RESULTS

1. Current-Voltage Relation in BLM (Unmodified):

Current-voltage relations in BLM of oxidized cholesterol and of lecithin are shown in Figures 14 and 15. In oxidized cholesterol BLM (Fig. 14), the relation is ohmic up to applied potential of 80 mV; for potentials above 80 mV there is a decrease in BLM resistance. Current-voltage relation in lecithin BLM (Fig. 15) is linear up to a potential of 60 mV, followed by a decrease in the BLM resistance at higher potentials. Previous workers D'Agostino and Smith (1965), Andreoli et al. (1967), Howard and Burton (1968), Ohki and Goldup (1968) and Kauffman and Mead (1970) have also reported a decrease in BLM resistance above certain applied potentials.

2. Effect of Ca^{2+} and pH on BLM Resistance:

The variation of BLM resistance with concentration of CaCl_2 is shown in Figure 16. The resistance increases from 1.15 \times 10^{-7} \text{ohm-cm}^2 \text{ at } 10^{-7} \text{M CaCl}_2 \text{ to } 1.25 \times 10^{-8} \text{ohm-cm}^2 \text{ at } 10^{-1} \text{M CaCl}_2. \text{ The rate of thinning of BLM was observed to decrease with increase in Ca}^{2+} \text{ concentration and at higher Ca}^{2+} \text{ concentrations (>10}^{-2}\text{M)} \text{ even a very small difference in hydrostatic pressure was observed to rupture the BLM.}

Figure 17 shows the effect of pH on the resistance of lecithin BLM. In the presence of NaCl (0.1 M) the resistance
Figure 14. Variation in current with applied potential across oxidized cholesterol ELM (4% oxidized cholesterol in dodecane). Bathing medium, 0.1 M NaCl.
Figure 15. Variation in current with applied potential across lecithin ELM (2% egg lecithin in dodecane). Bathing medium, 0.1 M NaCl.
Figure 16. Variation of BLM (2% egg lecithin in dodecane) resistance with CaCl$_2$ concentration. Bathing medium, 0.1 M NaCl.
Figure 17. Effect of pH on the resistance of lecithin
BLM (2% egg lecithin in dodecane). Bathing
medium, o--o 0.1 M NaCl and O-----o 0.1 M
CaCl$_2$. pH was adjusted by adding 0.01 M
HCl or NaOH to the bathing medium.
MEMBRANE RESISTANCE (OHMS-CM²)

- NaCl (0.1M)
- CaCl₂ (0.1M)

pH
becomes maximum at about pH 4.0 \((1.3 \times 10^8 \text{ohm-cm}^2)\) and decreases with further increase in pH. At pH 9.0 the resistance is \(5.6 \times 10^5 \text{ohm-cm}^2\) which is about three orders of magnitude lower than that at pH 4.0. In CaCl\(_2\) (0.1 M), the resistance increases with increasing pH, from \(5.2 \times 10^6 \text{ohm-cm}^2\) at pH 3.0 to \(8.6 \times 10^8 \text{ohm-cm}^2\) at pH 9.0. Our data is in fair agreement with that of Ohki and Goldup (1968). The formation of BLM was rapid (in less than 1 min) at both high and low pH in NaCl. In CaCl\(_2\) at pH 7, the rate of formation of BLM was very slow (>15 min).

Table 6 summarises the results on the variation of BLM resistance with pH and the concentration of Ca\(^{2+}\) in the medium. BLM of oxidized cholesterol and of stearic acid + oxidized cholesterol have resistances one order of magnitude higher than lecithin BLM. Lecithin BLM has maximum resistance at pH 4.0 and the resistance is dependent on the concentration of Ca\(^{2+}\) in the medium.

3. **Effect of BSA and Cytochrome c on the Electrical Properties of BLM**

Figure 18 shows the effect of incorporation of BSA and cytochrome c onto lecithin BLM on its resistance. Both proteins reduce the resistance of BLM only when they are present on both sides of BLM. The resistance of lecithin
<table>
<thead>
<tr>
<th>BLM Forming Solution</th>
<th>Bathing Medium</th>
<th>BLM Resistance (ohm • cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidized cholesterol (4% in dodecane)</td>
<td>0.1 M NaCl (pH 7.0)</td>
<td>1.3 x 10⁸</td>
</tr>
<tr>
<td>Oxidized cholesterol + stearic acid (1:1 in dodecane)</td>
<td>0.1 M NaCl (pH 7.0)</td>
<td>1.2 x 10⁸</td>
</tr>
<tr>
<td>Egg lecithin (2% in dodecane)</td>
<td>0.1 M NaCl (pH 3.0)</td>
<td>7.2 x 10⁷</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.1 M NaCl (pH 4.0)</td>
<td>1.3 x 10⁸</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.1 M NaCl (pH 9.0)</td>
<td>5.6 x 10⁵</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.1 M NaCl + 10⁻⁶ M CaCl₂ (pH 7.0)</td>
<td>2.1 x 10⁷</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.1 M NaCl + 10⁻³ M CaCl₂ (pH 7.0)</td>
<td>8.0 x 10⁷</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.1 M NaCl + 10⁻¹ M CaCl₂ (pH 7.0)</td>
<td>1.25 x 10⁸</td>
</tr>
</tbody>
</table>
Figure 18. Plots for the variation of ELM resistance with the concentration of proteins in the bathing medium. •——• BSA and o——o cytochrome c.
MEMBRANE RESISTANCE (OHMS CM²)

CONCENTRATION OF PROTEIN (µg/ml)

○○ CYTOCHROME C
○○ BSA
BLM in the absence of protein at pH 7.0 is $1.2 \times 10^7$ ohm-cm$^2$ which is decreased to $1.5 \times 10^6$ ohm-cm$^2$ in the presence of 14 µg/ml cytochrome c and to $2 \times 10^5$ ohm-cm$^2$ in the presence of 14 µg/ml BSA. When CaCl$_2$ is also present in the medium there is a further decrease in the BLM resistance (Fig. 19). In the absence of CaCl$_2$, BLM with adsorbed cytochrome c (10 µg/ml cytochrome c in the bathing medium) shows a resistance of $5 \times 10^6$ ohm-cm$^2$. When the medium also contains $10^{-3}$M CaCl$_2$ the resistance is decreased to $3.8 \times 10^4$ ohm-cm$^2$. Further increase in the concentration of Ca$^{2+}$ does not change the BLM resistance.

Resistance of BLM with adsorbed BSA (10 µg/ml BSA in the medium) also decreases with increase in concentration of Ca$^{2+}$ (Fig. 19). From $6.2 \times 10^5$ ohm-cm$^2$ in the absence of Ca$^{2+}$, the resistance decreases to $7.0 \times 10^3$ ohm-cm$^2$. There is no decrease in the resistance with further increase in the concentration of Ca$^{2+}$.

BLM were observed to lose stability when the concentration of protein in the bathing medium was increased beyond 10 µg/ml, and that of Ca$^{2+}$ higher than $10^{-3}$M. The rate of thinning is also slower in the presence of the proteins.

Variation of the capacitance of BLM of oxidized cholesterol with the concentration of cytochrome c is
Figure 19. Plots for the variation of the resistance of BLM with adsorbed proteins (o--o BSA, o--o cytochrome c) with change in Ca$^{2+}$ concentration in the bathing medium.
shown in Table 7. Our data show no change in the capacitance of BLM even for high (51.94 μg/ml) protein concentration. Presence of Ca²⁺ (13 mM) did not affect the capacitance.

4. Characterisation of Fluorescent Probes

We have studied the effect of various solvents on the fluorescent properties of ANS and NPN in an attempt to characterise the changes in the fluorescence properties with the changes in the polarity and viscosity of the medium. Figure 20 shows the emission spectra of ANS in 40 to 100% ethanol. The relative fluorescence intensity at λ_{Fmax} increase from 7.5 in 40% ethanol to 87 in absolute ethanol. λ_{Fmax} is blue-shifted from 502 nm in 40% ethanol to 470 nm in absolute ethanol.

Table 8 shows the variation of relative fluorescence intensity, relative quantum yield, λ_{Fmax} and emission transition energy (defined as the reciprocal of the wavelength of maximum emission) of ANS with polarity (Kosower, 1958) (Z-value), and viscosity of the solvent. As the Z-value of the solvent is decreased (by the addition of less polar solvents to water) there is an enhancement in the relative fluorescence intensity and the relative quantum yield. λ_{Fmax} is blue-shifted with decreasing polarity (Z-value) and there is an increase in the emission transition energy (Δ). With increase in viscosity of the solvent there is
### Table 2

CAPACITANCE OF OXIDIZED CHOLESTEROL BLM AT DIFFERENT CONCENTRATIONS OF CYTOCHROME C IN THE BATHING MEDIUM

<table>
<thead>
<tr>
<th>Bathing Medium</th>
<th>Concentration of Cytochrome C (μg/ml)</th>
<th>BLM Capacitance (μF/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M NaCl</td>
<td>-</td>
<td>0.59</td>
</tr>
<tr>
<td>+</td>
<td>5.33</td>
<td>0.54</td>
</tr>
<tr>
<td>+</td>
<td>13.33</td>
<td>0.53</td>
</tr>
<tr>
<td>+</td>
<td>26.32</td>
<td>0.52</td>
</tr>
<tr>
<td>+</td>
<td>51.94</td>
<td>0.56</td>
</tr>
<tr>
<td>0.1 M NaCl</td>
<td>51.94</td>
<td>0.58</td>
</tr>
<tr>
<td>+ 13 mM CaCl₂</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 20. Emission spectra of 1,8 ANS in (a) ethanol, (b) 80% ethanol, (c) 60% ethanol and (d) 40% ethanol. Excitation wavelength, 365 nm. Concentration of ANS, 8.3 μM.
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Z-value* (centipoise)</th>
<th>Fluorescence yield</th>
<th>Rel. Quantum yield</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Υ cm&lt;sup&gt;-1&lt;/sup&gt; x 10&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol 40%</td>
<td>90.5</td>
<td>-</td>
<td>7.5</td>
<td>0.03</td>
<td>502</td>
</tr>
<tr>
<td>* 60%</td>
<td>87.9</td>
<td>-</td>
<td>15.5</td>
<td>0.07</td>
<td>500</td>
</tr>
<tr>
<td>* 80%</td>
<td>84.8</td>
<td>-</td>
<td>31.0</td>
<td>0.14</td>
<td>488</td>
</tr>
<tr>
<td>* 100%</td>
<td>79.6</td>
<td>1.2</td>
<td>87.0</td>
<td>0.4</td>
<td>470</td>
</tr>
<tr>
<td>Methanol 60%</td>
<td>89.7</td>
<td>-</td>
<td>10.5</td>
<td>0.05</td>
<td>500</td>
</tr>
<tr>
<td>* 80%</td>
<td>87.3</td>
<td>-</td>
<td>21.5</td>
<td>0.10</td>
<td>495</td>
</tr>
<tr>
<td>* 100%</td>
<td>83.6</td>
<td>0.54</td>
<td>45.5</td>
<td>0.20</td>
<td>482</td>
</tr>
<tr>
<td>1,4 Dioxane 40%</td>
<td>88.4</td>
<td>-</td>
<td>11.0</td>
<td>0.51</td>
<td>500</td>
</tr>
<tr>
<td>* 60%</td>
<td>85.0</td>
<td>-</td>
<td>26.5</td>
<td>0.12</td>
<td>494</td>
</tr>
<tr>
<td>* 80%</td>
<td>80.2</td>
<td>-</td>
<td>56.0</td>
<td>0.26</td>
<td>480</td>
</tr>
<tr>
<td>* 100%</td>
<td>65.7</td>
<td>-</td>
<td>145.0</td>
<td>0.67</td>
<td>469</td>
</tr>
<tr>
<td>Acetone</td>
<td>65.7</td>
<td>0.209</td>
<td>130.0</td>
<td>0.59</td>
<td>464</td>
</tr>
<tr>
<td>Ethylene Glycol</td>
<td>85.1</td>
<td>19.9</td>
<td>18.0</td>
<td>0.08</td>
<td>494</td>
</tr>
</tbody>
</table>

*Z-values of solvents are taken from Turner and Brand, (1968)

#η-values of solvents are taken from Kaye and Laby, (1973).
an enhancement of relative fluorescence intensity and relative quantum yield.

Figure 21 shows the variation in the emission transition energy ($\tilde{\nu}$) of ANS with the polarity of the solvent. The emission transition energy increases with decrease in polarity. The linearity between $z$-value and $\tilde{\nu}$ exists for $z$-values in the range 80 to 90. It seems that at lower $z$-values ($<80$) factors other than polarity of the solvent may play a significant role, which is indicated by the non-linearity seen for $z$-value of 65.7.

The variation of relative fluorescence intensity of ANS with polarity of the solvent is shown in Figure 22. Fluorescence intensity increase linearly with decrease in $z$-value. Values obtained for different solvents fall on the same line showing a direct correlation of the polarity of the solvent with the relative fluorescence intensity of ANS.

Figure 23 shows the emission spectra of NPN in solvents of different polarities and viscosities. The linearity of fluorescence intensity with $z$-value is maintained except in acetone where some other factors like polarizability and interactions with the solvent may play a role which causes a decrease in fluorescence intensity. The fluorescence intensity in the solvent ethylene glycol (viscosity = 19.9
Figure 21. Plot of the variation of the emission transition energy ($\bar{\nu}$) of ANS with polarity (Z-value) of solvent. △-ethanol, e-methanol, □-1,4 dioxane, x-acetone and 0-ethylene glycol. Z-values of the solvents are taken from Turner and Brand (1968). Concentration of ANS, 8.3 μM.
\[ \sqrt{v} \times 10^4 \text{ cm}^{-1} \]

\[ \text{\(z\) VALUE} \]

- \(\Delta\) - ETHANOL
- \(\bullet\) - METHANOL
- \(\square\) - 1:4 DIOXANE
- \(\times\) - ACETONE
- \(\circ\) - ETHYLENE GLYCOL
Figure 22. Plot of the variation of the relative fluorescence intensity of ANS with polarity (Z-value) of solvents. △ - ethanol, o-methanol, □ - 1:4 dioxane, x - acetone and 0 - ethylene glycol. Concentration of ANS, 8.3 μM.
Figure 23. Emission spectra of NPN in different solvents.

•—• ethanol, o——o methanol, △——△ ethylene glycol and □——□ acetone. Excitation wavelength, 365 nm. Concentration of ANS, 8.3 μM.
NPN SPECTRA

Z-VALUE η

- ETHANOL 79.6 1.2
- METHANOL 83.6 0.54
- ETHYLENE GLYCOL 85.1 19.9
- ACETONE 65.7 0.309

WAVELENGTH (nm)

RELATIVE FLUORESCENCE INTENSITY

350 400 450 500 550
centipoise) shows its dependence on viscosity. The
dependence of $\lambda_{F_{\text{max}}}$ on the polarity is clearly demonstrated
by its position in various solvents.

Our data on ANS fluorescence in various solvents
is in agreement with those of Turner and Brand (1968) and
Prasad (1976). The relative fluorescence intensity and
$\lambda_{F_{\text{max}}}$ of ANS are both dependent on the polarity of the
solvent and ANS can hence be used as a probe for polarity.
The relative fluorescence intensity of NPN is dependent on
the viscosity and the polarity of the solvent whereas the
position of $\lambda_{F_{\text{max}}}$ is mainly influenced by polarity. Our
data would thus suggest the use of NPN as a probe for
viscosity and polarity.

5. Fractionation of Liposomes:

The void volume of the Sepharose 4B column was
determined from the elution profile of Blue Dextran a
high molecular weight compound. Figure 24A shows the
elution profile of Blue Dextran. The first peak which
contains the large molecules starts at 20 ml and ends
at 50 ml of the eluted volume. This volume (50 ml)
corresponds to the void volume of the column.

Figure 24B, shows the elution profile of egg
lecithin liposomes. The first peak which is within the
void volume contains the large multilameller vesicles.
Figure 24. Elution profile of (A) Blue Dextran and (B) egg lecithin liposomes on Sepharose 4B column. Lipid phosphate was assayed by the method of Eibl and Land (1969).
Elution profile of Blue Dextran on Sepharose 4B column.

Elution profile of egg lecithin liposomes on Sepharose 4B.

- Void volume
- Large vesicles
- Single shelled vesicles
The second peak which extends from 50 to 130 ml of the
elution volume contains largely unilamellar vesicles
(Batzri and Korn, 1973). The eluted fractions within this
peak from 60 to 100 ml were pooled together and
concentrated.

The elution profile of BSA-liposome complex is shown
in Figure 25. The protein and lipid-phosphate, assayed in
the various fractions, form two peaks. The first is
within the void volume and the second is within 50 to 80 ml
of the eluted volume. The peaks for protein and lipid-
phosphate overlap showing that the protein is bound to the
liposomes. The shift in the position of the peaks observed
in Figure 25 to lesser elution volumes as compared to
those in the case of liposomes without BSA (Fig. 24B)
indicates that the vesicles which contain adsorbed proteins
are larger in size than those without the protein. The
lipid to protein ratio of the second peak is about 50:1,
which suggests that about 50 lipid molecules are bound to
each molecule of BSA.

6. 90° Light Scattering by Liposomes & Liposome-Protein
Complexes:

The changes in the intensity of 90° light scattering
by liposomes with the concentration of Ca²⁺ is shown in
Figures 26 and 27 for negatively charged (dipalmitoyl-
phosphatidyl choline + dicetylphosphate) and positively
Figure 25. Elution profile of BSA-liposome complex on Sepharose 4B column. o--o Lipid phosphate (assayed by the method of Ribl and Land, 1969), e--e protein (assayed following the procedure of Lowry et al., 1951).
Elution Profile of BSA–Liposome Complex on Sepharose 4B Column

Phosphate (µM)

Protein (µM)

Elution Volume (ml)
Figure 26. Plots of the variations in the intensity of 90° light scattering at 405 nm, by negatively charged liposomes and liposome-protein complexes with concentration of Ca²⁺. e—liposome (DPPC + DCP), o—liposome + BSA and Δ—liposomes + cytochrome c.
90° LIGHT SCATTERING INTENSITY AT 405 nm (REL. UNITS)

○ ○ LIPOSOMES (UPPC + DCP)
○ ○ LIPOSOME + BSA
△ △ LIPOSOMES + CYTOCHROME C

CONCENTRATION OF Ca²⁺ (mM)
Figure 27: Plots of the variations in the intensity of 90° light scattering intensity, at 405 nm, by positively charged liposomes and liposome-protein complexes with concentration of Ca2+. 
- - - - - liposome (DPPC + BA), o----o liposome + BSA and △-----△ liposome + cytochrome c.
charged (dipalmitoylphosphatidyl choline + stearylamine) liposomes, respectively. Negatively charged liposome with and without bound BSA scatter maximum light at 0.6 mM Ca\(^{2+}\) (Fig. 26). At higher concentrations of (>0.6 mM) Ca\(^{2+}\) there is a sharp decrease in the intensity, which levels off after 2.5 mM. With cytochrome c adsorbed on negatively charge liposomes there is a slight increase in the intensity of scattered light with Ca\(^{2+}\) concentration. At Ca\(^{2+}\) concentration higher than 3.5 mM the intensity levels off and no change is observed up to 10.5 mM Ca\(^{2+}\).

In the case of positively charged liposomes the intensity of scattered light shows an initial decrease (Fig. 27), and then becomes flat beyond 0.6 mM Ca\(^{2+}\). When BSA is adsorbed on positively charged liposomes, the intensity of 90° scattered light increases gradually with Ca\(^{2+}\) concentration (from 0.28 at zero Ca\(^{2+}\) to 0.35 at 10.5 mM Ca\(^{2+}\)). In cytochrome c-positive liposome complex the intensity of scattered light shows an initial decrease up to 0.6 mM Ca\(^{2+}\) and then an increase.

6.1. Effect of pH

Figure 28 shows the change in the intensity of the scattered light with pH. In the absence of Ca\(^{2+}\), there is a decrease in the intensity between pH 5.0 and 6.0 and between pH 7.0 and 8.0. The plot shows two flat
Figure 28. Plots of the variations in the intensity of 90° light scattering, at 405 nm, by egg lecithin liposomes with pH, e—e in the absence of Ca^{2+} and o—o in the presence of 10.5 mM Ca^{2+}. Suspension medium, 0.1 M NaCl. pH was adjusted by the addition of 0.01 M HCl and/or NaCl to the liposome suspension.
90° LIGHT SCATTERING INTENSITY AT 405 nm (REL. UNITS)

- LIPOSOMES (EGG LECITHIN)
- LIPOSOMES + Ca²⁺
portions between 6.0 and 7.0 and between pH 8.0 and 9.0, when the plot is drawn on point to point basis. On addition of Ca\(^{2+}\) (Fig. 28) the intensity of scattered light shows at maximum at pH 7.0.

Figure 29 shows the changes in the intensity of scattered light with pH in liposomes with adsorbed proteins, in the absence and in the presence of Ca\(^{2+}\). When BSA is adsorbed on liposome (Fig. 29A), maximum scattering of light occurs at pH 7.0. On addition of Ca\(^{2+}\) (10 mM) maximum light scattering however, occurs at pH 5.0. With cytochrome c adsorbed on liposomes (Fig. 29B) there is only a slight enhancement in the intensity of scattered light between pH 4.0 and 7.0. When Ca\(^{2+}\) (10 mM) is added to the suspension there is no change in the intensity of scattered light in the pH range 4.0 to 9.0.


The fluorescence emission spectrum of ANS bound to egg-lecithin liposomes is shown in Figure 30. The peak is at 480 nm and the relative fluorescence intensity at \(\lambda_{F\text{max}}\) is 175. This indicates that the probe is bound at an hydrophobic site as discussed earlier. Figure 31
Figure 29. Plots of the variations in the intensity of 90° light scattering, at 405 nm, by (A) egg lecithin liposome - BSA and (B) egg lecithin liposome -cytochrome c complexes with pH. - liposome - protein complex in the absence of Ca$^{2+}$ and o----o in the presence of 10.5 mM Ca$^{2+}$. Suspension medium 0.1 M NaCl. pH adjustments were made as described in Materials and Methods.
Figure 30. Emission spectrum of ANS in egg lecithin liposome suspension. Excitation wavelength, 365 nm. ANS concentration, 8.3 µM; lecithin concentration 330 µg/ml. Suspension buffer 0.01 M Tris, HCl, pH 7.2.
ANS SPECTRUM
LIPOSOME (EGG LECITHIN)
Figure 31. Emission spectrum of NPN in egg lecithin liposome suspension. Excitation wavelength, 365 nm. ANS concentration, 8.3 μM; lecithin concentration 330 μg/ml. Suspension buffer, 0.01 M Tris HCl, pH 7.2.
NPN SPECTRUM
LIPOSOME (EGG LECITHIN)
shows the emission spectrum of NPN bound to lecithin vesicles. The position of \( \lambda_{\text{Fmax}} \) is blue-shifted from 470 nm in water (data of Prasad, 1976) to 425 nm on binding of lecithin vesicles which plausibly indicates that the probe is located in a hydrophobic and viscous region.

NPN bound to negatively charged liposomes (dipalmitoylphosphatidylcholine + dicetylphosphate) has \( \lambda_{\text{Fmax}} \) at 431 nm (Fig. 32a). This indicates that by the addition of dicetylphosphate the hydrophobicity at the binding site of the probe is probably decreased. Addition of Ca\(^{2+}\) (10.5 mM) induces a further red-shift (Fig. 32b), in \( \lambda_{\text{Fmax}} \) (from 431 nm to 434 nm) which plausibly implies that there is a further decrease in the site hydrophobicity. The decrease in the relative fluorescence intensity on the addition of Ca\(^{2+}\) from 48 to 17 supports the contention of a further decrease in hydrophobicity and/or a decrease in viscosity.

Figure 33 shows the emission spectra of NPN bound to positively charged liposomes (dipalmitoylphosphatidylcholine + stearylamine) in the absence (Fig. 33a) and presence of Ca\(^{2+}\) (10.5 mM) (Fig. 33b). The addition of stearylamine induces a red-shift in \( \lambda_{\text{Fmax}} \) from 425 nm (in neutral liposomes) to 430 nm. Presence of Ca\(^{2+}\) induces a further red-shift (\( \lambda_{\text{Fmax}} = 433 \) nm) and a slight increase in the relative fluorescence intensity.
Figure 32. Emission spectra of NPN in negatively charged liposomes (a) in the absence and (b) in the presence of 10.5 mM Ca$^{2+}$. NPN concentration, 8.3 μM; DPPC concentration 500 μM. Excitation wavelength, 365 nm.
NPN SPECTRA

(a) DPPC + DCP
(b) DPPC + DCP + Ca^{2+}

RELATIVE FLUORESCENCE INTENSITY

WAVELENGTH (nm)

431

434
Figure 33. Emission spectra of NPN in positively charged liposomes (a) in the absence and (b) in the presence of 10.5 mM Ca\(^{2+}\). NPN concentration 8.3 μM; DPPC concentration 500 μM. Excitation wavelength, 365 nm. Suspension buffer 0.01 M Tris HCl, pH 7.2.
NPN SPECTRA

(a) DPPC + SA
(b) DPPC + SA + Ca$^{2+}$

RELATIVE FLUORESCENCE INTENSITY

WAVELENGTH (nm)
7.1. Effect of pH

Figure 34 shows the effect of pH on the fluorescence intensity of ANS bound to liposomes (egg-lecithin). The fluorescence intensity of ANS is maximum at pH 7.0. For pH < 6.0 there is no change in fluorescence intensity with pH. Above pH 7.0 there is a decrease in the fluorescence intensity. On the addition of Ca\(^{2+}\), the fluorescence intensity decreases for pH > 4.0, reaching a minimum at pH 6.0. Above pH 6.0 there is an enhancement in fluorescence and it is maximum at pH 7.0. For pH > 7.0 there is a decline in the fluorescence intensity.

8. Fluorescence of Probes Bound to Protein-Liposome Complex

Figure 35 show the changes in the relative fluorescence intensity of ANS bound to liposomes, BSA-liposome complex and BSA with change in Ca\(^{2+}\) concentration. The fluorescence intensity increases with the increase in the concentration of Ca\(^{2+}\) for both liposomes and BSA-liposome complex, reaching a maximum at 10 mM Ca\(^{2+}\). With further increase in Ca\(^{2+}\) concentration there is a decrease in fluorescence intensity and beyond 15 mM Ca\(^{2+}\) there is no further change. In the case of BSA, there is practically no change in fluorescence with Ca\(^{2+}\). The increase of fluorescence of ANS bound to BSA-liposome complex is greater than the increase in the case of liposomes.
Figure 34. Plots of the variations in the relative fluorescence intensity of ANS in egg lecithin liposome suspension e—e in the absence and o—o in the presence of 10.5 mM Ca^{2+}, with pH. pH was adjusted by the addition of 0.01 M HCl or NaOH. Suspension medium 0.1 M NaCl. ANS concentration, 8.3 μM.
ANS FLUORESCENCE

- LIPOSOME (EGG LECITHIN)
- LIPOSOME $+\text{Ca}^{2+}$

RELATIVE FLUORESCENCE INTENSITY

pH

4.0  5.0  6.0  7.0  8.0  9.0
Figure 35. Plots of the variations in the relative fluorescence intensity of ANS in o-o liposome, △-△ BSA-liposome suspensions and □-□ solution of BSA with concentration of Ca\(^{2+}\). ANS concentration, 8.3 \(\mu\)M. Excitation wavelength, 365 nm.
\begin{align*}
\text{FLUORESCENCE INTENSITY (arbitrary units)} \\
\text{CaCl}_2 \text{ (mM)}
\end{align*}
Table 9 gives the values of relative quantum yield and binding parameters \( (n \text{ and } K_D) \) for ANS bound to liposome, liposome + Ca\(^{2+}\), liposome + BSA and liposome + BSA + Ca\(^{2+}\). The relative quantum yield is increased from 0.13 to 0.15 by the addition of Ca\(^{2+}\) to liposomes while in liposome + BSA it is increased from 0.27 to 0.30 by the addition of Ca\(^{2+}\). Ca\(^{2+}\) almost doubles the number of binding sites \( (n) \) for ANS on liposomes (from 3.54 to 6.60). While in BSA-liposome complex the addition of Ca\(^{2+}\) increases the number of binding sites only from 3.2 to 3.75. \( K_D \) in liposomes is increased from 1.49 mM to 3.47 mM, by Ca\(^{2+}\), i.e. it reduces the affinity for binding. In BSA-liposome complex there is an increase in the affinity of binding, with a decrease in \( K_D \) from 4.44 mM to 2.68 mM by the addition of Ca\(^{2+}\).

Figure 36 shows the fluorescence emission spectra of ANS bound to egg-locithin liposomes (containing bound cytochrome c) in the absence (Fig. 36a) and presence (Fig. 36b) of 10 mM Ca\(^{2+}\). The addition of Ca\(^{2+}\) causes a red-shift in the emission maximum from 478 to 482 nm and an increase in the relative fluorescence intensity from 105 to 140, an increase by 33%.

Figure 37 shows the changes in the relative fluorescence intensity of NPN bound to BSA-liposomes (dipalmiloylphosphatidylcholine + dicetylphosphate) and cytochrome c-
<table>
<thead>
<tr>
<th></th>
<th>Rel. Quantum yield</th>
<th>No. of Binding sites (n) per 100 lipid Molecules</th>
<th>Dissociation Constant $K_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposome</td>
<td>0.13</td>
<td>3.54</td>
<td>1.49</td>
</tr>
<tr>
<td>Liposome + Ca$^{2+}$ (10 mM)</td>
<td>0.15</td>
<td>6.60</td>
<td>3.47</td>
</tr>
<tr>
<td>Liposome + BSA</td>
<td>0.27</td>
<td>3.20</td>
<td>4.44</td>
</tr>
<tr>
<td>Liposome + BSA + Ca$^{2+}$ (10 mM)</td>
<td>0.30</td>
<td>3.75</td>
<td>2.68</td>
</tr>
</tbody>
</table>
Figure 36. Emission of ANS in suspension of cytochrome c-liposome complex (a) in the absence and (b) in the presence of 10 mM Ca\textsuperscript{2+}. Concentration of lecithin, 300 µg/ml. Concentration of ANS, 8.3 µM. Excitation wavelength, 365 nm.
ANS SPECTRA
(a) LIPOSOME + CYTOCHROME C
(b) LIPOSOME + CYTOCHROME C + Ca^{2+}
Figure 37. Plots of the variations in the relative fluorescence intensity of NFP in suspensions of negatively charged liposome-protein complexes. With concentration of Ca$^{2+}$, e-----e liposomes (DPPC + DCP), o-----o liposome + BSA, and △-----△ liposome + cytochrome c. Concentration of NFP, 8.3 μM; Concentrations of proteins 0.66 μg/ml and concentration of lipid, 500 μM. Excitation wavelength, 365 nm, and emission wavelength 425 nm.
NPN FLUORESCENCES

- LIPOSOMES (DPPC + DCP)
- LIPOSOMES + BSA
- LIPOSOMES + CYTOCHROME C

CONCENTRATION OF Ca^{2+} (mM)

RELATIVE FLUORESCENCE INTENSITY

0 2.5 5.0 7.5 10.0 12.0
0 10 20 30 40 50 60 70
liposome complexes. The fluorescence intensity in the absence of Ca$^{2+}$ is highest (58) for BSA-liposome complex and lowest (24) for cytochrome c-liposome complex. The fluorescence intensities decrease rapidly with increase in Ca$^{2+}$ concentration and level off beyond 2.5 mM Ca$^{2+}$. The Ca$^{2+}$ induced quenching of fluorescence is minimum for liposomes without any protein and maximum for liposomes with adsorbed BSA. BSA-liposome and cytochrome c-liposome complexes have almost the same relative fluorescence intensities for bound NPN at Ca$^{2+}$ concentrations higher than 2.5 mM.

The variation in the relative fluorescence intensity of NPN bound to positively charged liposomes (dipalmitoylphosphatidyl choline + stearylamine) with Ca$^{2+}$ concentration is shown in Figure 38. A gradual quenching of NPN fluorescence is observed with increasing concentration of Ca$^{2+}$. NPN bound to BSA-liposome and cytochrome c-liposome complexes show a similar type of quenching of fluorescence in the presence of Ca$^{2+}$.

8.1. Effect of pH

The fluorescence emission spectra of ANS bound to BSA-liposome (egg lecithin) complex is shown in Figure 39. The position of $\lambda_{\text{Fmax}}$ probably depends on the pH of the
Figure 38. Plots of the variations in the relative fluorescence intensity of NPN in suspensions of positively charged liposome-protein complexes with concentration of Ca$^{2+}$.

- - - - - - liposomes (DPPC + SA), o - - - liposome + BSA and △ - - - liposome + cytochrome c.

Concentration of BSA, 0.66 µg/ml; lecithin, 300 µg/ml and of ANS, 8.3 µM. Excitation wavelength, 365 nm and emission wavelength, 425 nm.
NPN FLUORESCENCE

- LIPOSOMES (DPPC+SA)
- LIPOSOMES + BSA
- LIPOSOMES + CYTOCHROME C

CONCENTRATION OF Ca^{2+} (mM)

RELATIVE FLUORESCENCE INTENSITY
Figure 39. Emission spectra of ANS in suspensions of BSA-liposome complex at pH (a) 5.0, (b) 7.0 and (c) 9.0, in (A) absence and (B) presence of 10.5 mM Ca$^{2+}$. Concentration of BSA, 0.66 µg/ml; lecithin 300 µg/ml and ANS, 8.3 µM.
ANS SPECTRA

A) LIPOSOME + BSA
B) LIPOSOME + BSA + Ca^{2+}

(a) pH 5.0
(b) pH 7.0
(c) pH 9.0

RELATIVE FLUORESCENCE INTENSITY

WAVELENGTH (nm)
medium 475 nm at pH 5.0 and 9.0 and 480 nm at pH 7.0 (Fig. 39A). The fluorescence intensity at $\lambda_{F_{\text{max}}}$ is maximum (66) at pH 5.0 while it is 62 at pH 7.0 and 54 at pH 9.0. On the addition of 10 mM Ca$^{2+}$, the position of $\lambda_{F_{\text{max}}}$ at the three pH values becomes the same (478 nm) (Fig. 39B).

Figure 40A shows the emission spectra of NPN bound to BSA-liposome complex in the absence of Ca$^{2+}$. $\lambda_{F_{\text{max}}}$ at pH 4.0 and 9.0 are 425 nm and 422 nm respectively, while that at pH 7.0 is 428 nm. On addition of Ca$^{2+}$ (Fig. 40B) $\lambda_{F_{\text{max}}}$ at pH 4.0 increases to 430 nm while that at pH 9.0 is at 425 nm. $\lambda_{F_{\text{max}}}$ in pH 7.0 is decreased from 428 nm to 420 nm by the addition of Ca$^{2+}$.

Figure 41 shows the emission spectra of ANS bound to cytochrome c-liposome (egg lecithin) complex at pH 4.0, 7.0 and 9.0. There is no change in $\lambda_{F_{\text{max}}}$ at different pH (Fig. 41A). With the addition of Ca$^{2+}$ (Fig. 41B), $\lambda_{F_{\text{max}}}$ at pH 7.0 is increased from 480 nm to 485 nm. There is no change in $\lambda_{F_{\text{max}}}$ for pH 5.0 and 9.0.

The emission spectra of NPN bound to cytochrome c-liposome complex is shown in Figure 42. The position of $\lambda_{F_{\text{max}}}$ at pH 4.0 and 7.0 are at 436 nm while that in pH 9.0 is at 442 nm (Fig. 42A). On the addition of Ca$^{2+}$ $\lambda_{F_{\text{max}}}$ at all three pH values is at 435 nm (Fig. 42B).
Figure 40A. Emission spectra of NPN in suspensions of BSA-liposome complex at pH (a) 4.0, (b) 7.0 and (c) 9.0; Concentration of BSA, 0.66 μg/ml, lecithin 300 μg/ml and NPN, 8.3 μM.
NPN SPECTRA
LIPOSOME + BSA

(a) pH 4.0
(b) pH 7.0
(c) pH 9.0
Figure 40B. Emission spectra of NPN in suspensions of BSA-liposome complex containing 10.5 mM Ca$^{2+}$, at pH (a) 4.0, (b) 7.0 and (c) 9.0. Concentration of BSA, 0.66 µg/ml, lecithin 300 µg/ml and NPN, 8.3 µM.
NPN SPECTRA
LIPOSOME BSA + Co²⁺
(a) pH 4.0
(b) pH 7.0
(c) pH 9.0
Figure 41: Emission spectra of ANS in suspensions of cytochrome c - liposome complex (A) in the absence and (B) in the presence of 10.5 mM Ca$^{2+}$ at pH (a) 4.0, (b) 7.0 and (c) 9.0. Concentration of cytochrome c 0.66 μg/ml, lecithin, 300 μg/ml and ANS, 8.3 μM.
ANS SPECTRA

A LIPOSOME + CYTOCHROME C
B LIPOSOME + CYTOCHROME C + Ca^{2+}

(a) pH 4.0
(b) pH 7.0
(c) pH 9.0
Figure 42. Emission spectra of NPN in suspensions of cytochrome c - liposome complex (A) in the absence and (B) in the presence of 10.5 mM Ca$^{2+}$ at pH (a) 4.0, (b) 7.0 and (c) 9.0. Concentration of cytochrome c 0.66 µg/ml lecithin, 300 µg/ml and NPN, 8.3 µM.
NPN SPECTRA

(A) LIPOSOME + CYTOCHROME C

(B) LIPOSOME + CYTOCHROME C + Ca^{2+}

(a) pH 4.0
(b) pH 7.0
(c) pH 9.0
The plots for pH 7.0 and 9.0 coincide.

Figure 43 shows the effect of pH on the fluorescence intensities of NPN (Fig. 43B) and ANS (Fig. 43A) bound to BSA-liposome complex. Only a slight decrease in the fluorescence intensity is observed with increase in pH from 4.0 to 9.0.

Figure 44 shows the effect of pH on the fluorescence intensities of ANS (Fig. 44A) and NPN (Fig. 44B) bound to cytochrome c-liposome complex. A decrease in ANS fluorescence is observed with increase in pH up to pH 7.0. Above pH 7.0 there is an enhancement in the fluorescence. An enhancement of fluorescence is observed in the presence of Ca\(^{2+}\) with increasing pH. Fluorescence of NPN (Fig. 44B) bound to cytochrome c-liposome complex shows a decrease with increasing pH. The addition of Ca\(^{2+}\) has no effect.

9. Phase-Transition of Dipalmitoylphosphatidylcholine:

Temperature induced phase transition of dipalmitoylphosphatidylcholine is shown in Figure 45. The relative fluorescence intensity of ANS increases with increase in temperature and reaches a maximum at the phase transition temperature (Tm). Increase in temperature above Tm causes a decrease in the relative fluorescence intensity. Tm in the absence of Ca\(^{2+}\) occurs at 42.5\(^{\circ}\)C (Fig. 45A) while in the presence of 10 mM Ca\(^{2+}\) it is at 43.0\(^{\circ}\)C (Fig. 45B). The transition is more sharp in the presence of Ca\(^{2+}\).
Figure 43. Plots of the variations in the relative fluorescence intensity of (A) ANS and (B) NPN in suspensions of BSA-liposome complex with pH. Concentration of BSA, 0.66 μg/ml, lecithin 300 μg/ml and ANS/NPN 8.3 μM. Suspension medium 0.1 M NaCl.
(A) ANS FLUORESCENCE
(B) NPN FLUORESCENCE

(a) LIPOSOME + BSA
(b) LIPOSOME + BSA + Ca^{2+}
Figure 144. Plots of the variations in the relative fluorescence intensity of (A) ANS and (B) NPN in suspensions of cytochrome c-liposome complex with pH. Concentration of cytochrome c 0.66 µg/ml, lecithin 300 µg/ml and ANS/NPN, 8.3 µM. Suspension medium 0.1 M NaCl.
A ANS FLUORESCENCE  
B NPN FLUORESCENCE  
(a) LIPOSOME+CYTOCHROME C  
(b) LIPOSOME+CYTOCHROME C+Ca$^{2+}$

![Graph showing fluorescence intensity vs pH for ANS and NPN fluorescence with different conditions.](image)
Figure 45: Phase transition profile of DPFC, (A) in the absence and (B) in the presence of 10.5 mM Ca$^{2+}$. Concentration of DPFC, 500 μM and ANS, 8.3 μM.
ANS FLUORESCENCE

DPPC  

\[ T_m = 42.5^\circ C \]

DPPC + Ca^{2+}  

\[ T_m = 43.0^\circ C \]
Figure 46 shows the phase transition profile of DPPC liposomes which contain decetylphosphate. Tm from the plot is 42.5°C. On addition of 10.5 mM Ca²⁺ the Tm shifts to 41°C and the transition begins at 38°C and ends at 43.5°C.

Phase transition profiles of positively charged liposome (dipalmitoylphosphatidylcholine + stearylamine) are shown in Figure 47. In the absence of Ca²⁺ the transition occurs in the range 41.5°C to 48.0°C and Tm is 44.5°C. In the presence of 10.5 mM Ca²⁺ the transition range is 42.0 to 51.5°C and Tm is 47.0°C.

Table 10 summarizes the results of phase transition studies on dipalmitoylphosphatidylcholine liposomes. Cooperativity index is given by the slope of plots for transition at Tm, and indicates the relative degree of cooperativity that exists between the fatty acid chains of the lipid. The per cent enhancement of fluorescence intensity on completion of transition may indicate the changes affecting the environment of the probe molecule. Dicetylphosphate does not change Tm of DPPC; stearylamine increases Tm from 42.5°C to 44.5°C. Ca²⁺ decreases Tm in liposomes of DPPC + dicetylphosphate, but increases it in liposomes of DPPC and those of DPPC + stearylamine. An increase in cooperativity occurs in the presence of Ca²⁺ in DPPC and DPPC + dicetylphosphate liposomes.
Figure 46. Phase transition profile of DPPC + DCP.

(A) in the absence and (B) in the presence of 10 mM Ca^{2+}. Concentration of DPPC, 500 μM and NPN, 8.3 μM.
The diagram shows the relative fluorescence intensity of NPN as a function of temperature for two different conditions: DPPC + DCP and DPPC + DCP + Ca²⁺.

- **Condition A (DPPC + DCP):**
  - Temperature range: 20 to 60 °C
  - Fluorescence intensity range: 0 to 80
  - Transition temperature ($T_m$): 42.5 °C

- **Condition B (DPPC + DCP + Ca²⁺):**
  - Temperature range: 20 to 60 °C
  - Fluorescence intensity range: 0 to 80
  - Transition temperature ($T_m$): 41 °C
Figure 47. Phase transition profile of DPPC + SA, (A) in the absence and (B) in the presence of 10.5 mM Ca$^{2+}$. Concentration of DPPC, 500 μM and NPN, 8.3 μM.
NPN FLUORESCENCE

DPPC + SA

\[ T_m = 44.5 \, ^\circ C \]

DPPC + SA + Ca^{2+}

\[ T_m = 47 \, ^\circ C \]
<table>
<thead>
<tr>
<th>Liposome composition</th>
<th>Transition temp. (Tm)°C</th>
<th>Transition range</th>
<th>Cooperativity index</th>
<th>% Fluorescence enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPFC</td>
<td>42.5</td>
<td>-</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>DPPC + Ca^{2+}</td>
<td>43.0</td>
<td>-</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>DPPC + dicetylphosphate</td>
<td>42.5</td>
<td>37.5 - 47.5°C</td>
<td>0.03</td>
<td>100</td>
</tr>
<tr>
<td>DPPC + dicetylphosphate + Ca^{2+}</td>
<td>41.0</td>
<td>38.0 - 43.5°C</td>
<td>0.055</td>
<td>4</td>
</tr>
<tr>
<td>DPFC + stearylamine</td>
<td>44.5</td>
<td>41.5 - 48.0°C</td>
<td>0.065</td>
<td>163</td>
</tr>
<tr>
<td>DPFC + Ca^{2+}Stearylamine</td>
<td>47.0</td>
<td>42.0 - 51.5°C</td>
<td>0.025</td>
<td>76</td>
</tr>
</tbody>
</table>

**TABLE - 10**

PHASE-TRANSITION PROPERTIES OF DIPALMITOYLPHOSPHATIDYLCHOLINE
whereas in liposomes of DPPC + stearylamine there is a decrease in cooperativity in the presence of Ca\textsuperscript{2+}. Ca\textsuperscript{2+} induces a decrease in the per cent enhancement of fluorescence in liposomes of DPPC + dicetylphosphate and those of DPPC + stearylamine, while in neutral liposomes there is an increase in fluorescence enhancement by Ca\textsuperscript{2+}.

10. Phase Transition Properties of Protein–Liposome Complexes

Figure 48 show the phase transition profile of BSA–liposome (DPPC) complex. The fluorescence intensity of ANS shows an increase with temperature and is maximum at the Tm (42.0°C) of the complex. The addition of 10 mM Ca\textsuperscript{2+} to suspension caused a decrease in Tm to 41.5°C and decreases the transition range.

Figure 49 shows the transition profile of negatively charged liposomes (Dipalmitoylphosphatidylcholine + dicetylphosphate) with adsorbed BSA. In the absence of Ca\textsuperscript{2+}, Tm of the complex is 43.5°C (Fig. 49A) with the transition range 38.5° to 48.5°C. The addition of Ca\textsuperscript{2+} changes the Tm to 49.0°C (Fig. 49B) and the new transition range is 37.0° to 54.0°C. In the case of positively charged liposomes (dipalmitoylphosphatidylcholine + stearylamine) with adsorbed BSA the Tm is 43.0°C (Fig. 50)
Figure 48. Phase transition profile of DPPC + BSA,
(A) in the absence and (B) in the presence of 10.5 mM Ca$^{2+}$. Concentration of DPPC,
500 μM, BSA, 0.66 μg/ml and NPN, 8.3 μM.
DPPC + BSA

Tm = 42.0°C

DPPC + BSA + Ca²⁺

Tm = 41.5 °C

RELATIVE FLUORESCENCE INTENSITY

TEMPERATURE (°C)

FLUORESCENCE
Figure 49. Phase transition profile of DPPC + DCP + BSA, (A) in the absence and (B) in the presence of 10.5 mM Ca$^{2+}$. Concentration of DPPC, 500 μM, BSA, 0.66 μg/ml and NPN, 8.3 μM.
Figure 50. Phase transition profile of DPPC + SA + BSA,
(A) in the absence and (B) in the presence
of 10.5 mM Ca^{2+}. Concentration of DPPC, 500 µM;
BSA, 0.66 µg/ml and NPN, 8.3 µM.
The figure shows the NPN fluorescence intensity as a function of temperature for two different systems:

**DPPC+SA+BSA**

- **Panel A**
  - Temperature range: 20°C to 60°C
  - Maximum fluorescence intensity at Tm = 43°C

- **DPPC+SA+BSA+Ca^{2+}**
  - Temperature range: 20°C to 60°C
  - Maximum fluorescence intensity at Tm = 50°C

The graphs depict the relative fluorescence intensity on the y-axis and temperature on the x-axis.
which is increased to 50°C (Fig. 50B) on the addition of Ca\(^{2+}\). The transition ranges in the absence and presence of Ca\(^{2+}\) are 41°-46.5°C and 44.5°-57.0°C respectively.

When cytochrome c is adsorbed to negatively charged liposomes (DPPC + DCP), the Tm of the complex in the absence of Ca\(^{2+}\) is 43.5°C (Fig. 51A) which decreases to 43.0°C (Fig. 51B) on the addition of 10.5 mM Ca\(^{2+}\). The addition of Ca\(^{2+}\) changes the transition range from 38.0°-48.5°C to 38.5°-47.5°C. The transition profiles of cytochrome c-positively charged liposome complex (DPPC + SA) is shown in Figure 52. The Tm of DPPC in this complex is 45°C (Fig. 52A) which on addition of Ca\(^{2+}\) increases to 49.0°C (Fig. 52B). Presence of Ca\(^{2+}\) changes the transition range of the complex from 43.0°-49.0°C to 42.5°-54.0°C.

The transition properties of liposome-protein complexes are summarized in Table 11. Ca\(^{2+}\) increases the cooperativity index (column 5) only in cytochrome c-negatively charged liposome complex, in all other it decreases the cooperativity index. The per cent fluorescence enhancement is increased by the addition of Ca\(^{2+}\) to cytochrome c-negatively charged liposome complex and in BSA-positively charged liposome complex. In others Ca\(^{2+}\) decreases the per cent fluorescence enhancement.
Figure 51. Phase transition profile of DPPC + DCP +
cytochrome c, (A) in the absence and (B) in the presence of 10.5 mM Ca\(^{2+}\). Concentration of DPPC, 500 µM; Cytochrome c, 0.66 µg/ml and NPN, 8.3 µM.
NP
FLUORESCENCE

DPPC+DCP+CYTOCHROME C

Tm = 43.5 °C

DPPC+DCP+CYTOCHROME C + Ca^{2+}

Tm = 43.0 °C
Figure 52. Phase transition profile of DCGP + SA + cytochrome c. (A) in the absence and (B) in the presence of 10.5 mM Ca$^{2+}$. Concentration of DPPC, 500 µM; cytochrome c, 0.66 µg/ml and NPN, 8.3 µM.
<table>
<thead>
<tr>
<th>Protein-Liposome complex</th>
<th>Transition Temperature ($T_m$)°C</th>
<th>Transition Range</th>
<th>Cooperativity Index</th>
<th>% Fluorescence Enhancement $\frac{F_{final} - F_{initial}}{F_{initial}} \times 100$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPC + BSA</td>
<td>42.0</td>
<td>-</td>
<td>-</td>
<td>26</td>
</tr>
<tr>
<td>DPPC + BSA + Ca$^{2+}$</td>
<td>41.5</td>
<td>-</td>
<td>-</td>
<td>18</td>
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<tr>
<td>DPPC + DCP + BSA</td>
<td>43.5</td>
<td>38.0° - 48.5°</td>
<td>0.113</td>
<td>331</td>
</tr>
<tr>
<td>DPPC + DCP + BSA + Ca$^{2+}$</td>
<td>49.0</td>
<td>37.0° - 54.0°</td>
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<td>109</td>
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<td>DPPC + SA + BSA</td>
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<td>41.0° - 46.5°</td>
<td>0.068</td>
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<tr>
<td>DPPC + DCP + Cytochrome c +</td>
<td>43.5</td>
<td>38.0° - 48.5°</td>
<td>0.024</td>
<td>96</td>
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<td>38.5° - 47.5°</td>
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<td>DPPC + SA + Cytochrome c</td>
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<td>43.0° - 49.0°</td>
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<td>DPPC + SA + Cytochrome c + Ca$^{2+}$</td>
<td>49.0</td>
<td>42.5° - 54.0°</td>
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<td>238</td>
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</tbody>
</table>
## DISCUSSION

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Resistance of ELM</td>
<td>167</td>
</tr>
<tr>
<td>1. Effect of Ca\textsuperscript{2+}</td>
<td>168</td>
</tr>
<tr>
<td>ii. Effect of pH</td>
<td>169</td>
</tr>
<tr>
<td>2. 90° Light Scattering by Liposomes</td>
<td>169</td>
</tr>
<tr>
<td>1. Effect of Ca\textsuperscript{2+}</td>
<td>169</td>
</tr>
<tr>
<td>ii. Effect of pH</td>
<td>170</td>
</tr>
<tr>
<td>3. Fluorescence Properties of Probes in Liposomes</td>
<td>171</td>
</tr>
<tr>
<td>1. Effect of Dicetylphosphate, Stearylamine and Ca\textsuperscript{2+}</td>
<td>171</td>
</tr>
<tr>
<td>ii. Effect of pH</td>
<td>173</td>
</tr>
<tr>
<td>4. Phase-Transition of Dipalmitoyl-phosphatidylcholine</td>
<td>173</td>
</tr>
<tr>
<td>1. Effect of Dicetylphosphate and Stearylamine on Tm of DPPC</td>
<td>175</td>
</tr>
<tr>
<td>5. Binding of Proteins to Lipid Membranes</td>
<td>176</td>
</tr>
<tr>
<td>1. Effect of Ca\textsuperscript{2+}</td>
<td>179</td>
</tr>
<tr>
<td>ii. Effect of pH</td>
<td>182</td>
</tr>
</tbody>
</table>