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Ibuprofen and the Phosphatidylcholine Bilayer: Membrane Water Permeability in the Presence and Absence of Cholesterol

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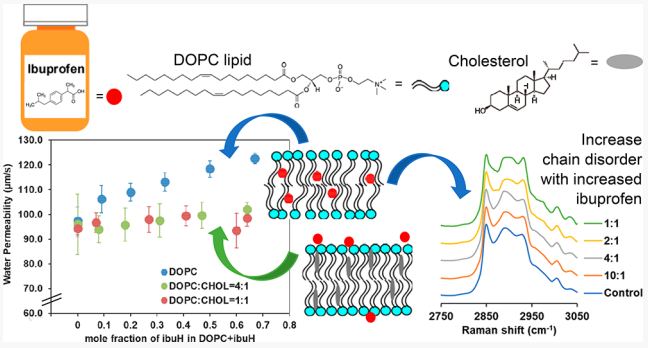
ABSTRACT: The interactions between drugs and cell membranes can modulate the structural and physical properties of membranes. The resultant perturbations of the membrane integrity may affect the conformation of the proteins inserted within the membrane, disturbing the membrane-hosted biological functions. In this study, the droplet interface bilayer (DIB), a model cell membrane, is used to examine the effects of ibuprofen, a nonsteroidal anti-inflammatory drug (NSAID), on transbilayer water permeability, which is a fundamental membrane biophysical property. Our results indicate that the presence of neutral ibuprofen (pH 3) increases the water permeability of the lipid membranes composed of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC). When cholesterol is present with the DOPC, however, the water permeability is not influenced by addition of ibuprofen, regardless of the cholesterol content in DOPC. Given the fact that cholesterol is generally considered to impact packing in the hydrocarbon chain regions, our findings suggest that a potential competition between opposing effects of ibuprofen molecules and cholesterol on the hydrocarbon core environment of the phospholipid assembly may influence the overall water transport phenomena. Results from confocal Raman microspectroscopy and interfacial tensiometry show that ibuprofen molecules induce substantial structural and dynamic changes in the DOPC lipid bilayer. These results, demonstrating that the presence of ibuprofen increases the water permeability of pure DOPC but not that of DOPC−cholesterol mixtures, provide insight into the differential effect of a representative NSAID on heterogeneous biological membranes, depending upon the local composition and structure, results which will signal increased understanding of the gastrointestinal damage and toxicity induced by these molecules.



INTRODUCTION

The study of drug−membrane interactions has significant implications in the field of medicine, contributing insight into drug design and optimized drug delivery.[1−3] An enhanced understanding of the interaction of drugs with the plasma membrane at the molecular level plays an essential role in monitoring their physiological activity and cytotoxicity. The first physical encounter of drug molecules with cells would be with their lipid bilayers, the fundamental scaffold of biological membranes. The lipid bilayers form a protective hydrophobic barrier between the cellular interior and the surrounding environment, perform the vital functions of maintaining homeostasis, and propagate cellular signaling processes.[4,5] Many biological processes are mediated by the lipid bilayer membrane and membrane-bound proteins, which require an adequate membrane as a matrix to ensure their structural and functional integrity.[6] The perturbation of the integrity in the lipid bilayer membrane may influence many physiological processes including the membrane protein conformation, which can lead to significant changes in their biological function.[6−8] For example, mechanosensitive channels and gramicidin channels have been shown to depend on the physical properties of their host lipid bilayer.[9,10] In addition, the stability and function of drug-metabolizing cytochrome P450 (CYP) enzymes, which play a major role in the metabolism of many drugs and are anchored to the membrane of the endoplasmic reticulum, have been reported to sensitively depend on the acyl-chain composition of the lipids and the fluidity of the membrane.[11] It has also been reported that the positioning of drugs on lipid bilayer membranes might affect their interaction with CYP enzymes and, as a consequence, affect the metabolism of drugs.[12,13] Considering the important potential influence of drugs on the collective structure and

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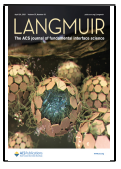

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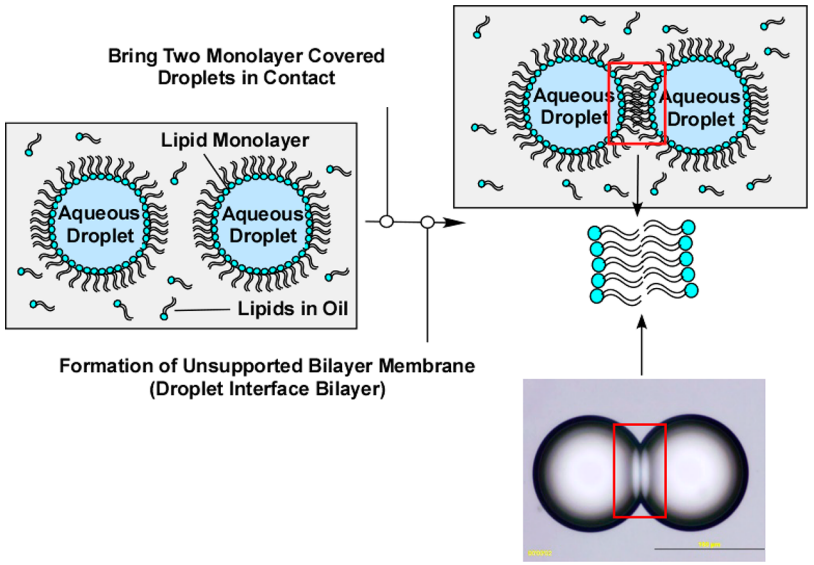

Figure 1. Schematics of the formation of a DIB: contacting apposed monolayers to form a bilayer when aqueous droplets are brought together in an immiscible oil medium, forming a structure simulating the cell membranes consisting of a double leaflet of lipids organized into a lipid bilayer structure. Scale bar on the videomicrograph represents 100 μm.

physical properties of lipid membranes and membrane-bound proteins, many experimental and computational techniques have been developed to study various aspects of drug–membrane interactions.[1,14−19]

Ibuprofen (2-(4-isobutylphenyl)propionic acid) is one among the most widely used nonsteroidal anti-inflammatory drugs (NSAIDs)[20] for its action in alleviating pain, fever, and inflammation. NSAIDs have shown a wide range of beneficial effects including on cardiovascular diseases,[21] cancer,[22] and Alzheimer's disease.[23] The mechanism by which ibuprofen relieves the aforementioned symptoms involves the blocking prostaglandin biosynthesis and neutrophil activation that are mediators of the inflammatory processes[24,25] through the nonselective inhibition of a monotopic membrane-associated protein, cyclooxygenase (COX).[26,27] In addition, NSAIDs have been shown to influence membrane lateral heterogeneity in both model and cell membranes, providing another pathway for modulating the biophysical properties of the membranes.[28] However, long-term use of ibuprofen (in common to other NSAIDs) has been reported to have toxic consequences,[20,29,30] including fatal ulcers and other gastrointestinal (GI) complications such as stomach bleeding, with approximately 16 500 deaths annually from NSAID-induced gastrointestinal side effects.[31] Although most of the biological activities of NSAIDs are related to COX-dependent mechanisms, there has been evidence that the direct interaction of ibuprofen with the zwitterionic phospholipid bilayer of gastric mucosal cells contributes to their local cytotoxic side effects.[24,26,32,33] For example, it has been demonstrated that NSAIDs have the capacity to destabilize the mucosal lining to lessen its surface hydrophobicity.[34]

Ibuprofen can be characterized as a small amphiphilic drug molecule with overall hydrophobic character that includes a phenyl ring with an isobutyl tail and having carboxylic −COOH functionality. Due to ibuprofen's hydrophobic nature and in addition to its direct action on specific protein targets, there is an array of evidence for the interaction of ibuprofen with lipid membranes partitioning into the interfacial region of the bilayer. Previous work has shown that the interaction of ibuprofen with the cellular membrane depends on diverse factors including the composition and physical state of the membrane, drug concentration, and charge state of the membrane and drugs and triggers profound alterations to the phospholipid bilayer biomechanical properties (elasticity, compressibility, microviscosity), structural properties (thickness and lipid packing), and thermodynamic properties (phase transition temperature and cooperativity).[12,14,16,35−49] These types of perturbations to the membrane physical properties are known to play an important role in physiological functions, including cell fusion, cell division, endocytosis, and exocytosis. While considerable effort has been made in developing both experimental and computational methods that provide qualitative insights into the impact of ibuprofen molecules with the lipid bilayer and its influence on the membrane structure, there are relatively few studies addressing the impact of ibuprofen molecules on the dynamics of the lipid membrane. Any changes in the membrane dynamics, such as fluidity and permeability, would have potential consequences upon the transport phenomena of lipid membranes, a set of fundamental biophysical properties that affect essential functions of the membrane. To our knowledge, there have been no reported studies of how the interaction of ibuprofen with lipid membranes would influence water permeability. As an additional matter, cholesterol has been reported to play an important role in the activity pathways of human cytochrome P450 (CYP). Cholesterol does so by modulating the physical properties of the biomembrane to affect the positioning and structural features of CYP, in turn affecting the drug metabolism process.[50] However, the detailed mechanism of action by which cholesterol may influence the drug behavior has not been fully explored.[2] The differential effect of ibuprofen on the lipid bilayer, including cholesterol-containing bilayers, has been previously reported but includes contradictions and thus still requires further elaboration.[35,38,41,51]

The goal of the present study is (1) to probe the changes in passive water permeability across the lipid membrane in the presence of ibuprofen molecules as a function of drug concentration, (2) discern the role of cholesterol on the effect

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of ibuprofen on water permeability, and (3) investigate these systems with diverse techniques designed to examine relevant structural properties. These goals will be facilitated through adoption of a flexible and configurable model membrane. The composition of various cell membranes is very complex and highly diverse, consisting of a wide variety of different constituents such as lipid, carbohydrates, and proteins.[52] There is also a large variety of different lipid species in terms of their headgroup, chain length, and degree and position of unsaturation of the hydrocarbon chain.[4,5] Due to the complexity of real biological membranes, model lipid membranes have been proposed as a useful platform to gain insight into the role of lipids in drug−membrane interactions in a defined and controlled way.[53,54]

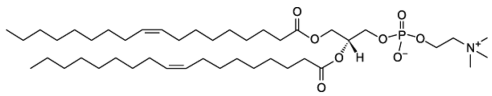
The passive transport of water molecules across bilayer membranes plays a significant role in understanding the cellular physiology and maintaining homeostasis and is of considerable importance for the overall functioning of the cellular plasma membrane.[55] Given that the water transport process is a function of the underlying lipid bilayer structure, it is desirable to establish a consistent and reliable method for quantifying water transport through bilayers, which could in turn shed light on the bilayer structure. Toward this end, in our earlier studies, we demonstrated that the droplet interface bilayer (DIB) represents a convenient model membrane to readily explore structural effects on bilayer water permeability.[56−61] A DIB can be constructed by contacting two individual aqueous droplets positioned in a surrounding immiscible oil medium, each covered with a lipid monolayer, to create a bilayer region. The region adopts a structure essentially the same as the double-leaflet lipid bilayer structure of cellular plasma membranes, as shown in Figure 1.[62,63] The DIB system has many new capabilities and uses, such as for studies of bioelectric phenomena,[64] transmembrane transport,[65] mechanotransduction,[66] 3D printing of model tissue,[67] and droplet crystallization.[68] The convenience and versatility of the DIB membrane system offer great opportunities for investigating membrane transport phenomena through a very well-defined and controlled model membrane.

In the present study, we specifically investigated the effect of ibuprofen molecules on the dynamic properties of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) droplet bilayer membranes as a function of ibuprofen concentration and function of varying lipid composition such as inclusion of cholesterol at pH 3. The phosphatidylcholine (PC) class of lipid is a good prototype for the study of the interaction of small drug molecules with a biological membrane, as this class makes up about 40% of the human endoplasmic reticulum membrane mass, the location where drug-metabolizing CYP enzymes are mostly situated. Cholesterol is widely distributed in mammalian cellular membranes as a high fraction of total lipid (∼20−50 mol %), serves as a major structural component and a critical regulator of protein function, and plays a crucial role in the functional, structural, and dynamical properties of membranes.[69]

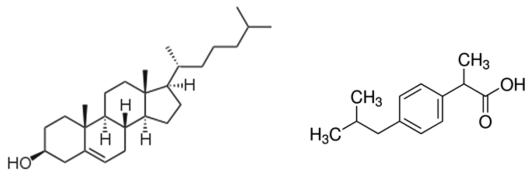
In order to facilitate investigation into the potential structural variations of the DOPC lipid bilayer due to interaction with ibuprofen, we applied confocal Raman microspectroscopy of supported bilayers as well as used interfacial tensiometry to scan the molecular organization of ibuprofen/lipid arrays. Raman spectroscopy is well suited to study phospholipid membranes, particularly so for investigating the structural, packing, and dynamic properties of membrane systems.[70−73] Raman spectroscopy has many advantages including simplicity of sample preparation, a noninvasive and nondestructive method that does not perturb the phospholipid environment, and relative insensitivity to aqueous media. The confocal Raman microspectroscopy technique has been increasingly used in probing the structural properties of substances on a microscopic scale, enabling acquisition of full spectral information with high spatial resolution.[74] The hydrocarbon chains in phospholipid membranes contribute to the characteristic features of Raman spectra, and the spectral frequency and intensity of their vibrational bands are extremely sensitive to structural alterations.[75] Specifically, changes in the relative peak intensities of Raman scattering in C−H or C−C stretching bands, which are sensitive to changes in intra- and intermolecular order, have been interpreted as being indicative of the level of molecular ordering of the self-assembly of the lipid and are used to investigate the differences in the hydrocarbon chain conformation and the corresponding intra- and intermolecular membrane order.[70−72]

EXPERIMENTAL SECTION

System of Study. Figure 2 shows the structures of the compounds employed in the present studies. As the basic component



1,2-dioleoyl-*sn*-glycero-3-phosphocholine (18:1 (Δ9 cis) PC, DOPC)



Cholesterol Ibuprofen

Figure 2. Structures of DOPC, cholesterol, and Ibuprofen molecules.

of a DIB model membrane, we used a neutral zwitterionic ester-linked glycerophosphocholine lipid commonly found in eukaryotic cell membranes, namely, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC). It comprises a headgroup portion with a negatively charged phosphate (PO_4^-) group and a positively charged ammonium ($N(CH_3)_3^+$) group. We also used mixtures of DOPC with cholesterol. Cholesterol consists of a rigid tetracyclic ring structure with a hydroxyl group at one end and a short hydrocarbon tail at the other. Ibuprofen, 2-(4-isobutylphenyl)propionic acid, is a molecule of overall hydrophobic character and consists of a large hydrophobic portion comprising of an aromatic ring and a hydrocarbon tail and a small hydrophilic headgroup consisting of a carboxyl group.

Materials and Sample Preparations. The lipids used in the current study were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL) with 99+% purity and used as received without further purification. DOPC was provided as a solution in chloroform, a solvent removable by high vacuum or stream of inert gas. Monoolein, GMO (1-oleoyl-*rac*-glycerol), was purchased from Nu-Chek Prep, Inc. and used as received (purity ≥ 99%). Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene; $C_{30}H_{50}$; "SqE") and squalane (2,6,10,15,19,23-hexamethyltetracosane; $C_{30}H_{62}$; "SqA"), which were used as the immiscible organic phase, and all other chemicals, including cholesterol (CHOL) and ibuprofen of the

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highest purity available, were purchased from Sigma-Aldrich and used without additional purification. SqA and SqE are chosen since these molecules are excluded from the bilayer to form essentially solvent-free DIB.[76] All lipid and cholesterol samples were stored at −20 °C until use and freshly prepared immediately before use in experiments. SqE was stored in the temperature range of 2−8 °C. In order to prepare an oil solution containing DOPC (with or without CHOL), the chloroform solution of DOPC (optionally with CHOL) is evaporated under inert gas to make a dried thin film of the lipid or lipid mixture followed by overnight vacuum drying for complete removal of any residual solvent. When ibuprofen is used, it is codissolved with the lipid or lipid mixture, instead of being provided in the aqueous droplet phase (due to its low water solubility of ∼0.011g/L at 25 °C). The appropriate amount of ibuprofen is codissolved with lipid (or lipid mixture with CHOL) in chloroform, followed by complete evaporation of the chloroform to generate a dried drug/lipid film of defined mole ratio. This dried drug/lipid film is then dissolved in SqE to a total lipid concentration of 5 mg/mL, which was the concentration used for all water permeability experiments. When mixtures of DOPC and CHOL were used, two different ratios were employed, namely, 4:1 and 1:1 mol ratio for DOPC:CHOL. For sample preparations used in Raman micro-spectroscopy, the dried drug/lipid film described above is subsequently rehydrated with an aqueous solution of pH 3 and vortexed vigorously for about 5 min to obtain a suspension of multilamellar liposomes, followed by seven freeze−thaw cycles using liquid nitrogen. For sample preparations used in interfacial tensiometry, an appropriate amount of monoolein was directly dissolved into SqA (at a concentration of 5 mg monolein/mL SqA), followed by brief vortexing and bath sonication. Aqueous solutions containing osmolytes (NaCl at nominally 0.1 M) were prepared from deionized water (18.2 MΩ·cm) purified in a Millipore water purification system (Direct Q-3). The pH of the solution was adjusted using dilute solutions of either HCl and/or NaOH. The osmolality (in mOsm/kg) of all solutions used was measured by a vapor pressure osmometer (VAPRO model 5600) immediately after fresh preparation of each solution as well as prior to use.

Experimental Details for Water Permeability Measurement Using a Droplet Interface Bilayer (DIB) as the Model Biological Membrane. Our experimental setup and procedure for water permeability measurement using the DIB method has been described in previous papers, and a similar setup has been used for this experiment.[59] Briefly, for the creation and manipulation of the micrometer-sized droplets, our setup consists of an inverted microscope (Nikon Eclipse Ti−S with halogen lamp) combined with two hydraulic micropipet manipulators (Narishige) supported on a vibration-isolated workstation (Newport) with a camera (Andor Zyla sCMOS) directly attached to the microscope for real-time recording of the microdroplets and their size changes. Figure 3A shows the schematics of the sample stage. The capability for manipulating two aqueous microdroplets is facilitated by two 3D hydraulic micromanipulators (three axis with joystick, Narishige MN4 and MO-202U) mounted rigidly on the opposing side of the microscope stage to provide two-droplet manipulation capability with continuous movement on the micrometer scale. The pool of oil (hydrocarbon solvent) in which aqueous droplets are dispensed is held in a chamber made of two parallel strips of coverslip glass. The oil medium (a volume usually of about 200−400 μL) was held by surface tension. The strips of coverslip glass were supported on a custom-built temperature-controlled stage. Glass micropipets protrude into the oil medium to introduce aqueous droplets. A pair of osmotically unbalanced aqueous droplets (one being an essentially pure water droplet and the other being a droplet of 0.1 M NaCl, both adjusted to pH 3 with HCl(aq)) is created in an immisible solvent, SqE, which contains ibuprofen and DOPC or ibuprofen and DOPC/CHOL at a given mole ratio. All water permeability experiments were carried out at 37 °C using a custom-built temperature-controlled microchamber which was thermostated via an external circulating water bath. The temperature of the microchamber containing drug/lipid mixtures is measured by a thermocouple wire and is accurate to ±0.1 °C.

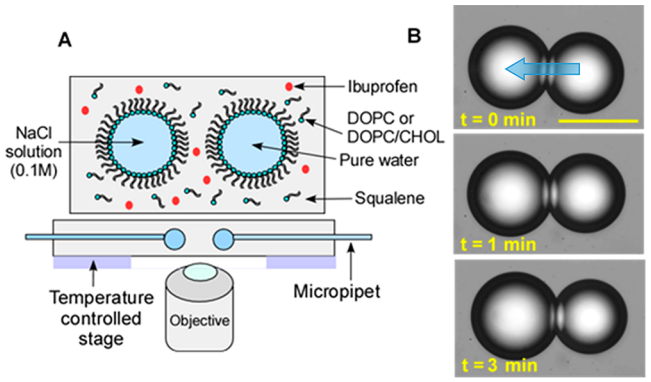

Figure 3. Schematics of the experimental setup showing (A) two aqueous microdroplets in a hydrocarbon dispersion of DOPC (or DOPC with CHOL mixture) containing ibuprofen using squalene solvent and (B) two aqueous microdroplets surrounded by a lipid monolayer form a droplet interface bilayer (DIB). When an imbalance in osmotic pressure is present between two aqueous droplets, subsequent osmotic water transport is monitored and recorded for ∼5 min (arrow, direction of water transport; bar, 100 μm), as shown in representative videomicrographs of the progression of the typical DIB permeability experiment.

When two osmotically unbalanced microdroplets, each covered with lipid monolayer, were made to adhere at a bilayer, osmotic water transport immediately commenced through the bilayer (contact zone), resulting in a visible change in the droplet diameter (Figure 3B). The leftmost droplet in Figure 3B contains NaCl (0.1 M) with an accurately known osmolality in the range of 200 mOsm/kg. The osmotic gradient drives water transport through the droplet bilayer, and the direction of water transport is shown with the arrow in the image. Any electrolyte flux is expected to be negligible compared to that of water, as ion permeation is typically almost 8 orders of magnitude slower than that of water.[77] Changes in droplet size due to this water transport were thus measured from the commencement of the process. Control experiments using osmotically balanced droplets were performed that confirm that no volume change in either droplet occurs without an osmotic imbalance. In addition, the sum of the volumes of the two droplets remains constant during the duration of each experiment (∼5 min), demonstrating that the water transport is occurring exclusively between the two droplets without loss to the surrounding hydrocarbon solvent.

The corresponding changes in droplet volume over time (dV/dt) are measured optically by microscopic observation, and the behavior of the system follows the expression of eq 1 based on Fick's law

$$\frac{dV(t)}{dt} = P_f A(t) v_w \Delta C(t) \quad (1)$$

where A is the geometric bilayer area, ν_w is the molar volume of water (18 mL/mol), $\Delta C(t)$ is the osmolality gradient between two droplets, and P_f is the bilayer permeability coefficient of water. Coupling knowledge of the bilayer contact area ($A(t)$) (observed microscopically) and the evolution of gradient $\Delta C(t)$ can yield P_f values directly through the integrated form of eq 1. When the bilayer contact area is constant, the time evolution of the swelling droplet can be obtained from the following equation derived from integration of eq 1 with the following simplifying assumption: since one of the droplets (the shrinking droplet) contains no osmotic agent, its concentration does not change with time[58,78]

$$\left(\frac{V}{V_o}\right)^2 = \left(\frac{2P_f A v_w C_o}{V_o}\right) t + 1 \quad (2)$$

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Using the measured values for the initial size of the osmotic (swelling) droplet, bilayer contact area (A), and initial osmolarity of the osmotic droplet (C_o), the coefficient P_f for the bilayer water permeability may be derived from eq 2 from the slope of the curve obtained by plotting $(V/V_o)^2$ as a function of time. All data points presented in this paper are an average ($n \geq 50$) of individual permeability runs, each of which took place over a time course (~5−10 min) for osmotic water movement across the droplet bilayer, during which time the droplet contact area (A) remains constant. The recorded videos and images were postanalyzed to measure the dimensions of the droplets and contact area using custom-built image analysis software. All droplet pairs had the same initial size relative to each other in the diameter range of 100 ± 5 μm diameter. All videos were collected with a pixel size of 0.16 μm using the entire field of 1920 × 1080 pixels. Thus, the uncertainty in the radius measurement is 0.32 μm (2 pixels × 0.16 μm/pixel).

Experimental Details for Raman Spectroscopic Analysis. Raman spectroscopic experiments were performed using an inverted confocal Raman microscope system (XploRA INV, Horiba) which consists of a Raman spectrometer directly coupled to an inverted microscope (Nikon Eclipse Ti-U). The Raman setup includes an internal laser kit operating at 532 nm (air-cooled solid-state laser) and a thermoelectrically-cooled CCD detector. A 40× microscope objective (N.A.0.60) was used for focusing a 532 nm wavelength laser beam and for collecting Raman scattered light, which was subsequently dispersed with a grating of 1200 lines/mL. The glass coverslips (#1.5) used as substrates for deposition of lipid films were rinsed with ethanol and blown dry with N_2. A sample (10−20 μL) of a lipid suspension immediately after the freeze−thaw process (as described in the sample preparation section) was spread on the surface of the cleaned coverslip, and the aqueous solvent was allowed to evaporate in a closed homemade chamber for ~≤30 min on top of a heating plate at ~30 °C to form a solid-supported lipid bilayer on a hydrophilic surface.[79,80] The coverslip was mounted to the custom-made chamber for secure support of the sample during the imaging and scanning. All Raman spectra described herein are obtained at ambient room temperature, ~25 °C. Typically, a data set of 5−10 scans was averaged with 20 accumulations.

Experimental Details for Interfacial Tension and Contact Angle Measurement. The interfacial (oil−water) tension was measured with the ramé-hart Advanced Goniometer/Tensiometer (model 590) in conjunction with image analysis software DROP-Image. In experiments intended to study the surface adsorption of monoolein (GMO) at SqA−aqueous interfaces, varying amounts of sodium ibuprofen were present in the aqueous phase. To confirm the validity of the optical calibration of the tensiometer, the surface and interfacial tensions of various known systems were tested before each experiment measurement series. Standards included water/air (72.7 mN/m), SqA/air (28.8 mN/m), and SqA/water (44 mN/m). Reported values are from the average of 10 or more measurements. Typically, ~2 mL of an oil phase containing GMO was used, into which was introduced a pendent drop of an aqueous volume of 1 μL. For the contact angle (θ) measurement, two apposing iso-osmotic droplets are brought into proximity using two micropipets and then touched together to make contact with each other. The droplets shown in Figure 4 were formed in a chamber containing 14 mM GMO in SqA. From the microscopic video images of the two adherent droplets, the contact angle can be measured by considering the geometry of the contacting spheres (as given in eq 3) based on the geometrical parameters shown in Figure 4

$$2\theta = \sin^{-1}\left(\frac{r}{R_1}\right) + \sin^{-1}\left(\frac{r}{R_2}\right) \quad (3)$$

where R_1 and R_2 are the radii of the respective two droplets and r is the radius of the contact zone between the droplets.[81,82]

RESULTS AND DISCUSSION

Water Permeability of DOPC Membranes Increases As a Function of Ibuprofen Concentration. Figure 5 and Table 1 display the osmotic water permeability coefficients (P_f) of DOPC-based membranes at 37 °C as a function of varying mole fraction of ibuprofen in the DOPC−ibuprofen mix. The droplets were at pH 3, a value chosen to suppress ibuprofen dissociation and emulate gastric acidity conditions. Ibuprofen is a propionic acid derivative with a pH-dependent charge state ($pK_a \approx 4.6$−4.9); hence, the carboxyl group of ibuprofen is fully protonated and in undissociated form at an acidic pH 3.[83,84] The results in Figure 5 indicate that P_f for water transport (at 37 °C) increases with increasing concentration of ibuprofen. When the latter P_f values are compared to that of a DIB formed from pure DOPC (as a control), there was an approximately 10% increase in water permeability (from 97.3 ± 5.6 to 106.2 ± 5.5 μm/s) in the presence of a 10:1 DOPC to ibuprofen mole ratio. The increase in water permeability is further enhanced with increasing concentration of ibuprofen, reaching 122.6 μm/s at a 1:2 DOPC to ibuprofen mole ratio, which is about a 26% increase from the P_f of the DIB formed from pure DOPC at the same temperature.

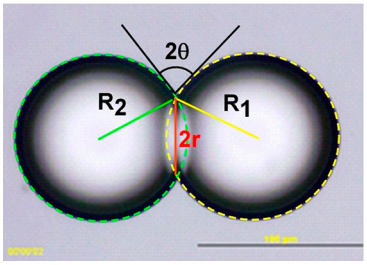

Figure 4. Microscopic picture of two adherent droplets in SqA in the presence of monoolein 14 mM. Contact angle (θ) is determined by eq 3. Scale bar on the image represents 100 μm.

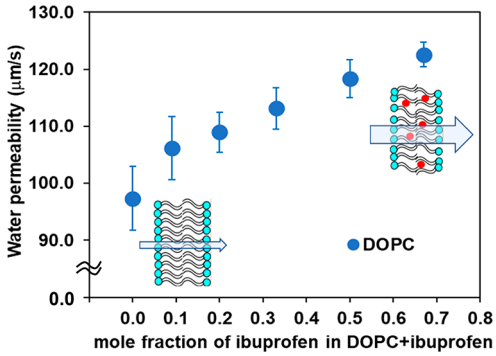

Figure 5. Osmotic water permeability coefficients (μm/s) of the lipid bilayer formed from DOPC at 37 °C with varying mole fraction of ibuprofen at pH 3. Each data point represents an average of individual permeability runs ($n \geq 50$) and standard deviation as error bars.

Table 1. Effect of Ibuprofen on Osmotic Water Permeability at 37 °C for the DOPC Membrane at pH 3

DOPC:ibuprofen mole ratio	P_f (μm/s) avg ± SD
1:0	97.3 ± 5.6
10:1	106.2 ± 5.5
4:1	109.0 ± 3.5
2:1	113.1 ± 3.6
1:1	118.4 ± 5.4
1:2	122.6 ± 2.2

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Langmuir 2021, 37, 4468−4480

Table 2. Surface Activities of Ibuprofen at the Water/Monoolein−SqA Interface at 25 °C[a]

ibuprofen conc. (mg/mL)	monolayer tension, γ_m (mN/m)	contact angle, θ (deg)	bilayer tension, $\gamma_B = 2\gamma_m \cos\theta$ (mN/m)	free energy of adhesion $E = 2\gamma_m(1 - \cos\theta)$ (mJ/m^2)	relative lateral pressure $\pi = \gamma_m(1 - \cos\theta)$ (mN/m)
0	1.32 ± 0.01	25.7 ± 0.2	2.38	0.261	0.131
0.5	1.08 ± 0.01	26.9 ± 0.5	1.93	0.235	0.118
1.0	0.89 ± 0.01	28.8 ± 0.2	1.55	0.220	0.110
2.0	0.44 ± 0.01	38.6 ± 0.3	0.69	0.194	0.097
3.0	0.14 ± 0.02	56.4 ± 0.9	0.15	0.121	0.060

[a]Each data point represents the average and standard deviation (SD) for $n \geq 10$ trials.

The evident increase in the water permeability coefficient of DOPC membranes containing progressively increasing quantities of ibuprofen may reflect the nature and extent of interaction of ibuprofen with the DOPC lipid bilayer. In general, water permeability has been shown to be dependent on the physical properties of the individual lipids and their aggregate bilayers, such as thickness, fluidity, and area per molecule.[85,86] Prior studies have suggested that changes in the thickness of and fluidity within the bilayer will influence the water permeability. Overall, fluidity in the bilayers is generally correlated with the packing density of the lipids,[87] and water permeability is indeed expected to depend on the perturbation of lipid packing and creation of porous voids in the bilayer region. The present findings, in which an increase in water permeability is correlated with increased ibuprofen content, are in general qualitative agreement with the many previous observations in the literature regarding the impact of ibuprofen on the overall membrane structural properties. For example, it has been reported that ibuprofen induces bilayer thinning and decreases both the bending rigidity and the compressibility modulus for 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) model lipid bilayers, as investigated by small-angle neutron scattering (SANS).[49] Through fluorescence anisotropy studies on the behavior of ibuprofen at lipid bilayers consisting of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), it has been shown that ibuprofen diminishes the ordering of the lipid hydrophobic chains.[36] Ibuprofen has also been reported to expand monolayers of DPPC and induce a large change in the monolayer elasticity and lipid packing, lowering the compressibility modulus.[42] X-ray scattering and electron density studies collectively demonstrated that a high concentration of ibuprofen (10−20 mol %) can induce disruption of the lamellar bilayer structure of DMPC and cause a transition from lamellar to cubic phase.[38] Molecular dynamics (MD) simulations in combination with neutron diffraction also have been used to report the thinning of the DOPC bilayer and increased hydration of the lipid polar headgroup with increasing ibuprofen molar fraction.[16] Recent detailed studies by Cremer et al. on the interactions of ibuprofen with lipid bilayers reveal that binding exhibits a multistep character involving adsorption and insertion in a concentration-dependent manner. At low concentrations, ibuprofen favors the lipid headgroup region, whereas at higher concentrations, it begins to penetrate more deeply into the lipid bilayer core region.[35] In addition, the effects of ibuprofen reactivity at varying pH have been reported both experimentally and computationally, demonstrating that ibuprofen localization in the cellular membrane differs depending on the pH.[37,43−45] These studies show that ibuprofen in its uncharged form (pH < pK_a) partitions into the hydrocarbon chain region and perturbs the bilayer structure, but when it is in its anionic form, it is positioned toward the hydrophilic headgroup interface (pH > pK_a).[16,43,45] It should be noted that, in our studies, the DIB became unstable if it was comprised of ibuprofen at too high a ratio relative to DOPC lipid. This indicates that ibuprofen at high concentrations solubilizes the lipid membrane, resulting in a detergent-like effect; thus, the maximum concentration we used in this study was a 1:2 mol ratio of DOPC to ibuprofen. Others have observed similar effects, albeit at concentrations not directly comparable due to the different applied techniques.[35] Collectively, all of these findings point to the penetration of ibuprofen into the hydrocarbon chain region of the lipid bilayer environment, resulting in various structural perturbations that support our findings of the increase in water permeability.

Lateral Pressure of the DIB Interface Decreases with Increased Ibuprofen. One basic parameter relevant to any discussion on the interaction of a drug with the lipid bilayer is the tensiometry of the bilayer, i.e., changes in bilayer tension concomitant to the adsorption of drug molecules. A biological membrane can be characterized by numerous physicochemical properties, an important one of which is the tension across the bilayer (i.e., membrane tension or bilayer tension). This property can be related to the rigidity of a biomembrane and in turn its stability.[88] The bilayer tension of biomembranes, which is a surface energy, can be correlated with the activity of cells in membrane fusion and protein function.[89] The interaction of many drug substances with a bilayer, at least those with amphiphilic character, should manifest in changes in the bilayer tension. Another concept which is closely related to the energetics of bilayer tension is the free energy of formation of the interdroplet bilayer formed at the point of adherent contact between droplets. It provides the driving force for the spontaneous formation of a lipid bilayer at the interface when apposing monolayers are brought together and droplets adhere. Its values can be readily extracted by knowledge of the relevant contact angle θ that the droplets make at their interface and the liquid−liquid interfacial tension γ_m for each monolayer (measured by a pendant drop tensiometer). By taking into account a balance of forces and the following version of the the Young−Dupré equation, eq 4

$$\Delta F = -2\gamma_m(1 - \cos\theta) \tag{4}$$

an energy of adhesion (ΔF) can be derived.[90,91]

In the present studies, we employed a bilayer system based upon the single-chain lipid monoolein (1-oleoyl-*rac*-glycerol). It rapidly forms long-lasting droplet interface bilayers with ease and has been extensively used for the study of bilayer tension and its changes, in the form of both black lipid membranes and droplet interface bilayers.[90,92,93] Thus, the monoolein bilayer should afford a reliable platform with which to observe any tensioactivity of drug substances which are bound to or incorporated in the bilayer.

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Langmuir 2021, 37, 4468−4480

The effects of varying concentrations of ibuprofen on the bilayer tension (γ_B) and energy of adhesion are shown in Table 2. The interfacial tension of the monolayer (γ_m) decreased from 1.32 to 0.14 with increased concentration of ibuprofen from 0 to 3 mg/mL. When the monolayer interfacial tension (γ_m) is combined with the value for the contact angle (θ) as measured between two adherent droplets (described in the Experimental Section), the bilayer tension γ_B can be determined from eq 5

$$\gamma_B = 2\gamma_m \cos\theta \quad (5)$$

We have shown that an increase of the ibuprofen concentration in the aqueous phase enhances the adsorption of ibuprofen at the bilayer interface, as shown by a reduction in the bilayer tension from 2.38 mN/m in the absence of ibuprofen to 0.15 mN/m in the presence of 3 mg/mL of ibuprofen. Increased inclusion of ibuprofen lowers the bilayer tension, possibly due to increased cohesion of the monoolein lipid bilayer assembly, via hydrogen bonding between headgroups. The reduction in bilayer tension was accompanied by a decrease in the free energy of adhesion. Prior reports have also shown evidence for the surface activity of ibuprofen. Surface tension measurements were performed on the water/air interface having a DPPC lipid monolayer.[42] When ibuprofen was present in the subphase or when cospread with DPPC,[42] the monolayer underwent expansion. Other reports have given somewhat contrary results in that dilute ibuprofen solutions (10^{-5}–10^{-6} M) led to a more compact and condensed monolayer and decrease in area per molecule.[35,47] The lowest concentration of ibuprofen in our studies, however, exceeds the reported concentration at which this condensation phenomenon has been observed. We worked in a millimolar concentration regime for ibuprofen, which was comparable to earlier studies in which ibuprofen exhibited a detergent-like behavior on the lipid bilayer, leading to overall fluidization of the hydrophobic region of the membrane.[35] Our data showing a reduction in the bilayer tension is also relevant to the passive transport properties for small molecules across the bilayer, as evidenced by a recent report studying the passive transport of small fluorophores across the DIB, in which the relative membrane lateral pressure ($\pi = \gamma_m(1 - \cos\theta)$) was used to demonstrate a relationship with the permeability: the weaker the lateral pressure, the greater the permeability.[94] This is consistent with our findings where an increased water permeability scaled with a decreased energy of adhesion and lateral pressure.

While we did not perform analogous tensiometric studies for DOPC membranes, some conclusions can be deduced. In a pristine DOPC bilayer, there should be a greater lateral pressure versus our monoglyceride bilayers owing to the contribution of increased tail–tail repulsion in a double-chained lipid and possibly also owing to a reduced hydrogen bonding at the headgroups. Given the detergent-like effect of ibuprofen on PC bilayers, as demonstrated by Cremer et al.,[35] the incorporation of ibuprofen into DOPC membranes would be likely to have at least as much of a decrease of the relative lateral pressure in DOPC since there is more internal lateral pressure to relieve.

Ibuprofen Has a Disordering Effect on DOPC Bilayers. Vibrational spectroscopy can be a powerful tool for investigating the structural and packing properties of membranes. We employed Raman microspectrosopy of supported bilayers in order to correlate the permeability parameters for the DOPC lipid bilayer and any changes that are caused by interaction with ibuprofen molecules. Figure 6 shows raw Raman spectra of DOPC-supported bilayers

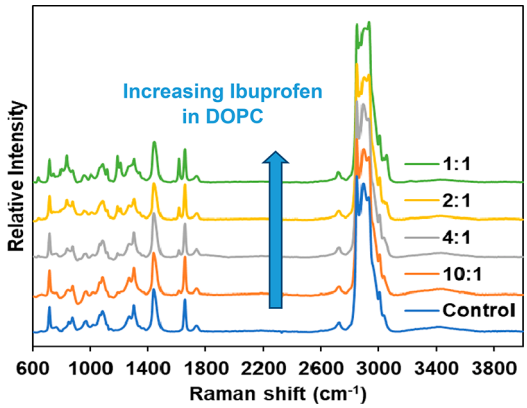

Figure 6. Raman spectra of DOPC:ibuprofen (mol ratio) mixtures of increasing ibuprofen concentration at pH 3 and at ambient temperature.

obtained from varying the degree of addition of ibuprofen at room temperature. All spectra are normalized to the intensity at 2849 cm^{-1} so that their scale shows a clear comparison. The characteristic vibration bands of DOPC are observed: CH_2 twist (~1300 cm^{-1}), CH_2 bend (~1440 cm^{-1}), C=C stretching (~1650 cm^{-1}), and C–H stretching (~2800–3100 cm^{-1}). A more detailed assignment of the characteristic Raman peaks for pure DOPC is shown in the Supporting Information (Figure S1, Table S1). A general trend of increasing intensity of the peak at 1611 cm^{-1} (aromatic C–C stretching from ibuprofen) is noticeable with increased concentration of ibuprofen in DOPC (SI, Figure S2).

Figure 7A shows the C–H stretching region (2750–3050 cm^{-1}), which exhibits strong Raman scattering for phospholipid molecules. It is thus the most well-studied region of the lipid spectrum for its correlations to hydrocarbon chain order.[70−72] Peaks centered at approximately 2850 and 2890 cm^{-1} are assigned to the methylene C–H symmetric and C–H asymmetric stretching modes, respectively, and the peak at ~2930 cm^{-1} is assigned to the symmetric stretching mode for the hydrocarbon chain terminal methyl C–H. The C–H stretching region (2750–3050 cm^{-1}) also has peaks from ibuprofen that interfere with the peaks from DOPC. Therefore, the spectra shown in Figure 7A are the result after subtraction of the ibuprofen-originating peaks from the spectra of the mixture of DOPC and ibuprofen. The detailed spectral subtraction method is shown in the SI (Figure S3). Much prior work has been performed establishing that certain ratios of relative intensities in the C–H stretching regions are useful indicators for determining the membrane properties, such as chain decoupling, rotational disorder, relative hydrocarbon chain order/disorder parameters, and packing effects.[48,70−72]

In this study, the ratios of the peak intensities of [$C–H_{term}$ (2930)/$C–H_{sym}$(2848)] and [$C–H_{term}$ (2930)/$C–H_{asym}$(2890)] are used as a measure of hydrocarbon chain disorder. As seen in Figure 7B, an increased concentration of ibuprofen in the DOPC leads to a monotonic increase in the peak intensity ratio of [$C–H_{term}$ (2930)/$C–H_{sym}$(2848)] and [$C–H_{term}$ (2930)/$C–H_{asym}$(2890)], which span the range from 0.84 to 0.92 and from 0.89 to 0.96, respectively. These specific peaks and their ratios have previously been established as being useful indicators for determining the membrane

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Langmuir 2021, 37, 4468–4480

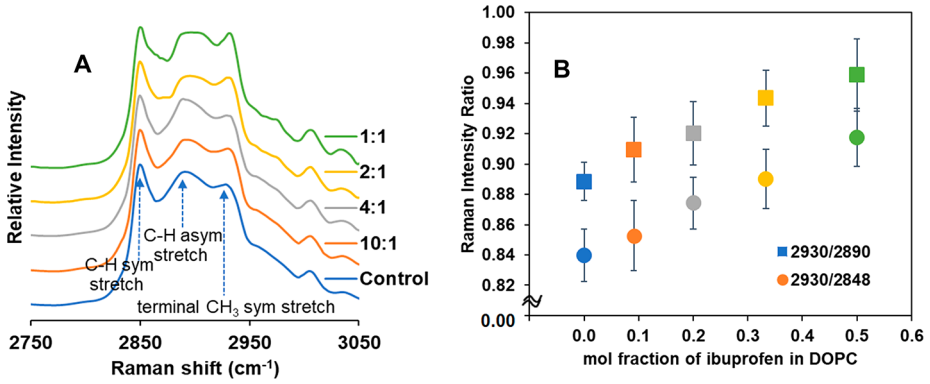

Figure 7. (A) Spectra of DOPC and ibuprofen lipid bilayer mixtures in the Raman shift region between 2750 and 3050 cm^{-1} after subtraction of the characteristic ibuprofen peaks. (B) Raman intensity ratios of [C−H_{term} (2930)/C−H_{sym} (2848)] and [C−H_{term} (2930)/C−H_{asym} (2890)] as a function of ibuprofen concentration in DOPC bilayer films at pH 3 and ambient temperature. Each data point represents the average and standard deviation (SD) for n = 5−10 trials.

structural properties.[70,72] Specifically, an *increase* in the [C−H_{term}/C−H_{sym}] intensity ratio indicates an *increase* in rotational disorder and freedom of motion. An *increase* in the [C−H_{term}/C−H_{asym}] ratio infers a *decrease* in both intramolecular (gauche/trans) and intermolecular (chain packing) interactions.

Since these ratios increase with increased ibuprofen content, it thus appears that a greater quantity of ibuprofen molecules in a DOPC lipid bilayer affects intermolecular interactions in the hydrocarbon chain region, progressively lessening the packing order, thereby having a disordering effect on DOPC lipid bilayers. With increased ibuprofen concentration, the hydrocarbon chains decouple (decrease in intermolecular interactions), thereby increasing the rotational and vibrational freedom of the terminal methyl group, resulting in increased ratios of [C−H_{term} (2930)/C−H_{sym}(2848)] and [C−H_{term} (2930)/C−H_{asym}(2890)]. Our findings are consistent with earlier vibrational studies revealing that the aromatic core of ibuprofen can be localized in the hydrocarbon chain, inducing defects in the hydrocarbon chain region, to disrupt lipid packing and provide the hydrocarbon chain with more room to move,[95] with an overall increase in membrane disorder and fluidizing of the lipid bilayer.[37,46,48]

Figure 8 shows the water permeability coefficient (P_f) values for the droplet interface bilayers comprised of varying amounts of ibuprofen in DOPC plotted against the value of the Raman intensity ratio [C−H_{term}/C−H_{sym}] for vesicle bilayers of the same drug composition. As discussed above, the increased intensity ratio of 2930/2840 reflects the decrease of intermolecular interactions.[72] It is seen that P_f scales with the Raman intensity ratio as ibuprofen concentration increases: the greater the degree of disorder and decreased packing density, the greater the water permeability. This finding, reflecting a strong correlation between the Raman order/disorder parameter and the osmotic water permeability in ibuprofen/DOPC mixtures, indicates that packing in the hydrocarbon chains of ibuprofen-containing DOPC plays a significant role in the passive water transport process.

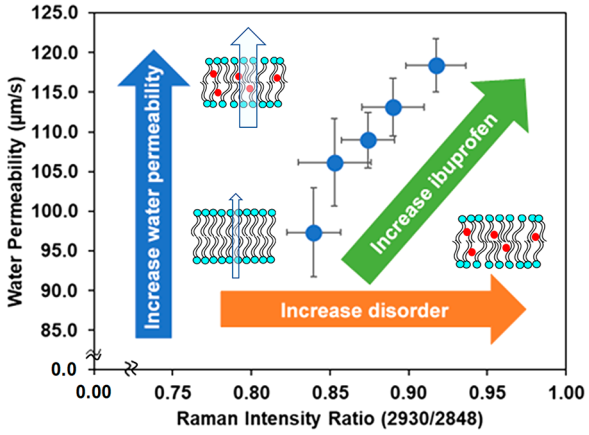

Figure 8. Dependence of osmotic water permeability P_f as a function of Raman intensity ratio [C−H_{term}/C−H_{sym}] in DOPC bilayers containing varying amounts of ibuprofen (R^2 = 0.9323).

Overall, our findings are consistent with those from other experimental and computational simulations in which ibuprofen at low pH (in its neutral form) strongly interacts with the fluid-phase DOPC bilayer (T_m = −18.3 °C). Our findings—of increased water permeability and increased disorder in the hydrocarbon chain—indicate that the ibuprofen molecules perturb the hydrocarbon chain region to decrease intermolecular forces (chain decoupling) and make the overall bilayer more fluidic. Note that analogous Raman structural analyses in the C−H stretching region cannot be extended to DOPC/cholesterol mixtures due to the fact that there are significant overlapping complex Raman bands around the C−H stretching region around 2800−3100 cm^{-1}.[96,97]

Cholesterol Suppresses Ability of Ibuprofen To Increase Water Permeability. We further incorporated cholesterol into the DOPC bilayer to determine the role of a more realistic lipid composition for investigating the influence which ibuprofen has upon the water permeability. Cholesterol's principal role in the membrane is considered to be its organizing effect upon other lipidic components of the membrane, leading to modulation of the structural, dynamical, and physicochemical properties of the plasma membrane lipid bilayer.[69] For example, increasing amounts of cholesterol in DOPC membranes have been reported to cause a corresponding decrease in the area occupied by the lipid and also correspond to a decrease in the water permeability across these membranes.[85] The condensing effect of cholesterol in DOPC lipid bilayers was also reported via atomistic MD simulation for a mole fraction of up to 0.66 at 323 K.[98] It was noted that while the DOPC−cholesterol mixture forms a phase-separated bilayer at a cholesterol mole fraction of 0.75, its mixtures do not exhibit such separation at mole fractions of 0.25 and 0.5.[99]

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Langmuir 2021, 37, 4468−4480

Also, it has been observed that DOPC, a possessor of two unsaturated acyl chains, interacts with cholesterol relatively weakly in comparison to PC lipids having saturated acyl chains. Our results, showing a negligible reduction in the water permeability for membranes containing cholesterol in DOPC, as seen in Figure 9 and Table 3, are not inconsistent with the foregoing. The water permeabilities of the lipid bilayer made of 4:1 and 1:1 mole ratios of DOPC:CHOL (without ibuprofen) are (respectively) 96 ± 12.2 and 94.4 ± 3.4 μm/s compared to 97.3 ± 5.6 μm/s for pure DOPC.

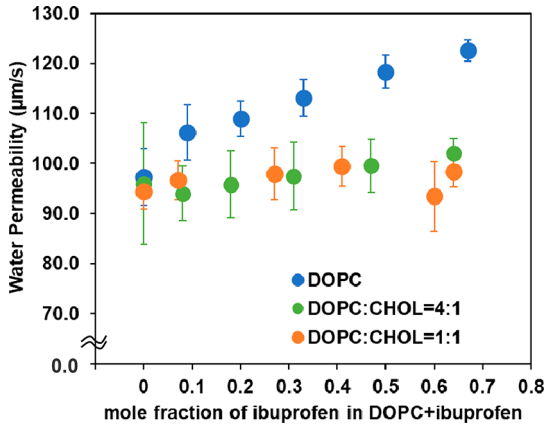

Figure 9. Osmotic water permeability coefficients (μm/s) for bilayers composed of DOPC with CHOL (4:1 and 1:1 mol, green and orange circles, respectively) at 37 °C and pH 3 with varying concentration of ibuprofen (abscissa). P_f for pure DOPC is also shown for comparison (blue circle). Each data point represents an average of individual permeability runs ($n \geq 50$) and standard deviation as error bars.

Figure 9 and Table 3 show the water permeability values for two different mole ratios of DOPC:CHOL (namely, 4:1 and 1:1), having concentrations of total lipid to ibuprofen in the relative mole range from 10:1 to 1:2. The cholesterol concentration is chosen to be below the solubility limit of cholesterol in DOPC.[99]

As seen in Figure 9, with increasing concentration of ibuprofen for both concentrations of cholesterol (4:1 (green circle) and 1:1 (orange circle) DOPC:CHOL mole ratio) there seems to be no significant differences in water permeability within the standard deviation. This observation is a sharp contrast to the pure DOPC scenario (in the absence of CHOL (blue circles)), where water permeability monotonically increased with increasing ibuprofen concentation. It appears that the presence of cholesterol in DOPC bilayers suppresses the ability for ibuprofen to increase the water permeability. Cholesterol's widely ascribed ability to increase chain rigidity and decrease membrane permeability seems to exert a counteracting effect upon ibuprofen's ability to increase the water permeability of the DOPC bilayer. This offset results in no significant changes in the water permeability.

Relatively few reports address the effect of ibuprofen on the cholesterol-containing membrane, and some are contradictory. For example, an X-ray scattering and electron density study on a DMPC bilayer containing 20 mol % of cholesterol shows that ibuprofen is expelled from the tail groups (which is a preferred position of ibuprofen in the absence of cholesterol) and partitioned in the head groups.[38] However, a recent study on the effect of ibuprofen on POPC/cholesterol bilayers using solid-state NMR spectroscopy and other biophysical assays reported that ibuprofen disturbs the molecular order of liquid crystalline phospholipid membranes and that the presence of cholesterol in membranes has only a very small effect on the localization of ibuprofen in the model membrane.[51] MD studies of DMPC-based bilayers by Khajeh et al. show that ibuprofen prefers to be located in the hydrophobic acyl chain region of DMPC/cholesterol bilayers and causes the ordering of hydrocarbon tails when cholesterol is less than 25 mol %, but when more than 50 mol % cholesterol is present, ibuprofen perturbs and disorders the flexible chains of DMPC and reduces the acyl chain order parameter.[41] Somewhat contrary to this is the work of Sun et al., which shows that introduction of 20 mol % cholesterol into the POPC membranes produced significant weakening (2 orders of magnitude) of ibuprofen binding to the hydrocarbon region.[35] The present results show a similar effect for ibuprofen, regardless of cholesterol concentration (for both 4:1 and 1:1 mol ratios of DOPC to cholesterol, no concentration-dependent water permeability changes were observed). This is dissimilar to the report of Khajeh et al. mentioned above, which shows a differential effect for ibuprofen depending on the concentration of cholesterol.[41] In the presence of cholesterol, ibuprofen molecules do not appear to be able to influence the membrane water permeability in the same manner as demonstrated with the pure DOPC bilayer. This may indicate that either cholesterol is competing with ibuprofen at a site of drug–membrane interaction or changes to the membrane induced by the presence of cholesterol in the DOPC bilayer hinder the interaction of ibuprofen with the membrane. The concentration of ibuprofen used in this study ranges from 10:1 to 1:2 mol ratio (DOPC to ibuprofen) with a total ibuprofen concentration from ~600 μM to 12 mM. This range is greater than the physiologically relevant concentrations that have been reported (mean plasma concentration of 100 and 200 μM).[100] However, it should be noted that the biological membrane environment may not always be in an averaged state with a static distribution of drug molecules. Rather, some computer simulations have indicated that partitioning drugs accumulate heterogeneously in the membrane,[19] with local concentrations that may be much higher than that of plasma concentrations.

Table 3. Osmotic Water Permeability at 37 °C with Increased Concentration of Ibuprofen at pH 3 for DOPC Lipid Bilayer Containing Two Different Concentrations of Cholesterol

(DOPC + CHOL):Ibuprofen mol ratio	DOPC:CHOL = 4:1 mol, P_f (μm/s) avg ± SD	DOPC:CHOL = 1:1 mol, P_f (μm/s) avg ± SD
1:0	96.0 ± 12.2	94.4 ± 3.4
10:1	94.0 ± 5.5	96.6 ± 3.9
4:1	95.8 ± 6.6	97.9 ± 5.2
2:1	97.5 ± 6.7	99.5 ± 4.0
1:1	99.6 ± 5.3	93.4 ± 7.0
1:2	102.0 ± 2.9	98.4 ± 3.1

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Langmuir 2021, 37, 4468–4480

Table 4. Surface Activities of Ibuprofen at the Water/Monoolein:Cholesterol (4:1 mol ratio) SqA Interface at 25 °C

ibuprofen conc. (mg/mL)	monolayer tension, γ_m (mN/m)	contact angle, θ (deg)	bilayer tension, $\gamma_B = 2\gamma_m \cos\theta$ (mN/m)	free energy of adhesion $E = 2\gamma_m(1 - \cos\theta)$ (mJ/m^2)	relative lateral pressure $\pi = \gamma_m(1 - \cos\theta)$ (mN/m)
0	1.11 ± 0.08	25.9 ± 0.2	2.00	0.224	0.112
0.5	0.90 ± 0.03	29.2 ± 0.1	1.58	0.230	0.115
1.0	0.72 ± 0.04	32.1 ± 0.5	1.22	0.221	0.111
2.0	0.48 ± 0.04	38.5 ± 0.4	0.76	0.210	0.105
3.0	0.23 ± 0.02	55.1 ± 1.1	0.26	0.197	0.098

In addition, the lateral distribution of the molecular components in the membranes is generally considered to be heterogeneous, corresponding to an organization having compositionally distinct domains and compartments.[101] In view of this, the heterogeneous accumulation of ibuprofen, leading to a high local concentration, is expected to occur in vivo, especially in the membranes of inflammatory cells, which are extremely fluid and unstable because of the presence of lysophospholipids and highly unsaturated tails.[102,103] Our observed composition-specific ibuprofen binding is consistent with this view.

Cholesterol Suppresses Lateral Pressure Reduction with Increased Ibuprofen. To further elucidate the biophysical effects of ibuprofen on cholesterol-containing membranes, we acquired tensiometric data for our model system. Table 4 shows the effects of varying concentrations of ibuprofen on the relative lateral pressure (π) and energy of adhesion in the presence of cholesterol (4:1 mol ratio of monoolein:cholesterol). Overall, the increased inclusion of ibuprofen has only a relatively moderate effect on these parameters, as compared to the case of ibuprofen in the absence of cholesterol (Table 2). A relatively lower reduction in the lateral pressure was observed in the presence of cholesterol, from 0.112 to 0.098 mN/m, compared to the change from 0.131 to 0.060 mN/m in the absence of cholesterol. Our data showing a relative insensitivity in the lateral pressure to the presence of ibuprofen molecules when the membrane contains cholesterol are in line with our observation of no appreciable change in the water permeability for the cholesterol-containing case.

CONCLUSIONS

The influence of drugs on the structural and dynamic properties of cell membranes plays a key role in membrane function, including membrane−protein interactions, cell signaling, membrane trafficking, cell division and fusion, among others. An enhanced understanding of drug−membrane interactions at the molecular level will provide important insight into the overall effectiveness of a drug and its toxicity. We systematically examined how ibuprofen molecules affect the dynamic properties of a model lipid bilayer, as sensed by water permeability across DIB membranes, structural variation using confocal Raman microspectroscopy, and bilayer tensiometry. The combined results from diverse experimental techniques provide evidence for differential nonspecific interaction between ibuprofen and DOPC membranes, relative to DOPC/CHOL membranes. The nature and extent of interactions greatly depend on the presence and absence of cholesterol as well as the concentration of ibuprofen. Our results suggest that progressive inclusion of ibuprofen in the DOPC membranes results in an enhancement of the osmotic water permeability with increased concentration of drug. This phenomenon of increased water permeability is dramatically modulated by the presence of cholesterol in the DOPC membrane: there is no change in the water permeability when CHOL is present regardless of the percentage of cholesterol in the DOPC membrane. The increase in water permeability is shown to scale with an increase in the Raman intensity ratios that are indicative of chain order/disorder. In addition, surface energy/tension data suggest that the adsorption of an ibuprofen molecule at the droplet−bilayer interface results in a decrease in the relative lateral pressure as well as a reduced adhesion energy between the two droplets, while this is less marked when cholesterol is present. These results could hold implications for the behavior of CYP enzymes given that CYP has been shown to be quite sensitive to membrane fluidity, as manifested by accelerated drug substrate binding when embedded in membranes with the highest fluidity and lowest packing density;[11] by imparting increased fluidity to low-cholesterol membranes, ibuprofen could thus similarly affect CYP dynamics. Our findings are consistent with previous reports showing ibuprofen's effect in decreasing the membrane thickness and imparting increased fluidity on the lipid bilayer, all of which are significant in the context of understanding the nature and extent of ibuprofen−membrane interactions that may lead to potential toxicity associated with gastrointestinal damage. Our studies build on a platform that uses water permeability parameters, a critical biophysical property of membranes, to probe lipid bilayer structural modulation induced by interaction with an exogenous molecule, ibuprofen. The present findings indicate that the study of the water permeability parameters based on the DIB model is a useful and versatile interrogative tool for understanding and evaluating drug−lipid membrane interactions and can be further elaborated to investigate the effects of an even wider range of exogenous molecules on the membrane structure.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.langmuir.0c03638.

Raman spectra of pure DOPC lipid bilayer and ibuprofen along with the detailed characteristic peak assignments; Raman spectra of DOPC obtained from varying the degree of addition of ibuprofen and detailed spectral subtraction method, allowing for elimination of the ibuprofen components (PDF)

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Notes

The authors declare no competing financial interest.

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