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Original Article

THE INFLUENCE OF CHLORPROMAZINE HYDROCHLORIDE ON THE THERMOTROPIC BEHAVIOR OF DIMYRISTOYL PHOSPHATIDYLCHOLINE LIPOSOMES AS REVEALED BY DIFFERENTIAL SCANNING CALORIMETRY

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ABSTRACT

Objective: The aim of this study is to investigate the influence of the model cationic, amphiphilic, drug chlorpromazine hydrochloride (CPZ-HCl) on the thermotropic behavior of dimyristoyl phosphatidylcholine (DMPC) liposomes, using differential scanning calorimetry (DSC). The effect of sonication, charged lipids and CPZ-HCl at concentrations known to cause anesthesia on the enthalpy (ΔHt), entropy (ΔSt), phase transition (Tc), pre-transition (pre-T_C) and half-height width **(**HHW) of DSC thermograms were examined.

Methods: The experiments conducted, using the Perkin Elmer (DSC-2C), include the effect of a wide range of CPZ-HCl concentrations on ΔH_t, ΔS_t, Tc, pre-Tc and HHW of DSC thermograms of DMPC liposomes. The effect of sonication on ΔH_t, ΔS_t, Tc and HHW of DSC thermograms of DMPC/CPZ-HCl liposomes as a function of sonication time. The effect of both positively charged stearyl amine (ST) and negatively charged diacetyl phosphate (DCP) lipids on ΔH_t, ΔS_t and Tc of DMPC/CPZ-HCl liposomes. In addition, the effect of CPZ-HCl at concentrations known to cause anesthesia on ΔH_t, ΔS_t and Tc of DMPC liposomes in the presence and absence of ST and DCP in phosphate buffer (pH 7.4), was also carried out.

Results: Using DSC, CPZ-HCl concentrations as low as 1×10^{-7}M were observed to alter the gel-liquid crystalline phase transition and thus to possess a membrane destabilizing effect. CPZ-HCl reduces ΔH_t, ΔS_t, T_C, the pre-T_C and increases HHW of DMPC liposomes. ΔH_t and ΔS_t of DMPC liposomes were observed to decrease with increasing CPZ-HCl concentrations, exhibiting an inflection point at 5×10^{-5}M. ΔHt of DMPC liposomes was observed to decrease linearly in the absence and presence of and CPZ-HCl as a function of sonication time. Both ΔH_t and ΔS_t of DMPC liposomes were observed to increase in the presence of cationic lipid (ST) and to decrease in the presence of anionic lipid (DCP). ΔSt and Tc of DMPC, DMPC/ST, DMPC/DCP liposomes, were found to decrease as a function of CPZ-HCl concentrations known to cause anesthesia.

Conclusion: Using DSC, CPZ-HCl concentrations, as low as 1×10^{-7} M were observed to influence the enthalpy, entropy, phase transition, pre-transition and half-height width of DSC thermograms of DMPC liposomes**,** altering the gel-liquid crystalline phase transition and thus possessing a membrane destabilizing effect. It can also be inferred that CPZ-HCl interacts with both the polar head group and the hydrophobic interior of the phospholipid bilayer. These results could support the hypothesis that the addition of local anesthetics might trigger a change in the lipid surrounding the sodium channel from the gel to the liquid crystalline state, allowing the sodium channel to close with the resulting anesthesia.

Keywords: CPZ-HCl, Liposomes, Transition temperature, Enthalpy, Entropy

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INTRODUCTION

Liposomes have been extensively used as drug delivery systems for a wide range of drugs [1-3]. In addition, aqueous phospholipid liposomal dispersions have been widely studied as model membranes because of their striking resemblance to biological membranes [4]. Liposomes composed of pure synthetic phospholipids exhibit a phase change from a close-packed, relatively immobile, L-β-gel-crystalline state to a disordered L-α-liquid crystalline state at a well-defined characteristic transition temperature (Tc). This transition temperature is readily observed as an endothermic peak by DSC and is mainly attributed to the melting of the phospholipid acyl chains from an all trans-configuration in the ordered gel-state at low temperatures to the more disordered liquid crystalline state at high temperatures [5-7].

The phase transition of phospholipids has been measured by a variety of physical techniques such as DSC [8], densitometry [9], dilatometry [10], fluorescent probe analysis [11], X-ray diffraction [12], ESR [13], NMR [14] and light scattering [15]. The examination of the phase transition is important in the design of liposomes as a controlled drug delivery system. This concept has been applied in localized tumors in mice. Liposomes of mixed phospholipid composition, with Tc slightly higher than body temperature containing the cytotoxic agent methotrexate, were injected into mice bearing solid tumors and the tumor region was locally heated by a microwave device to 42 °C, a temperature above the Tc of the liposomes. A four-fold increase in the concentration of the drug was observed in the heated tumors compared with the unheated ones [16].

The permeability of solutes in liposomes was observed to be affected by the phase transition. A large increase in the permeability of $Na^+$was observed in the vicinity of the phase transition [17]. Chlorpromazine, has been found to interact preferentially with bilayers containing phospholipids with a high proportion of phosphatidylserines and highly unsaturated acyl chains [18]. Furthermore, CPZ has been found to slightly increase lipid order when the bilayer is above Tc and to decrease lipid order when the bilayer is below Tc [19]. Recent study has shown that CPZ binds rapidly to phospholipid bilayers perturbing molecular ordering of phospholipids and causing membrane disruption as reported in hemolysis and changes in erythrocytes morphology. At low concentrations, CPZ penetrates the bilayer. At high concentrations, the drug disrupts the lipid bilayer and induces aggregation [20]. The cationic amphiphile, trimeprazine (a phenothiazine) was found to exhibit maximum efflux in the vicinity of the phase transition of DMPC liposomes [21]. The role of the phase transition on solute permeability is also reflected in the behavior of various biological membranes. Mycoplasma Laidlawii, for example, stops growing and eventually lyses if the environmental temperature is lowered below the Tc of the constituent lipids [22]. The characteristics of the phase transition depend on the nature of the polar head group and the length and degree of unsaturation of the fatty acyl chains of the phospholipid. It was found that the Tc of fully saturated diacyl phosphatidylcholine increases with the hydrocarbon

chain length [23]. DSC is a versatile technique that has been used for decades to study phospholipid membranes [24, 25]. For pure lipids, DSC can accurately determine the phase transition temperatures and the associated enthalpies (5). A DSC study of a number of synthetic phosphatidylcholines containing monosaturated hydrocarbon chains differing in the position of the unsaturated residue has shown that the Tc and the enthalpy for all the cis-unsaturated phospholipids were lower than those for the corresponding saturated phosphatidylcholines of similar chain length. In addition, the location of the cis-unsaturated bond was shown to affect the thermos-tropic behavior [26]. Using light scattering and fluorescent probe analysis, the effect of the polar head group of dimyristoyl-and dipalmitoyl phosphatidic acid on the phase transition at different pHs was studied. The Tc increases to a maximum value at pH 3.5, which corresponds to pKa_1 value for the phosphate group. With increasing pH up to pKa_2 (pH 9.5), there is only a small decrease in the Tc despite the substantial increase in the surface charge. At pHs above 9.5, the Tc decreases markedly [27]. The enthalpy values showed a similar pattern to that observed by the Tc and it was suggested that the reduction of Tc and ΔH_t at higher pH values reflects a significant decrease in the co-operativity of the transition as the phospholipid attains two negative charges [28]. Apart from the main endothermic transition, DSC thermograms show a smaller endothermic transition at pre-transition temperature. The pre-transition is thought to be associated with the conformational changes in the phosphorylcholine head group of the bilayer (5). Modification of the choline group has been shown to abolish the pre-transition [29].

In this study, the influence of the model cationic, amphiphilic, drug CPZ-HCl on the thermotropic behavior of DMPC liposomes in phosphate buffer (pH 6.0), was examined by DSC. The effect of sonication and charged lipids on the enthalpy, entropy, phase transition and pre-transition of phospholipid liposomes were also examined in the presence and absence of CPZ-HCl. Furthermore, the effect of CPZ-HCl at concentrations known to cause anesthesia on ΔH_t, ΔS_t and T_c of DMPC liposomes in the presence and absence of ionic lipids in phosphate buffer (pH 7.4) was investigated.

MATERIALS AND METHODS

Materials

Synthetic dimyristoyl phosphatidylcholine (not less than 98% pure), chlorpromazine hydrochloride, stearyl amine and dicetylphosphate were purchased from Sigma Co. Chloroform was purchased from BDH and was of Analar grade.

Methods

The thermotropic behavior of DMPC liposomes was examined using differential scanning calorimetry (DSC).

DSC method

A Perkin Elmer DSC-2C is used to examine the thermotropic behavior of DMPC liposomes. The instrument consists of two cells; one is an inert reference cell and the other containing the sample. If the sample is a solution or suspension, then the reference is the corresponding solvent. Both cells are heated at a programmed rate to maintain an equal temperature. Changes in the sample enthalpy of transition or melting are inferred from measurements of the difference in power necessary to keep the temperature of both the sample and the reference equal. The Tc appears as an endothermic peak.

Preparation of liposomes for DSC

50 mg DMPC were dissolved in 5 ml chloroform (Analar grade) in 25 ml round bottom flask. The solvent was evaporated to dryness using a rotary evaporator (Rotavapor-R, Buchii). Traces of chloroform were removed by blowing a jet of dry nitrogen. The dried film was stored under vacuum in the presence of phosphorous pentoxide to complete the drying process overnight. 0.5 ml of phosphate buffer (pH 6.0) or phosphate buffer (pH 7.4) was added to form a 10% W/V lipid dispersion. 0.5 ml of CPZ-HCl at the required concentration was added to the dry lipid film to form a 10% W/V lipid dispersion. The dispersions were hand-shaken in a water bath 10 °C above the Tc to form large multilamellar vesicles (LMV).

Approximately 6 mg accurately weighed samples of liposomes were sealed into aluminum pans and DSC thermograms were obtained using a Perkin Elmer DSC-2C at a scanning rate of 5 °k/min on a sensitivity range of 1 mcal/s. The temperature range was 280-305 °K. The instrument was calibrated using indium. Liquid nitrogen was used to cool the calorimeter cell holder and dry helium was passed over the sample during scanning. Each sample was scanned three times using fresh samples.

Determination of T_C, ΔH_t and ΔS_t from DSC thermograms

The T_C was determined from the peak of the DSC thermogram. The enthalpy of transition (ΔH_t) was calculated from the area under the DSC thermogram according to the following equation:

$$\Delta H = KAR/WS \quad \text{..... (1)}$$

Where ΔH= enthalpy of transition (cal/g).

K=calibration constant of the instrument determined using indium

R= sensitivity range (mcal/s)

A= the area under the peak ($inches^2$)

W=Sample weight in mg

S=Chart speed (inches/s)

The area under the peak was calculated from the calibration curve of area ($inches^2$) against weight (mg) using a standard weight paper. ΔH_t values were converted into J/mol by multiplying the calculated values by a factor of 4.2 and the molecular weight of DMPC.

The corresponding entropy of transition (ΔS_t) was calculated from:

$$\Delta S_t = \Delta H_t / Tc \quad \text{..... (2)}$$

DSC experimental

The following experiments were carried out using the DSC:

(a) The effect of a wide range of CPZ-HCl concentrations on the enthalpies (ΔH_t), entropies (ΔS_t), Tc, pre-T_c and half-height width (HHW) of DSC thermograms of DMPC liposomes (table 1).

(b) The effect of sonication time on ΔH_t, ΔS_t and T_c of DMPC/CPZ-HCl liposomes (table 2). Sonication was carried in a bath sonicator (Kerry Co.) under nitrogen at 40 °C.

(c) The effect of both positively charged (ST) and negatively charged (DCP) lipids on ΔH_t, ΔS_t and T_c of DMPC/CPZ-HCl liposomes (table 3).

(d) The effect of CPZ-HCl at concentrations known to cause anesthesia on ΔH_t, ΔS_t and T_c of DMPC liposomes in the presence and absence of ST and DCP in phosphate buffer (pH 7.4) (table 4).

RESULTS AND DISCUSSION

The effect of CPZ-HCl concentrations on the phase transition temperature (Tc) of DMPC liposomes in phosphate buffer (pH 6.0) using DSC is shown in table 1 and fig. 1.

The T_C value for DMPC liposomes was 23.7 °C (table 1), which agrees with the literature value (11). A shift of the T_C to lower temperatures occurred as a function of CPZ-HCl concentration over the entire range of 10^{-8}M to 10^{-3}M (table 1). The shift of the T_C to lower temperatures in the presence of CPZ-HCl may indicate that CPZ-HCl affects the mobility of the acyl fatty acid chains of the phospholipid and thus, the fluid state is more easily achieved.

The pre-transition temperature of DMPC liposomes was observed at 13.4 °C (table 1), which agrees with the literature value [30]. The pre-transition temperatures were found to be abolished at CPZ-HCl concentrations of 1×10^{-4}M, 1×10^{-3}M and 5×10^{-3}M, respectively (table 1).

The pre-transition is thought to be associated with the mobility of the polar head groups of the phospholipid. p-NMR spectra of phosphatidylcholine dispersions have shown increased mobility of the–$N(CH_3)_3$ group at the pre-transition temperature [31]. In addition, modification of the polar head groups abolishes the pre-transition [32]. However, X-ray diffraction has shown that the pre-transition temperature is associated with partial melting of the hydrocarbon chains

(10). The abolition of the pre-transition, whether associated with the inhibition of the polar head group or the tilting of the hydrocarbon chains prior to melting, in the presence of CPZ-HCl, reflects some degree of CPZ-HCl-liposomes interaction.

The calculated enthalpies (ΔH_t) (table 1) for DMPC liposomes agree with literature values [33]. It is apparent that Tc (fig. 1) and pre-T_C (table 1), ΔH_t (fig. 2) and ΔS_t (fig. 3) decrease steadily with increasing CPZ-HCl concentrations.

Table 1: The effect of CPZ-HCl concentrations on the enthalpies (ΔH_t), entropies (ΔS_t), transition temperature (T_C) and pre-transition temperature (pre-T_C) of DMPC liposomes (10%W/V) in phosphate buffer (pH 6.0) as determined by DSC

Phospholipid	CPZ-HCl (Molar Conc.)	ΔH_t (KJ. mol^{-1})	ΔS_t (J. mol^{-1}. K^{-1})	T_C (°C)	Pre-T_C (°C)
DMPC	0	26.65+0.6	89.52+1.7	23.7+0.02	13.4+0.01
	1×10^{-7}	25.17+0.7	85.09+1.4	22.8+0.01	13.0+0.01
	1×10^{-6}	23.87+0.5	80.86+1.5	22.2+0.02	12.2+0.02
	1×10^{-5}	23.17+0.8	78.68+1.4	21.5+0.01	a*
	1×10^{-4}	22.49+0.6	76.50+1.3	21.0+0.01	a*
	1×10^{-3}	20.94+0.8	71.32+1.8	20.6+0.02	a*
	5×10^{-3}	19.92+0.6	68.22+1.6	19.0+0.01	a*
	7.5×10^{-3}	17.07+0.5	58.54+1.3	18.6+0.02	a*
	1×10^{-2}	13.20+0.4	45.41+1.4	17.7+0.01	a*
	5×10^{-2}	9.09+0.2	31.35+1.5	17.0+0.01	a*
	1×10^{-1}	a*	a*	a*	a*

a* indicates peak abolished. ΔH, ΔS, Tc and pre-Tc values, shown in table 1 are the mean values of three experiments (n=3) with mean±SD.

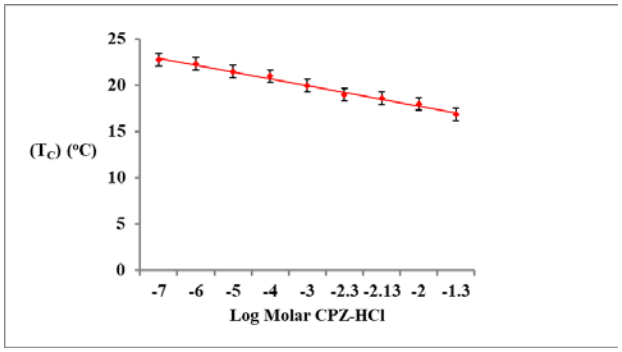

Fig. 1: Changes in phase transition (Tc) of CPZ-HCl/DMPC (10% W/V) liposomes in phosphate buffer (pH 6.0). The values obtained were the mean of three experiments (n= 3). SDs are shown as error bars and all values were in the range of+0.01-0.02

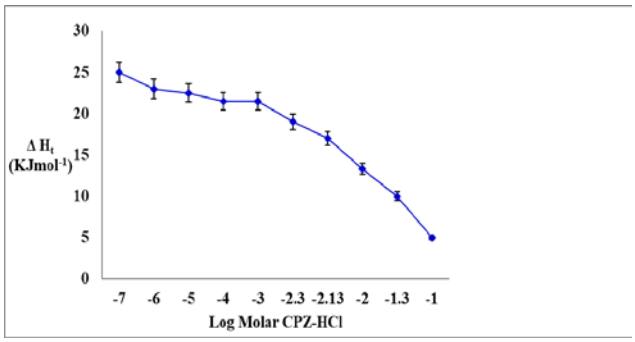

Fig. 2: Changes in the enthalpies of transition (ΔH) of 10% W/V DMPC liposomes as a function of log molar CPZ-HCl in phosphate buffer (pH 6.0). The values obtained were the mean of three experiments (n= 3). SDs are shown as error bars and all values were in the range of+0.2-0.8

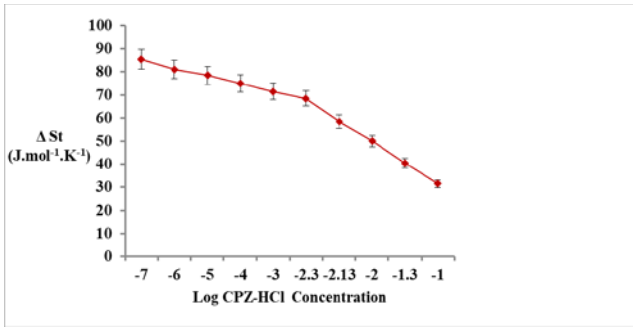

Fig. 3: Changes in the entropies of transition (ΔS) of 10% W/V DMPC liposomes as a function of log molar CPZ-HCl in phosphate buffer (pH 6.0). The values obtained were the mean of three experiments (n= 3). SDs are shown as error bars and all values were in the range of+1.3-1.8

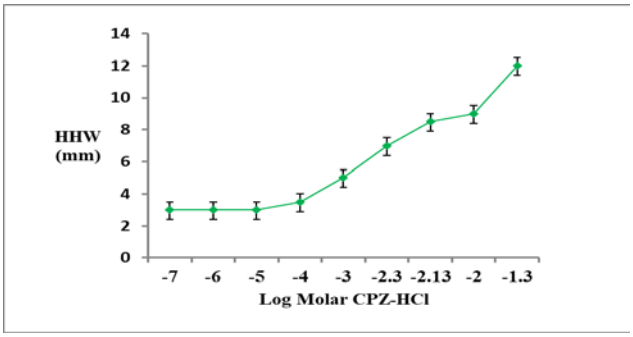

Fig. 4: Changes in the half-height width of DSC thermograms (HHW) of CPZ-HCl/DMPC (10% W/V) liposomes in phosphate buffer (pH 6.0). The values obtained were the mean of three experiments (n= 3). SD are shown as error bars and all values were in the range of +0.01-0.02

Above 1×10^{-5}M CPZ-HCl, the half-height width (HHW) of the DSC thermograms of DMPC liposomes increases with increasing CPZ-HCl concentrations (fig. 4).

Both ΔH_t (fig. 2) and ΔS_t (fig. 3) were observed to decrease with increasing CPZ-HCl concentrations, exhibiting an inflection point at 5×10^{-5}M. The inflection could not be explained on the basis of the formation of mixed lipid/CPZ-HCl micelles since this concentration is far below the CMC of CPZ-HCl in aqueous solution of 3.2×10^{-3}M at 25 °C [33]. Also, no such transition would be expected in a micellar phase. A study has shown that the partitioning of CPZ-HCl into DMPC liposomes was concentration dependent both above and below the T_C; increasing at low concentrations but decreasing at high concentrations with a maximum in partition coefficient at 2.8×10^{-4}M CPZ-HCl concentration [34]. This offers no explanation for the sharp decrease in ΔH_t and ΔS_t above 5×10^{-5}M CPZ-HCl. At a high concentration of 1×10^{-1}M CPZ-HCl, the phase transition of DMPC is completely abolished (table 1).

It may be inferred from these DSC studies that the ionized CPZ-HCl has penetrated the polar head group region to interact with the acyl chains of the hydrophobic membrane interior. Further, the data suggests a possible interaction with the polar head group of the phospholipid such that the positively charged tertiary amine group of CPZ-HCl interacts with the polar head group and the phenothiazine ring perturbs the hydrophobic interior of the bilayer. An NMR study has shown that both the phospholipid head group and the degree of phospholipid acyl chain unsaturation determine part of the CPZ interaction with the bilayer [35].

The effect of sonication time on ΔH_t, ΔS_t, T_C and half-height width (HHW) of DSC thermograms of DMPC liposomes in the absence and presence of CPZ-HCl is summarized in table 2.

Table 2: The effect of sonication time on the enthalpies (ΔHt), entropies (ΔSt), transition temperature (T_C) and half-height width (HHW) of DMPC/CPZ-HCl liposomes in phosphate buffer (pH 6.0) as determined by DSC

Time (min)	ΔH_t (KJ. mol^{-1})	ΔS_t (J. mol^{-1}. K^{-1})	T_C (°C)	HHW (mm)
(a) DMPC+0 M CPZ-HCl				
0	26.56+0.6	89.52+1.7	23.7+0.01	3.0+0.2
5	25.53+0.3	86.04+1.4	23.7+0.02	3.0+0.1
10	24.26+0.3	81.78+1.6	23.7+0.01	3.0+0.2
15	22.92+0.5	77.25+1.7	23.7+0.01	3.0+0.1
20	21.42+0.9	72.21+1.3	23.6+0.02	3.0+0.2
25	20.46+0.7	68.96+1.1	23.6+0.01	3.5+0.1
30	20.23+0.6	68.21+1.0	23.6+0.01	3.5+0.1
(b) DMPC+1×10^{-5} M CPZ-HCl				
0	23.17+0.9	78.68+0.6	21.5+0.02	3.0+0.1
5	22.72+0.6	77.15+0.5	21.5+0.01	3.0+0.1
10	21.54+0.4	73.13+0.8	21.5+0.02	3.5+0.2
15	20.79+0.7	70.58+0.6	21.5+0.01	3.5+0.1
20	20.07+0.6	68.16+0.4	21.5+0.02	4.0+0.2
25	19.21+0.8	65.23+0.6	21.4+0.01	4.0+0.1
30	18.10+0.5	61.48+0.5	21.4+0.01	4.0+0.1
(c) DMPC+1×10^{-3} M CPZ-HCl				
0	20.94+0.3	71.32+0.7	20.6+0.01	4.0+0.2
5	19.96+0.6	67.97+0.4	20.6+0.00	4.0+0.1
10	18.61+0.7	63.39+0.6	20.6+0.02	4.0+0.2
15	17.82+0.8	60.72+0.5	20.5+0.01	4.5+0.1
20	17.27+0.5	58.84+0.6	20.5+0.02	5.0+0.1
25	15.65+0.9	53.32+0.8	20.5+0.01	5.5+0.1
30	14.31+0.8	48.76+0.7	20.4+0.01	6.0+0.1
(d) DMPC+1×10^{-2} M CPZ-HCl				
0	13.20+0.5	45.41+0.9	17.7+0.02	9+0.1
5	11.74+0.7	40.37+0.7	17.5+0.01	10+0.2
10	10.43+0.8	36.00+0.8	16.8+0.00	11+0.1
15	9.72+0.6	33.60+0.4	16.3+0.01	11+0.2
20	8.93+0.8	30.87+0.3	16.3+0.01	11+0.1
25	7.47+0.5	25.84+0.5	16.0+0.02	12+0.2
30	6.84+0.7	23.66+0.6	16.0+0.01	12+0.1

ΔH, ΔS, Tc and HHW values, shown in table 2 are the mean values of three experiments (n=3) with mean±SD. ΔH_t values were observed to decrease linearly as a function of sonication time both in the absence and presence of CPZ-HCl (fig. 5).

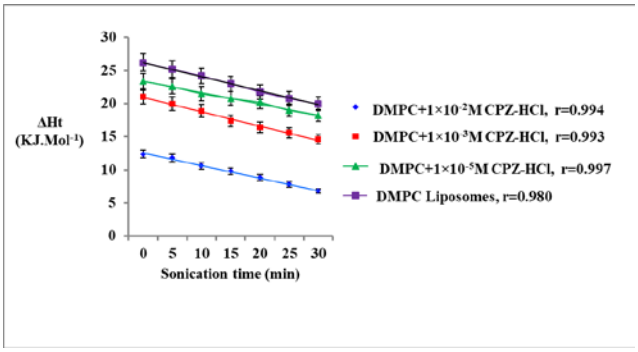

Fig. 5: The effect of sonication time on the enthalpies of transition (ΔH_t) of 10% W/V DMPC liposomes in the absence and presence of CPZ-HCl in phosphate buffer (pH6.0). The values obtained were the mean of three experiments (n= 3). SD are shown as error bars and all values were in the range of+0.3-0.9

The reduction in ΔHt, ΔSt, T_C and the increase in HHW as a result of sonication (table 2) implies a change in the organization of DMPC molecules upon sonication. The constraints imposed by the smaller radius of curvature of liposomes resulting from sonication reduce the energy requirements of the phase transition process. Addition of CPZ-HCl would tend to produce further constraints within the system, disrupting the regular molecular packing of the bilayer and hence reducing ΔHt.

The effect of charged lipids on ΔHt, ΔSt and T_C of DMPC liposomes was studied in the absence and presence of CPZ-HCl concentrations in phosphate buffer (pH6.0) (table 3).

Table 3: The effect of ionic lipid (ST and DCP) on the enthalpies (ΔHt), entropies (ΔSt) and transition temperature (Tc) of DMPC/CPZ-HCl liposomes in phosphate buffer (pH 6.0) as determined by DSC

Liposome	ΔH_t (KJ. mol^{-1})	ΔS_t (J. mol^{-1}. K^{-1})	T_C (°C)
10%W/V DMPC	26.56+0.6	89.52+1.7	23.7+0.01
10%W/V DMPC+1×10^{-5}M CPZ-HCl	23.87+2.3	81.01+1.6	21.5+0.02
10%W/V DMPC+1×10^{-3} M CPZ-HCl	20.94+2.6	71.29+1.4	20.6+0.01
10%W/V DMPC+1×10^{-2} M CPZ-HCl	13.20+2.1	45.38+1.6	17.7+0.01
10%W/V DMPC+1% ST	13.71+1.8	46.34+1.4	22.8+0.02
10%W/V DMPC+1%ST+1×10^{-5}M CPZ-HCl	16.56+2.0	55.99+1.1	21.6+0.01
10%W/V DMPC+1%ST+1×10^{-3}M CPZ-HCl	14.61+1.6	49.77+1.9	20.4+0.01
10%W/V DMPC+1%ST+1×10^{-2}M CPZ-HCl	11.34+1.4	39.04+1.8	17.3+0.02
10%W/V DMPC+1% DCP	10.87+2.2	36.98+1.8	20.8+0.01
10%W/V DMPC+1%DCP+1×10^{-5}M CPZ-HCl	9.16+2.0	31.32+1.0	19.3+0.01
10%W/V DMPC+1%DCP+1×10^{-3}M CPZ-HCl	7.54+1.5	25.84+1.3	18.6+0.02
10%W/V DMPC+1%DCP+1×10^{-2}M CPZ-HCl	6.63+1.6	22.87+1.5	16.7+0.02

ΔH, ΔS and Tc values, shown in table 3 are the mean values of three experiments (n=3) with mean±SD.

Table 4: The effect of ionic lipid (ST and DCP) on the enthalpies (ΔHt), entropies (ΔSt) and transition temperature (Tc) of DMPC/CPZ-HCl liposomes (at concentrations known to cause anesthesia) in phosphate buffer (pH 7.4) as determined by DSC

Liposome	ΔH_t (KJ. mol^{-1})	ΔS_t (J. mol^{-1}. K^{-1})	T_C (°C)
10%W/V DMPC	26.56+1.6	89.52+1.7	23.7+0.01
10%W/V DMPC+1×10^{-5}M CPZ-HCl	23.03+1.4	78.11+1.9	21.9+0.02
10%W/V DMPC+2.5×10^{-5} M CPZ-HCl	22.61+1.2	76.74+1.4	21.7+0.01
10%W/V DMPC+5×10^{-5} M CPZ-HCl	22.43+1.8	76.12+1.6	21.5+0.01
10%W/V DMPC+7.5×10^{-5} M CPZ-HCl	22.09+1.0	75.02+1.5	21.2+0.01
10%W/V DMPC+1×10^{-4} M CPZ-HCl	21.87+1.7	74.35+1.2	21.0+0.01
10%W/V DMPC+1% ST	13.71+1.8	46.43+1.0	22.8+0.02
10%W/V DMPC+1%ST+1×10^{-5}M CPZ-HCl	16.42+1.6	55.52+1.6	22.6+0.01
10%W/V DMPC+1%ST+2.5×10^{-5}M CPZ-HCl	16.27+1.7	55.00+1.4	22.4+0.01
10%W/V DMPC+1%ST+5×10^{-5}M CPZ-HCl	16.17+1.3	54.71+1.8	22.2+0.02
10%W/V DMPC+1%ST+7.5×10^{-5}M CPZ-HCl	15.91+1.2	53.85+1.9	22.1+0.01
10%W/V DMPC+1%ST+1×10^{-4}M CPZ-HCl	15.71+1.5	53.23+1.7	22.0+0.02
10%W/V DMPC+1% DCP	10.87+0.9	36.98+1.7	20.8+0.01
10%W/V DMPC+1%DCP+1×10^{-5}M CPZ-HCl	9.16+0.8	31.32+1.2	19.6+0.02
10%W/V DMPC+1%DCP+2.5×10^{-5}M CPZ-HCl	7.54+0.7	25.84+1.8	19.4+0.01
10%W/V DMPC+1%DCP+5×10^{-5}M CPZ-HCl	6.63+0.8	22.87+1.6	19.1+0.01
10%W/V DMPC+1%DCP+7.5×10^{-5}M CPZ-HCl	6.63+0.6	22.87+1.0	18.8+0.02
10%W/V DMPC+1%DCP+1×10^{-4}M CPZ-HCl	6.63+0.9	22.87+1.5	18.6+0.01

ΔH, ΔS and Tc values, shown in table 4 are the mean values of three experiments (n=3) with mean±SD.

Both positively charged ST and negatively charged DCP greatly reduce ΔH_t, ΔS_t and T_C (table 3). The reduction in the above parameters was more pronounced using DCP. The reduction in ΔH_t in the presence of ionic lipids may arise from an electrostatic and hydrogen bonding effect resulting from the interaction of the bulky choline head group with the charged ionic lipid. This will alter the

packing efficiency of the acyl chains in the bilayer and more disordered system results. The incorporation of CPZ-HCl into DMPC/ST liposomes was observed to increase ΔH_t and ΔS_t (table 3) which reflects a competition between CPZ-HCl and ST for sites within the bilayer. It also indicates that CPZ-HCl at low concentrations has a stabilizing effect on the hydrophobic bilayer in the presence of ST. The addition of CPZ-HCl to DMPC/DCP liposomes resulted in a greater reduction in ΔH_t, ΔS_t and T_C compared with DMPC/ST liposomes (table 3). This indicates an increase in the surface concentration of the positively charged CPZ ions, which is predominantly ionized at pH 6.0 as CPZ has a pKa of 9.2. An NMR study on phospholipid bilayer showed that CPZ exhibited low interaction with the acyl packing of liposomes made of neutral phospholipids, such as DMPC. However, the addition of anionic phospholipid such as phosphatidylserine to such neutral liposomes has perturbed the acyl packing of liposomes [36].

The effect of CPZ-HCl in phosphate buffer (pH 7.4) at concentrations known to cause anesthesia on ΔH_t, ΔS_t and T_C of DMPC liposomes was also investigated in the absence and presence of ionic lipids (table 4)

Both ST and DCP greatly reduce ΔH_t, ΔS_t and T_C of DMPC liposomes (table 4). The addition of CPZ-HCl to DMPC/ST liposomes increases ΔH_t and ΔS_t (table 4). On the other hand, the addition of CPZ-HCl to DMPC/DCP liposomes decreases ΔH_t, ΔS_t and T_C (table 3). A previous study has shown that the partitioning of CPZ-HCl into DMPC at concentrations known to cause anesthesia was observed to increase linearly as a function CPZ-HCl concentration in phosphate buffer (pH 7.4) at 37 °C [33]. In addition, another study has shown that the partitioning of CPZ-HCl into DMPC liposomes was concentration-dependent both above and below the TC [34].

Since CPZ-HCl perturbs the gel-liquid crystalline phase transition by decreasing ΔH_t, ΔS_t and T_C at concentrations known to cause anesthesia, these results could support the hypothesis that the addition of local anesthetics might trigger a change in the lipid surrounding the sodium channel from the gel to the liquid crystalline state, allowing the sodium channel to close with the resulting anesthesia [37].

CONCLUSION

CPZ-HCl concentrations as low as 1×10^{-7}M were observed to alter the gel-liquid crystalline phase transition and thus to possess a membrane destabilizing effect, using DSC. CPZ-HCl reduces ΔH_t, ΔS_t, T_C, the pre-T_C and increases HHW of DMPC liposomes. ΔH_t and ΔS_t of DMPC liposomes were observed to decrease linearly as a function of CPZ-HCl both below and above the concentration of 5×10^{-3}M. ΔH_t of DMPC liposomes was observed to decrease linearly in the absence and presence of and CPZ-HCl as a function of sonication time. ΔH_t and ΔS_t of DMPC liposomes were observed to increase in the presence of cationic lipid (ST) and to decrease in the presence of anionic lipid (DCP). ΔS_t and T_C of DMPC, DMPC/ST, DMPC/DCP liposomes were found to decrease linearly as a function of CPZ-HCl concentrations known to cause anesthesia. As CPZ-HCl influences the ΔH_t, ΔS_t, TC, pre-T_C and HHW, it can be inferred that CPZ-HCl interacts with both the polar head group and the hydrophobic interior of the phospholipid bilayer. Since CPZ-HCl perturbs the gel-liquid crystalline phase transition by decreasing ΔHt, ΔSt and T_C at concentrations known to cause anesthesia, these results could support the hypothesis that the addition of local anesthetics might trigger a change in the lipid surrounding the sodium channel from the gel to the liquid crystalline state, allowing the sodium channel to close with the resulting anesthesia.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Bonde S, Nair S. Advances in liposomal drug delivery system: fascinating types and potential applications. Int J App Pharm. 2017;9(3):1-7. doi: 10.22159/ijap.2017v9i3.17984.
2. Zylberberg C, Matosevic S. Pharmaceutical liposomal drug delivery: a review of new delivery systems and a look at the regulatory landscape. Drug Deliv. 2016;23(9):3319-29. doi: 10.1080/10717544.2016.1177136, PMID 27145899.
3. Sharma S, Kumar V. *In vitro* cytotoxicity effect on mcf-7cell line of co-encapsulated artesunate and curcumin liposome. Int J Pharm Pharm Sci. 2016;8:286-92.
4. Bozzuto G, Molinari A. Liposomes as nanomedical devices. Int J Nanomedicine. 2015;10:975-99. doi: 10.2147/IJN.S68861, PMID 25678787.
5. Chen J, Cheng D, Li J, Wang Y, Guo JX, Chen ZP, Cai BC, Yang T. Influence of lipid composition on the phase transition temperature of liposomes composed of both DPPC and HSPC. Drug Dev Ind Pharm. 2013;39(2):197-204. doi: 10.3109/03639045.2012.668912, PMID 22443684.
6. Popova AV, Hincha DK. Thermotropic phase behavior and headgroup interactions of the non-bilayer lipids phosphatidylethanolamine and monogalactosyldiacylglycerol in the dry state. BMC Biophys. 2011;4:1-11.
7. Chiu MH, Prenner EJ. Differential scanning calorimetry: an invaluable tool for a detailed thermodynamic characterization of macromolecules and their interactions. J Pharm Bioallied Sci. 2011;3(1):39-59. doi: 10.4103/0975-7406.76463, PMID 21430954.
8. Demetzos C. Differential scanning calorimetry (DSC): A tool to study the thermal behavior of lipid bilayers and liposomal stability. Journal of Liposome Research. 2008;18(3):159-73. doi: 10.1080/08982100802310261.
9. Koynova R, Caffrey M. Phases and phase transitions of the phosphatidylcholines. Biochim biophys Acta. 1998;1376(1):91-145. doi: 10.1016/s0304-4157(98)00006-9, PMID 9666088.
10. Epand RM, Epand RF. Studies of thermotropic phospholipid phase transitions using scanning densitometry. Chem Phys Lipids. 1980;27(2):139-50. doi: 10.1016/0009-3084(80)90019-5.
11. Melchior DL, Morowitz HJ. Dilatometry of dilute suspensions of synthetic lecithin aggregates. Biochemistry. 1972;11(24):4558-62. doi: 10.1021/bi00774a020, PMID 4654143.
12. Trauble H, Overath P. The structure of Escherichia coli membranes studied by fluorescence measurements of lipid phase transitions. Biochim biophys acta. 1973;307(3):491-512. doi: 10.1016/0005-2736(73)90296-4, PMID 4581497.
13. Small DM. Phase equilibria and structure of dry and hydrated egg lecithin. J Lipid Res. 1967;8(6):551-7. doi: 10.1016/S0022-2275(20)38874-X, PMID 6057484.
14. Kornberg RD, McConnell HM. Inside-outside transitions of phospholipids in vesicle membranes. Biochemistry. 1971;10(7):1111-20. doi: 10.1021/bi00783a003, PMID 4324203.
15. Veksli Z, Salsbury NJ, Chapman D. Physical studies of phospholipids. XII. Nuclear magnetic resonance studies of molecular motion in some pure lecithin-water systems. Biochim Biophys Acta. 1969;183(3):434-46. doi: 10.1016/0005-2736(69)90158-8, PMID 5822816.
16. Koynova R, Caffrey M. An index of lipid phase diagrams. Chem Phys Lipids. 2002;115(1-2):107-219. doi: 10.1016/s0009-3084(01)00200-6, PMID 12047902.
17. Weinstein JN, Magin RL, Yatvin MB, Zaharko DS. Liposomes and local hyperthermia: selective delivery of methotrexate to heated tumors. Science. 1979;204(4389):188-91. doi: 10.1126/science.432641, PMID 432641.
18. Papahadjopoulos D, Jacobson K, Nir S, Isac T. Phase transitions in phospholipid vesicles. Fluorescence polarization and permeability measurements concerning the effect of temperature and cholesterol. Biochim Biophys Acta. 1973;311(3):330-48. doi: 10.1016/0005-2736(73)90314-3, PMID 4729825.

19. Underhaug Gjerde A, Holmsen H, Nerdal W. Chlorpromazine interaction with phosphatidylserines: a ^{13}C and ^{31}P solid-state NMR study. Biochim Biophys Acta. 2004;1682(1-3):28-37. doi: 10.1016/j.bbalip.2004.01.004. PMID 15158753.
20. Wisniewska A, Wolnicka Glubisz A. ESR studies on the effect of cholesterol on chlorpromazine interaction with saturated and unsaturated liposome membranes. Biophys Chem. 2004;111(1):43-52. doi: 10.1016/j.bpc.2004.04.001, PMID 15450374.
21. Lucio M, Lima JLFC, Reis S. Drug-membrane interactions: significance for medicinal chemistry. Curr Med Chem. 2010;17(17):1795-809. doi: 10.2174/092986710791111233, PMID 20345343.
22. Ahmed M, Burton JS, Hadgraft J, Kellaway IW. Thermodynamics of partitioning and efflux of phenothiazines from liposomes. J Membr Biol. 1981;58(3):181-9. doi: 10.1007/BF01870904, PMID 7218338.
23. Razin S, Tourtellotte ME, McElhaney RN, Pollack JD. Influence of lipid components of Mycoplasma laidlawii membranes on osmotic fragility of cells. J Bacteriol. 1966;91(2):609-16. doi: 10.1128/jb.91.2.609-616.1966, PMID 5883100.
24. Silvius JR, Read BD, McElhaney RN. Thermotropic phase transitions of phosphatidylcholines with odd-numbered n-acyl chains. Biochim Biophys Acta. 1979;555(1):175-8. doi: 10.1016/0005-2736(79)90081-6, PMID 476097.
25. Nerdal W, Gundersen SA, Thorsen V, Høiland H, Holmsen H. Chlorpromazine interaction with glycerophospholipid liposomes studied by magic angle spinning solid state 13C-NMR and differential scanning calorimetry. Biochim Biophys Acta. 2000;1464(1):165-75. doi: 10.1016/s0005-2736(00)00125-5, PMID 10704930.
26. Chapman D, Byrne P, Shipley GG. The physical properties of phospholipids I. Solid state and mesomorphic properties of some 2,3-diacyl-DL-phosphatidylethanolamines. Proc R Soc Lond A. 1966;290:115.
27. Barton PG, Gunstone FD. Hydrocarbon chain packing and molecular motion in phospholipid bilayers formed from unsaturated lecithins. Synthesis and properties of sixteen positional isomers of 1,2-dioctadecenoyl-sn-glycero-3-phosphorylcholine. J Biol Chem. 1975;250(12):4470-6. doi: 10.1016/S0021-9258(19)41327-6, PMID 1141217.
28. Eibl H, Blume A. The influence of charge on phosphatidic acid bilayer membranes. Biochim Biophys Acta. 1979;553(3):476-88. doi: 10.1016/0005-2736(79)90303-1, PMID 36911.
29. Blume A, Eibl H. The influence of charge on bilayer membranes. Calorimetric investigations of phosphatidic acid bilayers. Biochim Biophys Acta. 1979;558(1):13-21. doi: 10.1016/0005-2736(79)90311-0, PMID 40599.
30. Brady GW, Fein DB. An analysis of the X-ray interchain peak profile in dipalmitoylglycerophosphocholine. Biochim Biophys Acta. 1977;464(2):249-59. doi: 10.1016/0005-2736(77)90001-3, PMID 831796.
31. Hinz HJ, Sturtevant JM. Calorimetric investigation of the influence of cholesterol on the transition properties of bilayers formed from synthetic L-lecithins in aqueous suspension. J Biol Chem. 1972;247(11):3697-700. doi: 10.1016/S0021-9258(19)45197-1, PMID 5030639.
32. Darke A, Finer EG, Flook AG, Phillips MC. Nuclear magnetic resonance study of lecithin-cholesterol interactions. J Mol Biol. 1972;63(2):265-79. doi: 10.1016/0022-2836(72)90374-9, PMID 4634508.
33. Saito YD, Tehrani S, Okamoto MM, Chang HH, Dea P. Calorimetry studies of chlorpromazine hydrochloride in solution. Langmuir. 2000;16(16):6391-5. doi: 10.1021/la991297l.
34. Ahmed AMS, Farah FH, Kellaway IW. The thermodynamics of partitioning of phenothiazines between phosphate buffer and the lipid phases of cyclohexane, n-octanol and DMPC liposomes. Pharm Res. 1985;2(3):119-24. doi: 10.1023/A:1016359215869, PMID 24272688.
35. Holte LL, Separovic F, Gawrisch K. Nuclear magnetic resonance investigation of hydrocarbon chain packing in bilayers of polyunsaturated phospholipids. Lipids. 1996;31Suppl:S199-203. doi: 10.1007/BF02637076, PMID 8729119.
36. Van den Besselaar AM, De Druijff B, Van den Bosch H, Van Deenen LL. Phosphatidylcholine mobility in liver microsomal membranes. Biochim Biophys Acta. 1978;510(2):242-55. doi: 10.1016/0005-2736(78)90024-x, PMID 667042.
37. Lee AG. Model for the action of local anaesthetics. Nature. 1976;262(5569):545-8. doi: 10.1038/262545a0, PMID 958412.