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Thermodynamics of Partitioning and Efflux of Phenothiazines from Liposomes

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Summary. The partitioning of nine phenothiazines between dimyristoylphosphatidylcholine (DMPC) liposomes and 0.9% wt/vol saline at pH 6 has been studied both below and above the phase transition temperature (T_c) of the phospholipid. Higher partitioning was observed above T_c. Both the entropy and enthalpy of partitioning were positive below and above T_c, and a linear relationship between the entropy and enthalpy has been derived. In general, the partitioning and transport of alkylaminophenothiazines in DMPC liposomes over the temperature range of 5 to 40 °C is entropically controlled. The entropies and enthalpies of partitioning of various groups in the phenothiazine structure have been calculated.

No relationship was found between particle size of the DMPC liposomes and the equilibrium partition coefficient at 25 °C. However, the particle size of liposomes did increase with increasing acyl chain length of the phospholipid.

Using differential scanning calorimetry, the enthalpy and entropy of transition of the DMPC liposomes in the absence and presence of phenothiazines has been calculated. The temperature dependence of the first-order rate constant of trimeprazine tartrate transport in DMPC liposomes was investigated and was found to be maximum at the T_c of the phospholipid.

The permeability of membranes to drug molecules is governed by physical factors such as the equilibrium partition coefficient and the diffusivity of the drug molecule across the membrane interface and the membrane interior. In the latter case, although a mean bulk coefficient may be employed, the rate of solute transport will vary across the structured membrane interior. Thermodynamics of partitioning can be employed to obtain an insight into transport processes and their control. Group contributions to the thermodynamics of partitioning have been studied in depth, using trace quantities of primary, secondary, and tertiary alcohols distributed into DMPC liposomes (Diamond & Katz, 1974; Katz & Diamond, 1974a–c). The measured equilibrium partition coefficients (K) of the alcohols both below and above T_c of the phospholipid were used to determine the enthalpy ($\Delta H_{w\to l}$), free energy ($\Delta G_{w\to l}$) and entropy ($\Delta S_{w\to l}$) of partitioning from water to lipid. It was shown that $\Delta H_{w\to l}$ for $-CH_2-$ varies between -5.3 to -6.8 kJ·mol^{-1}, $\Delta G_{w\to l}$ for $-CH_2-$ varies between -1.9 to -2.7 kJ·mol^{-1}, and $\Delta S_{w\to l}$ for $-CH_2-$ varies from -8.7 to -16.6 J·mol^{-1}K^{-1}. All these thermodynamic parameters of partitioning vary markedly for different model membrane systems, e.g. toad bladder, erythrocytes, liposomes or olive oil (Wright & Bindslev, 1976). It has been shown, using the toad urinary bladder, that on average the addition of one $-CH_2-$ group to a nonelectrolyte molecule increases the permeability coefficient fourfold, whereas the addition of one $-OH$ group reduces the permeability coefficient 500-fold. The partitioning of $-OH$ groups is governed by hydrogen bonding between the $-OH$ group and water.

Another important thermodynamic parameter is the activation energy of permeation and this has been considered in detail by Cohen (1975*a*). The activation energy values for the permeability across egg phosphatidylcholine liposomes vary according to the solute and range from 21.0 to 68.0 kJ mol^{-1}. The linear correlation between these activation energies and the ability of the solutes to form hydrogen bonds in

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water, shows clearly that dehydration of the solutes plays an important role in the process. The magnitude of the activation energies has been found to be related to the physical state of the hydrocarbon chains in the bilayers.

In this paper, the use of nine closely related phenothiazine derivatives made feasible a group contribution study of partitioning below and above the T_c of DMPC liposomes. The concept of group contribution in permeability studies was originally investigated by Overton (1896). DMPC, like many other phospholipids, exists in the L-β solid crystalline phase below the T_c and in the L-α liquid crystalline state above the T_c. Using differential scanning calorimetry, the enthalpy and entropy of transition of the DMPC liposomes were studied in the absence and presence of phenothiazine drugs. The amount of phenothiazine drug incorporated, as assessed by the equilibrium partition coefficient, and the change produced in the particle size of DMPC liposomes was also examined.

Materials and Methods

Materials

The following drug samples were kindly donated by May & Baker Ltd., Dagenham, Essex: 2-Valerylphenothiazine (VP), 10-(3-dimethylaminopropyl)-2-valeryl phenothiazine oxalate (DAVP.O), (±)-2-valeryl-10-(3-dimethylamino-2-methylpropyl) phenothiazine hydrochloride (VDMP.HCl), (±)-10-(3-methylamino-2-methylpropyl)-2-valeryl phenothiazine oxalate hemihydrate (MMVP.OH), (±)-10-(3-diethylamino-2-methylpropyl)-2-valeryl phenothiazine (DMVP), (±)-10-(2-dimethylaminopropyl)-2-valeryl phenothiazine oxalate hemihydrate (DAVP.OH), promethazine hydrochloride (PROM.HCl), and timeprazine tartrate (TRIM.T). Promazine hydrochloride (PRZ.HCl) was donated by Wyeth Laboratories, Maidenhead, Berks. All the phenothiazines were of pharmaceutical grade and were used as received.

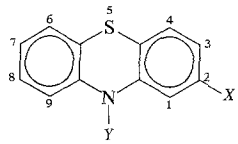
L-α-dimyristoylphosphatidylcholine (DMPC), L-α-dipalmitoylphosphatidylcholine (DPPC) and L-α-distearoylphosphatidylcholine (DSPC) were purchased from Sigma Chemicals Co., Poole, Dorset, England. All the synthetic phospholipids were not less than 98% pure. Egg phosphatidylcholine (EPC) was purified and recrystallized from the crude egg lecithin (BDH Chemicals) (Bangham, Hill & Miller, 1974) and was stored under acetone at 4 °C. The recrystallized EPC was found to be chromatographically pure with an R_f value 0.55 using chloroform/methanol/water, 14:6:1, as a solvent system.

Methods

Multilamellar liposomes were prepared by a technique similar to those previously described (Enoch & Strittmatter, 1979; Reeves & Dowben, 1968). The required amount of phospholipid was weighed and dissolved in the minimum amount of spectrophotometric grade ethanol. The phenothiazine derivatives of very low water solubility were added in the organic phase, whereas the more water-soluble derivatives were added to the aqueous phase. Ethanol was evaporated under vacuum using a rotary evaporator (Rotavapor-R, Buchi, Switzerland) so that the phospholipid remained as a thin film on the walls of the flask. Finally, the aqueous phase (0.9% wt/vol saline, pH 7.4) was added to give a final concentration of 1 mg ml^{-1} of phospholipid and 0.1 mg ml^{-1} of phenothiazine derivative. The flask was rotated until the film was dispersed to form multilamellar liposomes. The pH of the aqueous phase was adjusted by the addition of either 0.1 M hydrochloric acid or sodium hydroxide.

The liposomes were stirred for 28 hr at a constant speed and temperature which ranged from 5 to 40 °C at intervals of 5 °C. Equilibrium partition coefficients were determined by removing aliquots of the equilibrated suspension and separating the liposomes by centrifugation (M.S.E. 25 U.K.) at 70,000 × g for 1 hr. Centrifugation was carried out at the corresponding temperature of study. The supernatant was assayed by U.V. spectrophotometry (Beckman Model 25 U.S.A.) at the maximum wavelength of absorption (Table 1), for the free phenothiazine drug (Schwendener & Weder, 1978). The amount of residual lipid in the supernatant was not measured, but when the dispersions were centrifuged for periods longer than 1 hr, no further change in the U.V.

Table 1. Chemical structure, λ_{max}, and molar extinction coefficient (ε_{max}) of phenothiazines



Phenothiazine nucleus

Structure	Abbreviated name	λ_{max} (nm)	ε_{max}
a) $X-CO.(CH_2)_3.CH_3$ $Y-H$	VP.	244	0.99×10^4
b) $X-CO.(CH_2)_3.CH_3$ $Y-(CH_2)_3.N.(CH_3)_2$	DAVP.O	244	2.09×10^4
c) $X-CO.(CH_2)_3.CH_3$ $Y-CH_2.CH(CH_3).CH_2.N.(CH_3)_2$	VDMP.HCl	243	1.68×10^4
d) $X-CO.(CH_2)_3.CH_3$ $Y-CH_2.CH(CH_3).CH_2.NH.CH_3$	MMVP.OH	243	2.04×10^4
e) $X-CO.(CH_2)_3.CH_3$ $Y-CH_2.CH(CH_3).CH_2.N.(CH_2CH_3)_2$	DMVP	242	2.80×10^4
f) $X-CO.(CH_2)_3.CH_3$ $Y-CH_2.CH(CH_3).N.(CH_3)_2$	DAVP.OH	241	2.44×10^4
g) $X-H$ $Y-CH_2.CH(CH_3).N.(CH_3)_2$	PROM.HCl	249	2.85×10^4
h) $X-H$ $Y-CH_2.CH(CH_3).CH_2.N.(CH_3)_2$	TRIM.T.	252	5.53×10^4
i) $X-H$ $Y-CH_2CH_2CH_2.N(CH_3)_2$	PRZ.HCl	252	3.09×10^4

absorbance of the supernatant was observed. The amount of phenothiazine drug entrapped by liposomes was found by difference and the partition coefficient calculated.

The transport of TRIM.T was followed by diluting the equilibrated sample tenfold and taking samples over a 4-hr period, a technique previously found to be satisfactory for phenothiazines (Ahmed et al., 1980). Each sample was filtered using an ultrafiltration cell at 40 psi nitrogen pressure. The filtrate was assayed by U.V. at the maximum wavelength of absorption.

The particle size of the DMPC liposomes containing the phenothiazines and equilibrated for 28 hr at 25 °C was determined using a Coulter Counter Nano-Sizer™ (Lines & Miller, 1979).

For differential scanning calorimetry (DSC), the liposomes containing 10% wt/vol of DMPC in distilled water, pH 6, and varying concentrations of drugs were prepared (Keough & Davis, 1979). Approximately 6-mg samples were sealed into aluminium pans (Perkin-Elmer Alum 219-0062). DSC thermograms were obtained using a Perkin-Elmer DSC-2 calorimeter precalibrated using Indium. The preparation containing DMPC liposomes was scanned at a speed of 5 °C min^{-1} and a sensitivity range of 1 $Mcal \cdot s^{-1}$ over a temperature range of 280 to 310 °K. The half height width (HHW) of the DSC peaks and T_c in the absence and presence of drug were measured. The T_c values were determined from the mid-point of the transition peak. The enthalpy of transition was calculated from the area under the thermogram.

Results and Discussion

The temperature dependence of the equilibrium partition coefficient is given by the following relationship (Tinoco, Sauer & Wang, 1978).

$$\ln K = \text{constant} - \frac{\Delta H_{w\to l}}{RT} \quad (1)$$

where K is the equilibrium partition coefficient, $\Delta H_{w\to l}$ is the enthalpy of partitioning, T is the absolute temperature, and R is the gas constant. $\Delta H_{w\to l}$ can be found from the plot of $\ln K$ *vs.* T^{-1}, where the gradient of the line is $\Delta H_{w\to l}/R$ and the intercept is the constant. In calculating $\Delta H_{w\to l}$ from Eq. (1) it is assumed that $\Delta H_{w\to l}$ is independent of temperature over the range studied. $\Delta H_{w\to l}$ has the physical meaning of the change in enthalpy when one mole of solute is transferred from water to lipid at infinite dilution.

The free energy of partitioning and the equilibrium constant are related by the relationship shown in Eq. (2).

$$\Delta G_{w\to l} = -RT \ln K. \quad (2)$$

Once $\Delta H_{w\to l}$ and $\Delta G_{w\to l}$ are known, then $\Delta S_{w\to l}$ can be calculated from the following relationship.

$$\Delta S_{w\to l} = \frac{\Delta H_{w\to l} - \Delta G_{w\to l}}{T} \text{ Jmol}^{-1} K^{-1}. \quad (3)$$

From the DSC thermograms the enthalpy of transition (ΔH_t) can be calculated from the area under the peak. The entropy of transition (ΔS_t) is obtained using Eq. (3) where at the transition temperature, ΔG is assumed zero.

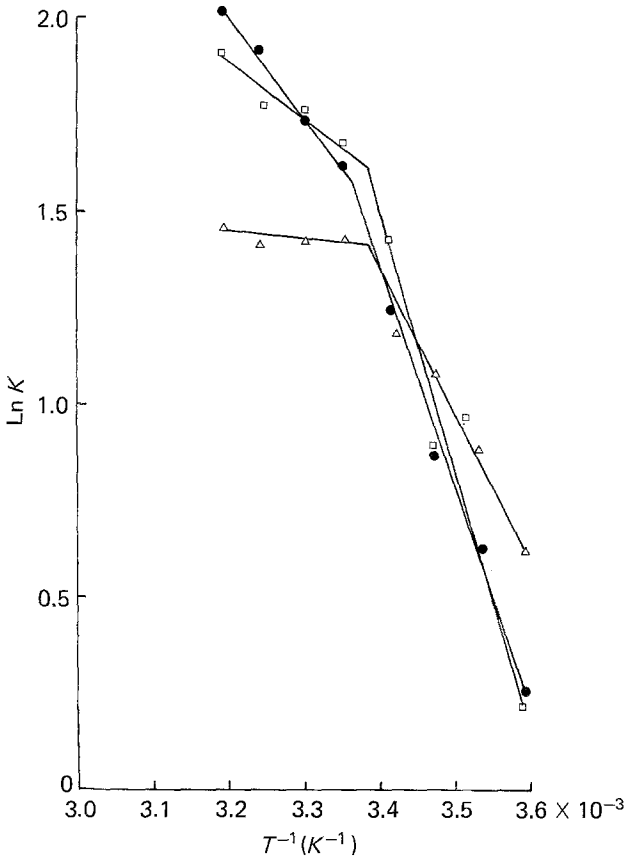


Fig. 1. Plot of natural log equilibrium partition coefficient (Ln K) between DMPC liposomes and water as a function of absolute temperature (K^{-1}); pH 6. Each point on the graph represents average of two readings. ●, VP; △, DAVP.O; □, VDMP.HCl

Partition coefficients were measured for the nine phenothiazines over the temperature range of 5 to 40 °C and the temperature dependence of K for these drugs is illustrated in Figs. 1–3. For each of the nine phenothiazines Table 2 gives the intercept (C), the slope ($-\Delta H/R$) of the straight line fitted by a least-squares analysis to points both below and above the T_c. The mean square error and the correlation coefficient are also tabulated. From Figs. 1–3 it is apparent that K for all nine phenothiazines increases with temperature both below and above the gel-liquid crystalline phase transition temperature. There is a distinct break at or near 23 °C in the plot of $\ln K$ *vs.* T^{-1} due to the change in phase of the liposomes from gel-crystalline to the liquid-crystalline state (Melchior & Steim, 1976).

It is also evident that Ks are higher above the T_c compared with values below the T_c (Cohen, 1975*a*). The break in the plot was taken to be the T_c of the DMPC liposomes in the presence of phenothiazines

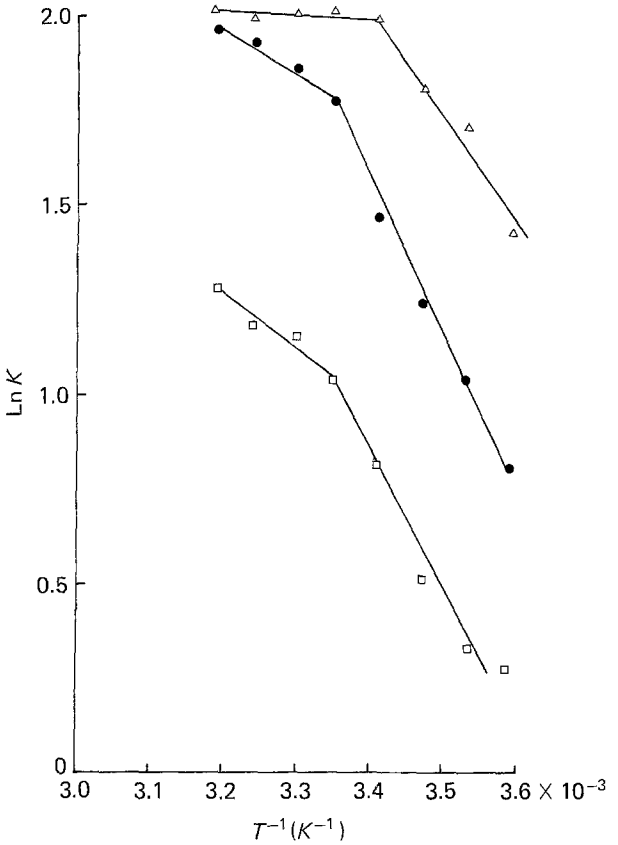


Fig. 2. Plot of Ln K between DMPC liposomes and water as a function of absolute temperature (K^{-1}); pH 6. ●, MMVP.OH; △, DMVP; □, DAVP.OH

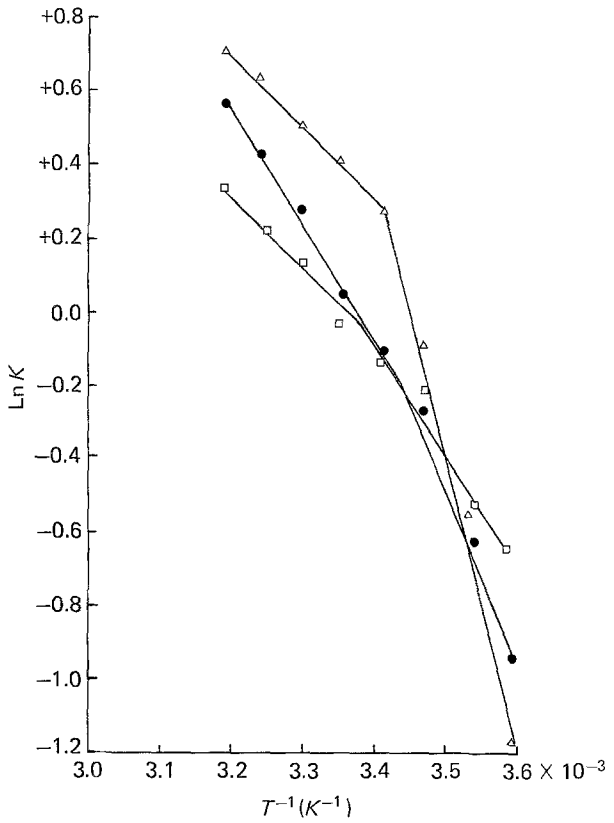


Fig. 3. Plot of Ln K between DMPC liposomes and water as a function of absolute temperature (K^{-1}). ●, PROM.HCl; △, TRIM.T; □, PRZ.HCl

Table 2. Linear dependence of LnK on T^{-1}

Phenothiazine drug	Below T_c (5–20 °C)				Above T_c (25–40 °C)			
	C	$-\Delta H/R$ (K)	r	M.S.E.	C	$-\Delta H/R$ (K)	r	M.S.E.
VP	19.7	-5.4×10^3	−0.99	0.015	11.4	-2.9×10^3	−0.99	0.009
DAVP.O	10.6	-2.8×10^3	−0.95	0.038	1.8	-0.1×10^3	−0.90	0.011
VDMP.HCl	21.8	-5.9×10^3	−0.91	0.102	5.8	-1.2×10^3	−0.93	0.018
MMVP.OH	14.5	-3.8×10^3	−0.99	0.015	5.7	-1.2×10^3	−0.97	0.006
DMVP	12.3	-3.0×10^3	−0.97	0.029	2.8	-0.2×10^3	−0.89	0.004
DAVP.OH	10.3	-2.8×10^3	−0.94	0.015	8.3	-2.2×10^3	−0.94	0.013
PROM.HCl	16.4	-4.8×10^3	−0.98	0.021	10.8	-3.2×10^3	−0.98	0.014
TRIM.T.	27.9	-8.1×10^3	−0.99	0.031	6.9	-1.9×10^3	−0.99	0.006
PRZ.HCl	10.3	-3.1×10^3	−0.96	0.026	8.7	-2.6×10^3	−0.97	0.007

For each phenothiazine drug a straight line, $\mathrm{Ln}K = C - \frac{\Delta H}{RT}$, was fitted by least mean squares to the experimental measurements of LnK below and above T_c. In the table C is intercept, $-\Delta H/R$ is gradient, r is correlation coefficient, M.S.E. is mean square error for each line. M.S.E. $=(\sum[((\mathrm{Ln}K)_{exp}-(\mathrm{Ln}K)_{fit})^2]^{1/2})/n$ where n is the number of measurements.

(Table 5). It is clear that the T_c of DMPC liposomes is increased in the presence of VP, MMVP. OH and DMVP but the addition of other phenothiazines decreased the T_c. The addition of some solutes may not affect the T_c of phospholipid, e.g., gramicidin-A (Cohen, 1975*b*).

Figure 4 shows that both $\Delta S_{w\to l}$ and $\Delta H_{w\to l}$ are positive and $\Delta S_{w\to l}$ does increase with increasing $\Delta H_{w\to l}$. There exists a linear relationship between $\Delta S_{w\to l}$ and $\Delta H_{w\to l}$ both below and above the T_c. $\Delta S_{w\to l}$ and $\Delta H_{w\to l}$ are related by Eqs. (4) and (5), respectively, for temperatures above and below T_c.

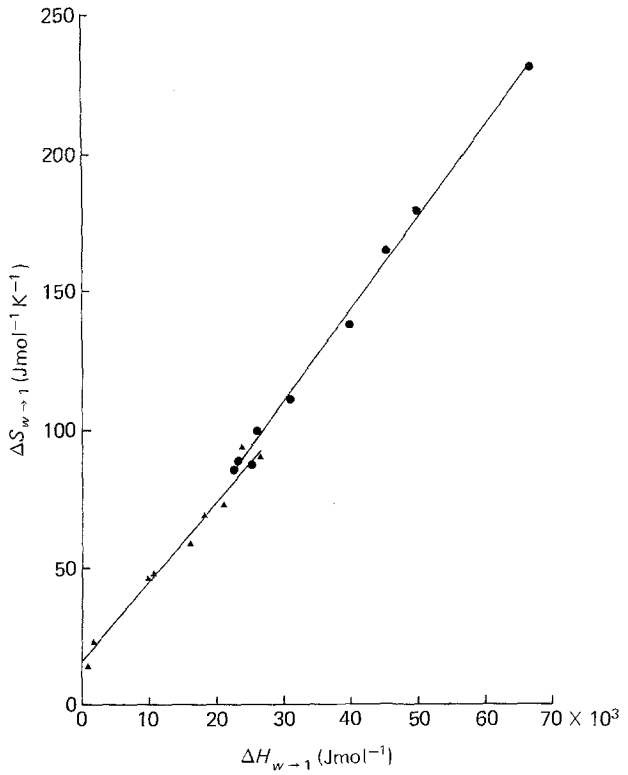


Fig. 4. The entropy of partitioning between DMPC liposomes and water ($\Delta S_{w\to l}$) below and above T_c plotted against the enthalpy of partitioning ($\Delta H_{w\to l}$). Each point represents one solute. The straight line gives the least mean squares fit. ●, below T_c; ▲, above T_c

$$\Delta S_{w\to l} = 0.00288\,\Delta H_{w\to l} + 15.99, \quad r = 0.9855 \qquad (4)$$

$$\Delta S_{w\to l} = 0.00334\,\Delta H_{w\to l} + 10.30, \quad r = 0.9929. \qquad (5)$$

A linear relationship between $\Delta S_{w\to l}$ and $\Delta H_{w\to l}$ is not normally expected, but it does occur under certain conditions; our results are analogous to those obtained by Katz & Diamond (1974*c*). It is also apparent from Fig. 4 that the $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$ are considerably higher below the T_c, which reflects the more rigid and ordered array of the phospholipid hydrocarbon chains in the crystalline state.

Table 3 shows that the $\Delta G_{w\to l}$ values are large and negative for all the phenothiazine drugs except for PROM.HCl, TRIM.T, and PRZ.HCl where $\Delta G_{w\to l}$ is positive below the T_c. It can be concluded in general that the partitioning and transport of alkylamino side chain phenothiazines into DMPC liposomes over the temperature range of 5 to 40 °C is predominantly entropically controlled, since the partitioning is said to be either enthalpy dominated or entropy dominated depending on whether $\Delta G_{w\to l} > 0$ or < 0. The partitioning of PROM.HCl, TRIM.T, and PRZ.HCl below the T_c can be considered to be enthalpically controlled. Table 4 shows the $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$ for partitioning of various groups in the

Table 3. Free energies ($\Delta G_{w\to l}$) of partitioning of phenothiazine drugs below T_c (15 °C) and above T_c (30 °C) in DMPC liposomes

Phenothiazine drug	Below T_c $\Delta G_{w\to l}$ (kJ mol^{-1})[a]	Above T_c $\Delta G_{w\to l}$ (kJ mol^{-1})[b]
VP	−2.20	−4.35
DAVP.O	−2.41	−3.59
VDMP.HCl	−2.86	−4.39
MMVP.OH	−3.05	−4.66
DMVP	−4.40	−5.08
DAVP.OH	−1.37	−2.90
PROM.HCl	+0.79	−0.63
TRIM.T.	+0.32	−1.61
PRZ.HCl	+0.78	−2.15

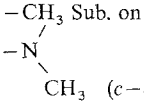
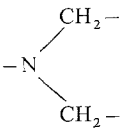
[a] Measured at 15 °C. [b] Measured at 30 °C.

phenothiazine molecules both below (15 °C) and above (30 °C) the T_c. A positive value in the table indicates that the $\Delta H_{w\to l}$ of partitioning is increased on the addition or substitution of a given group to the phenothiazine structure and vice versa.

For the $-CH_2$-group, two close and positive values of $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$ are obtained below the T_c. However, above the T_c the values of $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$ are more variable and negative (Wright & Bindslev, 1976), but the values for the $-CH_2$-group from (*c*–*f*) (*see* Table 4) are in reasonable agreement with literature values of $\Delta H_{w\to l} = -6.8$ kJ mol^{-1} and $\Delta S_{w\to l} = -16.6$ J mol^{-1} K^{-1} at 25 °C (Diamond & Katz, 1974). Below the T_c, $\Delta H_{w\to l}$ is +26.4 kJ mol^{-1} and $\Delta S_{w\to l}$ is +95.8 J mol^{-1} K^{-1} for the addition of the $-CH_2$-group. Similarly the values of $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$ for the $-CH_3$ group are in good agreement below the T_c whilst the values are more variable above the T_c. Unlike the $-CH_2$-group the $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$ values for the $-CO.(CH_2)_3.CH_3$ group are negative both below and above the T_c. It has been shown that the numerical values for various functional groups can vary considerably from system to system (Wright & Bindslev, 1976). There may be a number of reasons for this variation. The amount of energy required to insert a molecule into the membrane structure depends on (i) the number and strength of the bonds to be severed at the interface – the more polar the molecule the higher the energy required; (ii) the size and the shape of partitioning molecules; (iii) the structure of the membrane itself – such as the density of the lipids, the amount of free volume, and the presence or absence of "kinks" or structural defects (Cohen, 1975*a*, Traüble, 1971) between the hydrocarbon acyl chains. From the $-CH_3$ group data it can be concluded that branching increases the amount of energy required to partition into the lipid membranes (Diamond & Wright, 1969).

Table 5 attempts to correlate the particle size of liposomes, as a measure of phenothiazine drug in-

Table 4. Changes produced in $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$ of partitioning on the addition or substitution of the group, listed in the first column, to the alkylamino side chain or substitution at the number 2 position on the phenothiazine nucleus[a]

Addition or substitution of a group to phenothiazine structure	Below T_c		Above T_c	
	$\Delta H_{w\to l}$ (kJ mol^{-1})	$\Delta S_{w\to l}$ (J mol^{-1} K^{-1})	$\Delta H_{w\to l}$ (kJ mol^{-1})	$\Delta S_{w\to l}$ (J mol^{-1} K^{-1})
1) $-(CH_2)_3-N.(CH_3)_2$ Sub. on ring nitrogen				
$(b-a)$	−22.2	−76.3	−23.4	−79.7
2) $-CH_3$ Sub. on side chain.				
$(c-b)$	+26.7	+93.4	+ 9.5	+33.9
$(h-i)$	+41.6	+146.1	− 5.6	−31.2
3) $-CH_2-$ Add. to side chain.				
$(c-f)$	+26.4	+95.8	− 7.9	−21.2
$(h-g)$	+26.9	+95.2	−10.4	−31.2
4) $-CO-(CH_2)_3.CH_3$ Sub. at position 2 on the ring.				
$(f-g)$	−16.8	−51.0	− 8.3	−20.0
$(e-h)$	−17.3	−50.3	− 6.8	−10.0
5) $-CH_3$ Sub. on terminal nitrogen  $(c-d)$	+18.0	+60.9	+ 0.6	+ 1.2
6) Add. of two $-CH_2-$ groups on terminal nitrogen.  $(e-c)$	−24.6	−79.1	− 8.4	−25.4

[a] The alphabets in the parenthesis are the same as those in Table 1 and used to show how $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$ values are derived by subtraction. $+ve$ indicates that $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$ are increased on the addition or substitution of the group. $-ve$ values indicates that $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$ are decreased on the addition or substitution of the group.

Table 5. The effect of closely related phenothiazines on the particle size of liposomes made from DMPC at 25 °C, after 28-hr equilibrium period[a]

Phenothiazine drug in DMPC liposomes	Average liposome size (nm)	Polydispersity[b] factor	$K_{25\,°C}$	T_c
No drug	816	8.0	–	–
VP	3120	6.0	5.03	24.0
DAVP.O	1500	8.0	4.17	21.9
VDMP.HCl	843	6.5	5.34	22.4
MMVP.OH	1145	8.0	5.88	25.0
DMVP	2000	6.7	7.58	25.5
DAVP.OH	1100	7.0	2.79	21.0
PROM.HCl	1440	7.5	1.04	18.0
TRIM.T.	1427	8.0	1.49	19.4
PRZ.HCl	1192	7.5	0.91	22.8

[a] Fifth column in the table shows the T_c values which are derived from the break points in Figs. 1-3 plotted from temperature study data. Concentration of phospholipid − 0.1% wt/vol and that of drug 0.01% wt/vol. Each reading of the size is a mean of 4 × 2 min determination.

[b] A measure of the width of the particle size distribution given by the Coulter Nano-sizer.

corporation, with the partition coefficient of the same drugs in DMPC liposomes at 25 °C after a 28-hr equilibrium period. It is apparent from the table that all the phenothiazine drugs increase the particle size, some more than others. This change in size probably results in part from the charge-induced expansion of the aqueous channels in the multilamellar liposomes. The Coulter Counter Nano-sizer used in this study does not permit a size distribution to be determined. The polydispersity factor quoted is an indication of the width of the size distribution, with the smaller the factor, the less disperse the distribution. However, in the temperature dependence treatment of results previously described it was assumed that the system does not undergo physical change on partitioning and yet the particle size data shows changes in the size of liposomes. From the plot (not shown) of $K_{25\,°C}$ *vs.* change in particle size caused by the incorporation of phenothiazines, it appears that there is no correlation between partition coefficient and particle size (Rahman et al., 1978). From the partitioning data (Column 4, Table 5) it can be concluded that increasing the hydrocarbon chain length by a $-CH_2$-group enhance the partitioning of the drug molecule (Dia-

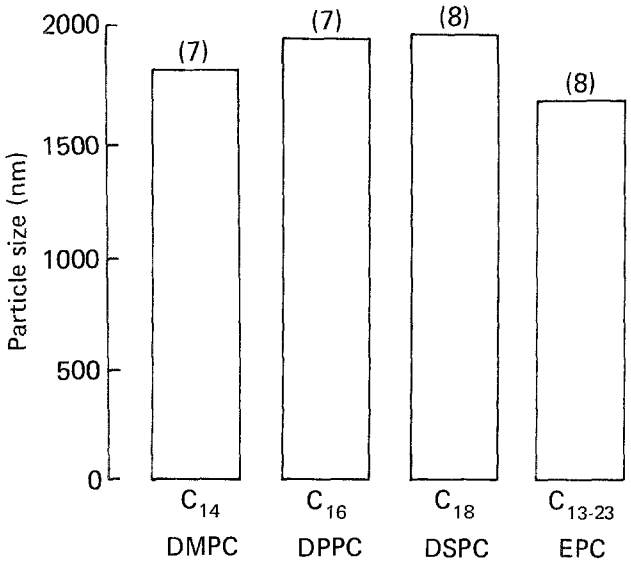


Fig. 5. Histogram showing the variation of particle size (nm) of hand-shaken liposomes with respect to chain length. Figures in brackets indicate polydispersity factor. Concentration of lipid, 0.1% wt/vol

mond & Wright, 1969). This is because the introduction of the $-CH_2$-groups promote lipid solubility of the drug molecule. The $-CH_3$ branching in the alkylamino side chain resulted in enhanced partition. This result was unexpected in view of the general concept that branching in solute molecules decreases their partitioning (Jain & Wray, 1978). Using the Coulter Nano-Sizer™, it was found that the increase in acyl hydrocarbon chain length in the phospholipid molecule produced an increase in the particle size of liposomes (Fig. 5). The results for EPC liposomes are ignored because of the variability of hydrocarbon chain length and degree of saturation in the EPC molecules.

Previously the incorporation of a series of phenothiazine drugs into the DPPC bilayer region has been reported by Jain and Wu (1978). Using DSC, it can be shown that closely related phenothiazines partition into the lipid bilayer of DMPC liposomes and are not simply associated with the surface of the liposomes. This is indicated by the increase in the half height width (HHW) of the DSC peaks at drug concentrations of 10^{-2} and 10^{-3} M (Table 6). Different drugs produce different anounts of broadening in the HHW. In the absence of drug the enthalpy of transition (ΔH_t) and entropy of transition (ΔS_t) are 31.49 kJ mol^{-1} and 106.2 J mol^{-1} K^{-1}, respectively. These values are higher than the literature values where for ΔH_t a value of 28.14 kJ mol^{-1} has been quoted (Papahadjopoulos & Kimelberg, 1973). In the presence of drug the ΔH_t and ΔS_t differ depending on both the amount and the nature of the incorporated

Table 6. The half height width (HHW), T_c, enthalpy (ΔH_t) and entropy (ΔS_t) of transition of DMPC liposomes in the presence of 10^{-2} and 10^{-3} M concentrations of phenothiazine derivatives[a]

Phenothiazine drug and concentration	HHW (mm)	T_c (°C)	ΔH_t (kJ mol^{-1})	ΔS_t (J mol^{-1} K^{-1})
No drug	3	23.5	31.49	106.20
10^{-2} M				
VP	4	23.0	26.61	89.75
DAVP.O	16	22.0	0.89	3.02
VDMP.HCl	8	21.0	1.96	6.67
MMVP.OH	16	23.0	0.70	2.37
DMVP	[b]	[b]	[b]	[b]
DAVP.OH	[b]	[b]	[b]	[b]
PROM.HCl	14	21.0	17.01	57.84
TRIM.T.	12	19.0	7.86	26.93
PRZ.HCl	10	20.5	6.51	22.19
10^{-3} M				
VP	4	23.5	37.98	128.77
DAVP.O	4	23.5	31.54	106.37
VDMP.HCl	5	23.5	41.87	141.22
MMVP.OH	4.5	23.0	30.92	104.29
DMVP	6	23.0	30.06	101.4
DAVP.OH	5	23.0	26.52	89.44
PROM.HCl	5	23.0	41.35	139.45
TRIM.T.	6	23.0	35.07	118.48
PRZ.HCl	4	23.0	37.94	127.97

[a] Concentration of phospholipid – 10% wt/vol.
[b] Indicates peak abolished.

drug. The T_c of the DMPC liposomes from DSC data was found to be 23.5 °C and is in close agreement with the literature value of 23 °C (Melchior & Steim, 1976). The incorporation of all the phenothiazines individually lower the T_c of the DMPC liposomes. Comparing the T_c values from the temperature-dependent partitioning study (Table 5, Column 5) it is seen that the incorporation of the phenothiazines also lowered the T_c of DMPC liposomes except in the case of VP, MMVP.OH, and DMVP where the T_c is raised. The reason for this rise in T_c is not clear, but it must be remembered that the temperature-dependent partitioning study is a very inaccurate method of determining T_c, since it relies upon extrapolation of two lines each of which is subject to error.

It has been shown that the release rates of solutes are greatest at or near the T_c of the phospholipid (Papahadjopoulos et al., 1973; Yatin et al., 1978). Therefore, the effect of temperature on the first-order rate constant (k, hr^{-1}) of TRIM.T from DMPC liposomes at pH 6 was studied. k values are obtained from the gradients of the first-order plots of $\log_{10}$% liposome associated TRIM.T *vs.* time (Fig. 6). It is possible to conclude from Fig. 6 that more drug is associated with liposomes at higher temperatures. Figure 7 shows the dependence of k on temperature,

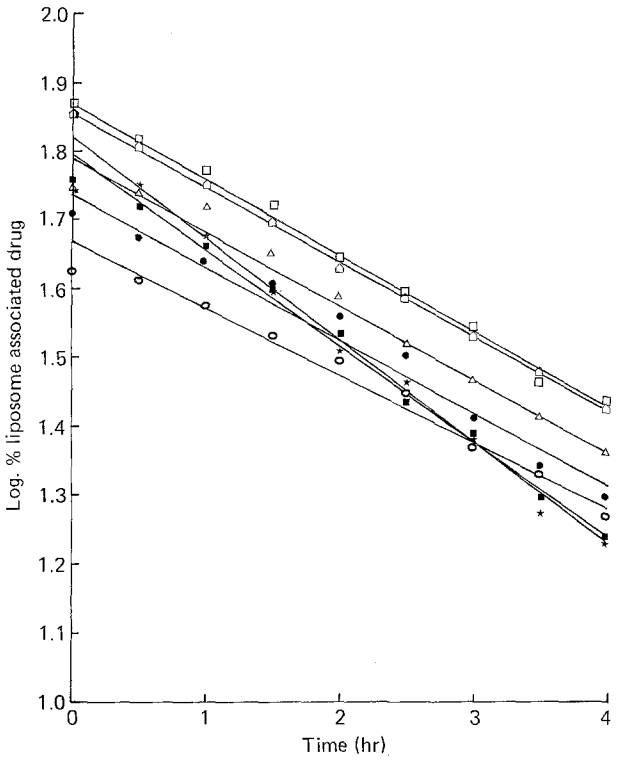


Fig. 6. Plot of $\log_{10}$% DMPC liposome associated trimeprazine tartrate *vs.* time (hr) at various temperatures; pH 6. ○, 10 °C; ●, 15 °C; ▲, 20 °C; ■, 23 °C; *, 25 °C; □, 30 °C; ⌂, 35 °C

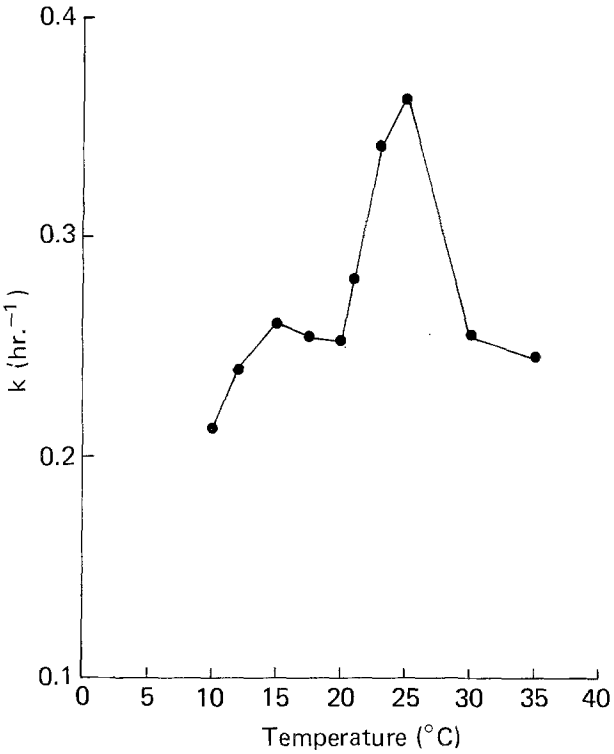


Fig. 7. The effect of temperature on the first-order rate constant (k, hr^{-1}) of trimeprazine tartrate transport from DMPC liposomes at pH 6

and it is apparent that k increases with temperature to 25 °C and thereafter decreases with increasing temperature. Maximum efflux which occurs in the region of T_c may reflect localized regions of extreme disorder. The reason for the reduction in efflux at higher temperatures is not known, but similar results have been reported using carboxyfluorescein as a solute in DPPC and DSPC liposomes (Yatin et al., 1978). The small peak at 15 °C may reflect the pretransition temperature of DMPC liposomes. Tsong (1975) showed that the maximum reaction rate of various fluorophores with DMPC vesicles occurred at 24 °C, a temperature in excess of the T_c determined by DSC. The objective of the permeability study was not to assess T_c values, but to examine the permeability of phenothiazines within phospholipid bilayers at temperatures above and below the T_c, as it may be possible to design liposomes of mixed phospholipid composition to release an entrapped drug at the optimum delivery rate. Weinstein et al. (1979) have suggested that locally induced hyperthermia in tumors could therefore result in improved targetting of cytotoxic agents. A fourfold increase in methotrexate concentration was shown in the heated compared with the unheated tumors.

Finally, no attempt is made to relate the differences in the partitioning of phenothiazine derivatives with their pharmacological efficacy, since only a few of these derivatives are used therapeutically. The emphasis of the present study is in assessing the contribution of various groups present in the phenothiazine molecule to membrane partition and transport processes.

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