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Research Article

Modulation of venlafaxine partitioning into zwitterionic and charged lipid bilayers via hydrophobic and electrostatic interactions

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ABSTRACT

The ability of a therapeutic agent to cross lipid membranes is crucial in terms of its pharmacodynamic properties. In this study, the partitioning of venlafaxine, a recently introduced antidepressant in the class of serotonin and norepinephrine reuptake inhibitors (SNRIs), into large unilamellar vesicles (LUVs) composed of zwitterionic 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and negatively charged 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG) was investigated using second derivative spectrophotometry. The partition coefficients of venlafaxine into DOPC and DOPG LUVs were determined at various temperatures (25, 37, and 45 °C) and pH values (7.4 and 9.5). The results indicate a positive correlation between temperature and the extent of venlafaxine partitioning into both zwitterionic and charged lipid bilayers; furthermore, the drug preferentially partitions into the zwitterionic lipid bilayer at all investigated temperatures. Thermodynamic analysis further reveals that the membrane partitioning of venlafaxine aligns with the classical hydrophobic effect. Varying the proportion of neutral versus charged venlafaxine further indicates that both electrostatic and hydrophobic interactions modulate the partitioning of the drug into the zwitterionic neutral bilayer. In the case of the negatively charged bilayer, electrostatic interactions are predominant over hydrophobic ones in modulating the partitioning of venlafaxine. These findings provide further insights into SNRI–lipid membrane interactions, potentially leading to the development of more effective antidepressants.

Key words: venlafaxine, serotonin and norepinephrine reuptake inhibitors, large unilamellar vesicles, partition coefficient, drug–lipid interaction

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INTRODUCTION

Most drugs exert pharmacological effects by directly binding to proteins or enzymes, with approximately half of the drugs in current use targeting integral membrane proteins [1]. Such drugs not only bind to the protein target but also exhibit strong interactions with lipid membranes, which can significantly affect their metabolism and bioavailability [1,2].

Serotonin and norepinephrine reuptake inhibitors (SNRIs) are among the more recently developed classes of antidepressant drugs, aiming to enhance treatment efficacy and provide novel therapeutic options for the treatment of mental disorders [3–5]. SNRIs selectively inhibit the reuptake of two neurotransmitters, serotonin (5-hydroxytryptamine [5-HT]) and noradrenaline (norepinephrine), at the nerve synapse [4]. Hence, this inhibition leads to an increased concentration of neurotransmitters, facilitating neurotransmission to regulate nervous system activity, including mood, cognition, and several physiological functions [5]. Venlafaxine, commercially known as Effexor, is a bicyclic phenylethylamine derivative belonging to the SNRI class and was initially launched in the United States market in 1993 for the pharmacological treatment of various psychiatric disorders, including major depressive disorder [5]. While not all research efforts acknowledge the effectiveness of venlafaxine compared to selective serotonin reuptake inhibitors (SSRIs) in achieving the remission of depressive symptoms, venlafaxine is considered a viable option for non-remitting patients. Additionally, studies have suggested that venlafaxine exhibits a favorable tolerability profile in comparison to other classes of antidepressant drugs [5]. Venlafaxine mainly targets the serotonin and noradrenalin transporters located on the cell membranes of neurons, resulting in the inhibition of serotonin and noradrenaline reuptake from the synaptic cleft [4,5]. For optimal efficacy, venlafaxine must successfully interact with lipid membranes and cross the blood–brain barrier to reach the neurons within the central nervous system.

The interactions between several classes of antidepressants and lipid membranes have been extensively characterized to reveal the role of lipid membranes in

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the therapeutic activities of antidepressants. A study on the interaction of monoamine oxidase inhibitors with dipalmitoylphosphatidylcholine vesicles at varying temperatures with different drug molecule substituents revealed that lipophilic drug molecules act as spacers within the lipid bilayer, disrupting its ordered structure, lowering the phase transition temperature, and enhancing membrane fluidity[6]. Similarly, tricyclic antidepressants exhibit preferential binding to the hydrophobic core of phosphatidylcholine and phosphatidylethanolamine via van der Waals forces and hydrophobic interactions[7]. Additionally, Coulombic or ion-induced dipole interactions contribute to the incorporation of both charged and uncharged tricyclic antidepressants into the phosphatidylserine bilayer. Similarly, the electrostatic interaction between cationic imipramine and anionic dipalmitoylphosphatidylglycerol has been suggested to promote the partitioning of antidepressants into the brain cell membrane via membrane thickening induced by the aggregation of the drugs at the membrane interface[8]. Another study revealed that fluoxetine, a common SSRI, exhibits a strong interaction with the anionic dipalmitoylphosphatidylglycerol membrane at neutral pH due to the electrostatic attraction between the drug and the lipid head group[9]. More recent evidence has suggested that the electrostatic interaction between fluoxetine and the dipalmitoylphosphatidylglycerol bilayer results in the accumulation of the drug within the lipid head group domain[10]. Current literature on antidepressant–lipid membrane interactions highlights the partitioning degree of drugs, the subsequent biophysical changes of the lipid bilayers, and the possible location of the drug within the lipid bilayers. Such insights are essential for a comprehensive understanding of the pharmacology of venlafaxine.

In this work, second derivative spectrophotometry is used to characterize the partitioning of venlafaxine into both zwitterionic 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and anionic 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG) lipid bilayers at physiological pH (7.4) and temperatures of 25, 37, and 45 °C. Additionally, the partitioning of venlafaxine into the DOPG lipid bilayer is also determined at pH 9.5 and 25 °C. The molecular interactions and thermodynamic profiles associated with the partitioning of venlafaxine into the zwitterionic and charged lipid bilayers are then elucidated. The chemical structures of venlafaxine, DOPC, and DOPG are depicted in Figure 1.

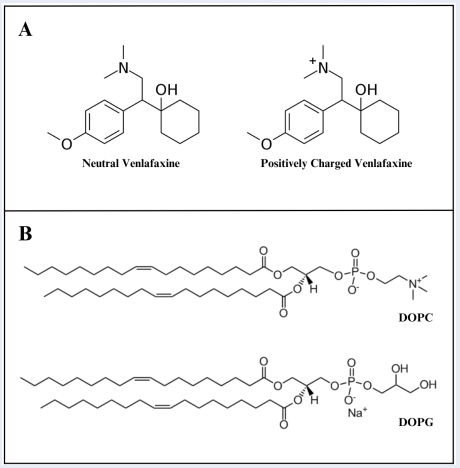

Figure 1: (A) Chemical structures of charged and neutral venlafaxine. (B) Chemical structure of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG).

MATERIALS AND METHODS

Materials

Venlafaxine hydrochloride and HEPES buffer, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, were purchased from Sigma Aldrich (USA). DOPC and DOPG were obtained from Avanti Polar Lipids (Alabaster, AL, USA) at a concentration of 25 mg/mL in chloroform and used without further purification. All lipid suspensions were prepared with 10 mM HEPES buffer containing 50 mM NaCl at pH 7.4 or 9.5. The pH of the drug solutions, lipid suspensions, and drug–lipid mixtures was measured before and after the addition of venlafaxine and during the lipid vesicle experiments. No significant changes in pH were observed under the experimental conditions, confirming the stability of the buffer system throughout the measurements.

Production of large unilamellar vesicles (LUVs)

The residual organic solvent in the lipid solution was gently evaporated using a stream of nitrogen, and the vessel was then placed in a vacuum chamber for at least 24 hours at room temperature. The resulting dried lipid films were then suspended in HEPES buffer and sonicated at a temperature above the T_m of the lipids to yield multilamellar vesicles. The lipid suspensions were then subjected to five freeze/thaw cycles to reduce their size and lamellarity. To produce the large unilamellar vesicles (LUVs), the lipid suspensions from the previous step were extruded

over 25 cycles through a 100-nm-pore polycarbonate membrane (Avanti Polar Lipids, USA) using a mini-extruder, yielding a monodisperse population of 100-nm LUVs.

Preparation of drug–liposome mixtures

Drug–liposome mixtures were prepared by adding venlafaxine at a fixed concentration (0.0675 mM) to lipid suspensions of different concentrations. The baseline suspensions were prepared using the same concentration of lipid suspensions of the drug–liposome mixtures without the addition of venlafaxine.

UV-Vis absorbance measurements

The drug–liposome and baseline suspensions were incubated at a certain temperature for 30 minutes before measuring their absorbance. The absorption spectra of the samples were acquired with an Agilent Cary 60 UV-Vis spectrophotometer (Agilent, USA) equipped with a thermostatic cell holder. The spectral window ranged from 200 to 300 nm, with a wavelength interval ($\Delta\lambda$) of 1 nm.

Determination of partition coefficients via second derivative spectrophotometry

Second derivative UV-Vis spectrophotometry has been extensively used to calculate the partition coefficients (K_p) of drugs partitioning into lipid bilayers[11–14]. Here, the K_p of venlafaxine between the lipid and aqueous phases is defined as in Eq. 1:

$$K_p = \frac{\left(\frac{[VLX]_m}{[VLX]_t}\right)/[\text{Lipid}]}{\left(\frac{[VLX]_w}{[VLX]_t}\right)/[\text{Water}]}, \quad (1)$$

where $[\text{VLX}]_t$ represents the total molar concentration (mM) of added venlafaxine, the sum of the theoretical venlafaxine concentrations in the lipid suspensions, $[\text{VLX}]_m$, and water, $[\text{VLX}]_w$; and [Lipid] and [Water] are the experimental molar concentrations (mM) of the lipid suspensions and water, respectively. The partition coefficients were calculated based on the change in drug absorbance induced by the interaction with the lipid bilayers. Additionally, the concentration of venlafaxine is proportional to its absorbance at a specific wavelength, as expressed in Eq. 2:

$$\text{A} = \varepsilon_\text{m}[\text{VLX}]_\text{m} + \varepsilon_\text{w}[\text{VLX}]_\text{w}, \quad (2)$$

where ε_m and ε_w are the drug extinction coefficients in liposomes and water, respectively. With $[\text{VLX}]_t = [\text{VLX}]_m + [\text{VLX}]_w$ and $\varepsilon = \varepsilon_m - \varepsilon_w$, the difference in absorbance in the presence and absence of the lipid bilayer (ΔA) can be related to the partition coefficient K_p using Eq. 3:

$$\triangle A = \frac{K_p \varepsilon [VLX]_t [Lipid]}{[Water] + K_p [Lipid]} \quad (3)$$

Similarly, the derivative intensity difference in absorbance in the presence and absence of liposomes (ΔD) is proportional to the venlafaxine concentration. From Eqs. 1–3, the relationship between K_p and ΔD can be described as in Eq. 4:

$$\triangle D = \frac{K_p \triangle D_{max} [Lipid]}{[Water] + K_p [Lipid]} \quad (4)$$

where $\triangle D = d^2A/d\lambda^2$ and $\triangle D_{max} = E[VLX]_t$, with $E = d^2\varepsilon/d\lambda^2$.

The ΔD values were derived by subtracting the second derivative intensity of the pure venlafaxine solution from the intensity of the solutions containing the drug and varying LUV concentrations, indicating the drug's tendency to penetrate the liposomes. ΔD reaches its maximum value (ΔD_{max}) when the entire drug fraction partitions into the lipid membranes at sufficiently high lipid concentrations. The K values and ΔD_{max} were obtained through the nonlinear least-squares fitting of Eq. 4 to the experimental data of the lipid molar concentration and the change in the second derivative of absorption in the presence and absence of lipids. A second-order polynomial with a Savitzky–Golay filter, with a window length of 20 points, was applied to the absorption spectrum to acquire the second derivative spectrum; this specific window length was selected, as increasing it to a higher number can result in the loss of spectral detail and introduce artifacts, while a window length that is too small may lead to insufficient background correction[15]. Regarding the thermodynamic characterization of SSRI partitioning into lipid vesicles, previous studies have also utilized a 20-point window length. In principle, K_p can be obtained using the ΔD value at any wavelength[12,16]. However, it is common practice to calculate K_p using ΔD values at the wavelength that leads to the smallest standard deviation. In the case of the current study, the ΔD values at 220 nm yielded K_p values with the smallest standard deviation; accordingly, this wavelength was selected for the final calculation.

Determination of thermodynamic parameters

The thermodynamics of the partitioning of venlafaxine from the aqueous to the lipid phase was analyzed through the correlation between the change in the Gibbs free energy (ΔG) and the natural logarithm of the partition coefficient (K_p), as shown in Eq. 5:

$$\triangle G = -RT \ln K_p, \quad (5)$$

where R is the ideal gas constant (8.312 $\text{J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$), and T is the temperature in Kelvin (K).

ΔG is defined as the difference between the change in enthalpy (ΔH) and the product of temperature (T) and the change in entropy (ΔS), as given by Eq. 6:

$$\triangle G = \triangle H - T \times \triangle S \quad (6)$$

Substituting Eq. 5 into Eq. 6 yields the Van't Hoff equation, as shown in Eq. 7:

$$\ln K_p = -\frac{\triangle H}{R} \times \frac{1}{T} + \frac{\triangle S}{R} \quad (7)$$

The ΔH and ΔS values can then be obtained from a linear regression plot of versus $\frac{1}{T}$.

RESULTS

Absorption and second derivative spectra of venlafaxine

The absorption spectra of venlafaxine at a sample concentration of 0.0675 mM are presented in Figure 2A, B, and C, illustrating the spectral changes induced by adding DOPC of various concentrations at 25, 37, and 45 °C, respectively, and pH 7.4. Similarly, Figure 3A–C depict the spectral changes resulting from the addition of varying DOPG concentrations at the same temperatures and pH. Moreover, Figure 3D reveals the spectral changes induced by varying DOPG concentrations at 25 °C and a pH of 9.5. These spectra were derived by subtracting the absorption spectra of the baseline suspensions from those of the venlafaxine–lipid suspensions. An increase in the lipid concentration results in a reduction of the absorption intensity (hypochromic effect) and a shift of the absorption peak of venlafaxine (λ_{max}) from 226 nm to longer wavelengths (bathochromic effect). The presence of a hypochromic shift may suggest a notable interaction between venlafaxine and the DOPC and DOPG LUVs. Additionally, the bathochromic shift of the venlafaxine peak in the presence of the DOPC and DOPG LUVs indicates a reduction in the polarity of the surrounding environment of the drug molecules. This shift demonstrates the movement of the drug from the aqueous phase into the lipid bilayers [9,17,18].

Light scattering from the lipid vesicles prevented the detection of an isosbestic point in the venlafaxine absorption spectra for both lipid vesicles; this occurred despite correcting for liposome absorption by subtracting its value from the venlafaxine absorption at each liposome concentration. To reduce background noise and improve the resolution of overlapping signals from the aqueous and lipid phases, the second derivative was applied to the absorption data. Figure 2D, E, and F present the second derivative spectra of the partitioning of venlafaxine into the DOPC bilayer—derived from the spectra in Figure 2A, B, and C, respectively. Similarly, the second derivative spectra of venlafaxine's partitioning into the DOPG bilayer (calculated from the spectra in Figure 3A, B, C, and D) are illustrated in Figure 3E, F, G, and H, respectively. With the increasing concentration of the DOPC and DOPG LUVs, the minima in the second derivative spectra increase in intensity and shift toward longer wavelengths. Moreover, venlafaxine exhibits an isosbestic point at 250 nm under all experimental conditions for both the DOPC and DOPG LUVs. The existence of the derivative isosbestic points reveals that the residual background signal (caused by light scattering from the lipid vesicles) is eliminated in the second derivative spectra [11,12]. Additionally, this indicates that venlafaxine equilibrates in two distinct states: the lipid phase and the aqueous phase [19].

Partition coefficients of venlafaxine

The ΔD values were calculated as the differences in the second derivative of venlafaxine absorption between the first spectrum and the remaining spectra at a wavelength of 220 nm for DOPC and DOPG. These ΔD values provide a basis for calculating the derivative intensity difference ($\Delta D/\Delta D_{max}$), which indicates the proportion of venlafaxine distributed within the DOPC and DOPG LUVs at various temperatures. The results were plotted against the lipid concentration, as illustrated in Figure 4. Similarly, for the DOPG LUVs at pH values of 7.4 and 9.5 at 25 °C, the $\Delta D/\Delta D_{max}$ values (obtained using the same methodology and wavelength) were plotted against the lipid concentration, as depicted in Figure 5.

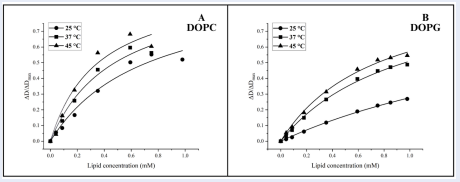

Figure 4: Fraction ($\Delta D/\Delta D_{max}$) of venlafaxine partitioning into varying concentrations of (A) DOPC and (B) DOPG at different temperatures.

The partition coefficients of venlafaxine partitioning into the DOPC and DOPG LUVs were determined via nonlinear least-squares fitting, as described by Eq. 4 in the "Materials and Methods". The K_p values of venlafaxine partitioning into the DOPC and DOPG LUVs at pH 7.4 for various temperatures (Table 1) and into the DOPG LUVs at pH values of 7.4 and 9.5 at 25 °C (Table 2) were calculated. Venlafaxine partitions into both types of LUVs to a greater extent at higher temperatures, as revealed by the increasing K_p values. The drug preferentially partitions into the zwitterionic DOPC bilayers over the negatively charged

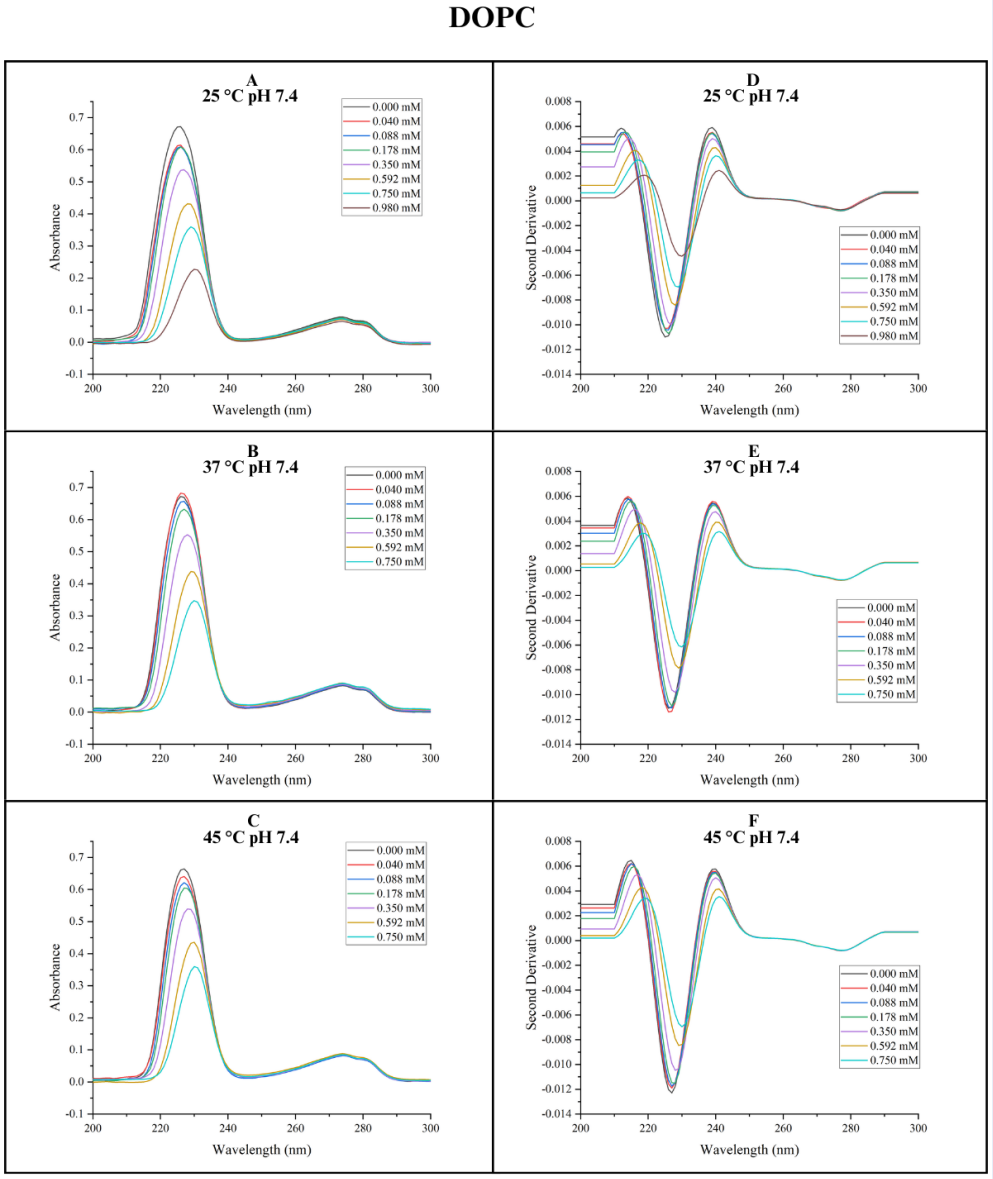

Figure 2: Absorption spectra (A, B and C) and the second derivatives of absorption spectra (D, E and F) of 0.0675 mM venlafaxine in HEPES buffer (pH 7.4) containing various concentrations (mM) of DOPC LUVs at 25, 37 and 45 °C.

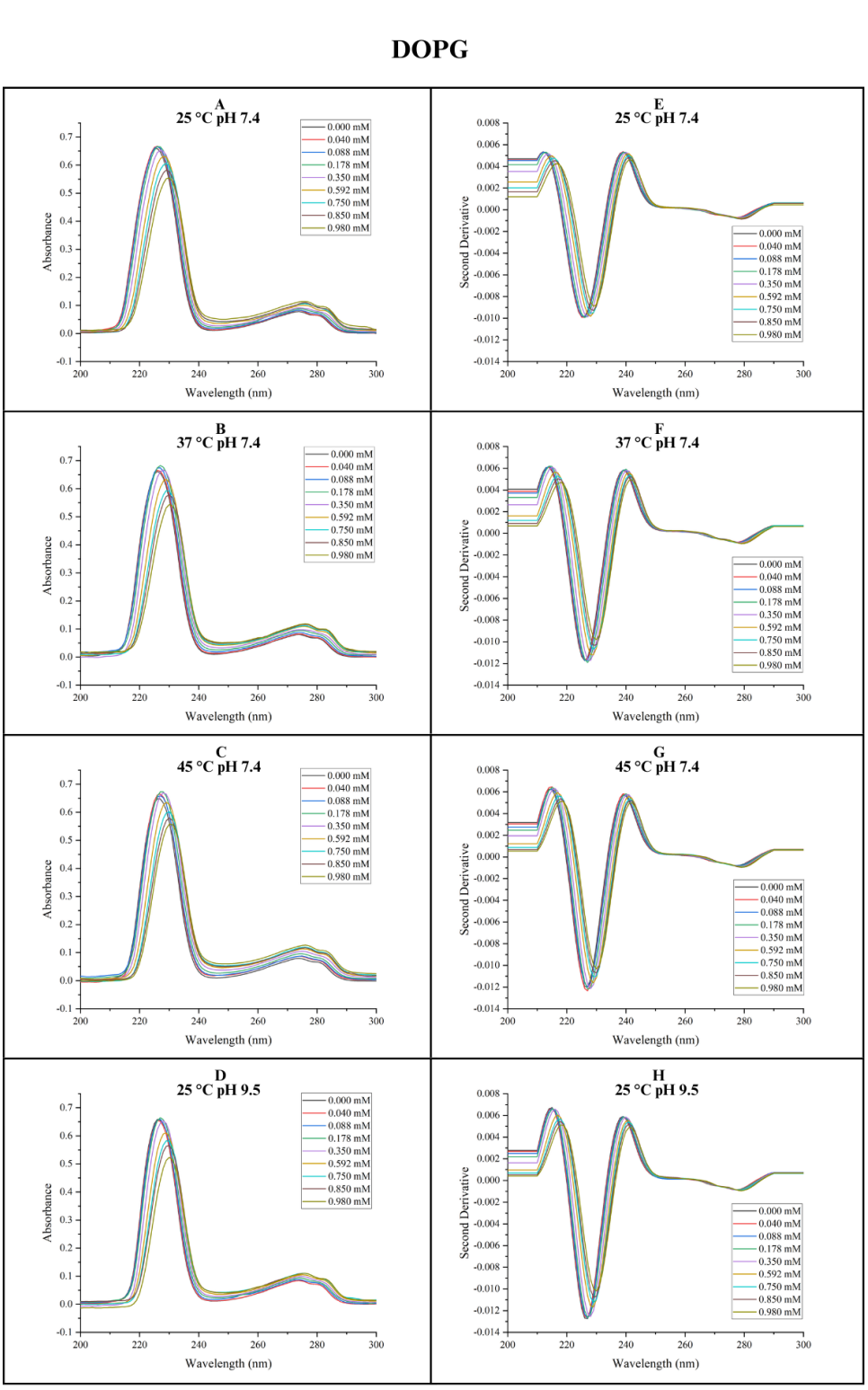

Figure 3: Absorption spectra (A, B, C and D) and the second derivatives of absorption spectra (E, F, G and H) of 0.0675 mM venlafaxine in HEPES buffer (pH 7.4 and 9.5) containing various concentrations (mM) of DOPG LUVs at 25, 37 and 45 °C.

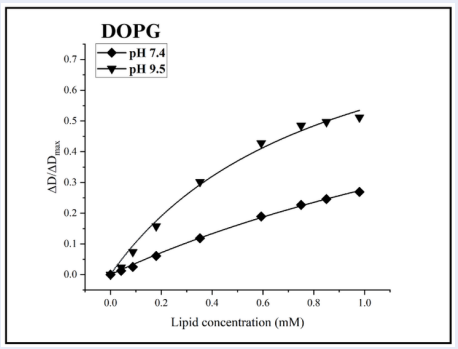

Figure 5: Fraction ($\Delta D/\Delta D_{max}$) of venlafaxine partitioning into varying concentrations of DOPG at pH 7.4 (◆) and 9.5 (▼), and 25 °C.

DOPG ones, as indicated by the higher K_p values for the DOPC bilayers over the investigated temperature range.

Table 1: Partition coefficient (K_p) values of 0.0675 mM venlafaxine into DOPC and DOPG LUVs at various temperatures.

Temperat	K_p $(\times 10^{-5})^a$	
	DOPC	DOPG
25 °C	0.75 ± 0.05	0.23 ± 0.04
37 °C	1.14 ± 0.26	0.61 ± 0.03
45 °C	1.57 ± 0.04	0.80 ± 0.07

[a]Mean ± standard deviation (N = 3)

Table 2: Partition coefficient (K_p) values of 0.0675 mM venlafaxine into DOPG LUVs at pH 7.4 and 9.5, and 25 °C

Temperature	Lipid	K_p $(\times 10^{-5})^a$	
		pH 7.4	pH 9.5
25 °C	DOPG	0.23 ± 0.04	0.58 ± 0.07

[a]Mean ± standard deviation (N = 3)

Thermodynamics of venlafaxine partitioning into DOPC and DOPG LUVs

Figure 6 presents the Van't Hoff plots for venlafaxine partitioning into the DOPC and DOPG LUVs at various temperatures, with the corresponding thermodynamic parameters presented in Table 3. The negative values of the Gibbs free energy change ($\Delta G_{w\to l}$) for venlafaxine indicate that its transfer from the aqueous phase into the DOPC and DOPG bilayers is thermodynamically favorable, signifying that the process occurs spontaneously under the experimental conditions. The Van't Hoff plots for venlafaxine partitioning into both lipid systems exhibit negative slopes, corresponding to positive enthalpy changes ($\Delta H_{w\to l}$); this confirms that the drug partitioning is an endothermic process. Considering these points together with the positive entropy changes ($\Delta S_{w\to l}$), the thermodynamic parameters highlight that the partitioning of venlafaxine into the lipid bilayers is predominantly entropy driven.

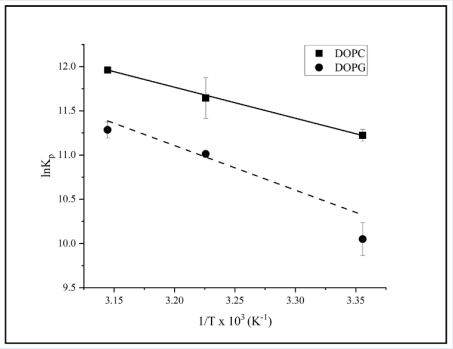

Figure 6: Van't Hoff plot of venlafaxine partitioning into DOPC (■) and DOPG (●) LUVs.

DISCUSSION

Increased temperature facilitates the membrane partitioning of venlafaxine

The partitioning of venlafaxine into the DOPC and DOPG bilayers is spontaneous, as indicated by the negative $\Delta G_{w\to l}$ values (Table 3). Higher temperatures can increase the kinetic energy of both the drug and lipid molecules, thus increasing the frequency and effectiveness of the drug–lipid interactions. Furthermore, an increase in temperature is associated with a decrease in membrane viscosity and, thus, a higher degree of freedom for the drug to migrate within the bilayer [1]. As expected, the temperature increase from 25 °C to 45 °C enhances the partitioning of venlafaxine into both the DOPC and DOPG bilayers, as indicated by the rise in the K_p values (Table 1). The results highlight the direct correlation between temperature and the membrane partitioning of venlafaxine and align with previous findings on SSRIs [9,17]. These antidepressants were shown to exhibit greater partitioning into the DOPC bilayer with increasing temperature from 25 °C to 37 °C for fluoxetine [9] and

Table 3: Thermodynamic parameters of venlafaxine partitioning into DOPC and DOPG LUVs

Lipid	Temperature	$\triangle G_{w\to l}\,(kjmol^{-1})$	$\triangle H_{w\to l}\,(kjmol^{-1})$	$\triangle S_{w\to l}\,(jmol^{-1}K^{-1})$
DOPC	25 °C	-27.78 ± 0.18	28.93 ± 0.18	190.3 ± 0.57
	37 °C	-30.07 ± 0.18		
	45 °C	-31.59 ± 0.18		
DOPG	25 °C	-25.03 ± 1.09	49.81 ± 1.09	251.12 ± 3.54
	37 °C	-28.04 ± 1.09		
	45 °C	-30.04 ± 1.09		

25 °C to 42 °C for paroxetine and sertraline[17]. This trend underscores the influence of temperature on the interaction between drugs and lipid bilayers, where higher temperatures facilitate the incorporation of drug molecules into lipid bilayers.

Membrane partitioning of venlafaxine adheres to the classical hydrophobic effect

The partitioning of amphiphilic small molecules aligns with either the classical or nonclassical hydrophobic effect[20–23]. Upon the partitioning of venlafaxine, various molecular interactions can contribute to the enthalpy change of the system. The value of $\Delta H_{w\to l}$ may be influenced by the displacement of water by the unbound drug at the interfacial region (i.e., the junction of the lipid head groups and the aqueous phase), cavity formation for drug insertion within the lipid bilayer (disruption to lipid packing), and the drug–lipid interaction itself (e.g., hydrogen bonding and hydrophobic/van der Waals interactions)[17,21,22]. The net positive $\Delta H_{w\to l}$ values for venlafaxine in both lipid systems suggest that the energy contributions of water displacement and lipid packing disruptions are predominant, as these processes generate an increase in enthalpy, while the drug–lipid interaction leads to a decrease in enthalpy. If the drug is able to perturb the inner hydrophobic core of the bilayer, the $\Delta S_{w\to l}$ of the system increases due to the disordering of the lipid acyl tails (induced by drug insertion)[17,21,22]. Additionally, the aforementioned displacement of water molecules by the drug at the interfacial region contributes to an increase in entropy. The $\Delta S_{w\to l}$ values for venlafaxine are significantly larger than the $\Delta H_{w\to l}$ values. This indicates that the partitioning of venlafaxine into the DOPC and DOPG bilayers results in the displacement of interfacial water molecules and the disordering of the lipid acyl tails.

Notably, the reported ΔH and ΔS values reflect a limited temperature range and are based on a linear Van't Hoff analysis, an approximation that assumes a negligible change in heat capacity ($\Delta C_p \approx 0$) over this interval. However, this type of analysis is standard and widely accepted, and the resulting parameters are considered a valid approximation for thermodynamic characterization within the limited temperature range studied[24,25].

In summary, the partitioning of venlafaxine into both the DOPC and DOPG bilayers is consistent with the classical hydrophobic effect. Previous works have also shown that the partitioning of SSRIs (paroxetine and sertraline)[17,18], antipsychotics (promethazine, trifluoperazine, and trimeprazine)[26] and beta-blockers (propranolol, alprenolol, and bupranolol)[27] aligns with the classical hydrophobic effect.

Electrostatic and hydrophobic interactions contribute to membrane partitioning of venlafaxine

At the physiological pH of 7.4, venlafaxine (pK_a = 9.4)[3] predominantly exists as a cationic species. DOPC is a zwitterionic lipid containing a positively charged choline moiety and a negatively charged phosphate group in its head group. In the transfer of venlafaxine from the aqueous to the lipid phase, electrostatic repulsion between the cationic venlafaxine and the positively charged choline group of the DOPC head group may occur. Simultaneously, electrostatic attraction between the cationic venlafaxine and the negatively charged phosphate of DOPC can arise. Within the hydrophobic core of the DOPC bilayer, venlafaxine engages in favorable hydrophobic interactions with the nonpolar acyl chains of the lipid. These van der Waals interactions between venlafaxine's hydrophobic region and the lipid acyl tails enhance drug stability within the bilayer environment. Overall, it could be stated that hydrophobic and electrostatic interactions modulate the partitioning of venlafaxine into the zwitterionic DOPC bilayer. Previous works have also revealed that the

combination of hydrophobic and electrostatic interactions is responsible for the partitioning of various amphiphilic small molecules[9,10,17]. Paroxetine and sertraline partition into the DOPC bilayer via electrostatic interactions with the lipid head groups and hydrophobic interactions with the lipid acyl tails[17]. Similarly, several studies have highlighted the contribution of electrostatic interactions in modulating drug–membrane partitioning for other cationic amphiphilic drugs[28–30].

DOPG is an anionic lipid containing a negatively charged phosphate and a neutral glycerol in its head group. Despite the electrostatic attraction between the cationic venlafaxine and the negatively charged phosphate group of DOPG, venlafaxine exhibits a lower degree of partitioning into the DOPG bilayer. This is evidenced by the approximately 3.3- and 1.9-fold lower K_p of venlafaxine partitioning into the DOPG bilayer compared to the DOPC bilayer at 25 °C and at 37 and 45 °C, respectively, as shown in Table 1. The limited partitioning of venlafaxine into the DOPG bilayer potentially arises from the strong association of venlafaxine with the head group region of DOPG. At first, the smaller volume of lipid head groups and the lower packing density of DOPG (indicated by the larger area per lipid head group) compared to DOPC are believed to correspond to a greater availability of voids for drug incorporation[31–34]. However, the repulsive electrostatic interactions among the DOPG head groups are attenuated by intra- and intermolecular hydrogen bonding and ion–lipid interactions[35], which are manifested as ion–lipid clusters that ultimately reduce the permeability of the DOPG bilayer compared to the DOPC bilayer[36,37]. Upon its partitioning into the DOPG bilayer, venlafaxine displaces the structured water molecules at the interface to a greater extent and disrupts the ion–lipid clusters in the head group region of the bilayer, resulting in a larger increase in the entropy of the system compared to DOPC (Table 3). The absence of this electrostatic attraction in the DOPC bilayer enables the progression of venlafaxine toward the acyl tail region of DOPC, leading to a greater extent of drug–lipid interaction. This results in a lower enthalpy change ($\Delta H_{w\to l}$) in the DOPC bilayer compared to the DOPG bilayer (Table 3). Considering these findings, one can speculate that the partitioning of venlafaxine into DOPG at pH 7.4 is primarily driven by electrostatic attraction, while hydrophobic interactions play a minor role.

To further validate this hypothesis, the contribution of electrostatic interactions to the partitioning of venlafaxine into the negatively charged DOPG bilayer was examined by lowering the amount of cationic venlafaxine species. At pH 7.4, venlafaxine predominantly exists as a cationic species, while at pH 9.5, approximately half exists in the cationic form, while the remaining half is neutral. Neutral venlafaxine is more lipophilic and can easily cross the lipid bilayer compared to its charged counterpart. Moreover, the reduced proportion of cationic venlafaxine could result in a lower extent of electrostatic interactions with the DOPG head groups and, consequently, diminished head group association. This is indicated by the 2.5-fold increase in the K_p of venlafaxine partitioning into the DOPG bilayer with an increase in the pH from 7.4 to 9.5 (Table 2). It is likely that at pH 9.5, both hydrophobic and electrostatic interactions contribute to the partitioning of venlafaxine into the charged DOPG bilayer; on the other hand, at pH 7.4, electrostatic interactions mainly contribute to the partitioning, with hydrophobic interactions playing a minor role. The findings correlate well with recent works on the interaction of fluoxetine with the negatively charged DPPG bilayer; here, cationic fluoxetine strongly associates with the DPPG head groups via electrostatic interactions, while neutral fluoxetine traverses the bilayer via hydrophobic interactions[10].

One should note that electrostatic interactions between cationic drugs and anionic lipid membranes are strongly influenced by the ionic strength of the medium. The present study employed 50 mM NaCl, which represents a moderate ionic strength. At this concentration, the partial (yet not complete) screening of electrostatic interactions is expected, allowing for measurable electrostatic contributions to remain. This interpretation is consistent with prior studies on drug–membrane interactions showing that moderate salt concentrations attenuate but do not eliminate charge-driven binding[38,39]. Our previous work on tamoxifen demonstrated that while high NaCl concentrations markedly reduced drug partitioning into anionic bilayers, moderate levels, such as 50 mM, still supported detectable electrostatic effects[40]. Therefore, the ionic strength applied in this study remains physiologically relevant and adequate in capturing the interplay between hydrophobic and electrostatic contributions in venlafaxine–lipid interactions.

In sum, the partitioning of venlafaxine into lipid bilayers at physiological pH is modulated by both hydrophobic and electrostatic interactions. In particular, in the zwitterionic DOPC bilayer, both hydrophobic and electrostatic interactions contribute to the partitioning of venlafaxine, while in the negatively charged DOPG bilayer, electrostatic interactions mainly drive venlafaxine partitioning, with a

smaller contribution from hydrophobic interactions. This study provides fundamental insights into the partitioning behavior of venlafaxine with regard to zwitterionic and anionic lipid bilayers, highlighting the distinct contributions of hydrophobic and electrostatic interactions. While our conclusions are based on partition coefficient analysis under controlled conditions, future work will integrate complementary approaches—including ζ-potential measurements, ionic strength dependence, and FTIR spectroscopy—to further elucidate the electrostatic contributions and the molecular locations of venlafaxine within lipid bilayers.

CONCLUSIONS

This study investigated the interaction between venlafaxine and lipid bilayers comprising zwitterionic DOPC and negatively charged DOPG using second derivative spectrophotometry. The findings reveal that temperature significantly enhances the partitioning of venlafaxine into both the DOPC and DOPG bilayers; furthermore, venlafaxine partitioning aligns with the classical hydrophobic effect and is spontaneous, endothermic, and entropy driven. Venlafaxine exhibits a pronounced preference for partitioning into the zwitterionic lipid bilayer compared to the charged bilayer at all investigated temperatures. Varying the proportion of the neutral or charged venlafaxine further indicates that both electrostatic and hydrophobic interactions modulate the partitioning of the drug into the charged bilayer. These findings provide insights into the molecular interactions between venlafaxine and lipid bilayers, which can aid the design and optimization of future antidepressants. For example, any change in the protonable or hydrophobic moiety of a drug can alter the membrane partitioning of the drug via its electrostatic and hydrophobic interactions with the lipid bilayer.

This study was conducted in vitro using model lipid bilayers composed of a single phospholipid. Further studies are needed to confirm the findings in more complex biological systems and clarify the effect of venlafaxine on membrane dynamics or the influence of protein transporters on venlafaxine's translocation across lipid membranes.

ABBREVIATIONS

SSRI: selective serotonin reuptake inhibitor

SNRI: serotonin and norepinephrine reuptake inhibitor

LUV: large unilamellar vesicle

DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine

DOPG: 1,2-dioleoyl-sn-glycero-3-phosphoglycerol

DPPG: 1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Phuong My Dang: Investigation, Formal analysis, Visualization, Writing – original draft. Dat Nguyen Tien Ngo: Writing – original draft, Writing – review & editing. Hieu Kim Huynh: Writing – review & editing. Trang Thao Nguyen: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. Phuong My Dang and Dat Nguyen Tien Ngo contributed equally to this work.

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