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Membrane Softening by Nonsteroidal Anti-inflammatory Drugs Investigated by Neutron Spin Echo

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Abstract

In spite of their well-known side effects, the nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly prescribed medications for their antipyretic and anti-inflammatory actions. Interaction of NSAIDs with the plasma membrane plays a vital role in their therapeutic actions and defines many of their side effects. In the present study, we investigate the effects of three NSAIDs, aspirin, ibuprofen, and indomethacin, on the structure and dynamics of a model plasma membrane using a combination of small angle neutron scattering (SANS) and neutron spin echo (NSE) techniques. The SANS and NSE measurements were carried out on 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) membrane, with and without NSAIDs, at two different temperatures, 11º C and 37º C, where DMPC membrane is in the gel and fluid phase, respectively. SANS data analysis shows that incorporation of NSAIDs leads to bilayer thinning of the membrane in both the phases. The dynamic properties of the membrane are represented by the intermediate scattering functions for NSE data, which are successfully described by the Zilman and Granek model. NSE data analysis shows that in both gel and fluid phases, addition of NSAID results in a decrease in the bending rigidity and compressibility modulus of the membrane, which is more prominent when the membrane is in the gel phase. The magnitude of the effect of NSAIDs on the bending rigidity and compressibility modulus of the membrane in the gel phase follows an order of ibuprofen > aspirin > indomethacin, whereas in the fluid phase it is in the order of aspirin > ibuprofen > indomethacin. We find that the interaction between NSAIDs and phospholipid membrane is strongly dependent on the chemical structure of the drugs and physical state of the membrane. Mechanical properties of the membrane can be quantified by membrane's bending rigidity. Hence, present study reveals that incorporation of NSAIDs modulate the mechanical properties of the membrane, which may affect several physiological processes, particularly those linked to the membrane curvature.

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INTRODUCTION

Despite their well-known side effects, nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their broad therapeutic actions. They are used to treat inflammation, mild-to-moderate pain, and fever. The common mechanism behind the therapeutic actions of NSAIDs is the inhibition of the biosynthesis of prostaglandins, which is a chemical messenger of pain [1]. This is achieved by obstructing the activity of cyclooxygenase (COX) membrane protein. NSAIDs block access of arachidonate to COX channel that obstructs the cyclic production of prostaglandins. However, use of NSAID is associated with the occurrence of a wide spectrum of side effects, such as cardiovascular and gastrointestinal toxicities [2-3]. These effects are dose-dependent and in many acute cases, severe enough to pose the risk of ulcer perforation, upper gastrointestinal bleeding and death, limiting the use of NSAID therapy. NSAIDs have also shown a potential for use in the main line therapy for chronic pathologies, such as arthritis or Crohn's disease, cancer, and Alzheimer's disease [2-3]. These therapeutic and side effects indicate that the actions of NSAIDs must be beyond the COX pathways. There has been an increasing body of evidence [2,4-5] that the interaction between NSAID and plasma membrane plays an important role in the therapeutic actions and side effects of NSAIDs.

Plasma membrane is the first biological structure encountered by the drug in its action pathway. It has a complex structure of a heterogeneous mixture of various lipids, membrane integral proteins and small molecules [6]. The main building block of plasma membrane is the lipid molecule. This makes the lipid bilayer membrane a suitable model system to examine basic aspects of the NSAID-membrane interaction. The main structural lipids for eukaryotic membranes are glycerophospholipids, among which zwitterionic phosphatidylcholine accounts for more than 50% of the membrane phospholipids [7]. In the present study, a saturated phosphatidylcholine, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) is used as a model membrane system. It has been suggested that direct interaction of NSAIDs with zwitterionic phospholipids is primarily responsible for gastrointestinal toxicity [8]. This has led to development of NSAIDs pre-associated with phosphatidylcholine (PC), as safer alternatives to unmodified NSAIDs. Hence, the study of NSAIDs–membrane interactions has paramount importance for not only rational development of new approaches to overcome NSAIDs side effects, but also to understand the pharmacological actions of these drugs. Various important phenomena, such as vesicles fusion and pore formation, have been observed upon interaction of NSAID with phospholipid membrane [9, 10]. It has been shown

that incorporation of NSAID alter the cooperatively of phospholipid phase transition [11]. The overarching goal of the present investigation is to study the effects of NSAIDs on the structure and dynamics of the phospholipid membrane.

Membrane dynamics is complex and includes an array of motions in a wide range of time scales; from vibration (femto-seconds) to trans-bilayer flip flop motion (few minutes), of the individual lipid molecules. Likewise, a wide range of length scales is involved, from undulations of micron-sized patches of the membrane to re-orientation of lipid molecules (~ Å) [12-16]. Effects of NSAIDs on the lateral and internal motion of membrane lipids have been studied recently by us [14,17]. The results revealed that addition of NSAID accelerates lateral and internal motion of the lipids. Moreover, the change in the dynamics is more prominent when the lipid bilayer is in the ordered phase. In the present study, our aim is to investigate the effects of NSAIDs on the undulation motion of the membrane. The undulation motion not only plays a vital role in the viscoelastic behavior of the membrane, but is also a key determinant in various important physiological processes, such as endocytosis, cellular uptake, and cell fusion and division. Undulation motion of the membrane is governed by the bending modulus, which depends on the bilayer thickness, compressibility modulus, surface charge density, and hydration of the head group [18-21]. It has been shown that contribution to the bending modulus, κ due to electrostatics is proportional to the square of surface charge density [20]. Recently, we have shown [21] that incorporation of melittin, a cationic antimicrobial peptide, into an anionic surfactant membrane leads to a decrease in the bending modulus of the membrane, which was explained based on the decrease in the surface charge density. Nagle and co-workers [22] have investigated effect of alamethicin on the bending modulus of the phospholipid membrane, which was found to be reduced due to incorporation of alamethicin. Previous studies [23-25] have indicated that impact of drugs on the mechanical properties of membrane is significant and related to the therapeutic actions and side effects of NSAIDs. A number of NSAIDs have been found to induce vesicle fusion [26,27], which is an indication of alteration in the mechanical properties of the membrane.

Neutron spin echo (NSE) [18,28-36] is a powerful experimental technique for studying undulation motion of the membrane, which has been employed widely to probe the impact of membrane-active molecules on the mechanical properties of the membrane. For example, a recent NSE study [33] has shown that the addition of HIV-1 fusion peptide makes membrane more rigid, which can be explained based on the increase in the bilayer thickness, as observed using small angle neutron scattering (SANS). Recently, Hellweg and co-workers

[34] have shown interesting results on effects of aescin on the mechanical properties of the bilayer and its dependence on the physical state of the membrane. In ordered phase, incorporation of aescin reduces the bending modulus of the membrane and softens the bilayer. However, in the fluid phase, it acts oppositely and increases the value of bending modulus which makes membrane more rigid.

NSAIDs comprise a large family of compounds classified into several subgroups, based on their chemical structure, such as salicylates (aspirin, diflunisal), profens (ibuprofen, ketoprofen), indoles (indomethacin), oxicams (piroxicam, meloxicam), and so forth. Three popular NSAIDs, aspirin, ibuprofen, and indomethacin, which belong to three different families of NSAID, namely salicylates, profens, and indole, respectively, were used in the present study. Chemical structures of these NSAIDs, along with the schematic of the lipid bilayer, are shown in Fig.1. Here we report the effect of these NSAIDs on the structure and undulation motion of DMPC membrane in the gel and fluid phases as investigated by SANS and NSE techniques. Our results show that NSAID strongly modulates the structural and mechanical properties of the phospholipid membrane. Incorporation of NSAID makes membrane softer, and the magnitude of the effect strongly depends on the chemical structure of the NSAID and the physical state of the membrane.

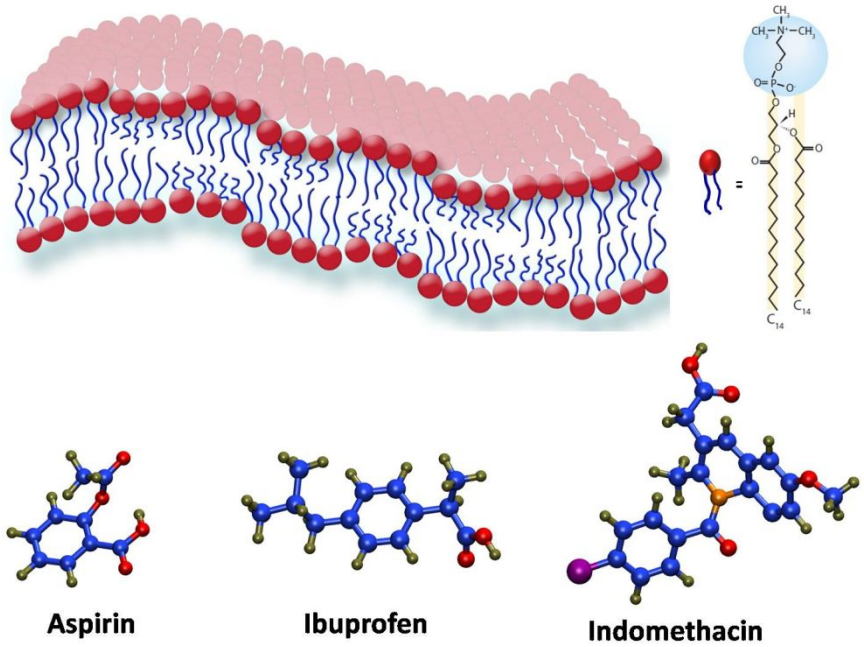

Fig. 1 Schematic of DMPC lipid bilayer. Chemical structures of the aspirin, ibuprofen, and indomethacin are also shown.

MATERIALS AND METHODS

Materials

Fully protonated DMPC and tail deuterated DMPC-d54 lipid powders were purchased from Avanti Polar Lipids (Alabaster, AL). Aspirin, ibuprofen and indomethacin were procured from Sigma Aldrich (St. Louis, MO). D_2O 99.9% was obtained from Cambridge Isotope Laboratories (Andover, MA). We have used 25 mol % NSAIDs, which is much less than the maximum concentration of NSAID used in the literature [4,5,18] to study the effect of drugs on the structure and dynamics of membrane.

Preparation of Unilamellar Vesicles

Unilamellar vesicles of DMPC were prepared using the method described by us [14,16]. In brief, DMPC powder with a molar fraction of 25 % NSAID were co-dissolved in chloroform, which was then evaporated using the dry nitrogen stream and keeping the sample under vacuum overnight. Dry lipid powder was suspended in the desired amount of D_2O at 310 K and underwent 3-freeze-thaw cycles. A mass fraction of 2 % DMPC unilamellar vesicles were prepared by extrusion method in which lipid suspension went through a mini-extruder (from Avanti Polar Lipids) with a porous polycarbonate membrane (pore diameter ~100 nm) more than 25 times.

SANS Experiment

Hellma quartz cells (Hellma, Germany) with 1mm path lengths were used to collect SANS data on ULVs at Bio-SANS instrument [37] at Oak Ridge National Laboratory. An instrument configuration with sample-to-detector distance of 1.13 m at a wavelength of 6 Å was used to cover the scattering vector range of $0.03 < Q$ (Å^{-1}) < 0.3, where $Q = \frac{4\pi}{\lambda}\sin\left(\frac{\theta}{2}\right)$ with λ and θ as the incident neutron wavelength and scattering angle, respectively. SANS measurements were performed at 11º C and 37º C.

The SANS data reduction was performed using standard reduction procedures to correct for detector sensitivity, instrument dark current, sample transmission, and solvent background [37]. A 1-D, *I(Q)* vs. *Q* SANS profile was generated from the reduced 2-D data by azimuthal averaging with respect to the incident direct beam. Unilamellar vesicles consisting of tail-deuterated lipid, DMPC-d54 were used for SANS measurements. DMPC-d54 provides a significant scattering contrast between the head groups and the acyl chains, in

addition to the contrast between the head group and D_2O, which results in enhancement in the bilayer structure features, especially at higher Q.

NSE Experiment

Fully protonated DMPC lipids were used in NSE experiments to enhance the scattering signal, especially at low Q (0.04 $Å^{-1}$ to 0.12 $Å^{-1}$). NSE experiments on DMPC membrane in absence and presence of NSAID's were carried out at a physiological temperature 37º C and 11º C, where pure DMPC membrane is in the fluid and gel phase, respectively. NSE experiments were carried out using NGA beam line NSE spectrometer [38] at the Center for High Resolution Neutron Scattering (CHRNS) at the NIST Center for Neutron Research (NCNR), Gaithersburg, Maryland. The bandwidth center wavelengths of the incident neutron beam (λ) were 8 Å and 11 Å, with a $\Delta\lambda/\lambda \approx 17.5\%$, which covers Fourier times of 0.07 ns to 100 ns. NSE sample cells designed at NIST with a thickness of 2 mm were used. A complete elastic scatterer made of carbon powder was employed as the standard sample for measuring the instrumental resolution. NSE measurements were also carried out on D_2O for reference. Three detector positions were used to cover a Q-range from 0.04 $Å^{-1}$ to 0.12 $Å^{-1}$. The raw data were reduced using the DAVE program provided by NCNR [39].

RESULTS AND DISCUSSION

Incorporation of NSAIDs Leads to Bilayer Thinning

SANS profiles for DMPC membrane with and without NSAID at 11 ˚C and 37 ˚C are shown in Fig. 2. SANS data were analyzed using a polydisperse core 3-shell model in the Q-range of 0.04 $Å^{-1}$ to 0.3 $Å^{-1}$, which is relevant to the size of bilayer structure features. A scattering length density (SLD) profile is composed of three shells, namely (1) the inner head (IH) group touching the D_2O polydisperse core on the inside and the hydrocarbon chains (HC) on outside, (2) the HC sandwiched within head groups on either side, and (3) the outer head (OH) group on the outside between D_2O solvent (approximated as infinite for dilute solvent condition) and HC regions on inside. The scattering intensity from dilute vesicles using a poly-core three-shell model is given by [40-43]:

$$I(Q) = A\left[\sum_{i=1}^{4} \frac{3V_i(\rho_i - \rho_{i-1})\, j_1(Qd_i)}{Qd_i}\right]^2 + bkg \qquad (1)$$

where A is the scaling factor, bkg is the constant background, V_i, d_i and ρ_i are the volume, thickness, and scattering length density, respectively, of each shell. The subscripts $i = 1,2,3,4$ represent the core, IH, HC, and OH regime, respectively. The SLDs, $\rho_i's$ for core and solvent were fixed to that of D_2O. The head regimes were not constrained to have a similar SLD or thickness profiles. The results from fitting the 3-shell model are given in Table-1. It is evident that incorporation of drugs significantly affects the structure of the bilayer. At both the temperatures, bilayer thinning is observed for all the drugs. Maximum bilayer thinning is observed for aspirin and minimum change is observed for indomethacin at both the temperatures.

Table-1. Structural parameters obtained by fitting the SANS data of DMPC bilayer in absence and presence of NSAIDs at 37 °C and 11 °C. The uncertainties are standard deviations.

Parameter (37˚C)	***DMPC***	***DMPC+Asp***	***DMPC+Ibu***	***DMPC+Indo***
Inner Head thickness, $d_{IH}\left(\text{Å}\right)$	*13.9±0.2*	*9.2±0.3*	*11.7±0.4*	*14.4±0.3*
Inner Head SLD, $\rho_{IH}\left(\times 10^{-6}\,\text{Å}^{-2}\right)$	*4.22±0.09*	*4.42±0.4*	*4.49±0.7*	*4.52±0.03*
Chain thickness, $d_t\left(\text{Å}\right)$	*23.1±0.1*	*28.5 ±0.2*	*25.1 ±0.3*	*22.8±0.2*
Chain SLD (Å-2), $\rho_{CH}\left(\times 10^{-6}\,\text{Å}^{-2}\right)$	*6.79±0.08*	*6.49±0.05*	*6.44±0.06*	*6.51±0.08*
Outer Head thickness, $d_{OH}\left(\text{Å}\right)$	*10.2±0.3*	*6.0±0.2*	*7.5±0.4*	*8.9±0.3*
Outer Head SLD (Å-2), $\rho_{OH}\left(\times 10^{-6}\,\text{Å}^{-2}\right)$	*4.53±0.05*	*4.53±0.04*	*4.57±0.4*	*4.57±0.07*
Total Thickness, $d_{Tot}\left(\text{Å}\right)$	*47.1±0.6*	*43.6±0.7*	*44.3±1*	*46.1±0.8*
Scale	*0.065±0.003*	*0.20±0.01*	*0.13±0.01*	*0.104±0.005*

Parameter (11˚C)	***DMPC***	***DMPC+Asp***	***DMPC+Ibu***	***DMPC+Indo***
Inner Head thickness, $d_{IH}\left(\text{Å}\right)$	*14.2±0.2*	*9.4±0.1*	*11.0±0.2*	*11.0±0.2*
Inner Head SLD, $\rho_{IH}\left(\times 10^{-6}\,\text{Å}^{-2}\right)$	*4.55±0.05*	*4.7±0.1*	*4.61±0.7*	*4.41±0.03*
Chain thickness, $d_t\left(\text{Å}\right)$	*29.4±0.1*	*33.1 ±0.1*	*31.7 ±0.2*	*31.6±0.2*
Chain SLD (Å-2), $\rho_{CH}\left(\times 10^{-6}\,\text{Å}^{-2}\right)$	*6.93±0.02*	*6.63±0.04*	*6.53±0.08*	*6.55±0.05*
Outer Head thickness, $d_{OH}\left(\text{Å}\right)$	*8.5±0.2*	*6.6±0.1*	*7.4±0.2*	*8.2±0.2*
Outer Head SLD (Å-2), $\rho_{OH}\left(\times 10^{-6}\,\text{Å}^{-2}\right)$	*4.26±0.08*	*4.75±0.09*	*4.87±0.7*	*4.78±0.03*
Total Thickness, $d_{Tot}\left(\text{Å}\right)$	*52.1±0.5*	*49.2±0.4*	*50.1±0.6*	*50.8±0.6*
Scale	*0.080±0.003*	*0.250±0.008*	*0.173±0.009*	*0.158±0.007*

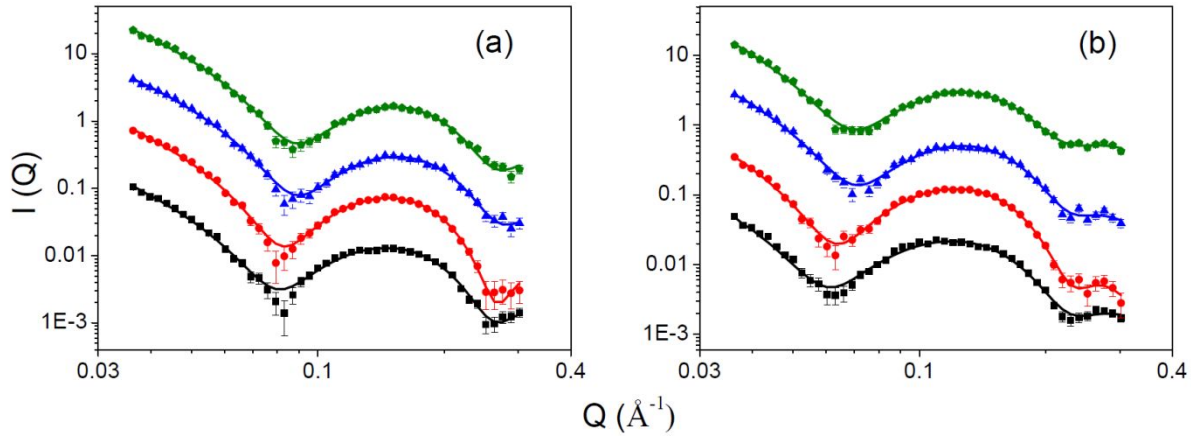

Fig. 2 SANS data on DMPC vesicles neat (black square) and with a mole fraction of 25 % aspirin (red circle), ibuprofen (blue triangle) and indomethacin (green pentagon) at (a) 37 °C and (b) 11 °C. For clarity, curves are offset in Y axis. Solid lines are the fits obtained using Core 3-shell model. Error bars throughout the text represent one standard deviation.

Membrane becomes Softer due to Incorporation of NSAIDs

Normalized intermediate scattering functions, $I(Q,t)/I(Q,0)$, as observed from NSE experiments for DMPC membrane in absence and presence of indomethacin, aspirin, and ibuprofen at physiological temperature of 37 °C are shown in Fig. 3. Zilman and Granek (ZG) [44] have given the theoretical formalism to describe the bending motion of free standing single membrane, according to which, due to thermal undulation motion, the intermediate scattering function describing the membrane's dynamics decays with time as a stretched exponential with an exponent 2/3, and the relaxation rate increases with Q^3. Intermediate scattering function for membrane according to ZG theory can be written as [44]

$$I(Q,t) = I(Q,0)\exp\left[-(\Gamma_{Bend}t)^{2/3}\right] \quad (2)$$

Here Γ_{Bend} is the relaxation rate for the bending motion given by

$$\Gamma_{Bend} = 0.025\gamma_k\left(\frac{k_BT}{\kappa}\right)^{1/2}\frac{k_BT}{\eta}Q^3 \quad (3)$$

Here, κ is the bending modulus of the membrane, η is the solvent viscosity, k_B is the Boltzmann's constant, T is the temperature, and γ_k is a pre-factor, which accounts for the angular averaging between $\boldsymbol{Q}$ and membrane plaquettes surface normal. Pre-factor γ_k is a

function of κ/k_BT and approaches unity for $\kappa/k_BT >>1$, which is valid for the lipid bilayer [18,19, 28-33].

ZG model has been successfully used to describe the experimental NSE data for various lipid membrane [28-33]. In addition to ZG model, centre of mass diffusion of whole vesicle has also been taken into consideration to describe the NSE data [34,36]. Recently, it has been shown [36] that, when centre of mass diffusion of whole vesicles is considered, the fit quality of the intermediate scattering function remains almost same. Moreover, the obtained value of membrane rigidity is unchanged when the solvent viscosity is replaced by the corresponding solution viscosity obtained from rheology. Hence, in the present analysis we have not accounted for centre of mass diffusion of whole vesicles and used solvent viscosity to estimate the bending modulus of the membrane, similar to earlier studies described in the literature [28-33]. Furthermore, a recent study of DMPC vesicles at 15 mg/ml concentration [34] has detected only a small contribution from the centre of mass motion to the NSE signal, which was explained as a result of a relatively high concentration of vesicles in the solution. Therefore, the contribution from the center of mass motion is expected to be even smaller for our present sample at 20 mg/ml concentration.

Stretched exponential function as described by Eq. (2) was used to describe the observed intermediate scattering functions for DMPC membrane with and without NSAIDs at 11 °C and 37 °C. We found that ZG model could describe the data well for all the membrane systems even in the gel phase. Relaxation rates for the bending motion were obtained for DMPC membrane, neat and with NSAIDs, and are shown in Fig. 4. We found that for the DMPC membrane with and without NSAIDs, relaxation rate varies linearly with Q^3, as suggested by ZG model, and from the slope one can extract the bending rigidity of the membrane. It has been shown [29] that bending modulus obtained using Eq. (3) is higher by a factor of about 9 compared to the expected value for lipid membrane. This discrepancy is mainly due to the local dissipation within the membrane, which was not considered in the ZG model. To account for this, an effective solvent viscosity, η_{eff}, which is three times of the bulk solvent viscosity, was generally used to estimate the value of bending modules [18,28,45]. However, this discrepancy was subsequently treated by Watson and Brown [46] considering slipping mode between monolayer leaflets as modelled by Seifert and Langer [47] in the framework of the ZG strategy. Basically, they have considered that dissipation of the energy stored in membrane is due to both solvent and membrane itself. Watson and Brown [46] have considered the effects of intermonolayer's friction on the length and time

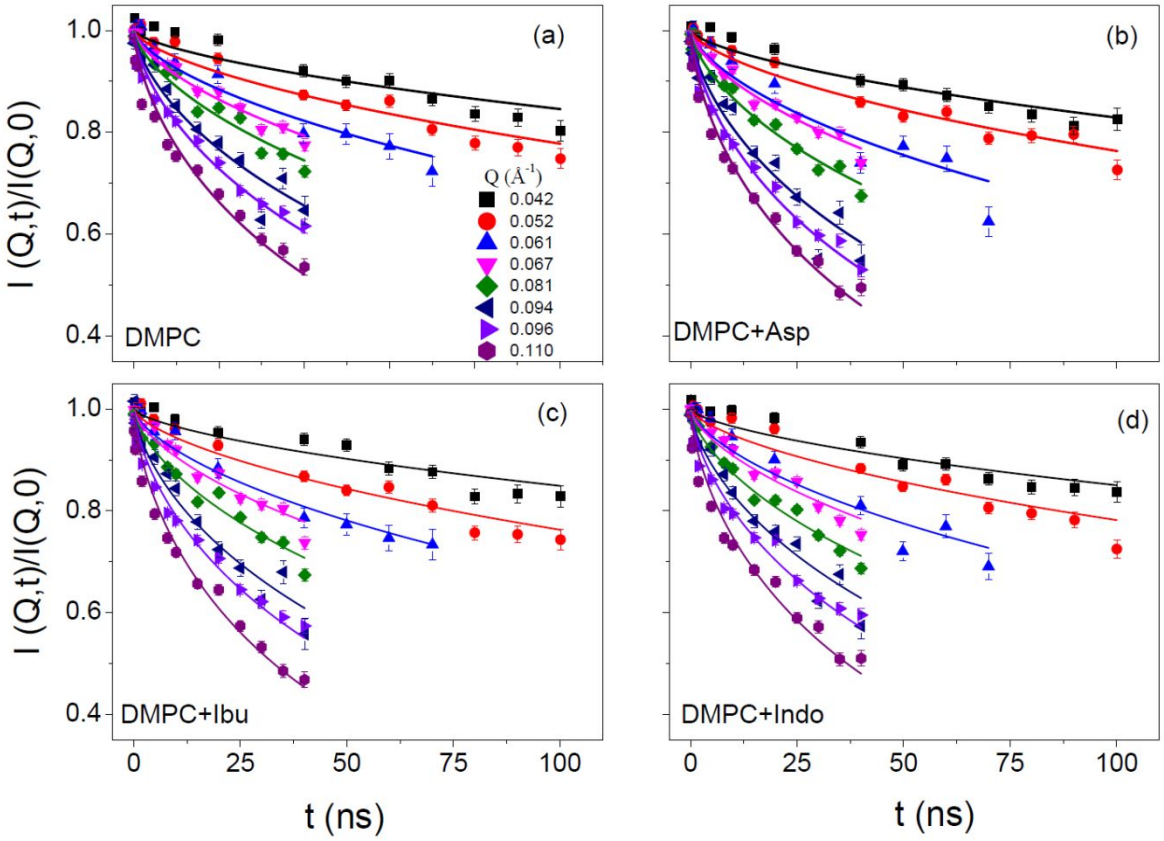

Fig. 3 Normalized intermediate scattering functions for DMPC membrane (a) neat and with a mole fraction of 25 % (b) aspirin (c) ibuprofen and (d) indomethacin at 37°C. Solid lines are the fits with Zilman and Granek model using Eq. (2).

scale observable with NSE. This consideration has enabled a direct comparison between theory and experiment without any scaling of the viscosity of the solvent. In the extended ZG model, bending modulus (κ) in Eq. (3) is replaced by an effective bending modulus ($\tilde{\kappa}$), which is related to κ and monolayer compressibility modulus (K_m) as

$$\tilde{\kappa} = \kappa + 2d^2K_m \qquad (4)$$

where d is the height of the neutral surface from the bilayer midplane [46]. Experimentally, exact value of d cannot be obtained. However, the value of d should be close to the half bilayer thickness. Bending of a bilayer involves a compression of one leaflet and an extension of the other. Monolayer compressibility modulus, K_m can be related to the bending modulus of the bilayer. Lee et al. [29] have used Eq. (4) in terms of bilayer compressibility modulus K_A (using K_A=2K_m) and employed polymer brush model [19], in which K_A can be written as

$$K_A = 24\frac{\kappa}{d_t^{\,2}} \tag{5}$$

where d_t is the thickness of the tail region of the bilayer. The values of d/d_t are found to be in a range from 0.25 to 0.6 [29, 32, 48, 49], and Lee et al. [29] have used $d/d_t \approx 0.6$, which gives

$$\Gamma_{Bend} = 0.008\left(\frac{k_BT}{\kappa}\right)^{1/2}\frac{k_BT}{\eta}Q^3 \tag{6}$$

A discrepancy in the numerical factor on the right hand side of Eq. (6) was found in the literature [15,30,31], where 0.0058 has also been used instead of 0.008. Recently, Nagao et al [32] refined this calculation and showed that one does not need to assume polymer brush model to relate compressibility modulus of monolayer (K_m) to the bending modulus (κ). For a monolayer (assuming a slab layer), compressibility modulus can be defined as $K_m = 12\frac{\kappa_m}{d_s^{\,2}}$, where κ_m is the monolayer bending modulus and d_s is the tail thickness of the monolayer. These monolayer parameters on the right hand side can be expressed in terms of bilayer parameter, since $\kappa_m=\kappa/2$ and $d_s=d_t/2$, which provides the effective bending modulus, $\tilde{\kappa} = \kappa\left\{1+48\left(\frac{d}{d_t}\right)^2\right\}$. A value of $d/d_t \approx 0.5$ was used to analyze the data. This value was selected as to keep the neutral surface of membrane at the interface between the hydrophilic head group and hydrophobic tail rather than in headgroup region of the bilayer. After these refinements, Eq. (3) can be written as [32]

$$\Gamma_{Bend} = 0.0069\left(\frac{k_BT}{\kappa}\right)^{1/2}\frac{k_BT}{\eta}Q^3 \tag{7}$$

Eq. (7) was used to describe the behavior of the relaxation rate with Q^3, and the bending modulus was obtained for DMPC membrane, neat and with NSAIDs, at both the temperatures. Obtained values of bending modulus are shown in Table-2. It is evident from Table-2 that bending modulus for DMPC membrane in the gel phase (11 °C) is higher by an order of magnitude compared to that in the fluid phase. At 37 °C, bending modulus (κ) of DMPC membrane is found to be 15.2 k_BT, which is consistent with the recent studies of Heller et al. [33] and R. Sreij et al [34]. Area compressibility modulus (K_A) characterizes the energy necessary for the increase of a bilayer surface area and can be calculated using Eq. (5), as the values of bending modulus (κ) and tails thickness (d_t) are known from NSE and SANS data, respectively. Obtained values of K_A for DMPC membrane in the absence and

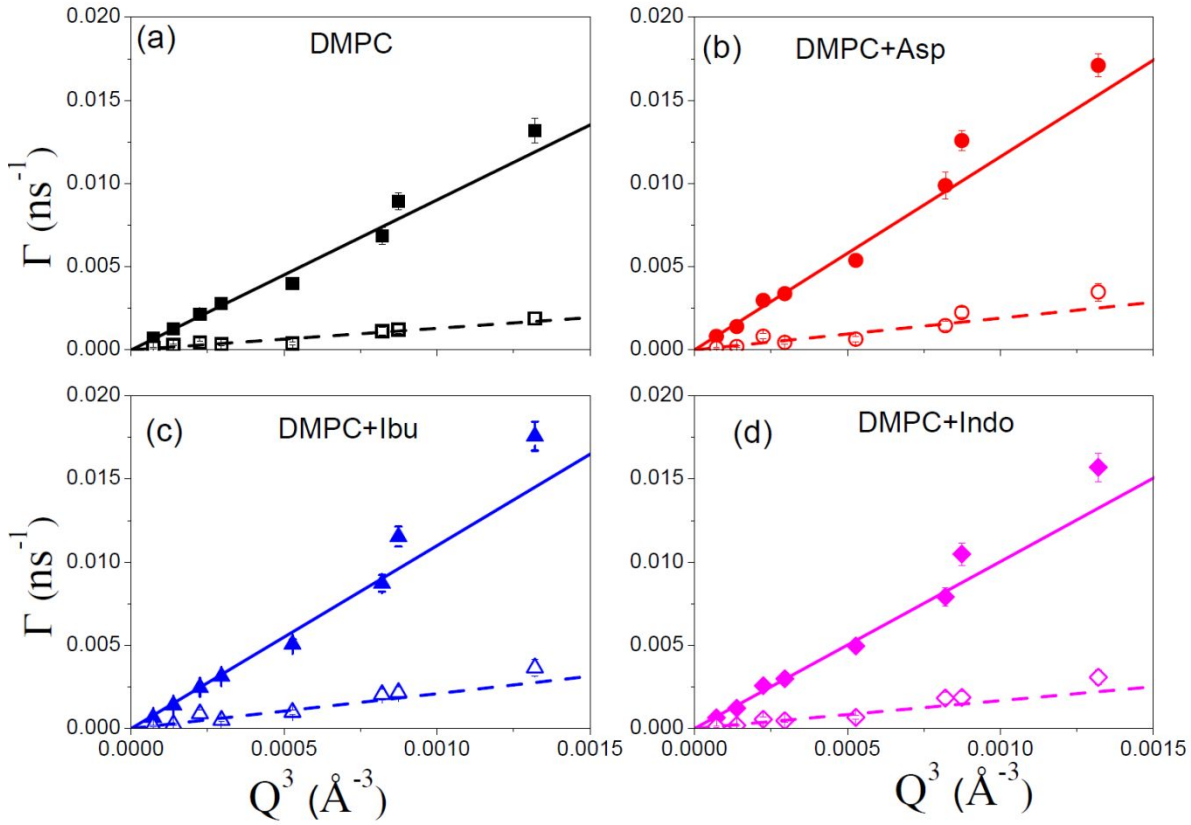

Fig. 4 Variation of relaxation rate for undulation motion with Q^3 for DMPC membrane (a) neat and with a mole fraction of 25 % (b) aspirin (c) ibuprofen and (d) indomethacin at 37 °C (filled symbol) and 11 °C (open symbol). Solid and dashed lines are the least squares fits at 37 °C and 11 °C, respectively, using Eq. (7).

presence of NSAIDs are shown in Table-2 at both the temperatures. For DMPC membrane, at 37 °C, compressibility modulus is found to be (292±14) mN/m. This value agrees well with the results of Boggara et al. [18] and Rawicz et al. [19]. It is evident from Table-2 that incorporation of NSAIDs significantly affects the bending modulus and compressibility modulus of the membrane. Irrespective of the type of a drug and physical state of the bilayer, addition of drugs leads to decrease of both bending and compressibility modulus, which indicates that the membrane becomes softer. This is an important observation, which indicates that incorporation of NSAID reduces the energy and stress required for the undulation and stretching of the membrane, respectively. Results are found to be consistent with the micropipette aspiration study on an antibiotic drug [25], which showed that addition

Table-2 Average values of Γ_{Bend}/Q^3, bending modulus (κ) and area compressibility modulus (K_A) for DMPC membrane in absence and presence of NSAIDs at both the temperatures.

T (°C)	System	Γ_{Bend}/Q^3 (Å^3/ns)	κ (k_BT)	K_A (mN/m)
37	*DMPC*	9.0±0.2	15.2±0.7	292±14
	DMPC+Asp	11.6±0.2	9.2±0.3	116±4
	DMPC+Ibu	11.0±0.2	10.2±0.4	166±8
	DMPC+Indo	10.0±0.2	12.3±0.5	243±11
11	*DMPC*	1.3±0.1	162±25	1765±273
	DMPC+Asp	1.9±0.1	76±8	652±69
	DMPC+Ibu	2.1±0.1	62±6	582±57
	DMPC+Indo	1.7±0.1	95±11	892±104

of azithromycin leads to decrease the bending modulus (from 23.1±3.5 k_BT to 10.6±4.5 k_BT) as well as the area compressibility modulus (from 176±35 mN/m to 113±25 mN/m) of DOPC membrane. Compressibility modulus of the membrane directly depends on the hydration of the headgroup and has a higher value for the well hydrated membrane. It has been shown [18] that at a neutral pH (~7) head group is well hydrated and zwitterionic for pure DMPC membrane, which results in a higher value of compressibility modulus. Incorporation of NSAID into the headgroup region results in a reduction in headgroup hydration, which leads to a decrease in the value of compressibility modulus [18]. We have employed the same drug to lipid molar ratio to examine comparatively the effects of these drugs on the mechanical properties of the membrane. We found that in the fluid phase aspirin reduces bending modulus and compressibility modulus of the membrane to the maximum extent. Upon incorporation of aspirin, the bending modulus, κ decreases by 40 % to (9.2±0.3) k_BT, and the compressibility modulus, K_A, is decreased by ~60 % to (116±4) mN/m. The magnitude of the effect of different NSAIDs on the bending rigidity and compressibility modulus of the membrane is found to be in same order, and in the fluid phase (37 °C) it is in the following sequence: aspirin > ibuprofen > indomethacin. In the ordered phase (11 °C), we found that the influence of drugs on the mechanical properties is more pronounced compared to that in the fluid phase, indicating that effects of NSAIDs depend on the physical state of the

membrane. Maximum effect on the bending modulus and compressibility modulus of the membrane in the gel phase is induced by addition of ibuprofen. For pure DMPC membrane at 11°C, the bending modulus (κ) and the compressibility modulus (K_A) are evaluated to be (162±25) k_BT and (1765±273) mN/m, respectively. Due to incorporation of ibuprofen, the bending modulus decreases by 62 % to (62±6) k_BT and compressibility modulus is decreased by 67 % to (582±57) mN/m. The magnitude of the effect of NSAIDs on the bending rigidity and compressibility modulus of the membrane in the gel phase is in the following sequence: ibuprofen > aspirin > indomethacin. Indomethacin shows the least pronounced effect on the bending modulus and compressibility modulus of the membrane in both gel and fluid phases. This might be attributed to the differences in the size, shape, and hydrophobicity of drugs, which in turn determine the location of NSAID molecules within the membrane core and associated interactions with the membrane. Indomethacin is relatively more hydrophobic than aspirin and ibuprofen and hence is mostly located deep in the hydrophobic core of the membrane, interacting mostly with the hydrophobic tails. On the other hand, aspirin and ibuprofen are relatively smaller in size and are mostly located at the interface of the membrane [50-51]. Therefore, they have a more significant effect on the bending motion of the membrane. Our study has shown that NSAID interacts with the lipid bilayer and modulates its structural and mechanical properties. Interaction between NSAID and lipid bilayer depends on the physical state of the membrane and chemical structure of the NSAID. A membrane containing NSAID has lower bending modulus, is thinner and, as a result, more permeable. These alterations in the membrane may affect several physiological processes, particularly those mediated by the membrane proteins.

CONCLUSIONS

We have investigated the effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on the structural and dynamical properties of a phospholipid membrane, dimyristoylphosphatidylcholine (DMPC), using small-angle neutron scattering and neutron spin echo. We found that representative NSAIDs from different drug families, aspirin, ibuprofen, and indomethacin, profoundly affect the thickness, bending rigidity, and compressibility modulus of the membrane, which are known to play an important role in physiological functions, including cell fusion, cell division, endocytosis and exocytosis. The bilayer thickness, bending rigidity, and compressibility modulus were invariably reduced upon incorporation of the drugs in both gel and fluid phase states of the membrane. The

bilayer thickness was most reduced by the aspirin and least reduced by the indomethacin, irrespective of the gel or fluid phase state. Similar trend in the bending rigidity and compressibility modulus reduction was observed in the fluid phase, but not in the gel phase, where the weakest effect was still induced by indomethacin, but the strongest effect was due to ibuprofen, and not aspirin. While all the studied NSAIDs make the membrane thinner, more disordered, and softer, it is evident that interactions between the incorporated drugs and lipid bilayer are strongly dependent on the chemical structure of the drug and the phase state of the membrane. Thus, while NSAIDs may share common features in their chemical mechanisms of therapeutic action, their influence on the plasma membrane may be highly drug-specific. This may need to be taken into account when considering possible effects of administering nonsteroidal anti-inflammatory drugs.

Conflicts of Interest

There are no conflicts of interest to declare.

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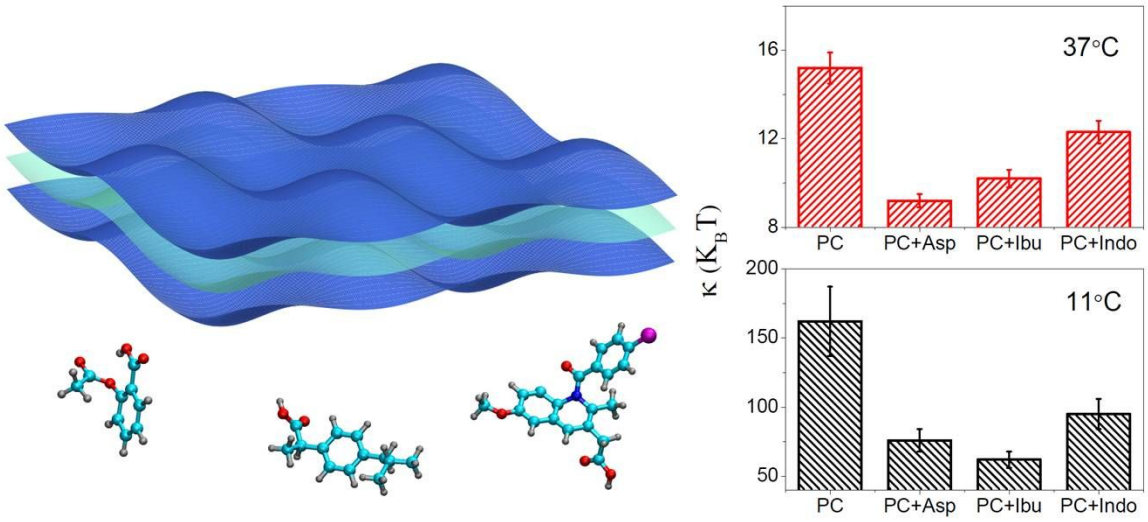
Table of Content (TOC)



Incorporation of drug makes membrane softer, and the magnitude of the effect depends on drug and the phase of the membrane.