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Elastic compliance as a tool to understand Hofmeister ion specific effect in DMPC liposomes

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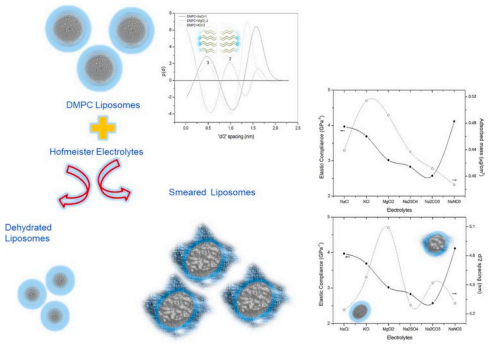
HIGHLIGHTS

- Elastic compliance as an indicator to study dehydration of liposomes.
- Hofmeister ions influence physical properties of membranes.
- Increased penetration of cations in the polar region compared to anions.
- Ions co-localize to the polar oxygen atoms of the phosphate and ester groups in the polar region of the lipid.

GRAPHICAL ABSTRACT



ABSTRACT

Elastic compliance of DMPC liposomes with Hofmeister electrolytes: NaCl, Na_2SO_4, Na_2CO_3, $NaNO_3$, KCl and $MgCl_2$ studied using Quartz crystal microbalance with dissipation has been correlated with changes in their lamellar spacing from SAXS. The study suggests that hydration water of the different ions has an effect on the overall packing of the lipid bilayer that results as either a dehydrated liposome or where water smears the surface of the liposomes. Ratio of hydrogen bonded carbonyl and phosphate of polar region of the liposomes from ATR-FTIR spectroscopy, suggests that the polar groups are less hydrated due to the displacement of water by the electrolytes compared to pure DMPC and ordered in the sequence for cations as: $K^+ < Na^+, Mg^{2+}$ and for anions as $SO_4^{2-} < CO_3^{2-} < Cl^- < NO_3^-$. These findings show the usefulness of Elastic compliance for structural studies of composite phospholipid bilayers, lipid–protein complexes and lipid systems of reduced dimensionalities.

1. Introduction

Membranes, constituent organelle in a cell, are important to exchange information and energy gradients that are necessary for sustaining living organisms. Among the different functional moieties that reside on the polar surface of the membranes, carbonyl, phosphate and choline from the lipids form the most common structural groups [1]. These help in mediating, molecular recognition and signal transduction [2–4]. The study of the self-organized formation of lipid bilayers in presence of water represents a major challenge for understanding the chemical and biological functions of cell membranes. Research, in recent years has focussed on the incoherent thermal motions of phospholipid model membranes [5–9] and on the dynamics of water molecules at the interface with membrane [10–12]. Molecular sub-picosecond motions of phospholipid bilayers at various hydrations have been analysed by D'Angelo et al. using far-infrared spectra. Their result

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suggest that the low frequency vibrations of lipid membrane resemble subpicosecond motions of liquid water and that resonance mechanisms are crucial to the dynamics coupling between membranes and their hydration water. [13] The restructuring of the hydration water plays a key role in a variety of structural and functional aspects such as coupling phenomena between membranes, adhesion, fusion and stacking [14]. Leontidis, has highlighted the specific ion effects (SIE) in hydrated lipid model systems through an interplay of their ionic size, shape and charge distribution [15].Song et al. in their work on decoupling ion-ion and ion-water interactions have demonstrated how ion-induced changes in surface energy is crucial to understanding the Hofmeister ion effect. [16]. Parsons et al. have highlighted the synergy between specific surface chemistry, surface charge density, pH, buffer, ion size and the relevance to Hofmeister effects [17]. Peschel et al. studied phase behavior in solid-supported lipid systems of different geometries (adsorbed liposomes and bilayers) using a custom-built Quartz crystal microbalance (QCM) with dissipation. Using Overtone dependence of the phase transition parameters, they demonstrated that hydrodynamic effects are important in adsorbed liposomes, and viscoelasticity is significant in supported bilayers [18]. Edvardsson et al. combined QCM- D with reflectometry measurements to study adsorption of bilayers and supported lipid structures [19].Richter et al. in an excellent review dealt with the process of SLB-formation and the physical properties of the surface-confined lipid bilayers [20]. Moratta et al. using nano indentation with AFM and some other research groups have studied the impact of cations on supported lipid bilayers. [21-23]

The study of Hofmeister electrolytes was initiated > 100 years ago, and today, these solutes are known to display recurring behavior for myriad biological and chemical processes. [24] Such behavior depends both on the nature and concentration of the species in solution. The free energy barrier for small ions like sodium or potassium to diffuse across such a membrane is as high as 40 kcal/mol. Due to low dielectric constant of the lipid bilayer (most oils and hydrocarbons have dielectric constant in the range 2–5), [25] Their transport across the membrane is therefore assisted by the ion channels, special proteins that are responsible for ion, as well as water and trans-membrane conductivity. [26] In addition to the degree of hydration, effect of mechanical stress on membranes is of primary importance in biophysics, since cells are known to perform their function under a complex combination of forces. The distribution of ions in the solution and their interaction with the membranes are factors that substantially modify the structure and dynamics of the cell membranes. In addition, many signalling processes are modified by the membrane capability of retaining ions.

In recent years, number of studies on ion-specific phenomena in lipid bilayer membranes using fluorescence correlation spectroscopy, [27,28] fluorescence solvent relaxation, membrane dipole potential measurements, [29] molecular dynamics (MD) simulations, [30,31], charge displacement techniques [32] and small angle X-ray scattering (SAXS) studies [33] have been reported. Measurements at zwitterionic lipid membranes have shown that several ions penetrate into the bilayer, while some may adsorb at the liquid/ membrane interface, and others do not even attach. [34]

The reasons for the partially contradictory results have been due to using a combination of van der Waals forces that mediate interactions of lipids with the ions at the interface with kosmotropic (structure forming) effects and water affinities. In the present study, we investigate the effect of different coexisting Hofmeister ions interacting with Dimyristoyl phosphatidyl choline (DMPC) liposomes. The salts chosen are cations: Na^+, K^+, Mg^{2+}, anions: Cl^-, SO_4^{2-}, NO_3^- and CO_3^{2-}. We have characterized the phase transitions in the DMPC liposomes using Differential scanning calorimetry (DSC) and evaluated the nature of hydration near the polar groups using ATR FTIR. The lamellar repeat distances were estimated by small-angle X-ray scattering (SAXS) against the different cations and anions. Further the elastic compliance of the liposomes incubated with the different ions adsorbing on gold coated quartz have been measured using a Quartz crystal microbalance (QCM) with dissipation (D) and correlated with the degree of hydration of the polar groups and the changing lamellar spacing. In this in situ method, the experiments are performed at a fixed percentage of relative humidity and degree of hydration and therefore closer to the real membrane process.

2. Materials and methods

1, 2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) from Avanti Polar Lipids Inc., was dissolved in chloroform to prepare thin lipid films of the desired concentration on the wall of a round bottom flask. Deionized Water (resistivity > 18 MΩ/cm) from a MilliQ plus unit (Millipore, France) was used for preparing the phosphate buffer (pH = 7.5, 10 mM) which was filtered and degassed before use. The solvent was removed, first under a gentle stream of N_2 to form the film, and secondly under vacuum overnight. The dried lipid was dispersed in buffer to a final concentration of 5 mM. After vortexing, the liposomal solution was extruded 5 times through a 100 nm and a 30 nm polycarbonate membrane.

2.1. Dynamic light scattering (DLS) for size and zeta potential

Vesicle solutions were characterized using dynamic light scattering (Malvern Zetasizer Nano ZS together with the Dispersion Technology software, Malvern Instruments., UK) for size and zeta potential at 25 °C using green laser (532 nm with the M3-PALS technique. Data processing was done by the Zetasizer software 6.32 (Malvern instruments). And zeta potential was calculated from the measured electrophoretic mobility by means of Henry's equation using the Smoluchowski approximation (f (Ka) = 1.5). Results here are reported as an average value of 3 independent measurements.

Electrolytes NaCl, $NaNO_3$, Na_2SO_4, Na_2CO_3, KCl and $MgCl_2$ were obtained from Merck, India and were 99.9% pure. All measurements were made before and after roasting the salts and the difference in d ($\Delta\gamma$)/dC were measured within permissible errors.

2.2. Differential scanning calorimetry

The phase behaviour of (DMPC) bilayers was studied using micro DSC (Q200, TA) equipped with T-zero cells with hermetic lids made for liquid samples. The capacity of the sample and reference cells is 1 cm^3. All the solutions before loading into the calorimetric cells have been degassed. A constant volume (0.80 cm^3) of the solution of interest has been maintained in the sample cell and the weight of the solutions in the sample and reference cells have been matched. The reference solution is buffer when the samples have been made in buffer or buffer with additive when the measurements have been made in the presence of any electrolyte. A heating rate of 0.5 °C/min has been used for all scans.

2.3. ATR-FTIR

ATR-FTIR for the liposomes and with the electrolytes has been carried out using JASCO 4700 spectrometer. All spectra were collected with a nominal resolution of 2 cm^{-1} and 50 scans. The hydrated DMPC films were mounted in a temperature-controlled demountable liquid cell equipped with (ZnSe) windows and a spacer of nominal length of 6 mm (Harrick). Temperature was regulated by refrigerated/heating circulator (Julabo, F12-ED) in 0.5 °C step and recorded at temperature = 25 °C.

The sample was held for approximately 10 min at the desired temperature. Peak positions were determined from the five baseline corrected absorbance points by calculating the maximum of the fitted 2nd order polynomial. Spectrum software was used to convert spectra from transmittance to absorbance, and to perform baseline correction.

Deconvolution and curve fitting of the acquired spectra for the

defined spectral regions have been Fourier deconvoluted using ORIGIN9.0. Parameters for deconvolution were 0.5 for gamma and 0.1 for smoothing. The peak analyzer tool was used to identify hidden peaks in the deconvoluted region. The multiple peak fit tool was used to fit the deconvoluted spectra assuming a Lorentzian curve fit.

2.4. Quartz crystal microbalance-dissipation (QCM-D)

In QCM-D experiments, the quartz crystal is excited at its fundamental frequency (5 MHz), and at n = 3, 5, 7, 9 or 11 corresponding respectively to 15, 25, 35, 45 and 55 MHz. Changes in the resonance frequencies (Δf) and in the relaxation of the vibration once the excitation is stopped are measured at these frequencies. The relaxation gives access to the dissipation D of the vibrational energy stored in the resonator.

The measurements have been conducted at 25 °C. This experiment with dissipation is independent of elastic properties of the adsorbing layer. A baseline for the buffer on the sensor surface has been established. Experiments have been carried out with pure DMPC liposomes wherein after equilibration with the buffer, the stock solution has been dropped and the adsorption is monitored. After adsorption equilibrium is reached, the buffer has been introduced into the chamber to remove any non-adsorbed liposomes.

QCM-D, monitors changes in mass m near the sensor surface as a shift in the resonance frequency (Δf or Δfreq.) of the sensor crystal according to Sauerbrey's Eqn. when a thin film is adsorbing onto the quartz surface.

$$\Delta f = f_0 (M/m_q) \quad (1)$$

Where m_q is a surface mass of the bare quartz crystal and f_0 is the resonant frequency. This experiment normally independent of elastic properties of the adsorbing layer exhibits a shear modulus when a hydrated layer is adsorbing on the surface and a shear elastic modulus G can be assigned to this layer. For soft matter as in the case of the liposomes, viscoelastic layers that include not only shear elasticity (storage modulus G_S) but also viscosity (loss modulus G_L) components of the complex shear modulus G* must be taken into account.

$$G* = G_S + iG_L \quad (2)$$

As simplification, D dissipation can be related to the rigidity of the film by

$$D = G''/2\pi G' \quad (3)$$

Here G″ is the loss modulus and G′ is the storage modulus.

Changes in frequency (Δfreq) and dissipation (Δdiss) induced by adsorbed film can be monitored simultaneously at different overtones (3rd, 5th, 7th, 9th, 11th etc.).

The ΔDiss/Δfreq ratio emphasizes the structural properties of the layer and a soft versus rigid layer can be uniquely characterized for the mechanical strength. For the various overtones used, values for the frequency shift are scaled by the overtone order, that is, Δf/n rather than Δf, if Δf/n is the same on all overtones for the frequency shift that is caused by gravimetric effects. Dissipation changes are not scaled by the overtone order because such a scaling by n is implicitly contained in the definition of D. By plotting ΔDiss/−Δfreq versus overtone n, one can obtain the film's elastic compliance J from the slope using the equation (η viscosity).

$$\Delta D/-\Delta f \approx \eta\varpi J \approx 2\pi\eta f_0 J \quad (4)$$

2.5. Small angle X-ray scattering studies (SAXS)

Lamellarity of liposomes was determined from Small Angle X-ray Scattering (SAXS). performed with SAXSess (Anton Paar KG, Graz, Austria) equipped with a sealed tube (Cu anode λ = 1.542°Ang.). The lipid concentrations were maintained at 10 mM and the samples were analysed in a quartz capillary (with an outer diameter of 1 mm, at 25 °C).The 2D scattering pattern in SAXS Quant software (Anton Paar), was integrated into the 1D scattering curves as a function of the magnitude of the scattering vector (q). Data were obtained in the range of 0.11–6 nm^{-1} and corrected for the (Buffer/Buffer + electrolyte)-filled capillary scattering. Since the beam incident is linear, desmearing treatment allows correcting the spectra.

3. Results and discussion

The size and zeta potential of the DMPC liposomes used in the present study show that pure DMPC liposomes have an average size of about 104.8 ± 1.2 nm with a zeta potential value of about −12.4 ± 0.34 mV. The sizes for the liposomes with the electrolytes vary from 92.5 ± 2.3 (with KCl) to a maximum of 170.6 ± 1.5 nm for NaCl. The values for the liposomes with $MgCl_2$, Na_2SO_4, $NaNO_3$ and Na_2CO_3 are 109.3 ± 1.5; 153.5 ± 2.2; 110.1 ± 1.8; 162.5 ± 2.1 nm respectively. The zeta potential of the liposomes varied slightly on dilution with the buffer. The zeta potential values for the liposomes with the electrolytes varied as −5.9 ± 0.22 mV (KCl), 7.5 ± 0.4 mV ($MgCl_2$); −6.4 ± 0.25 mV (Na_2SO_4); −4.1 ± 0.14 mV($NaNO_3$);-3.5 ± 0.23 mV (Na_2CO_3) respectively.

For DMPC liposomes, the lipid bilayer in an electrolyte solution can be thought of as an electrical double layer. The DMPC head group contains both an acidic and a basic moiety that should charge up equally in opposite directions at neutral pH. Therefore, considering the molecular structure only, one would expect an electrically neutral membrane. However, the zeta potential measurements for the pure DMPC liposomes and with the different electrolyte solutions revealed a negative membrane charge at pH = 7.5 and, rather independent of the ionic strength (ionic strength used varied from 1 mM to 20 mM). This may be due to the unsymmetrical adsorption of water ions, [35] i.e., the fact that OH^- ions are stronger adsorbed as compared to H_3O^+ ions. In order to quantify the Hofmeister ion effect on the thermotropic phase behaviour of (DMPC) bilayers, DSC thermograms have been used.

Fig. 1(a and b) show the DSC thermograms of the DMPC vesicles and with the different electrolytes. The changed character of the main transition at about 22 °C from sharp to gradual in vesicles with the electrolytes and the small shift of the main transition temperature in dispersions reflect the interactions of lipid head groups with the ions. In general, lipid membranes can exist in different phases, e.g., the liquid crystalline or fluid phase, gel phase, sub gel phase, and in some phospholipids a ripple phase. [36] The phase transition temperatures primarily depend on the composition of the membrane and the structure and degree of hydration of the polar groups of the lipids. The resulting electrostatic interactions within the lipid membranes as well as between lipid head groups and ions from solution are crucial for phase transition temperatures, [34] membrane fusion, [35] and transmembrane transport. [26]

Earlier measurements at zwitterionic lipid membranes have shown that several ions penetrate into the bilayer, that some are adsorbing at the liquid/ membrane interface, and that others do not even attach [27,28], [30]. The reasons for the partially contradictory results are still under debate and some have attributed it to a combination of Van der Waals forces that mediate interactions of lipids with the ions at the interface followed by kosmotropic (structure forming) effects and water affinities. In some of the studies, [28], [30], [33] it has been assumed that the acidic and basic moieties in zwitterionic lipid head groups bearing equal and opposite charge in the neutral pH range, should compensate each other (no net charge). However, recent electrophoretic and streaming currents measurements revealed a significant negative charge at neutral pH and an isoelectric point of about 4 for zwitterionic lipid membranes [29]. Our Zeta potential values for the DMPC vesicles in buffer and in the presence of most of the electrolytes show a similar negative value. For a divalent cation like Mg^{2+}, we see a positive potential. While the trends in changes in the zeta potential

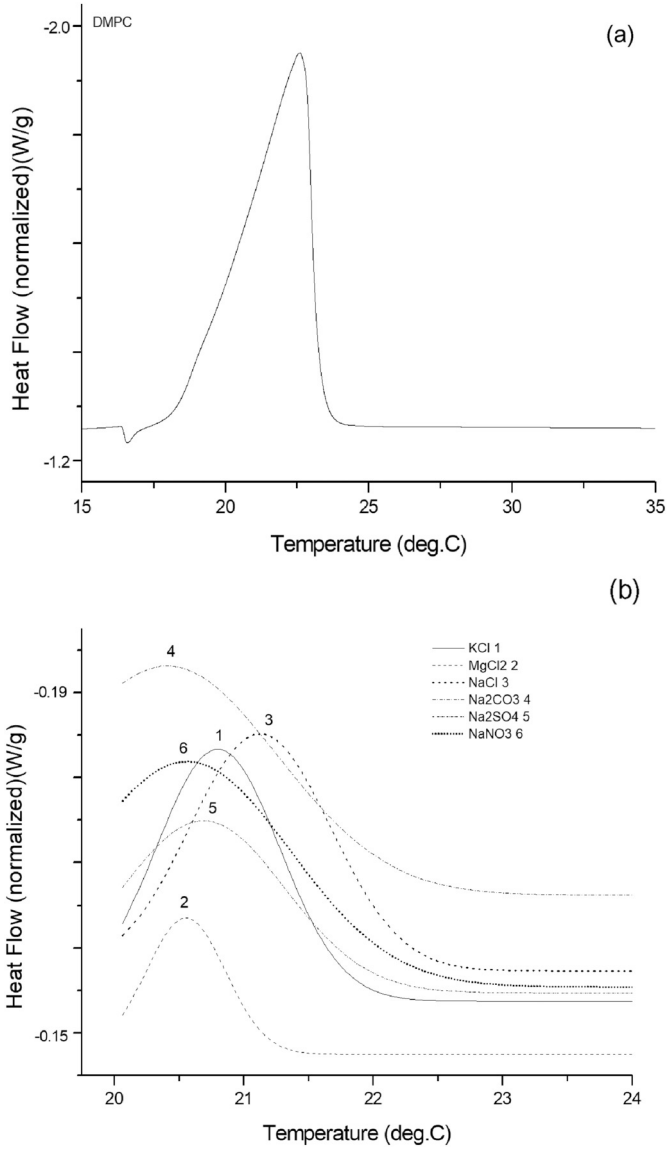

Fig. 1. DSC thermograms of (a) pure DMPC liposomes; (b) DMPC liposomes with Hofmeister ions.

values follow more or less the reverse Hofmeister order ($Na^+ > K^+$, $Cl^- > SO_4^{2-} < NO_3^- < CO_3^{2-}$), it should be noted that the zeta potential characterizes the amount of ions located in the bilayer vicinity and not necessarily the amount of bound ions. Adsorbing divalent metal ions are known to decrease the number of water molecules that can bind to the lipid [37]. Such cation binding can lead to an entropy gain due to release of water molecules into solution [38-41], and thus causing a more positive zeta potential value. This results in a charge-induced transition of the fluid bilayer in a gel/ordered phase bilayer. In order to confirm this, DSC thermograms of the DMPC vesicles and with the electrolytes have been carried out.

Table 1 shows the main phase transition temperature Tm for the pure DMPC vesicles and with the electrolytes. Increase in main transition enthalpy values attributable to the chain melting and molecular packing of the alkyl chains are observed with incorporation of the electrolytes in the DMPC liposomes. The ripple phase seen in the pure DMPC liposomes are not very evident in the liposomes with electrolytes This could indicate that ion−lipid interactions superimpose the primary charge formation processes and influence the fluidity of the bilayers. On the basis of the above experimental results, we discuss the impact of the different electrolyte ions on the lipid charge,

Table 1
T_m and Molar enthalpy of DMPC liposomes and with electrolytes.

DMPC liposomes	Pre T_m (°C)	T_m (°C)	Molar Enthalpy (J/g)
In Phosphate buffer (pH = 7.5)	16.58 ± 0.12	22.99 ± 0.18	6.795 ± 0.13
With electrolytes (10 mM)			
NaCl	21.11 ± 0.14		5.687 ± 0.24
Na_2SO_4	20.69 ± 0.12		5.838 ± 0.19
Na_2CO_3	20.48 ± 0.12		6.226 ± 0.24
$NaNO_3$	20.56 ± 0.12		6.204 ± 0.24
KCl	20.89 ± 0.10		6.723 ± 0.19
$MgCl_2$	20.56 ± 0.10		8.479 ± 0.17

conformation and hydration. In the pre-transition phase, the bilayer is not flat, but corrugated. Some lipids including DMPC exhibit this 'ripple' phase. From this phase, the bilayer undergoes the transition to the liquid crystalline or fluid L-phase, which is called the main transition or the chain order/disorder transition. In this phase, the bilayer is a two-dimensional fluid, meaning that the lipids are free to move in the plane of the bilayer, and therefore the transition to this phase is regarded as the melting of the bilayer. The Pre and main T_m for the DMPC vesicles seen here compare well with the reported values [42]. Normally, the pre- and main- transition temperatures decrease with increasing head group hydration and unsaturation of the alkyl chains.

Some research has suggested that in the phase behavior of PC's there could be an observed anomalous swelling (non linear increase of the lamellar repeat distance with temperature) near a phase transition. For long, this swelling was considered to be a key factor in the formation of the rippled phase. However, it is not clear what causes this anomalous swelling [43-46]. In this study, the molar enthalpies measured for DMPC with all the electrolytes, are positive (endothermic) indicating that this interaction is entropically driven. The binding of the cations to the lipids perturbs the hydration shells of both binding partners and leads to the liberation of water molecules and thus the positive endotherms.

Pure DMPC liposomes show the highest Tm while the samples incubated with the various electrolytes show a small decrease in the Tm. The decrease in Tm is an indicator of slight increase in disorder in the lipid tails arising from the change in the hydration of the polar head groups. The change in the main Transition T_m follows the trend $Mg^{2+} < K^+ < Na^+$ while for the anions $Cl^- > SO_4^{2-} > NO_3^- > CO_3^{2-}$. This trend follows the Hofmeister effect. Beyond the tendency of ions to interact with lipid molecules, the preferred adsorption site in the bilayer membrane depends on the structure of their hydration shell and thus influences the pattern of charge formation and compensation. In order to understand the nature of interaction with the polar head groups and or the alkyl tails, ATR-FTIR of the vesicles have been carried out. Fig. 2(a) shows a representative FTIR plot for the pure DMPC vesicles exhibiting the changes in the frequencies of alkyl stretch, the choline and the phosphate regions.

It is well known that hydration dynamics and lipid-water interaction strength are closely related to molecular organization and properties of lipids [47].Shifts in the asymmetric and symmetric CH_2 stretch from the alkyl chains of the lipid in the vesicles in the pure DMPC and with electrolytes are presented in Fig. 2(b, c). The spectra for the DMPC liposomes and DMPC with the electrolytes in the region 3000–2800 cm^{-1} are given in Supporting Information as Fig. S1.

It is well established that with increasing molar water/lipid ratio, the ν_{as} stretching frequency for CH_2 in the alkyl tails increases. In Fig. 2(b), it is seen that this frequency increases as $SO_4^{2-} < Cl^- < CO_3^{2-} < NO_3^-$ and for the cation the trend is $Na^+ < K^+ < Mg^{2+}$. These trends are again in keeping with the Hofmeister electrolytes.

DMPC molecules have two proton-accepting groups that can interact with water to form hydrogen bonds: the phosphate group in the

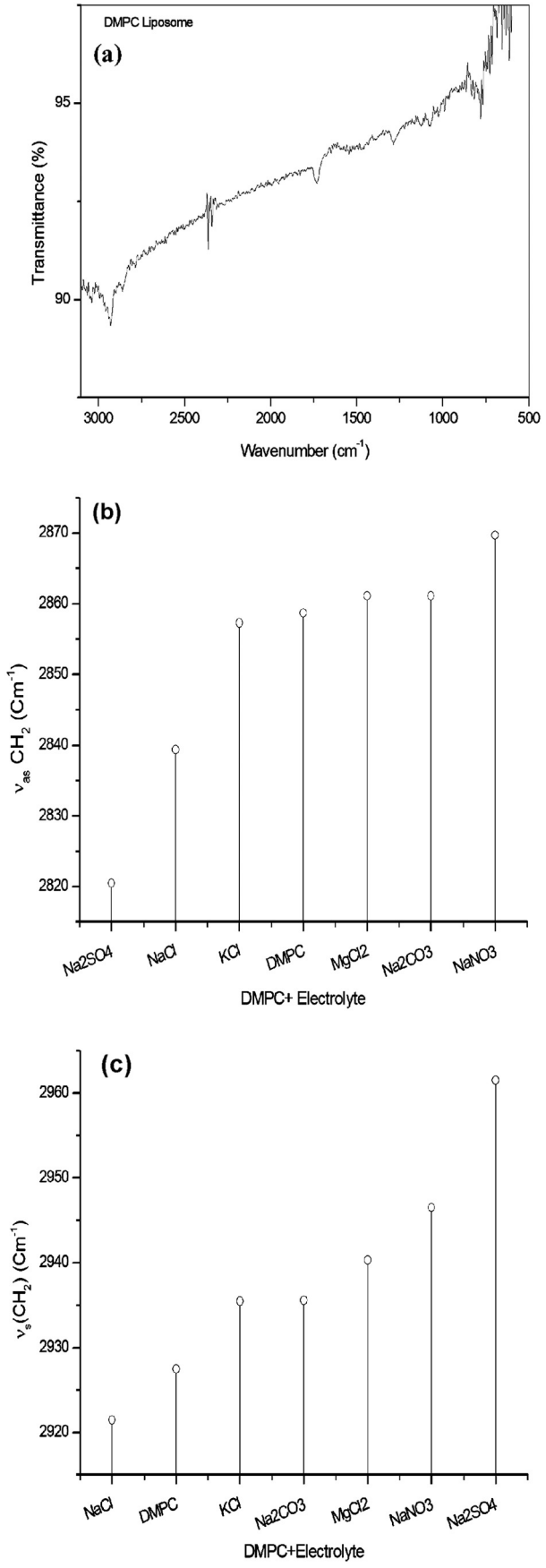

Fig. 2. (a) ATR-FTIR plot for pure DMPC liposomes exhibiting changes in the frequencies of alkyl stretch, the choline and the phosphate regions. CH_2 frequencies (b) asymmetric stretch ν_{asym}, (c) symmetric stretch ν_{sym} of the alkyl tails in DMPC liposomes.

polar head and the ester carbonyl groups in the interfacial region. Initially, during hydration, the water molecules interact with the phosphate group, and later with the carbonyl groups [48]. 1750 to 1700 cm^{-1} region in the spectra is dominated by the C═O stretching vibrations of the ester carbonyl group from the interfacial region, which give at least two strong bands, reflecting the conformational non equivalence of the two ester carbonyls. The higher frequency component is due to the non hydrogen bonded carbonyl group, whereas the lower frequency component band is associated with the hydrogen bonded carbonyl group[49,50]. The carbonyl stretching peak (νC═O) between 1770 cm^{-1} and 1700 cm^{-1} has been analysed by peak deconvolution and curve fitting using the peak-fitting module of Origin Pro 9.0 as described in detail previously [51]. Correlation coefficients for all fitted curves are higher than 0.999.

For a quantitative analysis of the interactions of the C═O groups, deconvolution has been used to obtain the peak frequencies of the component bands reported for the two populations of carbonyls: the non-hydrated (1737 cm^{-1}) and hydrated (1722 cm^{-1}) populations in the fluid state. The spectra of DMPC liposomes and with electrolytes are presented in Supporting Information: Fig. S2.

The corresponding wavenumbers that appear for the pure DMPC liposomes and those with the electrolytes are presented in Table 2 together with the integrated ratio of areas under the two bands. Compared to the pure DMPC liposomes, for the liposomes with the electrolytes, the H-bonded frequencies shift to slightly lower wave numbers suggesting the polar groups are less hydrated due to the displacement of water by the electrolytes. A precise examination of the IR spectra for DMPC and those with the electrolytes obtained suggest that the ratio of non H-bonded to H-bonded C═O appear systematically ordered according to the sequence for cations as: K^+,Na^+ < $Mg^{2+}$and for anions as SO_4^{2-} < Cl^- < NO_3^- < CO_3^{2-}. This sequence agrees with the oppositely directed order of the bound water for the same set of anions as well as the different cations. According to the atom–scale simulations, the main effect of monovalent cations is their binding to the carbonyl region [52]. In contrast, the mechanism of disruption and reformation of lipid–ion networks seems to be related to the attractive electrostatic forces occurring through the sharing of the positive charge of cations between the negatively charged phosphate groups [53]. SAXS measurements have been carried out on the pure DMPC liposomes and those with the electrolytes. The original plots of I(q) vs. q for the DMPC liposomes and those of liposomes with electrolytes are given in Supporting Information: Fig. S3.

An electron density profiles from SAXS pattern recorded from fully hydrated liposome dispersion of DMPC and DMPC with the electrolytes at a temperature of 25 °C is shown in Fig. 3(a, b). It can be seen that the scattering pattern can be fitted on the basis of lamellar phases and the lamellar d-spacings of pure DMPC and those with the electrolytes are given in Table 3. These d-spacings are significantly less than reported for dispersions of pure DOPC (4.91 nm) [54] and DPPC (6.40 nm) [55] respectively. The result suggests that the arrangement of the phospholipids in the mixed lipid bilayer leads to either a reduction in bilayer

Table 2
IR frequencies for non H bonded and H bonded C═O in the polar groups of the DMPC lipid bilayers.

Sample	Non H Bonded C═O (cm^{-1}) (area)	H Bonded C═O (cm^1) (area)	Ratio of area under the two bands
DMPC	1737	1731	1.006
With electrolytes			
NaCl	1744.6	1724.7	0.359
KCl	1748.1	1730.5	0.359
$MgCl_2$	1747.7	1725.5	0.383
$NaNO_3$	1747.6	1728	0.35
Na2SO4	1747.8	1726	0.304
Na_2CO_3	1747	1728	0.4

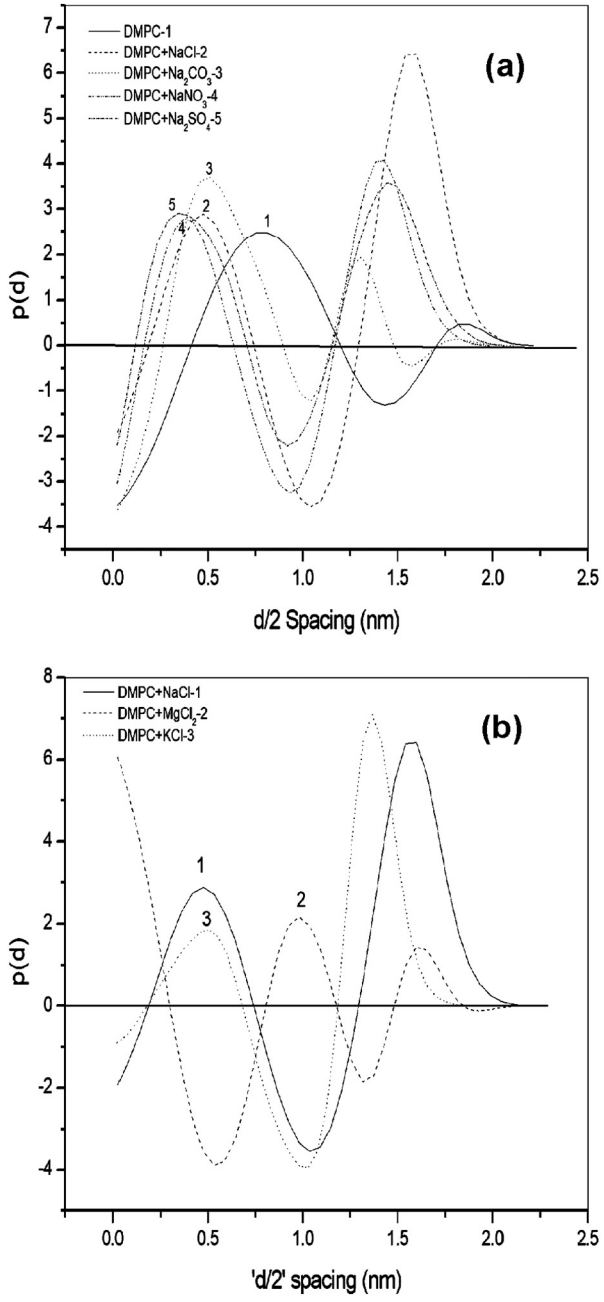

Fig. 3. Probability electron density p(d) versus d/2 spacing (Lamellar spacing /2) (a) DMPC liposomes with anions (b) with cations.

Table 3
Lamellar spacing and Thickness of hydrophobic core from SAXS, Elastic compliance and adsorbed mass from QCM-D of pure DMPC liposomes and with electrolytes.

DMPC liposomes with	Lamellar spacing (d) (nm)	Thickness of hydrophobic core dC(nm)	Elastic Compliance (GPa-1)	Mass (μg/cm2)
Buffer	4.42 ± 0.11	1.00 ± 0.01	1.406 ± 0.008	0.482 ± 0.008
NaCl	4.24 ± 0.09	1.12 ± 0.03	3.968 ± 0.002	0.439 ± 0.007
KCl	4.58 ± 0.07	1.01 ± 0.01	3.690 ± 0.001	0.514 ± 0.011
MgCl2	5.09 ± 0.09	1.40 ± 0.02	3.021 ± 0.001	0.492 ± 0.005
Na2SO4	4.52 ± 0.04	1.61 ± 0.03	2.832 ± 0.002	0.437 ± 0.004
Na2CO3	4.31 ± 0.03	1.07 ± 0.02	2.578 ± 0.002	0.412 ± 0.012
NaNO3	4.29 ± 0.01	1.19 ± 0.02	4.115 ± 0.001	0.387 ± 0.002

thickness, a decrease in hydration of the multilamellar dispersion or a combination of both factors.

The lamellar repeat distance d can be considered as that arising from a balance among four interactions- van der Waals interactions, short-range repulsion (decay length 1–2 Ång), long-range steric repulsion due to fluctuation of membranes (sometimes called Helfrich repulsion), and electrostatic interaction. From the SAXS measurements, the higher degree of hydration of the head groups for DMPC liposomes in the presence of the anions is seen from the higher p(d) values with CO_3^{2-} with the highest p(d) value. The p(d) values of the polar region show a trend $SO_4^{2-} > Cl^- > CO_3^{2-} > NO_3^-$ agreeing well with the IR results. The results suggest that for all the electrolytes added, there is marginal decrease in the lamellar periodicity for Cl^-, NO_3^- and SO_4^{2-} while for CO_3^{2-}, K+ and Mg^{2+} it increases. The reduction in the lamellar periodicity is possibly due to the entrained water being excluded from the intermembrane space. For the CO_3^{2-}, K^+ and Mg^{2+}, possibly due to a weak screening of the charges on the hydrated groups, the electrostatic repulsion between the neighbouring lipid bilayer becomes stronger. Accordingly, the larger water content increases the lamellar distance to larger extent.

It is well known that the bilayer structure formed by lipid aggregates is due to the hydrophobic effect [52], whereby non-polar molecules aggregate in such a fashion as to exclude water. The envisaged molecular structure from the profiles are only indicative, with the actual 3-dimensional structure folding up in different ways causing about a 10% shortening in length and giving rise to corrugated (i.e., not planar) surfaces. The head-group region in the presence of electrolytes are marked by slightly higher electron density, due to the presence of greater oxygen and phosphorous density plus more densely held water. The middle of the membrane shows slightly lower electron density due to the chain ends with associated vacant space caused by their poor interactions.

Based on the change in the degree of hydration, lamellar spacing and changes in the thickness of hydrophobic layer seen here agree quite well with the FTIR studies. In order to probe the effect of swelling or long range hydration effects on the mechanical properties of DMPC liposomes in the presence of the different electrolytes, QCM-D has been used to study the elastic compliance of the liposomes. Representative plots of Δ frequency and Δ dissipation as a function of time for the neat liposomes and liposomes with NaCl and $NaNO_3$ are shown in Fig. 4(a–c).

Based on the QCM-D measurements, the plots for decrease of Δf and corresponding increase of ΔD at the onset of adsorption are shown in the Figures. This change is caused by liposome attachment to the SiO_2 surface. Water trapped in the vesicles leads to the large decrease of Δf followed by the increased mass with increase in dissipation related to the viscoelastic properties of the liposome layer. The increasing frequency is because of water loss induced by vesicle rupture that eventually reaches a stable value corresponding to bilayer. Simultaneously, using the Voigt viscoelastic model instead of the Sauerbrey equation the data is fitted to a Voigt model where four parameters for the single layer consisting of the lipid bilayer, water film: density (ρ), thickness (d), elasticity (μ), and viscosity (η), the mass of the entrained water in the liposomes is evaluated and this is presented in the Table 3.

While most of the dissipation is in the water, the water flowing around the adsorbed liposomes causes them to shift slightly. The rest of the energy is dissipated in the contact zone between the liposomes and the surface, which deforms because of this motion. Increased dissipation occurs if the contact zone is narrow and thereby gives rise to a compliant element. Using the Δ Dissipation to Δ frequency values for the pure liposomes and those with the different electrolytes, the elastic compliance and adsorbed mass values were evaluated (presented in Table 3).

In general, the thickness best resolved by SAXS is the distance between the peaks in the electron density (ED) profile, which, corresponds to the distance between the lipid headgroup phosphates (DHH). In the

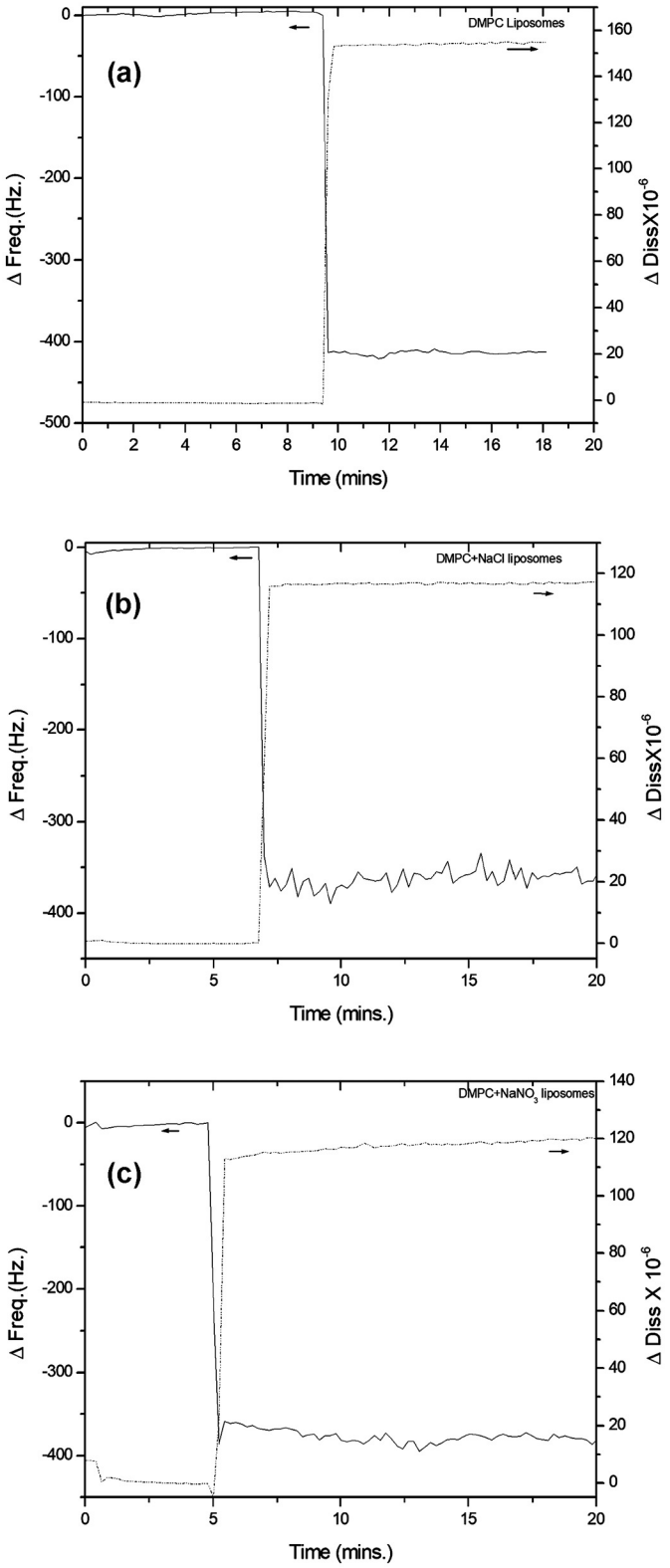

Fig. 4. Δfreq. and Δ Diss. Of (a) Pure DMPC liposomes; (b) DMPC liposomes with NaCl; (c) DMPC liposomes with $NaNO_3$.

SDP model developed by N. Kučerka et al. [56] the component groups are chosen on the basis that each group has the same functional form for all the different contrast conditions. The water distribution is not defined by any particular function, but is instead calculated based on the complementarity requirement. Terahertz spectroscopy has shown that three types of water are found among the lipid head groups; (1) bulk-like, (2) frozen held firm by two or more hydrogen bonds, and (3) molecules with only one (or perhaps no) hydrogen bond allowing rapid rotational diffusion in more hydrophobic areas [57]. Based on the ATR IR studies carried out in this study, for DMPC liposomes and with the electrolytes, the negatively charged phosphate groups form hydrogen bond with two water molecules strongly through each of their charged singly bonded oxygen atoms and one water molecule less strongly through each of their doubly single-bonded (ether) oxygen atoms. Most of these water molecules are likely to be so strongly bound as to be unfreezable. The deeply-located carboxylate ester groups within the head groups are likely to hydrogen bond weakly to atleast two water molecules through their double bonded (keto) oxygen atoms and one water more weakly through their doubly single-bonded (ether) oxygen atoms.

Based on the change in the bilayer thickness seen here for the different anions used, it is possible that the partitioning of anions into the polar region and interacting partially with the hydrophobic tail follows the Hofmeister effect. Aroti et al. have demonstrated a similar effect in DPPC monolayers at air/water interface [58], wherein the presence of anions near the interface seem to increase the surface pressure for a constant area/lipid molecule and this increase for the ions followed the Hofmeister series. In the DMPC liposomes with the different electrolytes, H_3O^+ ions partition into the water-lipid interface, displacing water and forming strong and long-lived hydrogen bonds with the lipid oxygen atoms. This results in a more compact surface area with an increased bilayer thickness. This trend is seen clearly in the values of bilayer thickness and changes in the coupled mass of the adsorbed lipid bilayers. When the water molecules lie close to the surface of the bilayer, some water can enter the membrane, due to their small size and their few or zero retaining hydrogen bonds when lying close to the interface. This process begins with hydrogen-bonding to the ester carbonyl groups, then moving between nano pools created from kinks in the lipid acyl chains. The surface packing in turn, may aid this process. This allows the possibility of the slow movement of water across membranes dependent on the osmotic pressure (water activity) difference between the solutions on opposite sides. This may result in a smearing of water molecules on the surface as well as across the bilayer. This in turn may give rise to the changes in the elastic compliance of the liposomes and also the changes in the bilayer packing and the lamellar spacings as shown in the scheme in Fig. 5(a). Such water layer over the membrane due to the counterions present near the lipid bilayers have been predicted by Le C TM et al. in their work on interaction of small ionic species with phospholipid membranes [59].

From the table the elastic moduli for the neat liposomes and those with electrolytes show that the presence of electrolytes, make the liposomes less rigid as seen by the decrease in elasticity. The values of elastic compliance agree fairly well with those reported for phospholipids [60].

Plots in Fig. 5(b, c) show the correlation between elastic compliance and d/2 spacing and against adsorbed mass/cm^2. The first plot shows a nearly inverse relationship between compliance and d spacing while the plot with adsorbed mass shows a direct correlation.

Takechi-Haraya et al. have in their recent work used AFM to study stiffness of nanosized liposomes as a function of saturation of acyl chains and also both charged and neutral lipids. Their results suggested that independently of the saturation degree of the lipid acyl chains, the measured stiffness of liposomes containing charged lipids are 30–60% lower than those of their neutral counterpart liposomes [61]. and overall the reported elastic compliance here agree with their observations.

In conclusion, the experimentally determined elastic compliance of DMPC liposomes and with Hofmeister electrolytes shows a correlation with both lamellar spacing and adsorbed mass, both of which are dependent on the degree of hydration of the polar region. It has been found that the modulus decreased by atleast 50% after interaction with

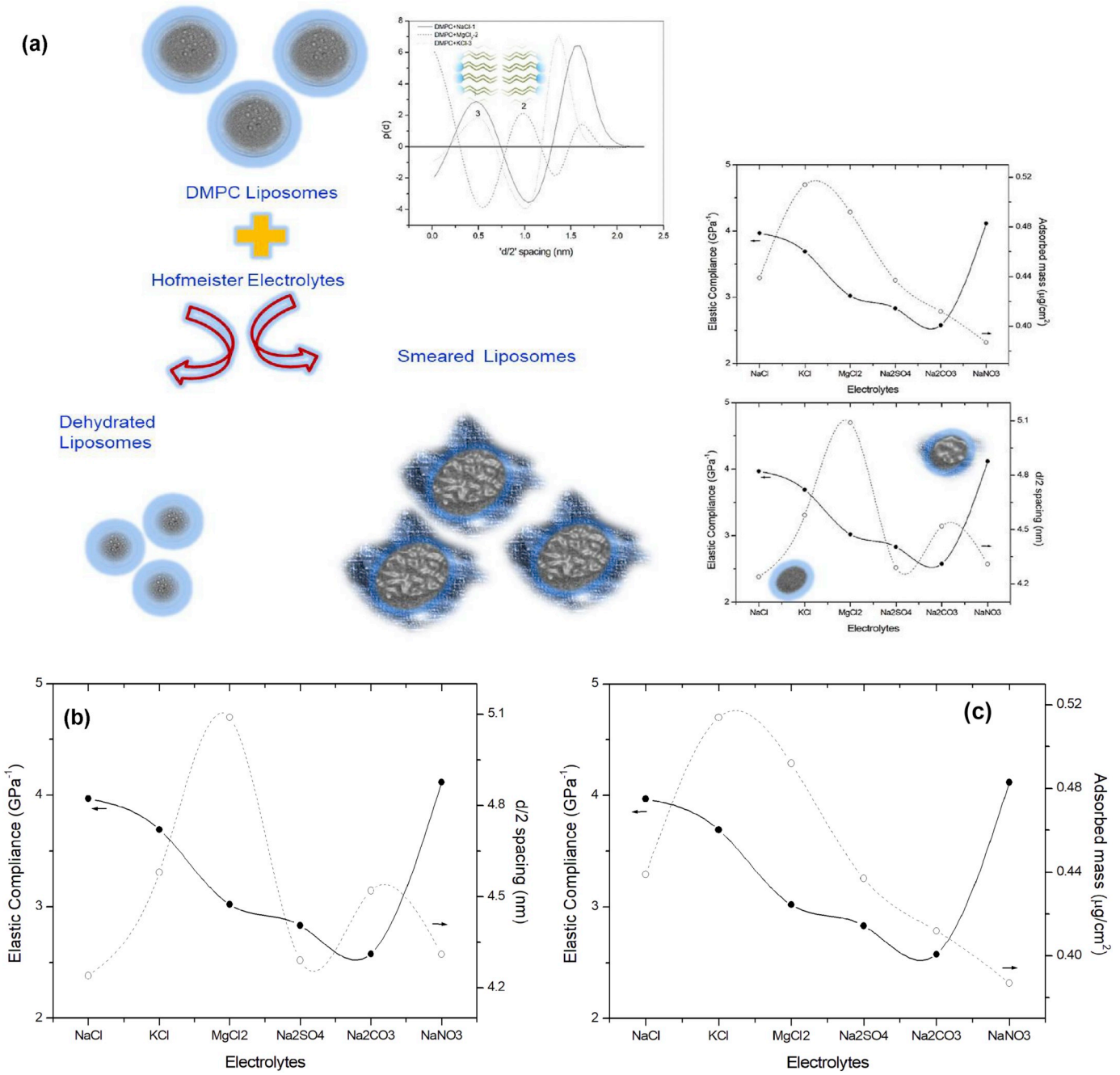

Fig. 5. (a) Scheme for DMPC liposomes and its elastic Compliance and lamellar spacing dependence on hydration. Elastic compliance and (b) d/2 spacing and (c) adsorbed mass for DMPC liposomes with Hofmeister electrolytes.

the electrolytes is attributed to the decreased rigidity of the vesicles and the decrease seem to follow the Hofmeister effect. The results indicate that the QCM can provide a direct method to measure the mechanical properties of immobilized small liposomes and to detect the stability change of liposomes. The result also showed that the stability of liposomes on substrate is significantly different from that in solution. These results are crucial for adhesion of liposomes which is an important essential step for mass transfer through large membrane. These experiments have implication for the behavior of water in biological systems, particularly water at the surfaces of cell membranes when ions get transported across the membranes. The proposed use of elastic compliance is promising for structural studies of mixtures of phospholipid bilayers, host–guest lipid–protein complexes and lipid systems of reduced dimensionalities.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bpc.2019.106148.

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