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Interaction of piroxicam and meloxicam with DMPG/DMPC mixed vesicles: Anomalous partitioning behavior

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Abstract

Small unilamellar vesicles (SUVs) formed from a mixture of dimyristoylphosphatidylcholine (zwitterionic lipid with bulkier headgroup) and dimyristoylphosphatidylglycerol (anionic lipid with relatively smaller headgroup) allows better modulation of the physical properties of lipid bilayers compared to SUVs formed by a single type of lipid, providing us with a better model system to study the effect of membrane parameters on the partitioning of small molecules. Membrane parameter like packing of the vesicles is more pronounced in the gel phase and hence the study was carried out in the gel phase. Mixed vesicles formed from DMPG and DMPC with the mole percent ratio of 100:0, 90:10 and 80:20 were used for this study. As examples of polar solutes, piroxicam and meloxicam, two Non Steroidal Anti-inflammatory Drugs (NSAIDs) were chosen. The pH was adjusted to 2.8 in order to eliminate the presence of anionic forms of the drugs that would not approach the vesicles containing negatively charged DMPG (50% deprotonated at pH 2.8). Surface potential measured by using TNS (2,6-*p*-toluidinonaphthalene sulfonate, sodium salt) as surface charge sensitive probe showed no significant changes in the surface electrostatics in increasing DMPC content from 0 to 20%. Transmission electron microscopy (TEM) was used to characterize SUVs of different composition at pH 2.8. The average diameter of the mixed vesicles was found to be smaller than that formed by DMPG and DMPC alone. Partition coefficient (K_P) of piroxicam and meloxicam was measured using intrinsic fluorescence of these molecules. K_P value of piroxicam decreases with increase in DMPC content whereas it increases with DMPC content in case of meloxicam. This anomalous behavior of partitioning is unexpected since there was no significant change in surface pH of the vesicles and has been explained in terms of lipid packing and water penetration in the lipid bilayer.

Keywords: Mixed vesicles; NSAIDs; Partition coefficient; TEM; Fluorescence

1. Introduction

One of the fundamental properties of bio-membranes is to put up barriers to the permeation of various chemical species. Partitioning and/or permeation of chemical species are important to initiate several biochemical processes in vivo. The partitioning of small molecules in membranes depends on the nature of the molecules themselves and also on the different membrane parameters like surface charge, lipid packing and water penetration [1–7]. Mixing of two lipids allows better modulation of the physical properties of lipid bilayers [8,9] compared to SUV formed by a single type of lipid, thereby, providing us with a better model system to study the effect of membrane parameters on the partitioning of small molecules. This is very important to understand the interaction between drug molecules and membranes. For drug molecules, whose target proteins sit within the membranes, the properties of the lipid bilayer is expected to guide the partitioning of the drugs and also which prototropic form of the drug will be preferentially presented to their target within the membrane. One such example is that of Non-steroidal Anti-inflammatory Drugs (NSAIDs) the most commonly used class of painkillers, whose target proteins are cyclooxygenases [10], which are membrane active enzymes. Drugs belonging to the oxicam group of NSAIDs are both chemically [11–14] and functionally [15–19] diverse molecules. Our work with simple membrane models like micelles have established the importance of surface charge of micelles for the incorporation of different prototropic forms of oxicam NSAIDs [14,20–22]. Studies on the partitioning of different prototropic

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forms of piroxicam [4-hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide1,1-dioxide] and meloxicam [4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide] (Fig. 1a and b) on dimyristoylphosphatidylcholine (DMPC) vesicles having no net surface charge showed that the neutral and anionic forms of both drugs were capable of partitioning within the DMPC vesicles. The higher partitioning of both forms of meloxicam over piroxicam is correlated with its higher lipophilicity [23]. The study established that the surface charge is important but not essential for the partitioning of these drug molecules. However using pure DMPC vesicles it is difficult to modulate different membrane parameters. Hence lipid bilayer formed by mixtures of DMPG (dimyristoylphosphatidylglycerol) and DMPC have been used in this study.

DMPG/DMPC lipid bilayer exhibit interesting changes in the physical properties of the bilayer, particularly lipid packing, which is strongly dependent on the mole percent of DMPG and DMPC. This is a result of a compromise between stress generated by charge–charge repulsion of negatively DMPG head groups and the stress due to steric crowding of DMPC head groups [9]. In acidic pH (~2.8) the situation becomes even more complex. At this pH DMPG is partially protonated (pK_a of DMPG is 2.5 [24]) and even DMPC is protonated only to a small amount (pK_a of DMPC is 0.8 [25]). Protonation of DMPG (at pH2.0 in 0.1 M NaCl) shifts T_m up to nearly 40 °C due to reduction in electrostatic repulsion and the increase in capacity for hydrogen bond formation between headgroup [8]. In this study we have formed SUVs containing mainly DMPG having small amount of DMPC at pH 2.8. The mole percent of DMPG and DMPC was varied as 100:0, 90:10 and 80:20. The amount of DMPC in DMPG vesicles was deliberately kept small so that there would be no large alteration of the electrostatics near the membrane surface. This will allow us to probe the effect of other membrane parameters in a better way, for example effect of presence of bulky headgroups of DMPC in a matrix of DMPG

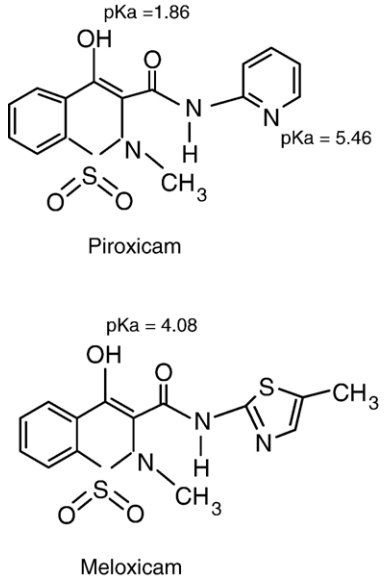

Fig. 1. Chemical structure of piroxicam and meloxicam.

headgroups. This steric mismatch of two types of headgroups can affect packing [26]. In general, solute permeation through lipid bilayer exhibits an inverse relation with the chain packing properties in the bilayer, irrespective of the way the chain ordering is varied [27]. Thus the extent of water penetration should also be modulated by the chain packing properties of the bilayer. For polar molecules, partitioning depends not only on the lipid chain packing but also on the extent of water penetration in the bilayer, which in turn modulates the hydrophobicity profiles across the bilayer [28,29]. Packing contribution becomes more pronounced in the gel phase than in the sol phase [27]. Hence in this work we have used a working temperature of 30 °C and pH 2.8 at which the DMPG/DMPC mixtures used are in the gel phase.

As example of solute molecules having varying degree of polarity, we have chosen two oxicam NSAIDs viz. piroxicam and meloxicam (Fig. 1a and b). Meloxicam shows only one pK_a that of the deprotonation of enolic –OH at pH 4.08, whereas piroxicam shows two pK_a values viz., 1.86 for the deprotonation of enolic –OH and 5.46 for the deprotonation of the pyridyl nitrogen [13]. At the working pH of 2.8 the predominant population of the two drugs would be neutral. For charged membranes, the surface pH varies considerably from that of the bulk pH [30–32]. We have first measured the surface potential of the DMPG/DMPC SUVs using the fluorescence property of the membrane probe 2,6-*p*-toluidinonaphthalene sulfonate, sodium salt (TNS), which is known to be sensitive to the surface potential of a charged membrane [30,33]. This was done to understand the variation of electrostatics of the environment with changing DMPG/DMPC ratio at the acidic pH used for the experiments. The average diameter of the vesicles formed with different mole percent ratio of DMPG and DMPC were determined using transmission electron microscope (TEM). The intrinsic fluorescence properties of the drug molecules were used to determine their partition coefficients (K_P) between aqueous buffer and SUVs. The partition coefficient of piroxicam and meloxicam is different with its value decreasing with increasing percentage of DMPC for piroxicam and vice versa for meloxicam. In this study we have tried to elucidate the reason behind this anomalous behavior in partitioning of the two drugs despite only small changes in the electrostatics of the vesicle surface.

2. Materials and methods

DMPC, DMPG and piroxicam were purchased from Sigma Chemicals (USA) and meloxicam from LKT Laboratories (USA) and were used without further purification. Water was distilled thrice before use. 2,6-*p*-toluidinonaphthalene sulfonate, sodium salt of TNS (Aldrich) is purified by repeated recrystallization from methanol–water mixture. Stock solution of piroxicam and meloxicam of concentration 0.5 mM were prepared in methanol (Merck, Germany) and the exact concentration was adjusted by diluting with the buffer. Each sample contains a maximum of 6% (v/v) of methanol. 25 mM glycine-HCl buffer was used for the measurement at pH2.8. It should be mentioned that presence of 6% (v/v) of methanol had

no effect on the vesicles [23] and this has also been verified in this study from TEM images of the vesicles in presence and absence of 6% (v/v) ethanol. The temperature was kept constant at 30 °C throughout all experiments to ensure that the gel phase is maintained [8]. Drug concentration was kept constant at 30 μM in all the experiments performed.

2.1. Preparation of SUVs

Small unilamellar vesicles (SUVs) of DMPC, DMPG and mixed DMPC and DMPG were prepared by the method of sonication [34]. To prepare SUVs of DMPC, DMPG and mixed DMPG/DMPC, the phospholipid was first dissolved in 2:1 (v/v) chloroform:methanol solution and the solvent was evaporated under a stream of nitrogen. The resultant lipid film was then dried overnight in vacuum desicator at −20 °C. The dry film was hydrated and swelled in the glycine–HCl buffer of pH 2.8 and the mixture was vortexed to disperse the lipids. The dispersion was then sonicated for 20 min using Lab Plant Ultrason 250 probe sonicator (Huddersfield, England) (70% pulse cycle). The samples were then allowed to stand for 30 min to be hydrated completely. The sonicated samples were centrifuged at 10,000 rpm for 15 min to remove titanium particles and aggregated phospholipids [35]. The titanium particles were introduced as impurity in the sample from the sonicator probe.

2.2. Estimation of phosphate

The phospholipid concentration was measured by following published protocol [36]. Vesicle sample of 0.2 ml was digested for 3 h with 0.8 ml perchloric acid at 180 °C. After cooling to room temperature, 5 ml of distilled water was added, then 0.5 ml of 5% ammonium molybdate solution was added followed by 0.5 ml ANSA (amminonaphthol sulphonic acid) reagent (prepared by dissolving 6 mg sodium metabisulfate, 1.2 mg of sodium sulfite and 100 mg of ANSA in 50 ml distilled water). Blue color developed after 20 min and the amount of phosphate was estimated from the absorbance measured at 660 nm.

2.3. Transmission electron microscopy (TEM) and characterization of SUVs by TEM

TEM was done with a Hitachi electron microscope model 600 operating at 75 kV with a resolution of 5 Å. Samples were spread over a copper grid coated with carbon. SUVs were negatively stained with heavy metal complex like phosphotungstic acid (PTA) using 2% (v/v) of its aqueous sample.

As is the general protocol [35], the formation of SUVs was ensured by TEM photograph at a magnification of 60,000×. The diameters of the vesicles were measured from the TEM photographs. Several TEM photographs from different films were taken for the diameter measurements.

2.4. Determination of the partition coefficient

Fluorescence measurements were performed using a Hitachi Spectrofluorimeter model F4010. All emission spectra were corrected for instrument response at each wavelength. A 2 × 10-mm^2 path length quartz cell was used for all fluorescence measurements to avoid any blue edge distortion of the spectrum due to inner filter effect [37].

The partition coefficient of the drugs between the lipid and aqueous phases, K_P, described by the equation

$$K_P = \frac{n_L/V_L}{n_W/V_W} \tag{1}$$

was determined in order to quantify the extent of interaction of the drugs with the membrane model system. In this equation, n is the moles of drug, $V$is the volume of the phase, and the subscripts L and W refer to the lipid and water phases, respectively. The partition coefficients of the drugs in the vesicular system can be obtained from fluorescence data, I, using Eq. (2).

Fluorescence emission intensities of the neutral forms of piroxicam and meloxicam have been used to calculate the partition coefficients of these drugs between the aqueous phase and the lipid phase (inside the vesicles) according to the following equation [38,39].

$$I = \frac{I_W + K_P \times \gamma \times I_{max} \times [L]}{1 + K_P \times \gamma \times [L]} \tag{2}$$

where, I is fluorescence intensity of drug at any concentration of lipid, I_W is the fluorescence intensity of drug in aqueous phase, K_P is the partition coefficient of the drug between lipid and aqueous phase, γ is the molar volume of lipid, $[L]$ is the concentration of lipid and I_{max} is the maximum fluorescence intensity of the drug at saturating lipid concentration.

We could use Eq. (2) for calculation of partition coefficient because for all cases, the fluorescence intensity increased in going from the aqueous phase to the lipid phase without any shift in their respective emission maximum. Non-linear least square fitting of I vs. $[L]$ plot was done using Eq. (2). The value of γ was considered as 0.95 [40].

The maximum fluorescence intensity I_{max} was obtained using the following equation[27]

$$\frac{1}{\Delta I} = \frac{1}{\Delta I_{max}} + \frac{1}{K_{app} \times \Delta I_{max}} \times \frac{1}{[L]-[\text{Drug}]} \tag{3}$$

where, ΔI is the change in fluorescence intensity at any concentration of lipid, ΔI_{max} is the maximum change in fluorescence intensity at the saturating molar concentration of lipid; K_{app} is the apparent partition coefficient of the drug between the lipid and aqueous phase. K_P was not used for the calculation of partition coefficient. A plot of $1/\Delta I$ vs. $1/([L]-[\text{Drug}])$ gave a straight line with correlation coefficient around 0.99, whose intercept on the y-axis gives the value of $1/\Delta I_{max}$. Knowing ΔI_{max}, I_{max} can be calculated from the initial fluorescence intensity at $[L]-[\text{Drug}]=0$.

3. Results

3.1. Characterization of SUVs by TEM

Small unilamellar vesicles (SUVs) of DMPG and mixed DMPG/DMPC were prepared by the method of sonication at

pH 2.8 (as mentioned in the Materials and methods section). The working pH was kept at 2.8 to investigate the interaction of the neutral form of the drugs with the negatively charged vesicles. At physiological pH (7.4) both the drugs would be in their anionic form [13] that cannot approach the negatively charged DMPG containing vesicles. The pK_a of DMPG vesicles is 2.5 which means that at the working pH of 2.8 almost 50% of DMPG molecules are deprotonated. The experiments were carried out at the gel phase because the packing contribution will be more pronounced in the gel phase than in the sol phase. We have prepared SUVs of pure DMPG and 90:10 and 80:20 DMPG:DMPC mixed vesicles. Variations of mole percent of DMPC between 0 and 20% is expected to cause only small alteration in surface potential compared to that of pure DMPG vesicles. Since our working pH is 2.8 it is important to characterize the pure and mixed vesicles at this low pH. The average diameters of three different types of vesicles having different mole percent of two lipids (100:0 DMPG:DMPC, 90:10 DMPG:DMPC, 80:20 DMPG:DMPC) at pH 2.8 were measured from TEM photographs. SUVs of pure DMPC were also characterized at pH 2.8, because DMPC is used as vesicles having no net surface charge (pK_a of DMPC is 0.8) in the surface potential measurement. Fig. 2 (a–d) show the representative TEM photographs of pure DMPG, 90:10 DMPG:DMPC, 80:20 DMPG:DMPC and pure DMPC vesicles. Fig. 3(a–d) demonstrate the bar diagrams of frequency vs. diameter corresponding to Fig. 2(a–d) respectively. Several TEM images were taken, and in all cases a high level of polydispersity were seen. The average diameter of pure DMPG and DMPC vesicles at pH 2.8 (Fig. 3a and d) is about 60–70 nm, whereas for mixed DMPG and DMPC vesicles used in this experiment (Fig. 3b and c), it is between 40–50 nm. It is of interest to note that vesicles formed from DMPG/DMPC have smaller

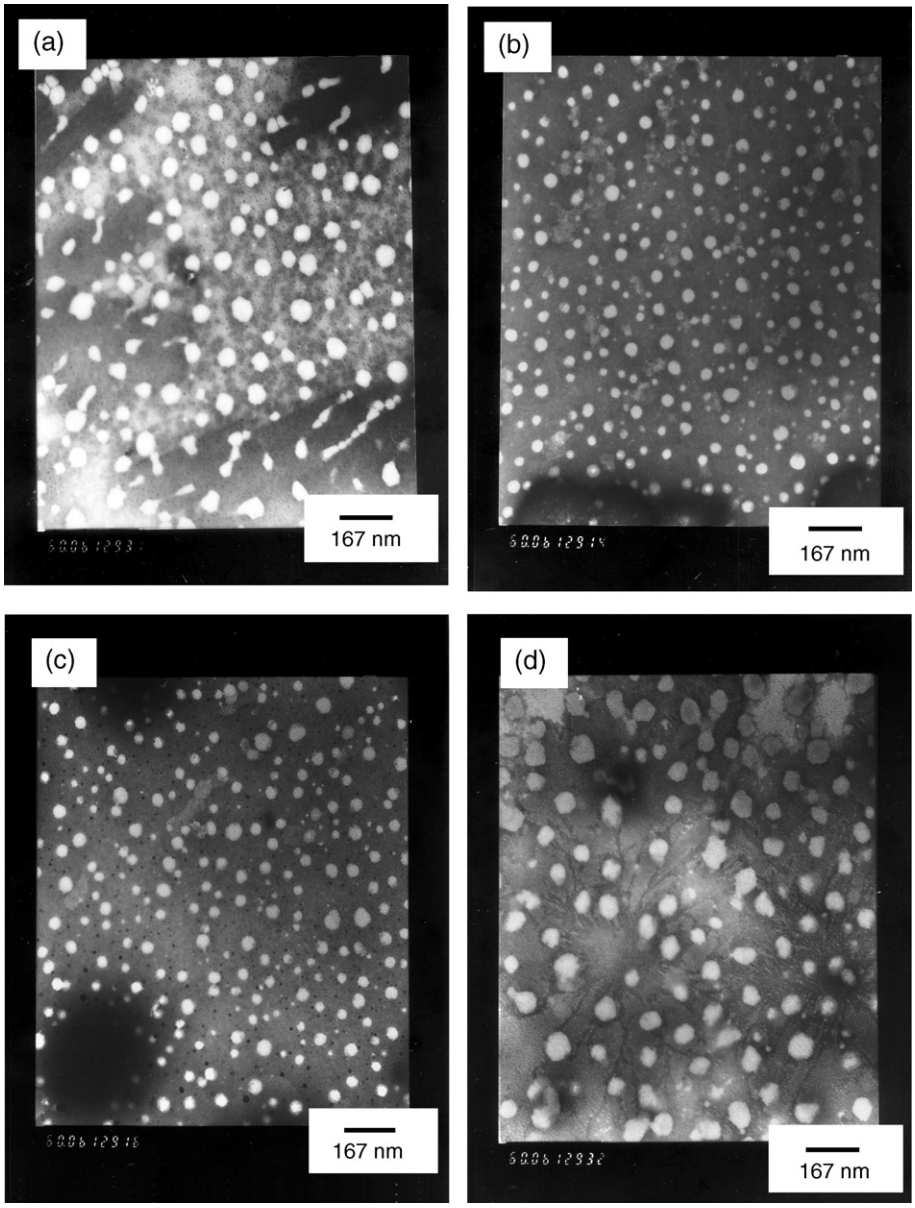

Fig. 2. Transmission Electron Microscope (TEM) photographs of mixed vesicles of (a) 100:0 DMPG:DMPC (mol%), (b) 90:10 DMPG:DMPC (mol%), (c) 80:20 DMPG:DMPC (mol%) and (d) 0:100 DMPG:DMPC (mol%) at pH 2.8.

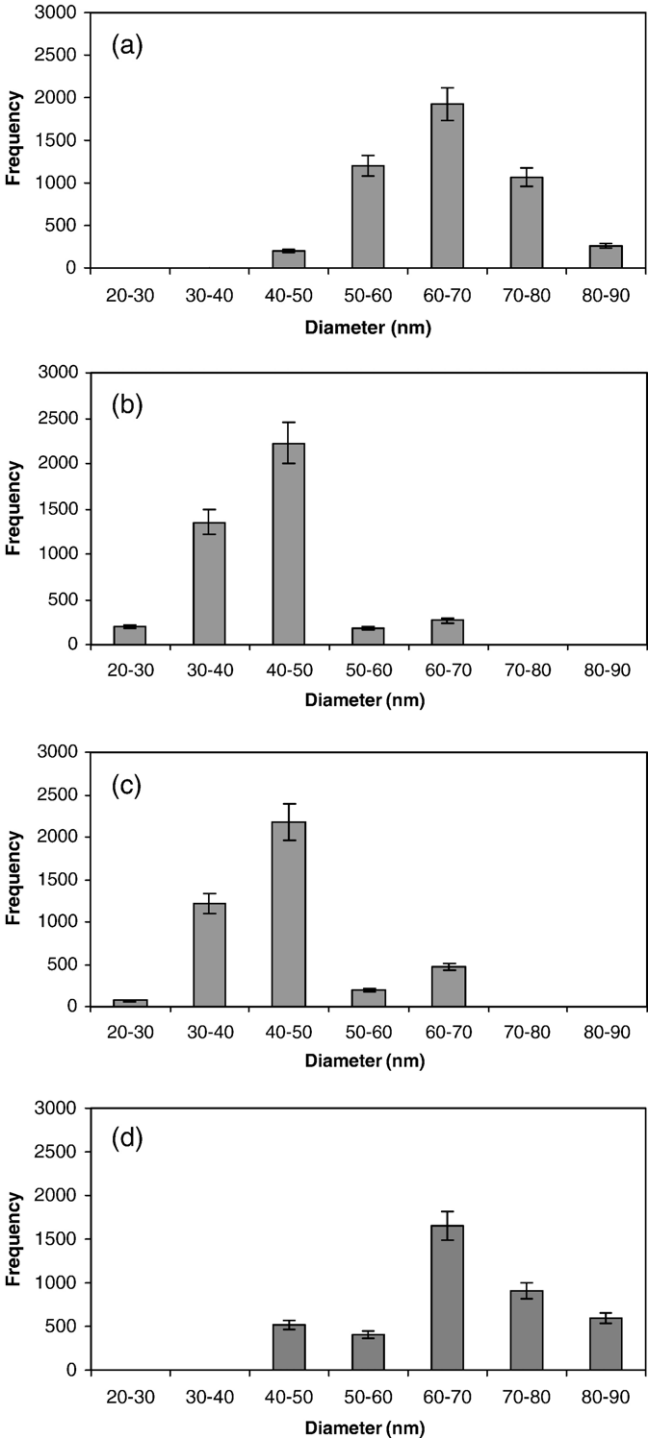

Fig. 3. (a–d) are the size distribution plots of vesicles of different compositions corresponding to Fig. 2(a–d) respectively.

diameter than vesicles containing pure DMPG or DMPC. The reason for this difference will be discussed later.

3.2. Measurement of surface potential and surface pH of the charged vesicles

The surface pH in an organized assembly differs notably from those in bulk water. The activity of hydrogen ion at a micellar surface, $a^i_{H^+}$ is related to the bulk value $a^w_{H^+}$ as [30]

$$a^i_{H^+} = a^w_{H^+} \exp\left(-\frac{F\psi}{RT}\right) \tag{4}$$

where F is Faraday's constant, R is the universal gas constant and T is the temperature in degree Kelvin. Hence the difference in pH between the surface of the vesicles and the bulk is given as follows,

$$\mathrm{pH}_{\mathrm{surface}} = \mathrm{pH}_{\mathrm{bulk}} + \frac{F\psi}{2.303RT} \tag{5}$$

To calculate the pH at the surface of the vesicles, the surface potential ψ needs to be determined. The surface potential of the negatively charged vesicles have been determined according to Winiski et al. [32] using TNS as probe. Binding of TNS is dependent on the surface potential of the lipid membrane and the net fluorescence is proportional to the number of TNS molecules adsorbed on the membrane. The ratio of the probe molecules adsorbed on a charged surface to those adsorbed on a neutral surface is related to the membrane-surface electrostatic potential, ψ, by

$$\psi = \frac{RT}{F} \ln\left(\frac{f}{f_0}\right) \tag{6}$$

where f and f_0 are the net fluorescence intensities of TNS adsorbed to the charged and uncharged vesicles, respectively. The excitation wavelength was set at 321 nm for TNS and the fluorescence intensity was measured at 446 nm [33]. The ψ value can be used to calculate the surface pH of the charged vesicles using Eq. (5).

To measure the surface potential of the charged vesicles, DMPC vesicles were used as vesicles having no net surface charge. The measured surface potentials were used in Eq. (5) to get the surface pH of the mixed vesicles, when the bulk pH is 2.8. For each composition the average results of three experiments have been shown in Table 1. From Table 1 it is seen that the surface potential varies by a small amount among the three different vesicles. This small change in surface potential alters the surface pH by only 0.1 unit from 0.57 in pure DMPG vesicles (0% DMPC) to 0.69 in vesicles containing DMPG:DMPC 80:20. The 0.1 unit difference in surface pH of the vesicles is within our experimental error limit. Hence it can be assumed that doping DMPG vesicles with DMPC up to 20% do not alter the electrostatics of the environments of these mixed vesicles. As has been mentioned earlier, piroxicam exhibits two

Table 1
Measured values of surface potential and calculated values of surface pH of different charged vesicles

Vesicles composition (DMPG: DMPC)	Surface potential (mV)	Bulk pH	Surface pH
100:0	−134±1.63	2.8	0.57±0.027
90:10	−129±1.41	2.8	0.64±0.023
80:20	−126±1.73	2.8	0.69±0.029

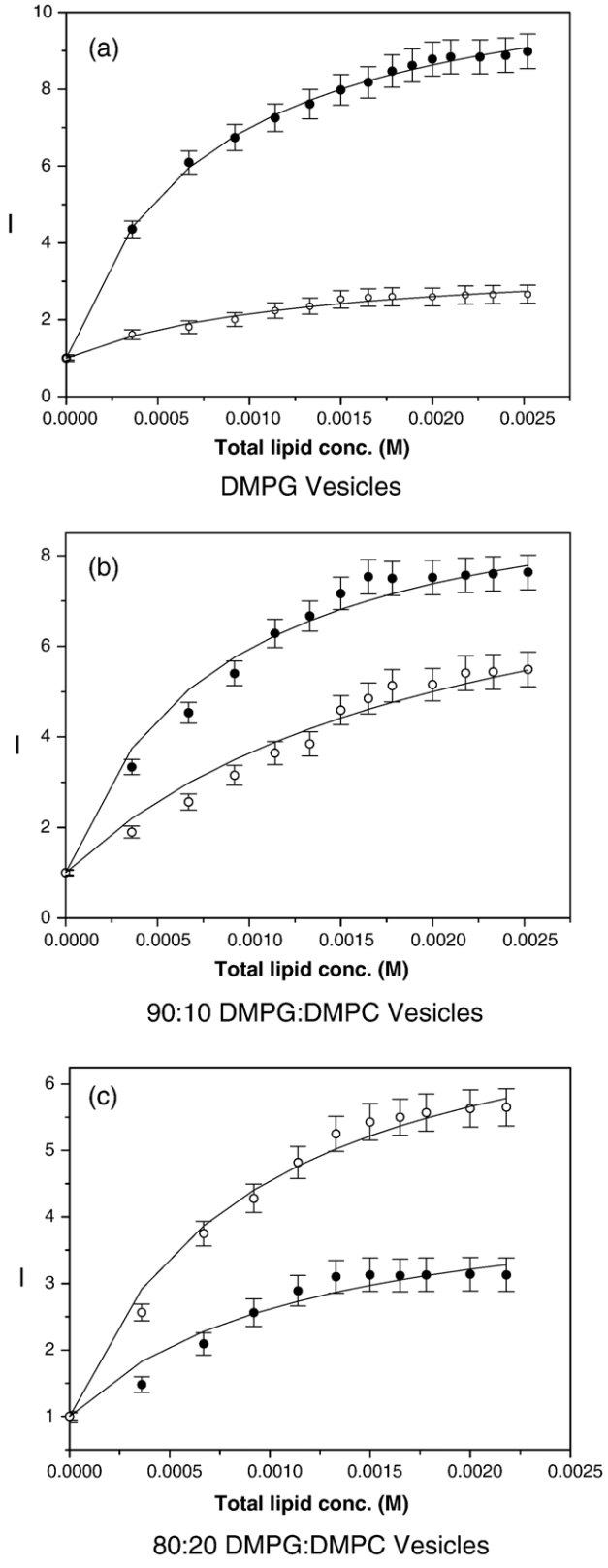

Fig. 4. Plot of relative fluorescence intensity (I) of piroxicam (●) and meloxicam (○) vs. total concentration of lipid. The composition of the lipid was varied in (a) 100:0 DMPG:DMPC (mol%), (b) 90:10 DMPG:DMPC (mol%) and (c) 80:20 DMPG:DMPC (mol%) at bulk pH 2.8. For all sets of experiments $\lambda_{excitation}$=335 nm and $\lambda_{emission}$=475 nm.

pK_a 1.86 and 5.46 for the deprotonation of the enolic –OH and pyridyl nitrogen respectively. Due to low surface pH of the vesicles (Table 1), piroxicam will exist in its cationic form near the surface. A change in pH of 0.1 unit is not expected to alter the population of cationic piroxicam. Meloxicam exhibits only one pK_a at 4.08 for the deprotonation of enolic –OH. Therefore even at such low surface pH of the vesicles, meloxicam will essentially be in its neutral form.

3.3. Interaction of piroxicam and meloxicam with pure DMPG and mixed vesicles

The surface potential measurement data (Table 1) shows that the surface charge does not change in a considerable amount in the three different vesicles having different DMPG and DMPC mole percent ratio and hence the change of surface pH is within a small window in which the population of the cationic form of piroxicam and neutral form of meloxicam does not change at all. The extent of interaction of a solute with a micro-heterogeneous system is quantitatively evaluated from its partition coefficient (K_P). Here we have calculated the partition coefficients of cationic form of piroxicam and neutral form of meloxicam from the changes in the fluorescence intensity profiles with increasing concentration of lipids. The absorption maximum of the cationic form of piroxicam is same as its neutral counterpart, hence the cationic form of piroxicam was excited at 335 nm and the neutral form of meloxicam was excited at 353 nm [41]. Fig. 4a shows the plot of fluorescence intensities (I) of the cationic form of piroxicam and neutral form of meloxicam with increasing concentration of DMPG. Fig. 4b and c are the same plot for cationic piroxicam and neutral meloxicam with increasing total lipid concentration in 90:10 DMPG:DMPC and 80:20 DMPG:DMPC vesicles respectively. The plots are hyperbolic in nature, which indicate a non-cooperative partitioning of both the different forms of two drugs. Partition coefficient (K_P) values were obtained by non-linear curve fitting of using Eq. (2). I_{max} values were obtained from y-intercept of the plot of $1/\Delta I$ vs. $1/[L]-[\text{Drug}]$ according Eq. (3). The K_P values obtained for both the drugs are given in Table 2. The K_P values are different for the two drugs. This is expected since piroxicam is in cationic form near the surface of the vesicles, whereas meloxicam is in the neutral form. What is not expected, is the change observed in the K_P values with increase in DMPC mole percent, since a pH change of 0.1 unit should neither change the electrostatics of the environment nor the population of cationic piroxicam and neutral meloxicam. With increasing DMPC from 0 to 20% K_P values for cationic piroxicam decreases to more than 50% while that of meloxicam increases (Table 1 and Fig. 4). The reason for this anomalous

Table 2
Calculated values of partition coefficient of piroxicam and meloxicam in the vesicles of different composition of DMPG and DMPC

Vesicles	K_P for piroxicam	K_P for meloxicam
100:0 DMPG:DMPC	1754.4±39.9	569.2±18.3
90:10 DMPG:DMPC	1115.2±67.3	803.3±60.4
80:20 DMPG:DMPC	806.3±48.7	1051.7±84.3

partitioning of the drugs in the DMPG/DMPC bilayer will be explained in the next section.

4. Discussion

The surface pH determined by TNS fluorescence of the DMPG vesicles is different (0.57 to 0.69) from that of the bulk pH2.8. However, the local pH of the mixed vesicles used in this study varied by only 0.1 units between pure DMPG vesicles and vesicles containing 20% DMPC. This small change in surface pH is within experimental error limit and hence for all practical purposes one can assume that the electrostatics of the local environment remains unaltered for the three vesicles studied. Even though piroxicam is in its cationic form and meloxicam in its neutral form near the vesicles surface, their total population should not vary for the three vesicles. With this data in mind, it is therefore expected that partitioning of piroxicam and meloxicam should be different from each other in all the vesicles. What is unexpected is that the value of K_P decreases with increasing DMPC concentration in case of piroxicam and vice versa for meloxicam. However, change in electrostatics of the environment cannot be made responsible for such changes in the K_P values of the drugs. To explain the changes in K_P values of the drugs, it is essential to understand the physical properties of DMPG/DMPC bilayers. The average area occupied by the DMPC headgroup is larger than that required for optimal packing of the hydrocarbon chains of the lipid [26]. Hence DMPC bilayers are subject to a destabilizing stress generated by the steric crowding of the bulky DMPC headgroups. On the other hand –PG headgroups are smaller in size, but the charge–charge repulsion between negatively charged DMPG headgroups exerts a destabilizing effect with the weakening of the lipid–lipid packing interaction in pure DMPG vesicles. In case of DMPG/DMPC mixed vesicles, the packing of the bilayer is dependent on the relief of stress generated by the charge repulsion of the –PG headgroups and the steric crowding of the –PC headgroups [9]. It should therefore be strongly dependent on the mole percent of DMPG/DMPC. In this study inclusion of small amount of DMPC (up to 20%) in DMPG vesicles results in relief of stress generated by charge repulsion of the –PG headgroups, thereby enhancing lipid/lipid packing interaction. The enhanced lipid packing of the DMPG/DMPC mixed vesicles are reflected by their smaller average size compared to the pure DMPG and DMPC vesicles as seen from the TEM photographs. It is also known that the DMPG vesicles are more hydrated than the DMPC vesicles [26,9]. So even though inclusion of a small amount of DMPC (up to 20%) in DMPG vesicles at pH 2.8 may not affect the electrostatics of the vesicles, but it enhances lipid packing, which in turn may decrease water penetration in the lipid bilayer [27]. This could explain the changes in the K_P values of the two drugs. Pure DMPG vesicles being more hydrated and with negative surface charge, favor the partitioning of cationic piroxicam over neutral meloxicam. Inclusion of a small percent of DMPC results in increase in lipid packing and decrease in hydration of the bilayer, which disfavors the partitioning of cationic piroxicam but favors the partitioning of neutral meloxicam, which is more lipophilic in nature [42]. As a result K_P of cationic piroxicam decreases with increase in DMPC content and vice versa in case of meloxicam. Our work demonstrates that in absence of significant changes in electrostatics, partitioning of drug molecules having different polar character is guided by other membrane parameters like lipid packing, which in turn affects water penetration thereby changing hydrophobicity of the lipid bilayer. Cell membranes are more complex system consisting of various types of phospholipids having different headgroup charges, proteins, sterols, etc. Interplay of these components determines the character of the membrane viz., its surface charge, stereometric properties, water penetration and hydrophobicity of the interior. All these parameters are expected to guide which solute molecule will partition into the membrane i.e., which drug will be presented to its target protein inside the membrane. Out of these parameters electrostatics plays the most important role. Our study demonstrates that for membranes where electrostatics takes a backseat, lipid packing and water penetration plays the guiding role in drug partitioning.

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