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REVIEW

Deciphering interactions of ionic liquids with biomembrane

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Abstract

Ionic liquids (ILs) are a special class of low-temperature (typically < 100 °C) molten salts, which have huge upsurge interest in the field of chemical synthesis, catalysis, electrochemistry, pharmacology, and biotechnology, mainly due to their highly tunable nature and exceptional properties. However, practical uses of ILs are restricted mainly due to their adverse actions on organisms. Understanding interactions of ILs with biomembrane is prerequisite to assimilate the actions of these ionic compounds on the organism. Here, we review different biophysical methods to characterize interactions between ILs and phospholipid membrane, a model biomembrane. All these studies indicate that ILs interact profoundly with the lipid bilayer and modulate the structure, microscopic dynamics, and phase behavior of the membrane, which could be the fundamental cause of the observed toxicity of ILs. Effects of ILs on the membrane are found to be strongly dependent on the lipophilicity of the IL and are found to increase with the alkyl chain length of IL. This can be correlated with the observed higher toxicity of IL with the longer alkyl chain length. These informations would be useful to tune the toxicity of IL which is required in designing environment-friendly nontoxic solvents of the so-called green chemistry for various practical applications.

Keywords Ionic liquids · Toxicity · Phospholipid membrane · Phase behavior · Structure and dynamics · Neutron scattering

Introduction

Ionic liquids (ILs) are a new class of organic salts in which the ions are poorly coordinated, which results in their melting point below 100 °C (Hayes et al. 2015; Welton 1999). Generally, they have an organic cation and either an organic or inorganic anion. Some of the ILs are liquid even at the room temperature and are known as room temperature ionic liquids (RTILs) (Welton 1999). Generally, ILs are designed with large organic cations, such as imidazolium or pyridinium, with alkyl chain substituent that alters the hydrophobicity of the molecule and an anion, viz. halide (X^-) and tetrafluoroborate (BF_4^-). Some examples of cations and anions commonly used in the ILs are given in Table 1. ILs are generally known as "designer solvents" and large combinations of potentially useful ILs have been made by variations in anions and cations (Hayes et al. 2015; Docherty and Kulpa Jr 2005). ILs have highly tailored structure, by which one can tune the chemical, physical, and biological properties of the ILs. They have low vapor pressure (Wilkes 2004) which has attracted a huge attention towards the solvents of choice of the so-called green chemistry (Ganske and Bornscheuer 2006). Green chemistry is defined as the design of chemical products and processes which diminish or eradicate the use and generation of hazardous substances (Anastas and Zimmerman 2003). Conventional organic solvents are generally toxic, flammable, and volatile and can have potentially devastating effects when released into the environment. ILs have the potential to replace many hazardous volatile organic solvents and have therefore been cited as an important element of green chemistry (Gilmore 2011). They are non-flammable and non-explosive and have good thermal stability and high ionic conductivity, hence are potential candidates for various industrial applications such as high-performance lubricants, advanced engineering materials, chemical and polymer synthesis, energy harvesting, food and cellulose processing, and waste recycling (Plechkova et al. 2009; Ghandi 2014; Plechkova and Seddon 2008; Lu et al. 2009). ILs have also been used for a variety of biotechnology applications, ranging from the

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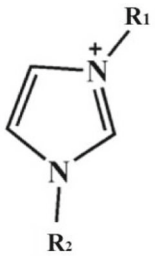
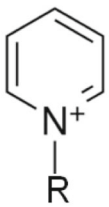
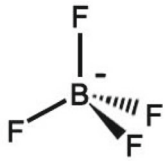
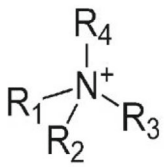
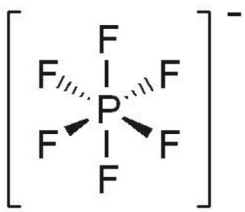
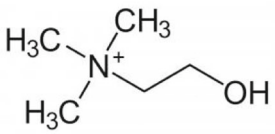
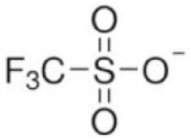
Table 1 Examples of cations and anions commonly used in the ILs

Cation	Anion
Imidazolium 	**Halide (X^-)** Cl^-, Br^-, I^-
Pyridinium 	**Tetrafluoroborate $[BF4]^-$** 
Quaternary Ammonium 	**Hexafluorophosphate $[PF_6]^-$** 
Choline 	**Trifluoromethansulfonate $[CF_3SO_3]^-$** 

formulation of biopharmaceuticals to media for biocatalysis (Weaver et al. 2013; Egorova et al. 2017). Due to their tunable antibacterial and antifungal properties, ILs are the potential candidate for novel antimicrobials that are required to keep pace with the growing challenge of bacterial resistance. Recent studies (Benedetto 2017; Gilmore 2011) suggest a potential positive role of ILs in pharmacology, biomedicine, and bionanotechnology. It has been already illustrated that ILs can kill bacteria (O'Toole et al. 2012), stabilize proteins and enzyme (Kumar et al. 2017), and dissolve cellulose and other complex polysaccharides (Zhang et al. 2017). However, release of ILs into aquatic environments would lead to pollution of water (Pham et al. 2010) and endanger life of aquatic species. Based on the number of recent studies (Docherty and Kulpa Jr 2005; Pham et al. 2010; Latała et al. 2009; Jeong et al. 2012; Matzke et al. 2007), toxicity of ILs to living organisms has become an emerging issue. It has been shown (Jeong et al. 2012, Liang et al. 2013; Matzke et al. 2007) that toxicity depends on the lipophilicity of the IL, which can be varied either by changing the hydrophobic part of cation or nature of anion of IL. A correlation between toxicity and alkyl chain length of ILs has been investigated (Matzke et al. 2007; Jeong et al. 2012). Three imidazolium-based ILs having different alkyl chain lengths, namely 1-ethyl-3-methylimidazolium tetrafluoroborate (EMIM[BF4]), 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM[BF4]), and 1-

methyl-3-octylimidazolium tetrafluoroborate (OMIM[BF4]), have been tested with a micro-organism, *Shewanella oneidensis* MR-1 (Jeong et al. 2012). These ILs differ only in terms of the alkyl chain length (C_nH_{2n+1}) of the cations. The EMIM(C_2mim), BMIM(C_4mim), and OMIM(C_8mim) cations have an alkyl chain length of two, four, and eight carbon atoms, respectively. Minimum inhibitory concentration (MIC) is the lowest concentration of a substance which prevents the visible growth of a bacterium, and has been observed for all these ILs (Jeong et al. 2012). It has been found that as the chain length increases, MIC decreases significantly (Jeong et al. 2012). MIC for EMIM[BF4] is 75 mM, which reduces to 25 mM for BMIM[BF4] and 0.48 mM for OMIM[BF4]. Observed results indicate the toxicity of ILs is greatly correlated with the alkyl chain length of the ILs and in general increases with the alkyl chain lengths. An exception has been observed recently (Drücker et al. 2017) on dialkylimidazolium-based ILs, which show that the IL with the shortest chain is the most toxic. It has also been shown that cytotoxicity depends on the counter-ions of the IL (Liang et al. 2013; Matzke et al. 2007). Effects of concentration of BMIM[BF4] on the growth of *Escherichia coli* (*E. coli*) have also been studied (Bhattacharya et al. 2017). It was found that bacterial growth hindered significantly due to the incorporation of ILs. The efficiency of bacterial inhibition was found to increase with the concentration of IL. In the most cases, the mechanism behind toxicity of ILs has not been understood well. Any foreign molecules encounter firstly with the plasma membrane before entering into the living cell. There have been increasing evidences of interactions of IL with cell membranes (Sharma et al. 2017a; Jeong et al. 2012; Bhattacharya et al. 2017; Benedetto et al. 2014, 2015; Benedetto 2017; Benedetto and Ballone 2016; Kontro et al. 2016; Yoo et al. 2014, 2016a, b). It has been hypothesized that the hydrophobic portions of the ILs penetrate into the membrane through hydrophobic interactions and are mainly responsible for the toxicity of the IL (Jeong et al. 2012). Hence, to understand the fundamental mechanism of the toxicity of ILs, the physical and biological interactions between the cell membrane and ILs must be studied in details. The main aim of the subject area is to assess and tune the toxicity of IL in such a way that one can achieve environment-friendly solvents.

Cell membranes play an essential role in the cellular protection as well as in the control and the transport of nutrients. It regulates the diffusion of chemical species into cells, either through protein channels or simply by absorption into their phospholipids. It contains a multitude of lipids, proteins, and carbohydrates unique for any given cell or organism. Various cell cultures have been used to understand these physiological processes at the membrane level and to investigate interactions of ILs (Matzke et al. 2007; Jeong et al. 2012; Bhattacharya et al. 2017). Despite the wealth of information and experimental data, fundamental mechanisms of membrane disruptions by ILs are not fully understood. Therefore, model membranes mimicking the more complex biological membranes have attracted vast attentions (Pabst et al. 2014; Sharma et al. 2017a; Bhattacharya et al. 2017; Benedetto et al. 2014, 2015; Benedetto 2017). Phospholipid bilayers are a well-accepted first-order model of cell membranes (Pabst et al. 2014). Structure and dynamics of these membrane mimicking systems are a prerequisite to understand the fundamental mechanism of interaction between ILs and membrane. Pure phospholipid membrane in general exists among three different phases: (i) solid gel, (ii) ripple, and (iii) fluid phases (Heimburg 2000). In solid gel phase, alkyl chains are more ordered and have almost *trans* conformation. In this phase, phospholipid has lower area per lipid and hence less fluidity. Upon heating, a periodic ripple is formed in the ordered phase which is called ripple phase. Further heating, ripple phase goes into the fluid phase, which is a complete disordered phase. In this phase, alkyl chains are disordered and have large gauche defects. The phospholipid molecules have large area per lipid and more fluidity. Schematic of a lipid bilayer and molecular arrangements in solid gel, ripple, and fluid phases is shown in Fig. 1. Pure phospholipid membranes generally go under pre-transition (solid gel to ripple) and main transition (ripple to fluid) as shown in Fig. 1.

In this article, we provide an overview on different biophysical methods suitable to study interactions between ILs and phospholipid membrane. We will also summarize the recent results on the interactions between ILs and phospholipid membrane using these methods. Imidazolium-based cations are one of the most popular classes of ILs (Jessop 2018) and have shown toxicity against living organisms (Yu and Nie 2011; Pendleton and Gilmore 2015). Chemical structures of four different imidazolium-based cations discussed in this review are shown in Fig. 2. 1-Ethyl-3-methylimidazolium, EMIM(C_2MIM), 1-butyl-3-methylimidazolium, BMIM(C_4MIM), 1-octyl-3-methylimidazolium, OMIM(C_8MIM), and 1-decyl-3-methylimidazolium (DMIM; C_{10}MIM) differ only in terms of alkyl chain length. Phosphatidylcholines are the main structural lipids of the eukaryotic membranes (Eeman and Deleu 2010). Two saturated phosphatidylcholines, dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC), have been used as a model biological membrane to study the interaction with IL. DMPC and DPPC have same choline head group and differ only in terms of the acyl chain length as shown in Fig. 2. DMPC has two acyl chains of 14 carbon atoms while DPPC have acyl chains of 16 carbon atoms.

Results and discussion

Different experimental and computational methods have been employed to study the interactions between ILs and

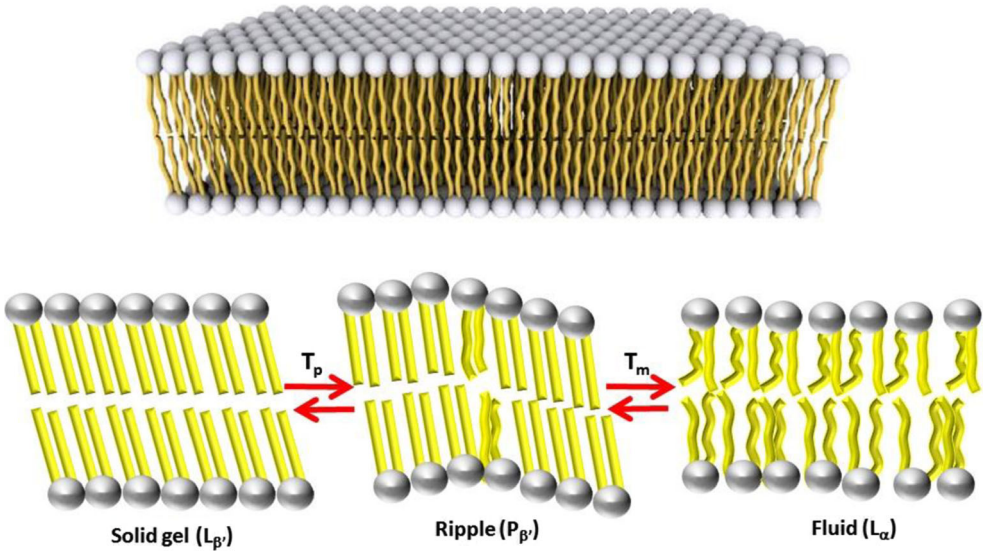


Fig. 1 Schematic of phospholipid bilayer and molecular arrangement in the different phases of the bilayer as discussed in the text. T_p and T_m are the temperatures corresponding to the pre- and main transitions respectively

phospholipid membrane (Sharma et al. 2017a; Jeong et al. 2012; Bhattacharya et al. 2017; Benedetto et al. 2014, 2015; Benedetto 2017; Benedetto and Ballone 2016; Kontro et al. 2016; Yoo et al. 2014, 2016a, b). Here we would review some of the biophysical techniques such as pressure area isotherm, differential scanning calorimetry (DSC), reflectivity, neutron scattering, and molecular dynamics (MD) simulation and discuss the interesting finding on the interactions between ILs and phospholipid membrane.

Pressure area isotherm

The pressure (π)–area (A) isotherm measurement of the Langmuir monolayer is a simple technique to follow the interaction of any foreign macromolecules with the self-assembled lipid monolayer formed at the air–water interface (Maget-Dana 1999; Giri et al. 2017a, 2017b; Sharma et al. 2017a). Recently, it has been used to investigate interaction of ILs with the phospholipid monolayer (Bhattacharya et al. 2017; Sharma et al. 2017a). In this method, phospholipids with and without ILs were dissolved in a common solvent say chloroform and about few hundred microliters of the solution is spread on the deionized water in Langmuir trough. After 15 min of waiting (complete evaporation of chloroform), spread monolayers are compressed by means of movable barriers (as shown in the inset of Fig. 3) while the thermodynamic parameters, viz. surface pressure and the area, are continuously recorded. Pressure area isotherms of pure DMPC membrane and in the presence of two different ILs having different alkyl chain length, BMIM[BF4] (C_4MIM[BF4]) and DMIM[BF4] (C_{10}MIM[BF4]), are measured at 20 °C and are shown in Fig. 3a (Sharma et al. 2017a). Both ILs have been used at the same molar concentration (20 mol%), so that one can directly compare the effects of the alkyl chain length of ILs. It is evident that presence of 20 mol% of ILs in the monolayer forming lipid solutions has

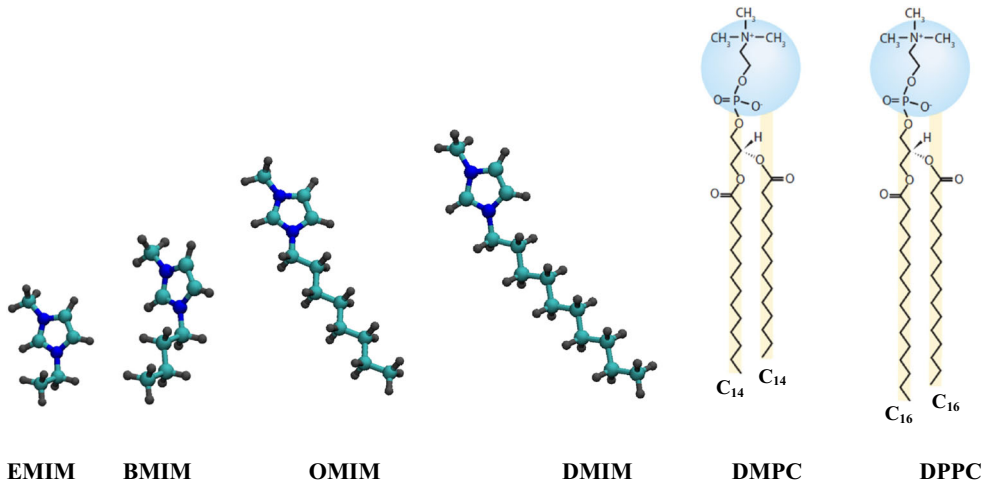


Fig. 2 Chemical structures of the imidazolium-based ionic liquids having different alkyl chain lengths and saturated phosphatidylcholine lipids discussed in the text

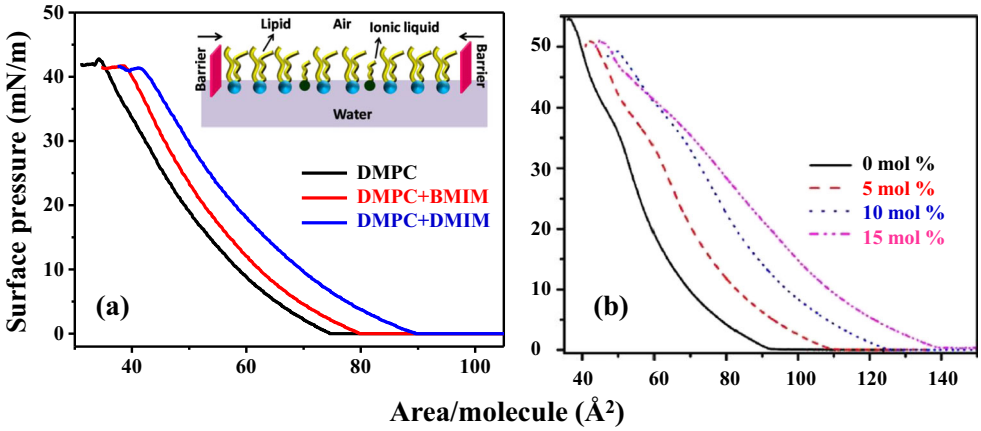


Fig. 3 Pressure–area isotherm measured for **a** DMPC in the absence and presence of 20 mol% BMIM[BF4] and DMIM[BF4] at 20 °C (figure taken from Sharma et al. 2017a, with the permission from the publisher) and **b** DPPC with different concentrations of BMIM[BF4] at 35 °C (figure taken from Bhattacharya et al. 2017, with the permission from the publisher). Schematic design of the experimental setup is shown in the inset

considerable effect on the phase behavior of pure phospholipid membrane. The isotherms shift towards higher area per lipid molecule indicating the penetration of ILs into the phospholipid monolayer. It is evident that DMIM[BF4] has a stronger effect compared to BMIM[BF4]. For example, at a surface pressure 10 mN m^{-1}, the area/lipid changes from 58.7 to 69.6 $Å^2$ (increase of 19%) due to addition of DMIM[BF4], whereas the value increases to 62.3 $Å^2$ (increase of 6%) for BMIM[BF4]. This indicates that in DMIM[BF4], there exists a stronger lateral thrust compared to BMIM[BF4] resulting in higher effective surface area for a lipid molecule. To investigate the effect of the concentration of IL on the pressure area isotherm, measurements were also carried out on membrane of another saturated phosphatidylcholine, DPPC, with different concentrations of BMIM[BF4] (Bhattacharya et al. 2017) at 35 °C and observed isotherms are shown in Fig. 3b. It is evident as the concentration of IL is increased, the isotherms are shifted towards a higher area per lipid. Observed data indicates that initially at low concentration of IL, there is a large change in the area/lipid molecules. However, rate of increment in area/lipid slows down with the increasing concentration of IL. The results of the pressure area isotherm clearly indicate that interaction of IL with phospholipid membrane depends on the alkyl chain length as well as the concentration of the IL.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a suitable technique to investigate the phase behavior of the phospholipid membrane (Lewis et al. (2007); Sharma et al. 2016b, 2017a, b; Jain and Wu 1977). As mentioned earlier, while heating pure phospholipid membrane undergoes a main phase transition from ordered phase to fluid phase, the main phase transition of phospholipid membrane is highly cooperative in nature and a sharp peak is observed in the DSC thermogram (Lewis et al. (2007); Sharma et al. 2016b, 2017a, b). Molecules that interact or intercalate with the acyl chains can significantly affect the cooperativeness of this transition (Jain and Wu 1977). Hence, by observing the change in this transition, one can infer about the interaction of the additive molecules with the phospholipid membrane. DSC measurements (Sharma et al. 2016b, 2017a, b, Lewis et al. 2007) are generally carried out on lipid vesicles (multilamellar or unilamellar), which can be prepared using the method as described earlier (Szoka Jr. and Papahadjopoulos 1980; Sharma et al. 2015a, b, 2016a, b, 2017a, b). In brief, required amount of lipid powder is co-dissolve in chloroform in a glass vial, which results in a transparent solution. Chloroform is evaporated using the gentle stream of nitrogen gas to obtain films of phospholipid. To remove the residual chloroform, the lipid films are dried by placing the samples under vacuum (10^{-3} atm) about 12 h. Dry lipid films are suspended in the desired amount of water at a temperature well above the main phase transition of the phospholipid and vortex mixed for ~ 30 min. It results in multilamellar vesicles (MLV) of the lipid. For the preparation of unilamellar vesicles (ULVs), there are two different methods: (i) sonication and (ii) extrusion. Extrusion method has very good control on the size of ULV and hence is widely used to prepare ULV. In extrusion method, lipid suspension went through a mini-extruder fitted with polycarbonate membrane. By changing the pore diameter of this polycarbonate membrane, one can tune the size of ULV. During the extrusion process, the temperature of extruder should be kept above the main phase transition to ensure that lipids are in the fluid phase.

A systematic DSC study was carried out by Jeong et al. (2012) to investigate role of alkyl chain length and concentration of IL on the interaction of IL with the phospholipid membrane. DPPC liposome was used for this study which undergoes solid gel to fluid phase transition ~ 41 °C (Heimburg 2000). Three imidazolium-based ILs having different alkyl chain lengths, namely EMIM[BF4], BMIM[BF4], and OMIM[BF4], as shown in Fig. 2 have been used. All experimental conditions such as concentration of IL and heating/cooling rates are kept constant such that one can investigate the effects mainly due to the variation in the alkyl chain length.

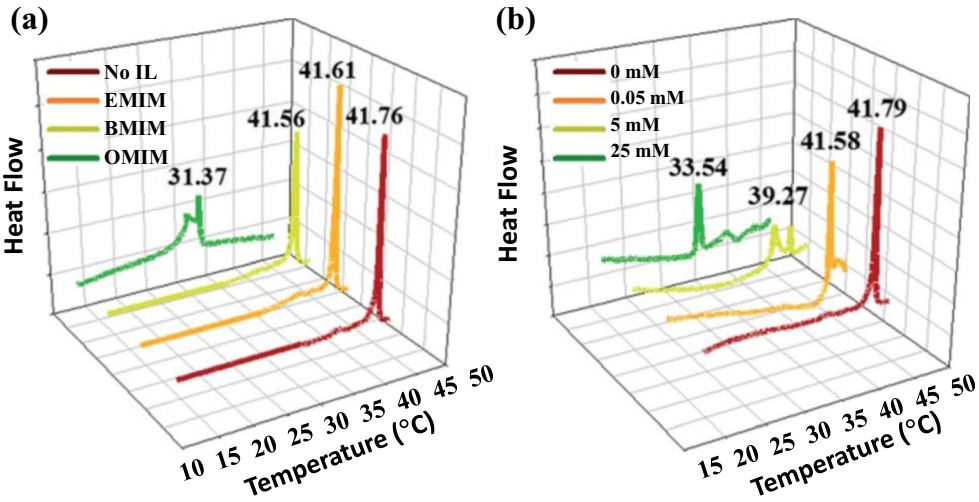


Fig. 4 DSC thermogram for **a** DPPC liposome in the absence and presence of 50 mM EMIM[BF4], BMIM[BF4] and OMIM[BF4] ILs and for **b** DPPC liposome with different concentrations of OMIM[BF4] (figure taken from Jeong et al. 2012, with the permission from the publisher). Concentration of DPPC in all the samples was kept constant at 20 mM

It is evident from Fig. 4a that IL OMIM[BF4], having the longest chain length, affects the phase behavior of the DPPC membrane most significantly. As chain length decreases, the effect of the IL on the phase behavior of the phospholipid membrane reduces. For the EMIM, it is almost unaffected indicating that IL having shorter chain length did not penetrate well in the membrane. Mixing of ILs in the fluid bilayer increases the entropy (i.e., creates disorder) and thus decreases the Gibbs free energy, which is the main origin of the reduction in the main phase transition temperature. These observations indicate that greater disorder created by the longer alkyl chain IL is mainly responsible for such large change in the main phase transition temperature. Effects of the concentration of IL have also been studied for OMIM[BF4] and the observed thermograms are shown in Fig.4b. It is evident that as the concentration of IL increases, it disrupts the phase behavior of the phospholipid membrane more significantly. The main phase transition shifted towards lower temperature, and for addition of 25 mM OMIM[BF4], it shifted about 8 °C towards the lower temperature.

Effects of choline-based ILs on DPPC were also studied using DSC technique and found that as the concentration of choline Bis(2-ethylhexyl)phosphate (CBEH) IL increases, the main phase transition gets broadened and shifted towards the lower temperature, indicating increasing disorder in the bilayer structure (Weaver et al. 2013). Recently, we have carried out DSC measurements on another saturated phosphatidylcholine DMPC membrane to investigate effects of imidazolium-based IL, DMIM[BF4] (Sharma et al. 2017a). DMPC membrane shows a sharp peak at ~ 24 °C, which corresponds to the main phase transition (Heimburg 2000). It is found that due to incorporation of 20 mol% (with respect to DMPC) DMIM[BF4], this peak gets broadened and shifted towards lower temperature (~ 18 °C). Broadening in the peak indicates that DMIM[BF4] interacts strongly with the phospholipid membrane and affected cooperatively of the phospholipids. Shift in the transition temperature at lower temperature indicates that incorporation of DMIM[BF4] creates disorder and enhances the fluidity of the membrane.

These studies have suggested that ILs are penetrated into the cell membrane depending on their hydrophobic alkyl chain length and result in the perturbation of lipid membrane structure. The effect of ILs on the phase behavior of the membrane increases with the alkyl chain length and the concentration of IL.

Reflectivity

Reflectivity is a useful technique to investigate structure and stability of the phospholipid bilayer (Johnson et al. 1991; Miller et al. 2005; Giri et al. 2017a, b). It gives in-depth structural information like thickness, electron/scattering length density, and roughness of the surface and interface from the samples. Recently, Bhattacharya et al. (2017) have used X-ray reflectivity to investigate change in the structure of the DPPC membrane due to the interaction of BMIM[BF4] IL. X-ray reflectivity measurements were carried out on polymer-supported DPPC bilayer in both gel (35 °C) and fluid phases (48 °C) of the membrane, in which lipid bilayer was deposited on a soft support formed by poly(acrylic acid) as explained Bhattacharya et al. (2017). In short, silicon wafers were extensively cleaned to remove organic dirt and oxide layers from the surface. These substrates were kept into a toluene solution of (aminopropyl)triethoxysilane (APTES) with a volume ratio of 50:1 (toluene/APTES) and then the substrates were heated at 100 °C for 2 h. Poly(acrylic acid) solution in methanol (3 mg/mL) was then spin coated on the top of APTES layer and heated at 200 °C for 3 h. In these steps, annealing was done to make the layers more stable and uniform. Detailed description of the substrate preparation for the bilayer deposition has been discussed by El-khouri et al. (2011). Polymer cushioned silicon wafers were then employed further for the bilayer deposition from the Langmuir monolayer of lipid

molecules using Langmuir–Blodgett (LB) trough. The first monolayer was deposited by LB technique followed by inverted Langmuir-Schaefer (LS) technique for the next layer. A schematic of polymer-supported lipid bilayer is shown in Fig. 5a. This bilayer configuration would be stable only under water; hence, this substrate containing bilayer is transferred to a specially designed cell (Giri et al. 2017a) so that the membrane always remains under water and the XRR measurements have been carried out. The layer model had been used to describe the X-ray reflectivity data, where a single layer was used to describe the whole lipid bilayer. Observed data showed strong perturbation in the bilayer structure due to the incorporation of ILs. Figure 5b shows variation in the bilayer thickness with the concentration of BMIM[BF4] in the fluid phase (Bhattacharya et al. 2017). Change in the bilayer thickness due to addition of BMIM[BF4] in the gel phase is shown in the inset of Fig. 5b (Bhattacharya et al. 2017). It is evident that in both the phases, incorporation of BMIM[BF4] leads to decrease in the bilayer thickness considerably (~ several Å). A more detailed insight on the IL–lipid bilayer interaction can be obtained using neutron reflectivity in which scattering length density (SLD) contrast between a lipid bilayer and the aqueous environment can be varied (Benedetto et al. 2014; Benedetto 2017). Here, fundamental difference from X-ray reflectivity is mainly due to the fact that neutron is scattered mainly through the interaction with nuclei. Hence, isotopes have different scattering lengths (b) for neutron. Especially, there is a big difference in the scattering length between the hydrogen (H, $b = -3.74 \times 10^{-15}$ m) and its isotope, deuterium (D, $b = +6.67 \times 10^{-15}$ m), and this gives a big advantage for soft material researches that possess many hydrogen atoms. Benedetto and his co-workers (Benedetto et al. 2014; Benedetto and Ballone 2016) have carried out neutron reflectometry measurements to investigate interaction of ILs with supported phospholipid bilayers. Measurements were carried out on two model biomembranes, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and DMPC phospholipid bilayers, in the absence and presence of two ILs, namely [BMIM][Cl] and choline chloride [Chol][Cl]. All measurements were carried out in the fluid phase of the membrane. For scattering contrast, tail-deuterated lipid and heavy water were used in neutron reflectivity measurements. It was found that for all the systems, IL cations enter into the bilayer and located in between the tail and head region. The volume fraction of cations located into the membrane depends on the nature of phospholipid but not on the choice of cations. For POPC, which is an unsaturated phosphatidylcholine membrane, IL cations occupy about ~ 5% of the bilayer volume which increases to ~ 10% in DMPC, a saturated phosphatidylcholine membrane.

These studies indicate that ILs penetrate into the membrane and profoundly affect the bilayer structure. Incorporation of IL leads to decrease in the bilayer thickness which indicates increase in disorder in the membrane. It has been shown that these ILs are mostly located in between the tail and head region.

Molecular dynamics simulation

Classical molecular dynamics (MD) simulation is an excellent tool to gain microscopic insights into the interaction between ILs and membranes and has been employed widely (Yoo et al. 2014, 2016a, b; Benedetto et al. 2015; Lim et al. 2014). For example, Yoo et al. (2014) have carried out MD simulation on a POPC membrane with different imidazolium-based ILs. To investigate the effects of alkyl chain length of cations, simulations have been carried out with 1-n-alkyl-3-methylimidazolium-based ILs (C_nMIM[Cl]) of alkyl chain with $n = 4$, 8, and 12 having different alkyl chain lengths. POPC lipid bilayer consisting of 128 lipids, with 64 lipids on the upper and lower leaflets, was used in the simulation studies (Yoo et al. 2014). More detailed information about the simulation such as the starting configuration for a hydrated lipid bilayer and force field parameters have been discussed by Yoo et al. 2014. Figure 6a shows molecular configurations of POPC phospholipid and the C_nMIM[Cl] ILs having different alkyl chain lengths. A snapshot of a representative IL/POPC/water system is also shown in Fig. 6a. MD simulation indicated that IL cations were inserted into the phospholipid membrane spontaneously irrespective of the alkyl chain length which is consistent with the observed reflectometry data (Benedetto et al. 2014; Bhattacharya et al. 2017).

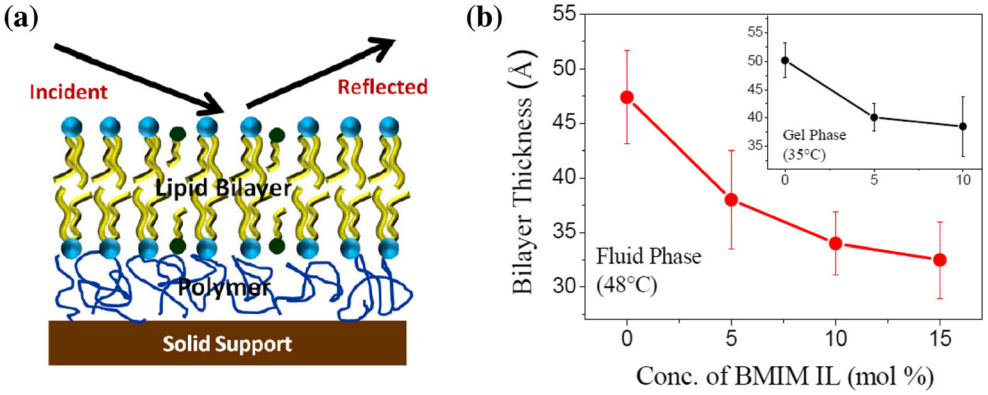


Fig. 5 **a** Schematic of the lipid bilayer sample deposited on a polymer cushion with spectrin attached to the bilayer. **b** Thickness of the DPPC lipid bilayer as a function of [BMIM][BF4] IL in the fluid phase ($T = 48$ °C) (figure taken from Bhattacharya et al. 2017, with the permission from the publisher). For the gel phase ($T = 35$ °C), variation in the bilayer thickness is shown in the inset

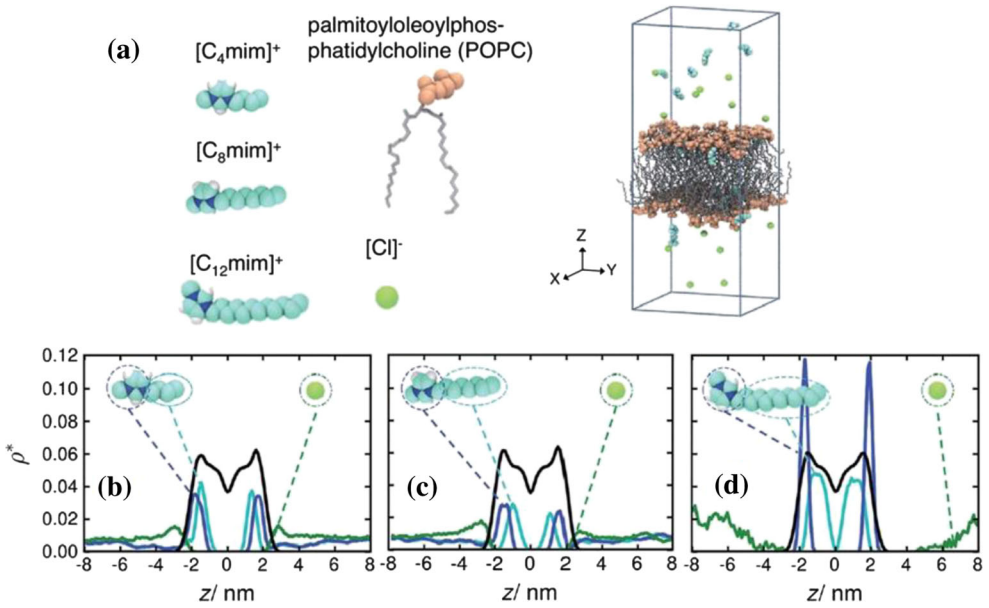


Fig. 6 **a** Representation of the imidazolium-based cations with different alkyl chain lengths: $[C_4mim]^+$, $[C_8mim]^+$, $[C_{12}mim]^+$, and Cl^- anion. The POPC lipid head (orange), lipid tail (gray), and IL/POPC/water bilayer system (water omitted for clarity) are also shown. Normalized density ρ* profiles along the *Z*-axis for the POPC lipid bilayer systems in the presence of **b** $C_4MIM[Cl]$, **c** $C_8MIM[Cl]$, and **d** $C_{12}MIM[Cl]$. Density profiles for the IL cation head, i.e., imidazolium (blue), and tail, i.e., alkyl chain length (cyan), are plotted separately. Those for anions are also plotted (green) (figure taken from Yoo et al. 2014, with the permission from the publisher)

Moreover, IL cations have preferred orientation in the membrane in which the alkyl chain goes deeply into the hydrophobic region of the bilayer, while the imidazolium ring interacts with the lipid head groups. Normalized density profiles of the different imidazolium-based ILs in POPC membrane ρ* along the *Z*-axis are shown in Fig. 6b–d. The density profiles have been averaged based on the last 20 ns of the simulation run. It is evident from Fig. 6b–d that cations of the IL exist within the bilayer and have preferred orientation as described above. For the longer alkyl chain cations, the density profiles show deeper penetration of the alkyl tails into the membrane with density maxima closer to the center of the lipid bilayer ($Z \sim 0$), while the imidazolium rings stay at the water–lipid interface. It was also shown that free energy of the system decreases due to the penetration of alkyl chain IL cation; however, it increases due to insertion of a hydrophilic chloride anion (Yoo et al. 2014). This indicates that hydrophilic anions are mostly located outside the phospholipid membrane. Density profiles of the hydrophilic Cl^- anion do not show any indication of penetration into the bilayer. Structural analysis of the lipid bilayer has shown that insertion of IL cations induces roughening of the bilayer surface, which might be a precursor to bilayer disruption. Lim et al. (2014) have carried out MD simulation to investigate different imidazolium-based ILs on the properties of a bacterial plasma membrane. The atomistic model of the membrane bilayer is designed to reproduce the lipid composition of the plasma membrane of *E. coli*. All the IL cations were inserted spontaneously into the membrane, whereas anions neither adsorb onto the membrane surface nor diffuse into the bilayer. Cations in the membrane were preferably oriented parallel to lipids with cation alkyl tails embedded into the hydrophobic membrane core, while the imidazolium ring remains mostly exposed to the solvent. It was shown that insertion of cations of ILs induces overall disorder in the membrane and average membrane thickness decreases. These results are found to be consistent with results obtained from pressure area isotherm, calorimetry, and reflectometry measurements as discussed above.

Recently (Yoo et al. 2016a, b), it has been shown that higher cytotoxicity of IL having a longer alkyl chain length might be due to inability of IL to diffuse from the outer leaflet to the inner leaflet of the lipid bilayer. Benedetto and coworkers (Benedetto et al. 2015) have carried out MD simulations to investigate the effects of ILs on the structure and dynamics of POPC lipid bilayer. It was found that the cation of the IL incorporated in the membrane was very quickly driven by the Coulomb attraction between the cation and most electronegative oxygen atoms in the head group of the phosphocholine membrane. Adsorption of cations into the bilayer favors the penetration of water into the membrane that results in bilayer thinning. However, no clear trend on the effects of ILs on the dynamics of the membrane is established.

Elastic intensity scan

Elastic intensity scan or fixed elastic window scan (FEWS) is a suitable technique to investigate microscopic dynamics and phase behavior of the phospholipid membrane (Toppozini

et al. 2012; Wanderlingh et al. 2008; Sharma et al. 2015a, b, 2016a, b, 2017a, b). In elastic intensity scan, elastic intensity (within the energy resolution of the spectrometer) is measured with variation of temperature. Any microscopic mobility in the sample shifts the scattering intensity away from the elastic (zero energy transfer) scattering, thereby making the elastic intensity sensitive to the microscopic dynamics. An abrupt loss or gain of intensity in an elastic scan measurement is a strong signature of a phase transition, which is associated with a change in the microscopic dynamics at the transition temperature. One can also calculate average mean square displacement (MSD), $<u^2>$, from the Q-dependence of the elastic intensity (Q is the magnitude of the scattering vector) using the Gaussian approximation (Yi et al. 2012) or self-distribution function (Magazù et al. 2008, 2010). It must be noted that the observed elastic intensity scan or obtained MSD is dependent on the energy resolution of the spectrometer. Hence, it is always advisable to use the same spectrometer with the same configuration while comparing the results of two different samples. For example, to investigate the effects of ILs on the dynamical and phase behavior of the membrane, one should measure pure membrane and then membrane in presence of IL with the same spectrometer for direct comparison. Aforementioned, scattered intensity at zero energy transfer within the instrumental resolution can be regarded as purely elastic. Hence, for higher sensitivity, it is customary to measure elastic intensity scan on a backscattering spectrometer as it has high energy resolution. In comparison to spallation neutron source, a reactor-based backscattering spectrometer is the better choice for elastic intensity scan measurements because in reactor-based source, purely elastic intensities can be recorded in Doppler-driven monochromator stopped mode (for example, IN16B spectrometer at ILL, Grenoble).

As mentioned earlier, pure phospholipid membrane in general exists among three different phases and undergoes pre-transition (solid gel to ripple) and main transition (ripple to fluid) upon heating. Pre-transition is rather a weak transition compare to main phase transition (ripple to fluid) and generally not so prominent in the elastic fixed window scan (Sharma et al. 2015a, b, 2017a). Recently (Sharma et al. 2015b, 2016a, b, 2017a, b; Toppozini et al. 2012; Wanderlingh et al. 2008), it has been shown that elastic intensity scan measurements are an invaluable tool which can observe effects of small additives such as ethanol, drugs, and peptides on the dynamical and phase behavior of the phospholipid membrane. In principle, one can get both qualitative and quantitative information from direct comparison of the elastic intensity scan observed from phospholipid membrane in the presence and absence of additives. Qualitative information pertains about the change in the temperature and nature of the phase transition while quantitative information pertains about the change in the microscopic dynamics of the membrane by the direct comparison of the extracted MSD. Theoretically, the difference in the Q-averaged normalized elastic intensity from phospholipid membrane with and without a small amount of additive also indicates information about the microscopic dynamics present in the system. It must be noted while comparing the Q-averaged elastic intensities, one must be careful to (i) keep the concentration and sample volume the same for the systems and (ii) observed elastic intensity should be normalized to monitor counts to take care of the incident neutron flux and (iii) additive should be in small amount and preferably deuterated or non-hydrogenated so that scattering signal due to additive itself is not significant. In the recent times, a number of systems have been studied (Toppozini et al. 2012; Wanderlingh et al. 2008; Sharma et al. 2015b, 2016a, b, 2017a, b) to investigate the effects of additives on the dynamical and phase behavior of the membrane that have led to very interesting information. For example, it has been shown (Sharma et al. 2015b) that inclusion of 0.2 mol% melittin, an antimicrobial peptide, abolishes the main phase transition of the phospholipid membrane. In the gel phase, it acts as a plasticizer, but in the fluid phase, it acts as a stiffening agent. To investigate the effects of ILs on the dynamical and phase behavior of phospholipid membrane, FEWS measurements have been carried out on 100 mM DMPC ULV, prepared by extrusion method in the presence and absence of 20 mM BMIM[BF4] and DMIM[BF4] ILs in the heating and cooling cycles (Sharma et al. 2017a). Our earlier small-angle neutron scattering (SANS) measurements on DMPC vesicles (Sharma et al. 2016a) prepared employing the same extrusion method and the lipid concentration have shown that DMPC vesicles are unilamellar in nature. Elastic intensity scan measurements were carried out in the same sample cell to maintain identical volumes. Q-averaged (0.32 to 1.88 $Å^{-1}$) monitor normalized elastic intensity scans for DMPC membrane with and without DMIM[BF4] are shown in Fig. 7.

It is evident that elastic intensity scan for DMPC membrane with DMIM IL differs than the observed for pure membrane due to interaction of IL with the phospholipid membrane. In the heating cycle (Fig. 7a), it is evident that for both the systems, elastic intensity decreases with the increase in the temperature indicating enhance in disorder. For pure DMPC membrane, a sharp fall in elastic intensity is observed at 24 °C which corresponds to the main phase transition (ordered to fluid) of DMPC membrane. However, in the presence of DMIM[BF4] IL, elastic intensity scan is found to be affected and main phase transition occurs at 6 °C lower and it is weaker and broader. In the presence of BMIM[BF4] IL, main phase transition shifts about 2 °C towards the lower temperature (Sharma et al. 2017a), indicating interaction between IL and phospholipid membrane strongly depends on the alkyl chain length of the cation of IL. In the cooling cycle (Fig. 7b), elastic intensity scans for DMPC membrane with and without ILs are observed to be similar to the elastic scans in the heating cycle indicating reversibility of the transitions for membrane with and without ILs. While in direct comparison of elastic

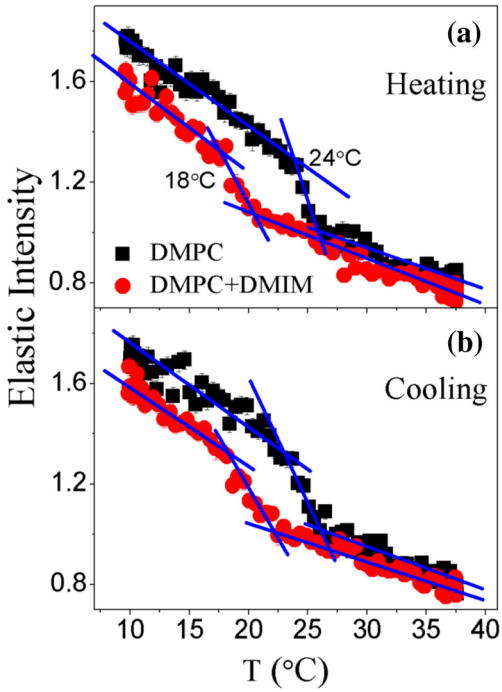


Fig. 7 Q-averaged elastic intensity scan data (figure taken from Sharma et al. 2017a, with the permission from the publisher) for DMPC unilamellar vesicles with and without DMIM[BF4] measured in the **a** heating and **b** cooling cycles. Solid lines are drawn as a guide to the eye

intensity, it is evident that the elastic intensity observed from DMPC with DMIM[BF4] is always lower compared to that from pure DMPC indicating that DMIM acts as a plasticizer, which enhances the fluidity of the membrane. This can be confirmed by the detailed quasielastic neutron scattering (QENS) measurement which is discussed in the next section.

Quasielastic neutron scattering

Quasielastic neutron scattering is a suitable technique to study stochastic molecular motions in condensed matter in the time scale of picoseconds to nanoseconds and length scale of few Angstroms to nanometer and has been widely employed to study dynamics in self-assembled surfactants/lipid aggregates (Bee 1988; Rheinstädter et al. 2005; Doxastakis et al. 2007; Busch et al. 2010, 2012; Toppozini et al. 2012; Rai et al. 2016; Sharma et al. 2010a, b, 2012a, b, 2013, 2015a, b, 2017c, d; Trapp et al. 2010). QENS has a significant advantage over other complementary experimental techniques (such as fluorescence spectroscopy and nuclear magnetic resonance), as it also provides spatial information related to the geometry of the motion in addition to temporal information. QENS is very suitable for hydrogenous system due to large incoherent scattering cross section of hydrogen. Both qualitative and quantitative information can be extracted through QENS. Qualitative information pertains to the geometrical mechanism of the motion, while quantitative information relates to the correlation times and length scales of the motion. Membrane dynamics plays a key role in the fluidity and viscoelastic behavior of the membrane and is a prime determinant in a number of processes, such as cell signaling, membrane trafficking, cell division, fusion, and permeability. Hence, it is of key interest to detect subtle changes of the membrane dynamics caused by any foreign molecules. Phospholipid membrane shows complex dynamical behavior characterized by a superposition of different motions of lipid such as vibrational, rotational, lateral, and flip flop. These motions cover a broad range of time scales, from femtoseconds for molecular vibrations to a few minutes for the transbilayer flip flop. QENS is one of the most suitable techniques to study the lateral and internal motions of lipid molecules within the bilayer. QENS experiments have been carried out on hydrated lipid powder (Busch et al. 2010), supported membranes (Rheinstädter et al. 2005; Armstrong 2013), free-standing membranes (Busch et al. 2012; Sharma et al. 2015a, b), etc. It is found that dynamical behavior of lipid molecules in bilayer systems is affected by various factors such as hydration, temperature, physical state of the membrane, and supported vs. free-standing membrane (Tocanne et al. 1994; Macháň and Hof 2010; Trapp et al. 2010). Lateral diffusion coefficient has been found to increase with the hydration (Tocanne et al. 1994). In the case of supported membrane, the bilayer experienced the presence of the substrate which may give some artifact in the results such as suppression of the main phase transition and enhanced diffusion at the nearest neighbor distance of the lipid molecules (Armstrong 2013). Moreover, this system does not mimic the biologically relevant scenario. Even for free-standing membranes, mobility of the phospholipid molecules has been shown to enhance from multilayers to single bilayers in ULVs to monolayers in emulsions (Busch et al. 2012). We have carried out QENS experiments on ULV which is the most alluring model for the living cell. Since the system of interest here is phospholipid, deuterated water (D_2O) was used to minimize the scattering contribution from the solvent. Spectra from the pure solvent were recorded and subtracted from that measured for the unilamellar vesicles, rather than modeling it with a separate scattering function. We have been using QENS widely on unilamellar vesicles to investigate interactions of foreign molecules (such as membrane-active peptides, drugs, cholesterol, and vitamin) with the phospholipid membrane (Sharma et al. 2015a, b, 2016a, b, 2017a, b). It was found that interaction of membrane-active agents strongly depends on the composition as well as the physical state of the membrane. Recently, interactions of two imidazolium-based ILs having different alkyl chain lengths, BMIM[BF4] and DMIM[BF4] with the DMPC membrane, have been studied by us using QENS technique (Sharma et al. 2017a). QENS experiments have been carried out on 100 mM DMPC ULV with and without 20 mM BMIM[BF4] and DMIM[BF4] ILs at 10, 20, and 30 °C where pure DMPC membrane is in solid

gel, ripple, and fluid phase respectively (Heimburg 2000). It is found that ILs affect the dynamics of the membrane significantly in all the phases. Irrespective of the physical state of the membrane, IL acts as a plasticizer, i.e., enhances the membrane dynamics. Results are consistent with the elastic intensity measurements as described above. Two distinct motions of phospholipid, namely lateral motion in which the whole phospholipid molecule diffuses within a leaflet and relatively faster internal motion of the phospholipid molecule, have been observed (Sharma et al. 2017a). Typical fitted QENS spectra for DMPC membrane with and without DMIM[BF4] at 30 °C at a typical, $Q = 1.2$ Å^{-1}, are shown in Fig. 8. Lateral motion of phospholipid is found to be Fickian in nature, whereas internal motion of phospholipid has been described using localized translational diffusion model (Sharma et al. 2017a). It is found that both ILs enhance lateral as well as the internal motions of the phospholipid. This could be understood with the fact that incorporation of ILs into the membrane disrupts the bilayer structure which results higher area per lipid. Higher area per lipid enhances the lateral and internal motions of the lipids. Diffusion coefficients that correspond to lateral and internal motions of phospholipid in the absence and presence of two different ILs BMIM[BF4] and DMIM[BF4] are shown in Fig. 8c, d. For pure DMPC membrane at 20 °C, diffusion coefficient corresponds to lateral motion, D_{lat} for pure DMPC membrane is found to be $1.6 \pm 0.1 \times 10^{-7}$ cm^2 s^{-1} which increases to $3.6 \pm 0.2 \times 10^{-7}$ cm^2 s^{-1} due to addition of DMIM[BF4]. For DMPC membrane with BMIM[BF4], D_{lat} is found to be $1.7 \pm 0.1 \times 10^{-7}$ cm^2 s^{-1}. Diffusion coefficient corresponds to internal motion; D_{int} for pure DMPC membrane is found to be $28 \pm 1 \times 10^{-7}$ cm^2 s^{-1} which increases to $46 \pm 2 \times 10^{-7}$ cm^2 s^{-1} due to addition of DMIM[BF4]. For DMPC membrane with BMIM[BF4], D_{int} is found to be $31 \pm 2 \times 10^{-7}$ cm^2 s^{-1}. It must be noted that concentration of ILs in the phospholipid membrane was kept to be the same so that direct comparison between the effects of these two ILs can be made. It is evident that the effects of ILs on the dynamics of phospholipid membrane strongly depend on the alkyl chain length of the ILs. For higher chain length IL, DMIM enhancement in the dynamics is stronger than BMIM[BF4].

Observed results are consistent with the recent studies using DSC, reflectivity, pressure area isotherms, and MD simulation, which indicates that addition of IL creates disorder in the membrane which results in bilayer thinning and increase in the area per lipid. This study indicates that interaction between IL and phospholipid membrane depends on the alkyl chain length of IL and stronger for higher alkyl chain length. As mentioned earlier, toxicity of the IL strongly depends on the alkyl chain length (Matzke et al. 2007; Jeong et al. 2012; Yoo et al. 2014). These observations can be correlated with the higher toxicity of the IL with longer chain length.

Conclusion

Study of interaction of IL with cell membrane is necessary to understand fundamental mechanism of the toxicity of IL. This can help to design IL with no toxicity and also very useful for various pharmaceutical applications such as design for novel antibiotic. Here, we have provided an overview on the various biophysical techniques that can be used to investigate complex IL–biomembrane interactions. Results obtained by these

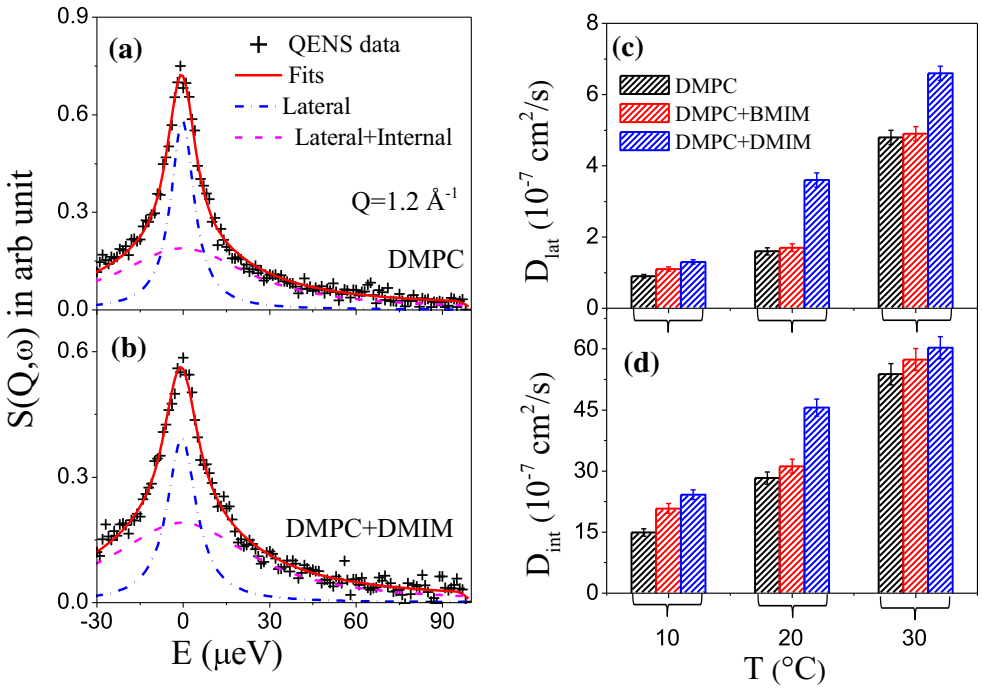


Fig. 8 Typical fitted QENS spectra for DMPC membrane **a** pure and **b** with DMIM[BF4] at $Q = 1.2$ Å^{-1} at 30 °C. Diffusion coefficients (Sharma et al. 2017a) correspond to **c** lateral and **d** internal motions for DMPC membrane with and without BMIM and DMIM ILs at different temperatures

methods on the interaction of various ILs on the phosphatidylcholine membrane, a model biomembrane system, have been discussed. All the observations indicate that ILs penetrate into the cell membrane and perturbed the structural, dynamical, and phase behavior of the membrane. Incorporation of IL creates disorder in the membrane; main phase transition shifted towards the lower temperature and gets broadened. IL inhibits the cooperative action of the phospholipid membrane. Reflectometry measurements show that in the addition of IL, the bilayer shrinks, which is consistent with the molecular dynamics simulation results. Elastic intensity scan and QENS measurements give experimental evidence that IL acts as a plasticizer which enhances the lateral and internal motions of the phospholipid membrane. Interactions of IL with the membrane strongly depend on the alkyl chain length of IL and increase for longer alkyl chain length. As the concentration of IL increases, more ILs are incorporated into the cell membrane and effects of IL on the biophysical properties of the membrane become stronger.

Membrane exhibits complex dynamical behavior and undergoes different kinds of motions including bending and thickness fluctuation, which can be studied using neutron spin echo (Woodka et al. 2012). In this article, we have discussed the effects of IL only on the lateral and internal motions of the phospholipids in the membrane as studied using quasielastic neutron scattering. It is of our interest to investigate effects of ILs on the bending motion and thickness fluctuation of the membrane, which is being pursued by our group. In the present study, we have focused mainly on the interaction between imidazolium-based ILs and zwitterionic phosphatidylcholine membrane. The cell membrane is much complex and is a mixture of multitude of different lipids along with proteins and carbohydrates. For more close resemblance to biomembrane, it would be better to work on a multicomponent system with varying lipid heads (PC, PG, PE, etc.) and alkyl chain lengths. One should also use combinations of saturated and unsaturated phospholipids. We are pursuing the study on the interactions of ILs with a multi-component system having similar lipid composition corresponding to selective organs. On the other hand, there are various different interesting ILs, and one has to investigate the effects of different groups of ILs such as pyridinium and ammonium in addition to imidazolium-based ILs. This has also been taken up by our group.

Compliance with ethical standards

Conflicts of interest V. K. Sharma declares that he has no conflict of interest. R. Mukhopadhyay declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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