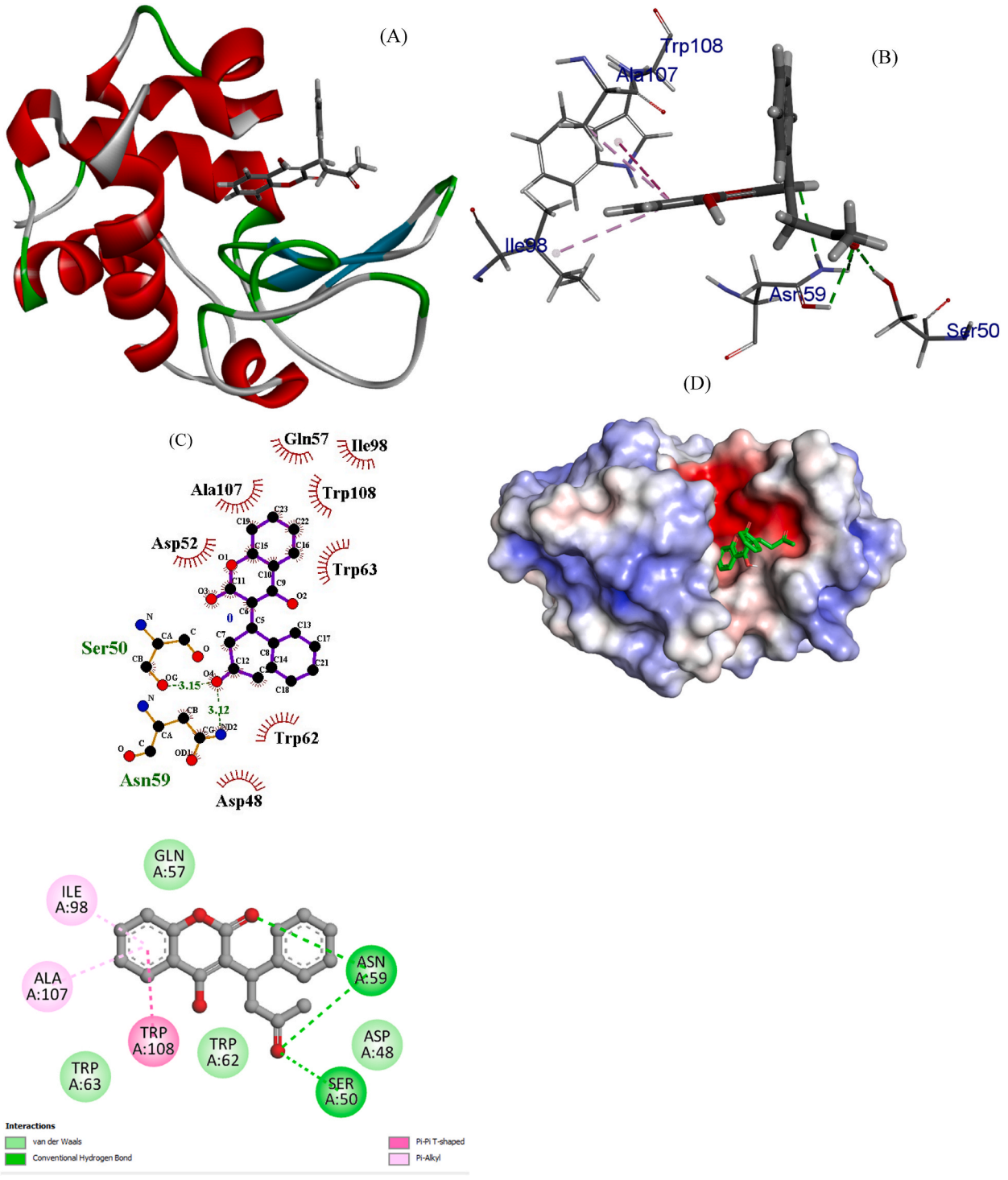


Fig. 5. (A) Docking poses of lysozyme with warfarin. (B) binding pocket of warfarin inside lysozyme showing interaction amino acids (C) Ligplot + diagram of lysozyme-warfarin interaction (D) Electrostatic surface potential map of lysozyme-warfarin complex.

emission wavelengths remain the same and a simultaneous excitation and emission at 350 nm provides the information of the relative size of the protein [68,69]. A huge increase in the scattering can be ascribed to the unfolding or aggregation of the proteins whereas a decrease can be due to the stabilization and compaction of the proteins [41]. Partial unfolding can also be judged by a slight increase in the RRS intensity [37]. The relative fluorescence intensities (RFIs) of RRS of lysozyme in absence and presence of warfarin are given in Fig. 4 (B). The small increase in the RFIs suggest that the size of protein is loosening slightly. These results are in excellent agreement with CD observations.

3.6. Molecular docking

The use of computational methods in drug designing has gained too much attention in recent years. Molecular docking is the basic tool in computer aided drug design to study.

the ligand-protein binding which can predict the potential binding modes of the ligand within the protein. In addition, it provides the information about types of interactions (hydrophobic, polar, electrostatic, etc.) involved in the binding. The docked pose of warfarin within lysozyme is given in Fig. 5 (A) warfarin binds inside the big hydrophobic cavity of lysozyme which is also the customary binding site for most of the ligands. The interacting amino acids inside the binding pocket of lysozyme are given in Fig. 5 (B) and the Ligplot diagram showing hydrophobic and hydrogen bonding interactions is displayed in Fig. 5 (C). Amino acids such as Ile98, Ala107 and Trp108 interacted through hydrophobic forces while hydrogen bonding interaction was reported with Ser50 and Asr59 residues. In addition to LigPlot, we have also used discovery studio program to visualize the various non-covalent interactions between warfarin and lysozyme. The electrostatic surface potential map of the lysozyme-warfarin complex (Fig. 5(D)) reveals that the ligand molecule binds to negatively charged or basic amino acid residues of the lysozyme.

3.7. Frontier molecular orbitals

To obtain more insight on the interaction between lysozyme and warfarin, frontier molecular orbitals (FMOs) were computed using DFT method. The aim of these calculations was to see the changes in the orientations of the bonds and atoms along with the electron densities of FMOs of warfarin before and after its binding with lysozyme to see influence of the active site of the lysozyme on these properties. The HOMOs and LUMOs diagrams of free and complexed warfarin (which was obtained from the docked complex) are given in Fig. 6. The IUPAC name of warfarin is 4-hydroxy-3-(3-oxo-1-phenylbutyl)-1-benzopyran-2-one, typically it is the derivative of 4-hydroxycoumarin substituted at position 3 by a 1-phenyl-3-oxo-1-butyl group. The HOMOs in the free warfarin are distributed all

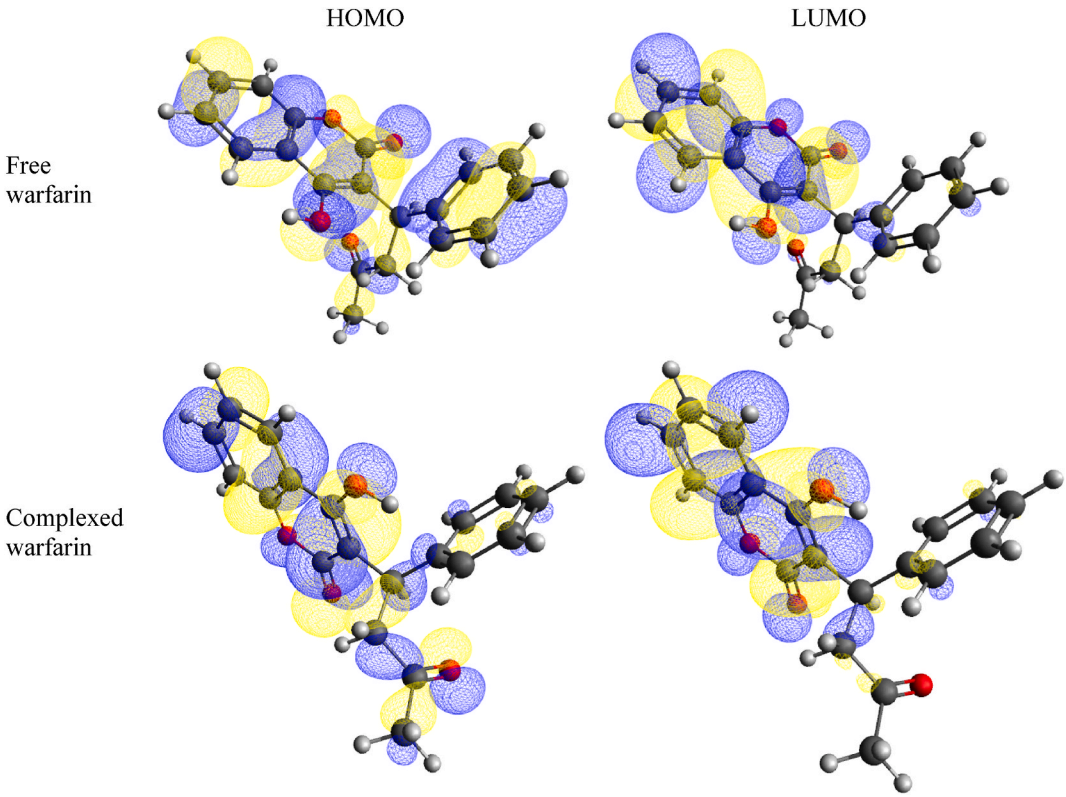


Fig. 6. Frontier molecular orbitals diagrams of free and complexed warfarin.

along the molecule whereas the LUMOs were distributed mainly on 4-hydroxycoumarin moiety. In complexed warfarin case a significant change in the orientation of the atoms can be seen and HOMOs, with slightly low electron density, were distributed all over the molecule except the phenyl group at position 3. The LUMOs were in the complexed form were also located at the same positions as existed in the free warfarin but with low electron density.

The values of energy parameters obtained from equations (1)–(5) are given in Table 2. A large energy gap (ΔE) between HOMOs and LUMOs is related to the stability of the molecule, therefore, it can be deduced that warfarin after binding with lysozyme occupy a conformation which is more stable than the free one which is accountable for the strong interaction between them. A slight increase of η value of the complexed warfarin is also an indication of the stable complex formation because a strong bonding leads to the increase in hardness [70]. The chemical potential of complexed warfarin decreases slightly in comparison to the free one whereas the electronegativity which is actually minus one times the chemical potential [71] increases slightly for the former. After the binding of warfarin with lysozyme, its ionization potential increases whereas electron affinity decreases. The electrophilicity index which is the ability of a molecule to add electron density [49] decreased for the complexed warfarin. From the changes in all quantum mechanical parameters obtained in current study it can be deduced that the interaction between warfarin and lysozyme causes the changes in the electronic structure of the former.

3.8. Molecular dynamics simulations

The molecular dynamics (MD) simulations were performed using GROMACS Version 2021.3 program [50] for comparing unbound lysozyme with the warfarin-bound lysozyme complex offer a detailed understanding of the protein's dynamic behavior and structural stability, flexibility, compactness, and folding properties when interacting with the ligand molecule, namely, warfarin. The Root Mean Square Deviation (RMSD) analysis (Fig. 7, Table 3) reveals that the warfarin-bound lysozyme complex experiences slightly higher RMSD values (range between 0.2 and 0.3 nm and average RMSD value: 0.23 nm) compared to the unbound form of lysozyme (range between 0.1 and 0.2 nm and average RMSD value: 0.12 nm) throughout MD simulations. Despite these higher values, the consistent RMSD pattern suggests a relatively stable complex, indicating that warfarin induces slight alterations in the structural stability of lysozyme. In the Root Mean Square Fluctuation (RMSF) (Fig. 7, Table 3) analysis, higher RMSF values, particularly a peak at 0.49 nm between amino acid residues of 70–75, are observed in the warfarin-bound lysozyme complex, possibly attributed to the influence of warfarin binding. Higher values of RMSF analysis suggest lower stability [72]. Notably, despite these fluctuations and structural deviations, the radius of gyration (Rg) analysis (Fig. 7, Table 3) shows a consistent pattern (average Rg value of unbound and warfarin bound lysozyme complex: 1.43 nm), suggesting that the overall compactness and folding properties of lysozyme remain largely unaffected by the binding of warfarin molecule.

The Solvent Accessible Surface Area (SASA) analysis (Fig. 7, Table 3) of warfarin bound lysozyme complex indicates an slightly altered SASA pattern, implying that the warfarin molecule's binding influences the lysozyme conformation [72]. This slight change in the SASA pattern and the SASA values suggests (average SASA value of warfarin bound lysozyme complex: 73.11 nm^2 and average SASA value of lysozyme: 72.38 nm^2) a potential modification in the exposure of lysozyme to the solvent due to the warfarin binding. Interestingly, despite these alterations, intermolecular hydrogen bond analysis (Fig. 8) shows the presence of a maximum of three hydrogen bonds in several time points of MD simulations, signifying a strong and stable interaction between warfarin and the lysozyme.

Beyond the analysis of the global dynamics, essential dynamics analysis using Principal Component Analysis (PCA) [73] (Fig. 8) was performed on unbound and warfarin-bound lysozyme complexes. PCA, a dimensionality reduction technique, reveals that warfarin bound lysozyme complex covered a larger conformational space region than the unbound form. The trace of covariance matrix values for unbound and warfarin-lysozyme complexes is 36.45 nm^2 and 49.56 nm^2, respectively. This result suggests that while warfarin induces changes in the protein, it does not largely alter the conformation of the protein. From the results obtained from secondary structural analysis (Fig. 9 and Table 4), it has been observed that there are minor differences between lysozyme and lysozyme-warfarin complex. For instance, slight variations in the percentages of residues in coil, bend, and 3_{10}-helix conformations exist, but the differences are generally small. The observed results from this analysis corraborate with CD spectroscopy result. In addition to analyzing global and essential dynamics, we have calculated the binding free energy between Lysozyme and warfarin using g_mmpbsa package [51], yielding a value of −13.3471 kcal/mol. A negative binding free energy suggests a strong binding affinity of the warfarin molecule to the Lysozyme protein target. The detailed results of the MM/PBSA-based binding free energy analysis was

Table 2
Frontier molecular orbitals and corresponding parameters obtained through DFT calculations.

	Free warfarin	Warfarin complexed with lysozyme
HOMO	−6.127	−6.244
LUMO	−1.679	−1.575
Energy gap (ΔE)	4.448	4.669
Chemical potential (μ)	−3.903	−3.9095
Global hardness (η)	2.224	2.3345
Ionization potential ($I = -E_{HOMO}$)	6.127	6.244
Electron affinity ($A = -E_{LUMO}$)	1.679	1.575
Electronegativity ($\chi = (I + A)/2$))	3.903	3.9095
Electrophilicity ($\omega = \mu^2/2\eta$))	3.424777203	3.273546852

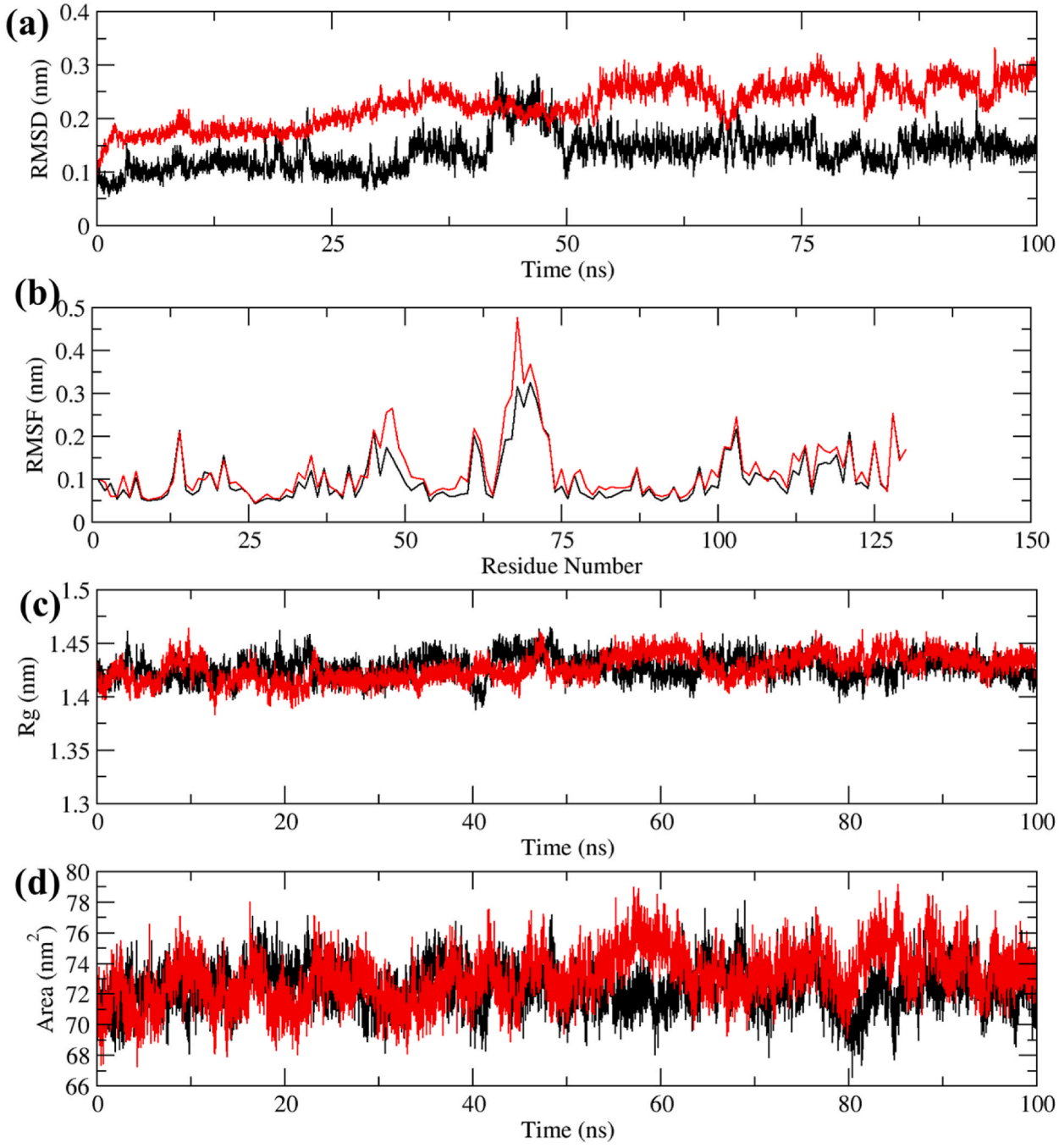


Fig. 7. Molecular dynamics simulation results of unbound and warfarin bound lysozyme complex. (A) Root Mean Square Deviation, (B) Root Mean Square Fluctuation, (C) Radius of Gyration, and (D) Solvent Accessible Surface Area (Color codes: Black: Lysozyme; Red: Lysozyme-warfarin complex.

given in Table 5 and Fig. 10.

4. Conclusions

we have studied the interaction between lysozyme and warfarin using spectroscopic and molecular docking methods. The binding between lysozyme and warfarin was a strong one that takes place mainly via hydrophobic interaction with additional support of hydrogen bonding. Warfarin also induced partial unfolding to the protein. The MD simulations provide a comprehensive

Table 3
Time averaged structural properties obtained from MD simulations of lysozyme and lysozyme-warfarin complex.

Structural parameters	Lysozyme_Native	Lysozyme_Warfarin
RMSD(nm)	0.14	0.23
RMSF(nm)	0.10	0.12
SASA(nm^2)	72.38	73.11
Rg (nm)	1.43	1.43

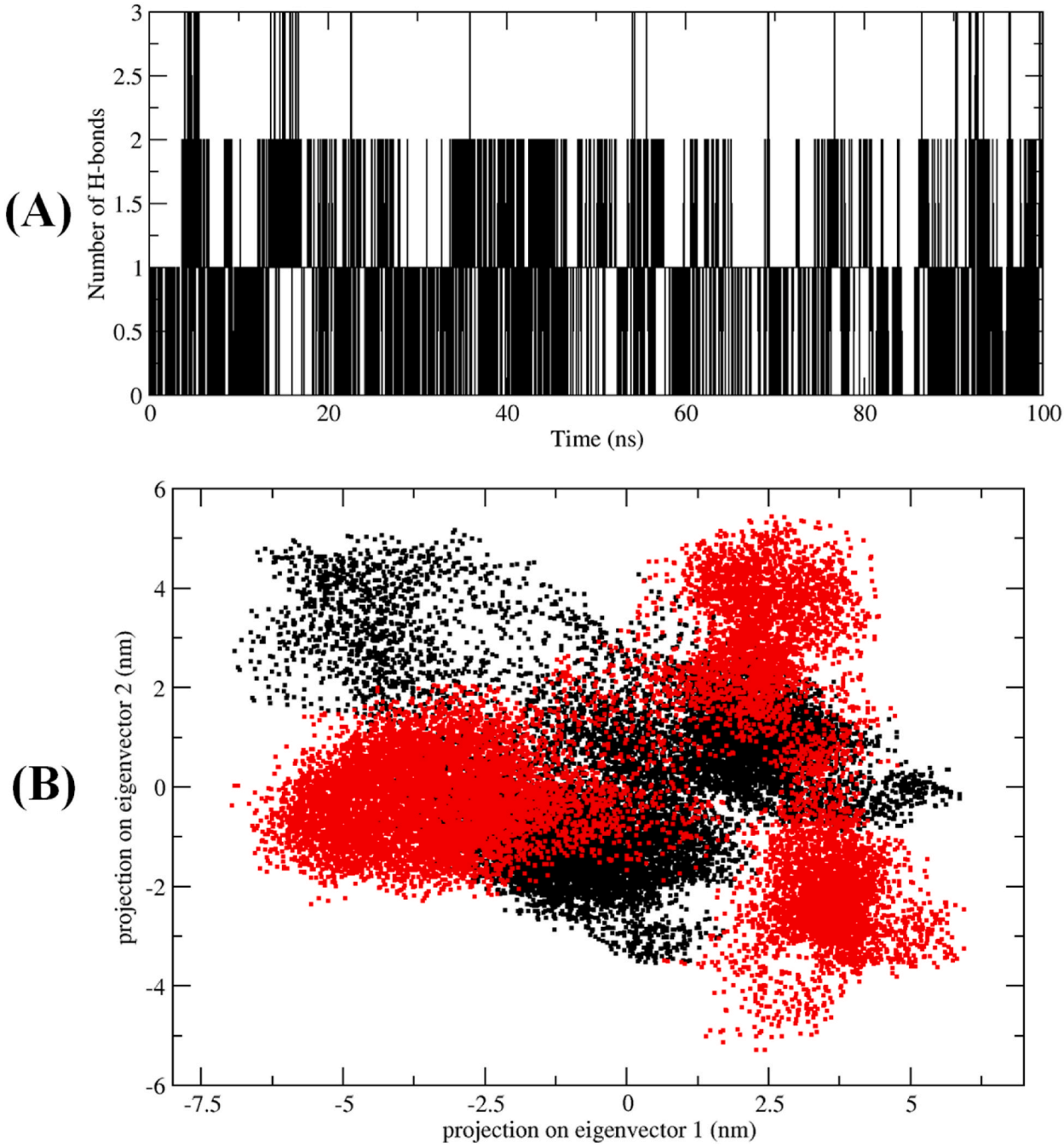


Fig. 8. ☹A) Intermolecular Hydrogen bonding and (b) Essential dynamics analysis of unbound and warfarin bound lysozyme complex (Color codes: Black: Lysozyme; Red: Lysozyme-warfarin complex; For HBonds, Black: Lysozyme-warfarin complex).

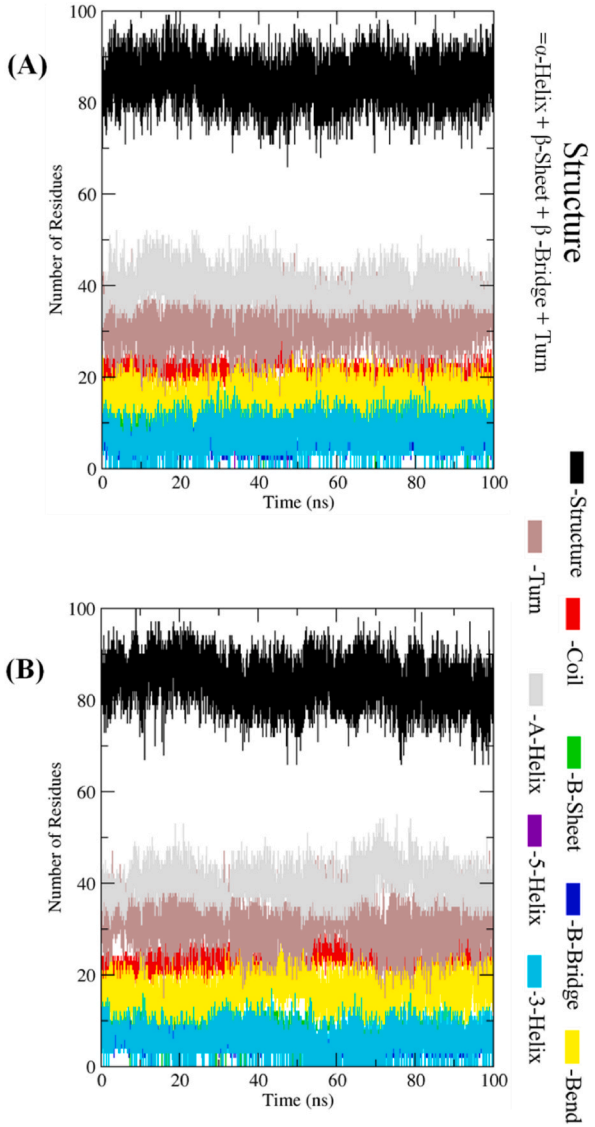


Fig. 9. Secondary structural analysis of (A) lysozyme and (B) lysozyme-warfarin complex.

Table 4
Secondary structural composition of lysozyme and lysozyme-warfarin complex.

System	Coil	β-sheet	β-bridge	Bend	Turn	α-helix	3_{10}-helix
Lysozyme	0.16	0.06	0.04	0.12	0.24	0.31	0.06
Lysozyme-warfarin complex	0.18	0.06	0.04	0.13	0.24	0.31	0.05

Table 5
MM/PBSA based binding free energy analysis between Lysozyme and warfarin.

Protein-Ligand complex	van der Waal energy	Electrostattic energy	SASA energy	Binding energy
Lysozyme_warfarin	−28.3189 kcal/mol	−4.3516	−3.3872 kcal/mol	−13.3471 kcal/mol

understanding of the dynamic behavior of lysozyme in the presence of warfarin molecule. Despite observable changes in RMSD, RMSF, and SASA values, lysozyme's overall stability, compactness, and folding properties are not largely affected. The consistent Rg pattern and the maintenance of the maximum of three hydrogen bonds suggest strong and stable binding of warfarin to the active site of

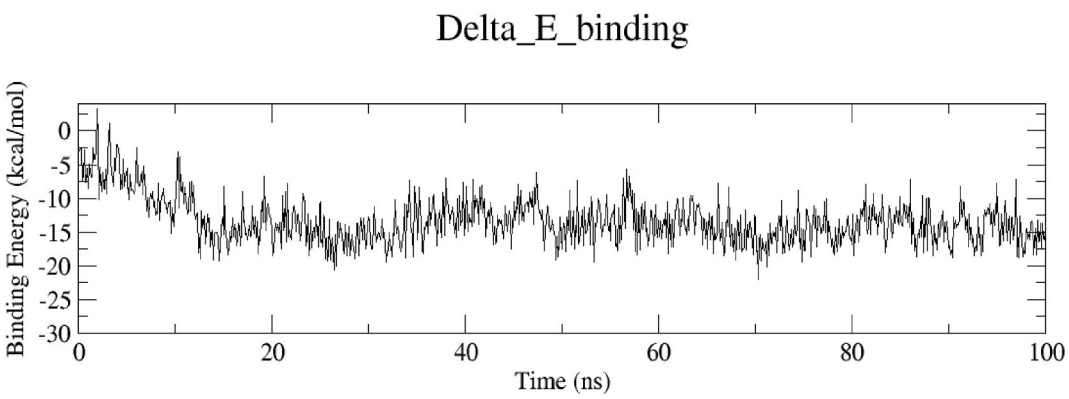


Fig. 10. MM/PBSA based binding freeenergy analysis of lysozyme-warfarin complex.

lysozyme. Since this work deduced that there is a possibility of binding between the lysozyme and warfarin, it could be helpful in designing the suitable carrier for warfarin or similar drugs using lysozyme. However, it requires more attention of the researchers for designing the suitable delivery systems using lysozyme as a carrier for the related drugs as a drug delivery system includes many components depending on various factors and this work is limited to the simple binding between the proposed carrier with a common drug.

CRediT authorship contribution statement

Mohd Sajid Ali: Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hamad A. Al-Lohedan:** Writing – review & editing, Writing – original draft, Resources, Project administration. **Rittik Bhati:** Writing – original draft, Visualization, Validation, Software, Resources, Formal analysis, Data curation. **Jayaraman Muthukumaran:** Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] J.J. Coleman, S.K. Pontefract, Adverse Drug Reactions, vol. 16, 2016, pp. 481–485.
[2] A. Odiba, C. Ukegbu, O. Anunobi, I. Chukwunonyelum, J. Esemonu, Making Drugs Safer: Improving Drug Delivery and Reducing the Side Effect of Drugs on the Human Biochemical System, vol. 5, 2016, pp. 183–194.
[3] T.C. Ezike, U.S. Okpala, U.L. Onoja, C.P. Nwike, E.C. Ezeako, O.J. Okpara, C.C. Okoroafor, S.C. Eze, O.L. Kalu, E.C. Odoh, U.G. Nwadike, J.O. Ogbodo, B. U. Umeh, E.C. Ossai, B.C. Nwanguma, Advances in drug delivery systems, challenges and future directions, Heliyon 9 (2023) e17488.
[4] L. Dang, E.J.M. Van Damme, Toxic proteins in plants, Phytochemistry 117 (2015) 51–64.
[5] R.N. Brey, N.J. Mantis, Chapter 55 - Ricin: A type II ribosome-inactivating protein, in: A.D.T. Barrett, L.R. Stanberry (Eds.), Vaccines for Biodefense and Emerging and Neglected Diseases, Academic Press, London, 2009, pp. 1099–1120.
[6] K. Urich, Toxic proteins and peptides, in: Comparative Animal Biochemistry, Springer Berlin Heidelberg, Berlin, Heidelberg, 1994, pp. 319–330.
[7] G. Klebe, Protein modeling and structure-based drug design, in: G. Klebe (Ed.), Drug Design: Methodology, Concepts, and Mode-Of-Action, Springer Berlin Heidelberg, Berlin, Heidelberg, 2013, pp. 429–448.
[8] L. Jiang, Y. Li, L. Wang, J. Guo, W. Liu, G. Meng, L. Zhang, M. Li, L. Cong, M. Sun, Recent insights into the prognostic and therapeutic applications of lysozymes, Front. Pharmacol. 12 (2021).
[9] P.D. Varma, Y.D. Shahu, S. Yende, S.K. Arora, P. Mishra, S. Jain, A. Mishra, A brief review on lysozyme's pharmacology and drug-carrying capacity, Res. J. Pharm. Technol. 15 (2022) 5886–5894.

[10] R.J. Kok, M. Haas, F. Moolenaar, D. de Zeeuw, D.K.F. Meijer, Drug delivery to the kidneys and the bladder with the low molecular weight protein lysozyme, Ren. Fail. 20 (1998) 211–217.
[11] E.J.F. Franssen, R.G.M. van Amsterdam, J. Visser, F. Moolenaar, D. de Zeeuw, D.K.F. Meijer, Low molecular weight proteins as carriers for renal drug targeting: naproxen–lysozyme, Pharmaceut. Res. 8 (1991) 1223–1230.
[12] J. Prakash, A.M.v. Loenen-Weemaes, M. Haas, J.H. Proost, D.K.F. Meijer, F. Moolenaar, K. Poelstra, R.J. Kok, RENAL-SELECTIVE DELIVERY AND ANGIOTENSIN-CONVERTING ENZYME INHIBITION BY SUBCUTANEOUSLY ADMINISTERED CAPTOPRIL-LYSOZYME, vol. 33, 2005, pp. 683–688.
[13] C.N. Nassab, M. Arooj, I.A. Shehadi, J.B.M. Parambath, S.M. Kanan, A.A. Mohamed, Lysozyme and human serum albumin proteins as potential nitric oxide cardiovascular drug carriers: theoretical and experimental investigation, J. Phys. Chem. B 125 (2021) 7750–7762.
[14] M.I. Hoq, K. Mitsuno, Y. Tsujino, T. Aoki, H.R. Ibrahim, Triclosan–lysozyme complex as novel antimicrobial macromolecule: a new potential of lysozyme as phenolic drug-targeting molecule, Int. J. Biol. Macromol. 42 (2008) 468–477.
[15] B. Bhargawa, V. Sharma, M.-R. Ganesh, F. Cavalieri, M. Ashokkumar, B. Neppolian, A. Sundaramurthy, Lysozyme microspheres incorporated with anisotropic gold nanorods for ultrasound activated drug delivery, Ultrason. Sonochem. 86 (2022) 106016.
[16] F. Cavalieri, M. Ashokkumar, F. Grieser, F. Caruso, Ultrasonic synthesis of stable, functional lysozyme microbubbles, Langmuir 24 (2008) 10078–10083.
[17] L. Lin, W. Xu, H. Liang, L. He, S. Liu, Y. Li, B. Li, Y. Chen, Construction of pH-sensitive lysozyme/pectin nanogel for tumor methotrexate delivery, Colloids Surf. B Biointerfaces 126 (2015) 459–466.
[18] D.K. Wysowski, P. Nourjah, L. Swartz, Bleeding complications with warfarin use: a prevalent adverse effect resulting in regulatory action, Arch. Intern. Med. 167 (2007) 1414–1419.
[19] S. Mendonca, D. Gupta, A. Valsan, R. Tewari, Warfarin related acute kidney injury: a case report, Indian J. Nephrol. 27 (2017) 78–80.
[20] S.E. Dolinina, V.E. Parfenyuk, Sol-gel derived warfarin – silica composites for controlled drug release, Curr. Drug Deliv. 14 (2017) 734–740.
[21] I. Msolli, R. Belibel, F. Chaubet, R.M. Maaroufi, C. Barbaud, Synthesis of nanoparticles based on PDMMLA derivative copolymers and study of warfarin encapsulation and controlled release, RSC Adv. 7 (2017) 6704–6711.
[22] Y. Zhu, M. Shennan, K.K. Reynolds, N.A. Johnson, M.R. Herrnberger, R. Valdes Jr., M.W. Linder, Estimation of warfarin maintenance dose based on VKORC1 (−1639 G>A) and CYP2C9 genotypes, Clin. Chem. 53 (2007) 1199–1205.
[23] A. Rulcova, I. Prokopova, L. Krausova, M. Bitman, R. Vrzal, Z. Dvorak, J. Blahos, P. Pavek, Stereoselective interactions of warfarin enantiomers with the pregnane X nuclear receptor in gene regulation of major drug-metabolizing cytochrome P450 enzymes, J. Thromb. Haemostasis 8 (2010) 2708–2717.
[24] A.K. Daly, A.E. Rettie, D.M. Fowler, J.O. Miners, Pharmacogenomics of CYP2C9: Functional and Clinical Considerations, vol. 8, 2018, p. 1.
[25] S.F. Zhou, Z.W. Zhou, L.P. Yang, J.P. Cai, Substrates, inducers, inhibitors and structure-activity relationships of human cytochrome P450 2C9 and implications in drug development, Curr. Med. Chem. 16 (2009) 3480–3675.
[26] M. Ufer, Comparative pharmacokinetics of vitamin K antagonists, Clin. Pharmacokinet. 44 (2005) 1227–1246.
[27] S. Wu, X. Chen, D.-Y. Jin, D.W. Stafford, L.G. Pedersen, J.-K. Tie, Warfarin and vitamin K epoxide reductase: a molecular accounting for observed inhibition, Blood 132 (2018) 647–657.
[28] M. Otagiri, T. Maruyama, T. Imai, A. Suenaga, Y. Imamura, A Comparative Study of the Interaction of Warfarin with Human α1-acid Glycoprotein and Human Albumin, vol. 39, 1987, pp. 416–420.
[29] Q. Li, W.-y. Yang, L.-l. Qu, H.-Y. Qi, Y. Huang, Z. Zhang, Interaction of warfarin with human serum albumin and effect of ferulic acid on the binding, Journal of Spectroscopy 2014 (2014) 834501.
[30] J. Wilting, W.F. van der Giesen, L.H. Janssen, M.M. Weideman, M. Otagiri, J.H. Perrin, The effect of albumin conformation on the binding of warfarin to human serum albumin. The dependence of the binding of warfarin to human serum albumin on the hydrogen, calcium, and chloride ion concentrations as studied by circular dichroism, fluorescence, and equilibrium dialysis, J. Biol. Chem. 255 (1980) 3032–3037.
[31] R.A. O'Reilly, Studies on the coumarin anticoagulant drugs: interaction of human plasma albumin and warfarin sodium, J. Clin. Investig. 46 (1967) 829–837.
[32] F.G. Larsen, C.G. Larsen, P. Jakobsen, R. Brodersen, Interaction of warfarin with human serum albumin, A stoichiometric description 27 (1985) 263–270.
[33] T.C. Pinkerton, K.A. Koeplinger, Determination of warfarin-human serum albumin protein binding parameters by an improved Hummel-Dreyer high-performance liquid chromatographic method using internal surface reversed-phase columns, Anal. Chem. 62 (1990) 2114–2122.
[34] J. Ghuman, P.A. Zunszain, I. Petitpas, A.A. Bhattacharya, M. Otagiri, S. Curry, Structural basis of the drug-binding specificity of human serum albumin, J. Mol. Biol. 353 (2005) 38–52.
[35] S. Urien, F. Brée, B. Testa, J.P. Tillement, pH-dependence of warfarin binding to α1-acid glycoprotein (orosomucoid), Biochem. J. 289 (1993) 767–770.
[36] M.S. Ali, H.A. Al-Lohedan, Interaction of biocompatible sugar based surfactant n-dodecyl beta-D-maltoside with lysozyme, J. Mol. Liq. 209 (2015) 662–668.
[37] M.S. Ali, H.A. Al-Lohedan, Spectroscopic and molecular docking investigation on the noncovalent interaction of lysozyme with saffron constituent "Safranal", ACS Omega 5 (2020) 9131–9141.
[38] M.S. Ali, M. Waseem, N. Subbarao, H.A. Al-Lohedan, Dynamic interaction between lysozyme and ceftazidime: experimental and molecular simulation approaches, J. Mol. Liq. (2021) 115412.
[39] M.S. Ali, M. Waseem, N. Subbarao, H.A. Al-Lohedan, Noncovalent molecular interactions between antineoplastic drug gemcitabine and a carrier protein identified through spectroscopic and in silico methods, Int. J. Biol. Macromol. 182 (2021) 993–1002.
[40] M. Sajid Ali, H.A. Al-Lohedan, Interactions of lysozyme with hydrophobic and hydrophilic non-steroidal anti-inflammatory drugs: spectroscopic and molecular docking analyses, J. Mol. Liq. (2023) 123020.
[41] M.S. Ali, H.A. Al-Lohedan, Spectroscopic and molecular docking studies of the interaction of non-steroidal anti-inflammatory drugs with a carrier protein: an interesting case of inner filter effect and intensity enhancement in protein fluorescence, J. Fluoresc. (2023).
[42] M.S. Ali, M. Waseem, N. Subbarao, A.N. Alahamed, H.A. Al-Lohedan, Probing the interaction of cephalosporin antibiotic "cefoperazone" with lysozyme using spectroscopic and in silico methods: effect of paracetamol on binding, Int. J. Biol. Macromol. 252 (2023) 126568.
[43] O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31 (2010) 455–461.
[44] F. Neese, F. Wennmohs, U. Becker, C. Riplinger, The ORCA quantum chemistry program package, J. Chem. Phys. 152 (2020).
[45] R.G. Parr, R.A. Donnelly, M. Levy, W.E. Palke, Electronegativity: the density functional viewpoint, J. Chem. Phys. 68 (1978) 3801–3807.
[46] R.G. Pearson, Maximum chemical and physical hardness, J. Chem. Educ. 76 (1999) 267.
[47] T. Koopmans, Über die Zuordnung von Wellenfunktionen und Eigenwerten zu den Einzelnen Elektronen Eines Atoms, Physica 1 (1934) 104–113.
[48] R.S. Mulliken, A new electroaffinity scale; together with data on valence states and on valence ionization potentials and electron affinities, J. Chem. Phys. 2 (1934) 782–793.
[49] R.G. Parr, L.v. Szentpály, S. Liu, Electrophilicity index, J. Am. Chem. Soc. 121 (1999) 1922–1924.
[50] D. Van Der Spoel, E. Lindahl, B. Hess, G. Groenhof, A.E. Mark, H.J.C. Berendsen, GROMACS: Fast, Flexible, and Free, vol. 26, 2005, pp. 1701–1718.
[51] R. Kumari, R. Kumar, A. Lynn, g_mmpbsa—a GROMACS tool for high-throughput MM-PBSA calculations, J. Chem. Inf. Model. 54 (2014) 1951–1962.
[52] M.S. Ali, M. Amina, H.A. Al-Lohedan, N.M. Al Musayeib, Human serum albumin binding to the biologically active labdane diterpene "leoheterin": spectroscopic and in silico analysis, J. Photochem. Photobiol. B Biol. 182 (2018) 9–17.
[53] M.S. Ali, J. Muthukumaran, M. Jain, T. Santos-Silva, H.A. Al-Lohedan, N.S. Al-Shuail, Molecular interactions of cefoperazone with bovine serum albumin: extensive experimental and computational investigations, J. Mol. Liq. 337 (2021) 116354.
[54] B.A. Shirley, Protein Stability and Folding: Theory and Practice, Humana Press, 1995.
[55] L.Z. Zhao, Y.S. Zhao, H.H. Teng, S.Y. Shi, B.X. Ren, Spectroscopic investigation on the interaction of titanate nanotubes with bovine serum albumin, J. Appl. Spectrosc. 81 (2014) 719–724.
[56] J. Bhattacharyya, M. Bhattacharyya, A.S. Chakrabarty, U. Chaudhuri, R.K. Poddar, Interaction of chlorpromazine with myoglobin and hemoglobin - a comparative-study, Biochem. Pharmacol. 47 (1994) 2049–2053.

[57] S. Valdebenito, G. Zapata-Torres, E. Lemp, A.L. Zanocco, Experimental and theoretical studies of the photophysics of 7-amino-3-phenyl-2H-benzo[b] [1,4] oxazin-2-one in homogeneous solvents and beta-cyclodextrin aqueous solutions, Afinidad 72 (2015) 53–59.
[58] W.Y. He, Y. Li, H.Z. Si, Y.M. Dong, F.L. Sheng, X.J. Yao, Z.D. Hu, Molecular modeling and spectroscopic studies on the binding of guaiacol to human serum albumin, J. Photochem. Photobiol., A 182 (2006) 158–167.
[59] M.S. Ali, H.A. Al-Lohedan, R. Bhati, J. Muthukumaran, Probing the Interaction of Lysozyme with Cardiac Glycoside Digitoxin: Experimental and in Silico Analyses, 2023, p. 10.
[60] S. Parveen, M.S. Ali, H.A. Al-Lohedan, N. Hoti, S. Tabassum, Molecular interaction of lysozyme with therapeutic drug azithromycin: effect of sodium dodecyl sulfate on binding profile, Int. J. Biol. Macromol. 242 (2023).
[61] P.D. Ross, S. Subramanian, Thermodynamics of protein association reactions: forces contributing to stability, Biochemistry 20 (1981) 3096–3102.
[62] I. Petitpas, A.A. Bhattacharya, S. Twine, M. East, S. Curry, Crystal structure analysis of warfarin binding to human serum albumin: anatomy of drug site I, J. Biol. Chem. 276 (2001) 22804–22809.
[63] Y.-H. Chen, J.T. Yang, H.M. Martinez, Determination of the secondary structures of proteins by circular dichroism and optical rotatory dispersion, Biochemistry 11 (1972) 4120–4131.
[64] G. Anderle, R. Mendelsohn, Thermal denaturation of globular proteins. Fourier transform-infrared studies of the amide III spectral region, Biophys. J. 52 (1987) 69–74.
[65] S. Cai, B.R. Singh, Identification of β-turn and random coil amide III infrared bands for secondary structure estimation of proteins, Biophys. Chem. 80 (1999) 7–20.
[66] C.Q. Ma, K.A. Li, S.Y. Tong, Microdetermination of proteins by resonance light scattering spectroscopy with bromophenol blue, Anal. Biochem. 239 (1996) 86–91.
[67] C.Q. Ma, K.A. Li, S.Y. Tong, Microdetermination of proteins by resonance light scattering spectroscopy with tetraiodo phenol sulfonaphthalein, Fresen. J. Anal. Chem. 357 (1997) 915–920.
[68] M.S. Ali, H.A. Al-Lohedan, M.Z.A. Rafiquee, A.M. Atta, A.O. Ezzat, Spectroscopic studies on the interaction between novel polyvinylthiol-functionalized silver nanoparticles with lysozyme, Spectrochim. Acta Mol. Biomol. Spectrosc. 135 (2015) 147–152.
[69] M.S. Ali, H.A. Al-Lohedan, Multi-technique approach on the interaction between sugar-based surfactant n-dodecyl β-d-maltoside and bovine serum albumin, J. Lumin. 169 (2016) 35–42.
[70] R.G. Pearson, Chemical hardness and density functional theory, J. Chem. Sci. 117 (2005) 369–377.
[71] R.G. Parr, R.G. Pearson, Absolute hardness: companion parameter to absolute electronegativity, J. Am. Chem. Soc. 105 (1983) 7512–7516.
[72] D. Jones, J.E. Allen, Y. Yang, W.F. Drew Bennett, M. Gokhale, N. Moshiri, T.S. Rosing, Accelerators for classical molecular dynamics simulations of biomolecules, J. Chem. Theor. Comput. 18 (2022) 4047–4069.
[73] C.C. David, D.J. Jacobs, Principal component analysis: a method for determining the essential dynamics of proteins, in: D.R. Livesay (Ed.), Protein Dynamics: Methods and Protocols, Humana Press, Totowa, NJ, 2014, pp. 193–226.