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Prototropism of 1-Hydroxypyrene in Liposome Suspensions: Implications towards Fluorescence Probing of Lipid Bilayers in Alkaline Medium

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1. Determination of partition coefficient of 1-hydroxypyrene in lipid bilayers at pH 11

The success of a fluorescent molecule as a membrane probe depends on the ease of its incorporation into the membrane. Before proceeding into any work on liposome it is desirable to have an idea of the partition behaviour and partition coefficient value of the membrane-associated probe. From this the lipid to probe ratio should be decided in order to have minimum interference from the probe remaining in the bulk water. The partition coefficient (K_p) value of any fluorescent probe between the aqueous and liposome medium can be evaluated by fluorescence spectroscopy as long as there is a difference in a fluorescence parameter of the partitioning molecule (*e.g.*, quantum yield, fluorescence anisotropy or fluorescence lifetime) when in the aqueous solution and after incorporation in the membrane [Ref 1].

The partition coefficient of a membrane probe (K_p) is defined as [Ref 2],

$$K_p = (P_L / L) / (P_W / W) \quad \text{.....(1)}$$

where, P_L = molar concentration of membrane associated probe

P_W = molar concentration of free probe in aqueous phase

L = molar concentration of lipid

W = molar concentration of water (55.6 M)

At pH 11 the fluorescence intensity of the neutral form (F) of 1-hydroxypyrene is proportional to the fraction of it associated with the membrane hence

$$F = \alpha P_L \quad \text{.....(2)}$$

where, α is proportionality constant. Considering the total probe concentration $P = P_L + P_W$ and inserting equation 2 in equation 1 and on rearranging we get

$$F = F_0 L / (55.6 / K_p + L) \quad \text{.....(3)}$$

where, $F_0 = \alpha P$, the maximum fluorescence resulting from total probe incorporation into membrane. Equation 3 gives a hyperbolic-like dependence of F on L and from the fitting parameter the value of K_p can be calculated.

Figure 1 shows the plot of neutral form fluorescence intensity of 1-hydroxypyrene as a function of lipid concentration along with the fitting curves at temperatures 30, 42 and 50°C. The K_p values were calculated to be equal to 1.98×10^5, 3.9×10^5 and 3.1×10^5 at 30°C, 42°C and 50°C, respectively.

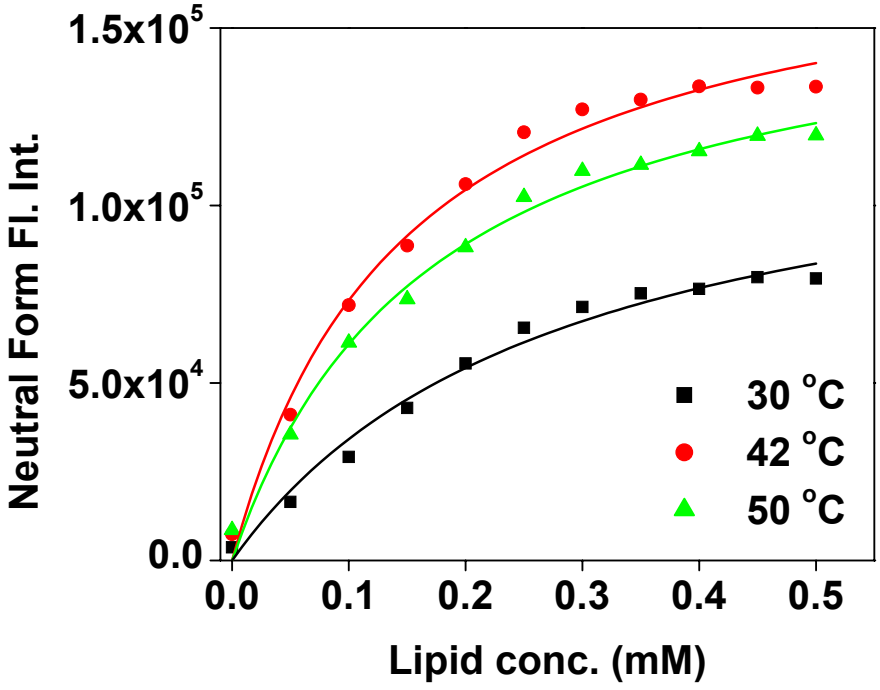

Figure 1. Plot of neutral form fluorescence intensity of 1-hydroxypyrene (1×10^{-6}M) versus DPPC concentration.

References:

1. N. C. Santos, M. Prieto and M. A. R. B. Castanho, *Biochim. Biophys. Acta*, 2003, 1612, 123.
2. H. Zhijian and R. P. Haugland, *Biochem. Biophys. Res. Commun.*, 1991, 181, 166.

2. Effect of EtOH on the emission spectra of 1-hydroxypyrene in homogeneous and liposome medium.

The emission spectra of 1-hydroxypyrene in ethanol-buffer (Tris-NaOH buffer at pH 11) mixture are shown in Fig 2A. In pure ethanol only the neutral form emission is seen, whereas in pH 11 buffer only the anion form emission is observed. With increasing addition of ethanol to the buffer there is a gradual increase in the neutral form intensity, which is clearly seen in the inset in Fig 2A, with corresponding decrease in the anionic emission. But when ethanol is added to1-hydroxypyrene labelled liposome, a gradual decrease in the neutral form intensity is observed which is in contrast to that observed in the homogeneous solution of buffer and ethanol (Fig 2B).

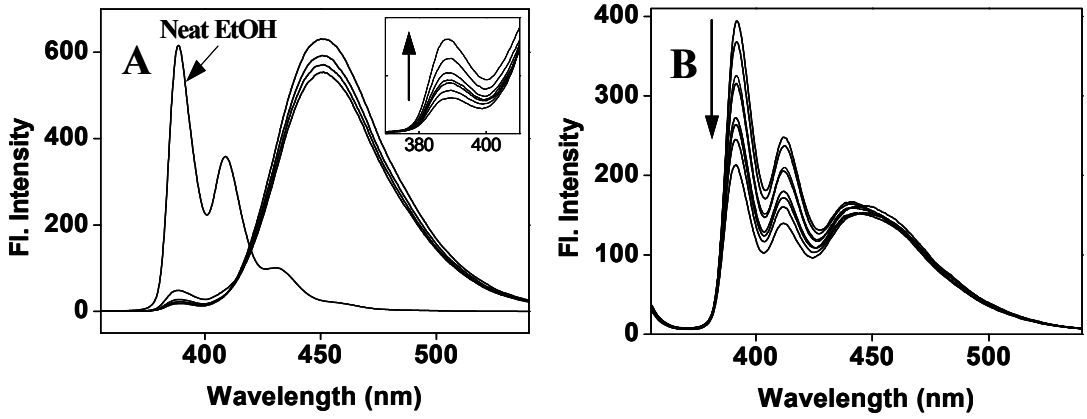

Figure 2. (A) Emission spectra of 1-hydroxypyrene ($1x10^{-6}$M) in water-ethanol mixture with increasing ethanol concentration (0-$2x10^{-3}$M) and in pure ethanol (the intensity is reduced by a factor of 6 for comparison). Inset shows the expanded spectra of the increase of neutral form intensity of 1-hydroxypyrene in the mixture with increasing ethanol concentration. (B) Emission spectra of 1-hydroxypyrene ($1x10^{-6}$M) in DPPC liposome ($3x10^{-4}$M) in presence of different concentration of ethanol (0-$2x10^{-3}$M).