

Effect of Salt on Phosphorylcholine-based Zwitterionic Polymer Brushes

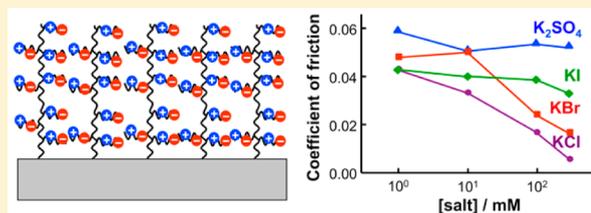
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ABSTRACT: A quantitative investigation of the responses of surface-grown biocompatible brushes of poly(2-(methacryloyloxy)ethyl phosphorylcholine) (PMPC) to different types of salt has been carried out using ellipsometry, quartz crystal microbalance (QCM) measurements, and friction force microscopy. Both cations and anions of varying valency over a wide range of concentrations were examined. Ellipsometry shows that the height of the brushes is largely independent of the ionic strength, confirming that the degree of swelling of the polymer is independent of the ionic character of the medium. In contrast, QCM measurements reveal significant changes in mass and dissipation to the PMPC brush layer, suggesting that ions bind to phosphorylcholine (PC) groups in PMPC molecules, which results in changes in the stiffness of the brush layer, and the binding affinity varies with salt type. Nanotribological measurements made using friction force microscopy show that the coefficient of friction decreases with increasing ionic strength for a variety of salts, supporting the conclusion drawn from QCM measurements. It is proposed that the binding of ions to the PMPC molecules does not change their hydration state, and hence the height of the surface-grown polymeric brushes. However, the balance of the intra- and intermolecular interactions is strongly dependent upon the ionic character of the medium between the hydrated chains, modulating the interactions between the zwitterionic PC pendant groups and, consequently, the stiffness of the PMPC molecules in the brush layer.



INTRODUCTION

In contrast to most polyelectrolytes that contain solely anionic or cationic groups, polyzwitterions comprise both positive and negative charges in each repeat unit. The coexistence of opposite charges in the polymer structure causes strong intra- and intermolecular interactions and gives rise to a range of unique properties that are the foundation for the great potential of polyzwitterions in a number of applications, including nonfouling coatings, drug delivery, and biosensor development.^{1–3} Poly(2-(methacryloyloxy)ethyl phosphorylcholine) (PMPC) is a particularly promising material for biomedical applications.⁴ The surfaces of phosphorylcholine-based polymers are highly biocompatible and remarkably resistant to protein adsorption and cell adhesion.^{5,6} Research shows that PMPC-coated surfaces can effectively prevent periprosthetic osteolysis (bone loss in the vicinity of a prosthesis) by significantly reducing the friction and the amount of wear in an artificial joint system while effectively suppressing biological reactions.^{7,8}

One of the critical factors that needs to be taken into account when designing systems for use in a biological context is the impact of electrolytes on the surrounding medium because ions are crucial to the function of many cellular processes.⁹ Because of the coexistence of opposite charges, the response of polyzwitterions to ion concentration and type is of significant interest.^{10–12} For example, viscosity and light-scattering

measurements suggested that the lower critical salt concentration of poly(vinyl imidazolium sulfobetaine)s is strongly dependent on the nature of the salt, which in turn may be related to the ion binding capabilities of the ions.¹⁰ The surface-grafted polyzwitterionic brush could form a completely collapsed self-associated state when the counterions are replaced by ions with high affinity to the charges on the brushes.¹³ Furthermore, in a recent study, poly(3-(1-(4-vinylbenzyl)-1H-imidazol-3-ium-3-yl)propane-1-sulfonate) (polyVBIPS) brushes exhibited a clear and distinct salt responsive thickness transition.³

For enhancing the performance of PMPC in biological applications, it is important to understand the effect of salt on PMPC, something that has been debated over the past decade. PMPC molecules in different forms, e.g., free polymer chains, microgels, and surface-grafted polymer brushes, have been examined using a range of techniques that include size exclusion chromatography, light scattering, neutron reflectometry, and surface force measurements.^{14–22} However, the results of many of these studies are not consistent. Some work suggests that, unlike conventional polyelectrolytes, PMPC chains remain highly solvated in the presence of high

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concentrations of salt: their dimensions and affinity for water are independent of the ionic strength.^{14–18} In contrast, other studies have suggested that salt has a noticeable impact on PMPC, such as its polymerization rate and hydrodynamic volume.^{19,20} As of yet there is no clear consensus on the effect of salt on PMPC in aqueous solution. In the present study, a resolution to this conflict is sought by drawing on the results of a study of the effect of salt on PMPC by a combination of nanotribological methods, quartz crystal microbalance measurements, and ellipsometry.

Surface-grown PMPC chains, so-called polymer brushes, were used to examine the effect of salt in the present study. The use of polymer brushes to control surface properties is an important strategy for developing stimuli-responsive surfaces such as biocompatible substrates, surfaces exhibiting switchable adhesion, and self-lubricating surfaces.^{23,24} For example, the friction coefficient between mica surfaces covered with PMPC was reported as 0.0004 at a pressure of 7.5 MPa, which is five times lower than that between natural synovial joints.²¹

Friction force microscopy (FFM), a variant of atomic force microscopy (AFM), was used to examine the nanotribology of PMPC brushes. FFM can yield quantitative insights into various phenomena, including the effect of surface composition, brush molar mass, and molecular organization on the structures and properties of polymer brushes.^{25–28} Quartz crystal microbalance (QCM) measurements were performed to examine the interactions between ions and phosphorylcholine (PC) groups. During QCM measurements, the change in the resonant frequency of an oscillating crystal is linearly proportional to the mass change of the surface layer, which evaluates the binding of ions to the PMPC molecules.^{29,30} QCM also provides quantitative insight into the viscoelastic properties of the bound film.^{31,32} Ellipsometry was used³³ to measure the thickness and structure of the PMPC brush layer in situ.

The properties of PMPC in aqueous ionic media are important in determining its performance in biomedical devices that are often exposed to physiological environments. Fundamental understanding of the interaction between PMPC and inorganic electrolytes developed from this work will not only provide design principles when using PMPC in biomedical applications but will also enhance our understanding of the phosphorylcholine group that is an essential building block in biological systems.

EXPERIMENTAL SECTION

Materials. Silicon wafers ((100) orientation, boron-doped, 0–100 Ω cm) were purchased from Compart Technology (Peterborough, UK). 2-(Methacryloyloxy)ethyl phosphorylcholine monomer (MPC, >99% purity) was donated by Biocompatibles UK Ltd. and used as received. Aminopropyl triethoxysilane (APTES, 99%), 2-bromoisobutyl bromide (BIBB, 98%), triethylamine (TEA, 99%), copper(I) bromide [Cu(I)Br, 99.9999%], copper(II) bromide [Cu(II)Br₂, 99.9999%], and 2,2'-bipyridine (bpy, 99%) were purchased from Sigma Aldrich UK. All chemicals were used as received apart from TEA, which was refluxed over calcium hydride, distilled, and stored over molecular sieves (4 Å). Hydrogen peroxide, ammonium hydroxide, sulfuric acid, and absolute ethanol (Fisher Scientific, Loughborough, UK) were used as received. Dichloromethane (DCM) was supplied by the Grubbs dry solvent system. All of the salts used in the present study, including lithium chloride (LiCl), sodium chloride (NaCl), potassium chloride (KCl), potassium bromide (KBr), potassium iodide (KI), calcium chloride (CaCl₂), and potassium sulfate (K₂SO₄), were purchased from Sigma and used as received.

HPLC grade water (CHROMASOLV, 2.5L), purchased from Sigma, was used to prepare the salt solutions used in this work.

Preparation of Initiator-Functionalized Silicon Wafers.

Silicon wafers were sonicated for 15 min in toluene, acetone, and then ultrapure deionized water (Elga Pure Nanopore, 18.2 M Ω cm). The substrates were then immersed in piranha solution, a mixture of 30% hydrogen peroxide and 98% concentrated sulfuric acid at a ratio of 3:7 for 1 h. (**Caution!** Piranha solution is a strong oxidizing agent that has been known to detonate spontaneously on contact with organic material and should be handled with extreme care.) The substrates were then rinsed thoroughly with ultrapure deionized water obtained from an Elgastat Option 3 system and subsequently immersed in an RCA-1 (Radio Corporation of America) cleaning solution (a mixture of ultrapure deionized water, hydrogen peroxide, and ammonium hydroxide at a ratio of 5:1:1 at 80 °C) for 40 min. The substrates were again rinsed thoroughly with ultrapure deionized water and finally dried in an oven. The cleaned substrates were immersed in a 1% solution of APTES in ethanol for 30 min. They were then rinsed and sonicated with ethanol, dried under a stream of nitrogen, and placed in a vacuum oven at 120 °C for 1 h. Substrates were immersed in a solution of 150 mM BIBB with the same molar quantity of TEA in DCM under nitrogen for 30 min. They were then rinsed with ethanol, sonicated in DCM for 10 min, and dried under a stream of nitrogen.

Preparation of PMPC Brushes. PMPC brushes were grafted from silicon surfaces using atom transfer radical polymerization (ATRP). Prepared substrates were cut into 1 \times 1 cm² pieces and placed under nitrogen using three pump-refill cycles of up to 12 tubes in a Radley's Carousel 12 Plus Reaction Station (Radleys Discovery Technologies, Essex, UK). Methanol and ultrapure deionized water were deoxygenated via a nitrogen purge for at least 30 min. MPC (2.0 g, 6.8 mmol) was weighed into a flask, stoppered with a rubber septum, and placed under nitrogen using three pump-refill cycles. The deoxygenated methanol (4.0 mL) and water (4.0 mL) were added and then stirred to allow the MPC to dissolve. Cu(I)Br (16.2 mg, 0.11 mmol), Cu(II)Br₂ (7.4 mg, 0.03 mmol), and bpy (49.5 mg, 0.32 mmol) were added to the solution; sonication was used to aid in dissolution of the solids (relative molar ratios were MPC:Cu(I)-Br:Cu(II)Br₂:bpy = 60:1:0.3:2.8). The prepared solution was then injected into the reaction tubes, making sure to cover the substrates completely. The polymerization was terminated after 2.5 h by the introduction of oxygen (air); the substrates were rinsed with methanol and ultrapure water and then sonicated for 15 min in both ultrapure water and then methanol. The substrates were then dried under a stream of nitrogen.

Friction Force Microscopy. Silicon nitride triangular AFM probes (NP-10, Bruker, Cambridge, U.K.) were cleaned by submersion in piranha solution, rinsed thoroughly with ultrapure water, and dried in an oven at approximately 80 °C. For coating the cantilevers with gold, an Edwards Auto 306 bell jar vacuum coater system was first used to deposit a 1 nm thick layer of chromium with a deposition rate of \sim 0.02 nm s⁻¹. Following deposition of the adhesion layer, the system was allowed to cool down for \sim 20 min prior to deposition of a 15 nm thick gold coating at a typical deposition rate of 0.03 nm s⁻¹. These gold-coated probes were stored in a vacuum desiccator prior to use.

Friction force measurements were performed using a Multimode atomic force microscope with a Nanoscope IV controller (Veeco, Cambridge, U.K.) operating in contact mode. The size of the scan was maintained at 1.0 μ m, and the scan rate was kept at 3.05 Hz. The nominal spring constant of the tip used in the present study was 0.06 N m⁻¹. The spring constant of each cantilever was determined from its thermal spectrum following the method of Hutter and Bechhoeffer.³⁴ The friction response was measured from friction loops acquired by obtaining forward–reverse scan cycles with the microscope employed in scope mode. The friction signal was obtained by subtracting the mean signals in both directions, giving a resultant force that is twice the frictional force. The lateral signal was converted from volts to nN by using the wedge method³⁵ in which the cantilever is scanned across a calibration grating (TGF11, MikroMasch, Tallinn, Estonia), and the friction signal is measured as a function of applied load.

Quartz Crystal Microbalance–Dissipation (QCM-D). QCM-D measurements were conducted using a Q-Sense D300 system (Q-Sense AB, Gothenburg, Sweden). Details of the QCM can be found elsewhere.^{30,36} PMPC brush layers were grown on silicon-coated quartz crystals (5 MHz, AT-cut), which were subsequently mounted in the QCM flow chamber (QAFC301) in which the polymer layer was exposed to a series of salt solutions. Changes in the resonant frequency and dissipation factor of the crystal associated with mass variation on the surface were monitored simultaneously at four frequencies (the fundamental and three overtones). In the present work, PMPC molecules are tethered at one end to the silica substrate; therefore, any change in mass is attributed to the binding of water molecules or ions. The dissipation factor, D , measures the sum of energy losses during the oscillation cycle and hence reflects the viscoelastic properties of the brush layer.

The shifts from the measurements achieved at 15 MHz (third overtone) are presented here due to the increased sensitivity of the signal at this frequency. Changes in mass sensed by the QCM crystal could be calculated according to the Sauerbrey equation²⁹

$$\Delta m = -\frac{C\Delta f_n}{n} \quad (1)$$

where C is constant, n is the overtone number, and Δf_n is the corresponding frequency change observed. Frequency and dissipation changes acquired from the fundamental frequency were discarded, as they were usually noisy due to insufficient energy trapping.³⁷ Changes in frequency and the dissipation factor caused by density and viscosity of salt solutions were quantified by^{30,38}

$$\Delta f = -n^{1/2}f_0^{3/2} \left(\frac{\rho_L \eta_L}{\pi \mu_q \rho_q} \right)^{1/2} \quad \text{and} \quad (2)$$

$$\Delta D = 2 \left(\frac{f_0}{n} \right)^{1/2} \left(\frac{\rho_L \eta_L}{\pi \mu_q \rho_q} \right)^{1/2} \quad (3)$$

respectively, where n is the overtone number, f_0 is the resonant frequency, ρ_L and η_L are the density and viscosity of the liquid, respectively, and μ_q and ρ_q are the shear modulus and density of quartz, respectively. This method was then verified with reference crystals that were exposed to NaCl or CaCl₂ solutions. Values calculated for each salt solution were then subtracted from raw data, and the presented shifts in frequency and dissipation are solely induced by the impact of aqueous medium on the PMPC brush layer.

Ellipsometry. A phase-modulated spectroscopic ellipsometer (M-2000 V, J. A. Woollam Inc., USA) was used to monitor the thickness of PMPC brush layers in salt solutions. A silicon substrate covered with PMPC brushes was positioned in a chamber filled with 15 mL of ultrapure water. Concentrated salt solution (1 M) was added to adjust the ionic strength from 1 to 10, 100, and 300 mM. The refractive index for PMPC was approximated using Cauchy parameters of $A_n = 1.4778$ and $B_n = 0.004775 \mu\text{m}^2$ obtained from a previous study.³³ The thickness of the brush layer was modeled as a single slab of variable thickness using the completeEASE software package (J. A. Woolam Inc.). The solvent-swollen PMPC layer was modeled using a linear effective medium approximation (EMA) between polymer and solvent to approximate the refractive index of the swollen layer. The refractive indices of solvents were approximated using Cauchy parameters of $A_n = 1.3242$ and $B_n = 0.003064 \mu\text{m}^2$ for water. With the concentration range examined in the present work, the impact of the addition of salt on the refractive index of the aqueous media is negligible.³⁹

RESULTS

Ellipsometry. When exposed to a good solvent, e.g., aqueous solution for PMPC, polymer brushes adopt an extended conformation because the chains try to maximize contact with solvent molecules; they thus swell away from the surface and avoid contact with each other. In contrast to

polyelectrolyte brushes that take a less extended conformation in solutions of high ionic strength, most polyelectrolytes become more extended in solutions of high salt concentration because attractive intra- and intermolecular interactions are screened.¹ With a constant grafting density, the thickness of a polymer brush layer can effectively reveal its hydration state.

A series of PMPC brush samples with thickness between 18 and 23 nm in ambient conditions were examined in salt solutions with concentrations from 1 to 300 mM. Figure 1

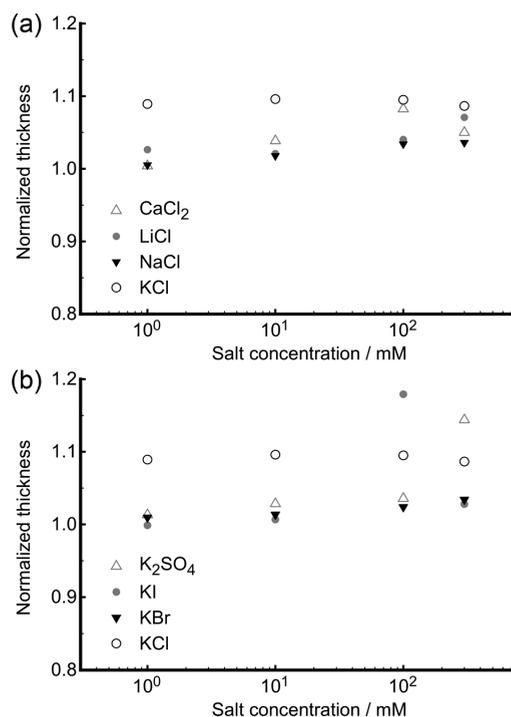


Figure 1. Normalized thicknesses of PMPC brushes as a function of the concentrations of a range of salts. Because a selection of samples with varying absolute thicknesses was used during ellipsometric measurements, fitted thicknesses have been normalized against the value of each specimen in deionized water. Error bars are similar in magnitude to the dimensions of the symbols used in the graph and are not shown.

presents the thickness of the swollen PMPC brush layer as a function of salt concentration and salt type. The thicknesses of the swollen brushes, measured by ellipsometry, were in the range 98–115 nm; for comparing the effect of salt on chains with slightly varying lengths, the thickness for each sample was normalized to the value measured in pure water for the same sample. The raw data were fitted with a single slab linear effective medium approximation (EMA) model according to a previous work.³³ Measuring the thickness of swollen PMPC brushes immersed in a wide range of salt solutions confirmed that addition of salt has little effect on the thickness of PMPC brushes. The layer remained swollen in the salt solutions examined with a relatively constant thickness at all salt concentrations, which suggests that the hydration state of PMPC brushes was not affected. Although single-slab models may not be the best approach to describe polymer brushes in wet conditions, as argued in the previous study,³³ the purpose of the present work is to consider the effect of salt on the thickness of the PMPC brushes. Consequently, changes in the relative thickness are more important than the absolute values.

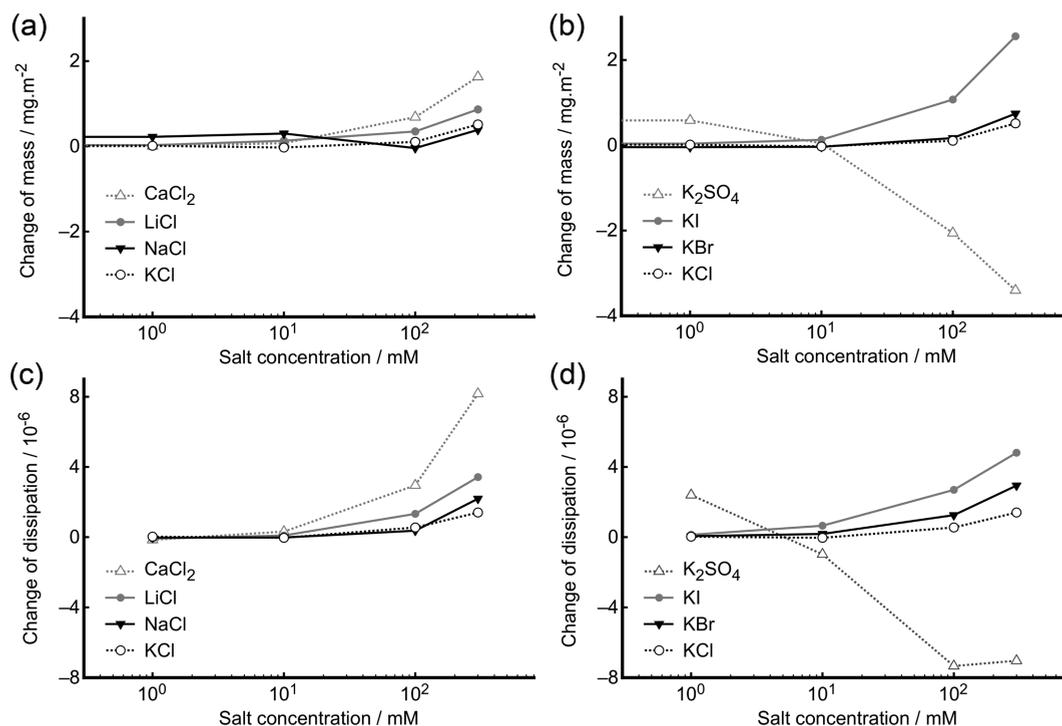


Figure 2. Changes in mass (a,b) and dissipation (c,d) during QCM measurements for PMPC brush-coated crystals exposed to solutions of chlorides (a,c) and potassium salts (b,d). Error bars were similar in magnitude to the dimensions of the symbols used in the graph and are not shown for clarity.

The ellipsometric data are consistent with recent work⁴⁰ in which a negligible discrepancy was found between the hydration state of PMPC brushes in NaCl solutions of different concentrations when the swollen PMPC brushes were compressed by an AFM cantilever in the normal direction. The finding is also in good agreement with previous studies: measurements of the dimensions of both free linear PMPC and silica nanoparticles coated with PMPC suggested that the dimensions of the free PMPC chain are independent of salt concentration;¹⁶ the size of free PMPC chain was investigated using light scattering techniques, and a similar conclusion was reached.¹⁷

Quartz Crystal Microbalance Measurements. For QCM measurements, QCM crystals grafted with PMPC brushes were left in pure water and allowed to equilibrate. These equilibrated specimens were used as the calibration baseline. For present purposes, the brush layer can be treated as a viscoelastic “hydrogel” composed of polymer chains and their associated water molecules. For testing the interaction between specific salt and PMPC molecules, the corresponding aqueous solution was introduced to the measurement chamber, retained until the brush layer reached equilibrium, and then replaced by a solution of higher concentration. Changes in both the frequency (Δf) and energy dissipation (ΔD) of the quartz crystal, caused by the interactions between the salt solution and the PMPC brushes, were measured in situ. According to the Sauerbrey equation, a decrease in frequency effectively indicates that more dynamic mass is sensed by the crystal, whereas an increase in dissipation suggests additional energy is being dissipated in each oscillation cycle by the more viscoelastic brush layer. Changes in mass (Δm) sensed by the quartz crystal were then calculated using eq 1 and are shown in Figure 2 alongside those in energy dissipation (ΔD). Values acquired in

pure water were set as the baseline and therefore are not shown here.

Panels a and b in Figure 2 show that there is a gradual but noticeable increase of mass (Δm) with increasing concentration for all of the salts except K₂SO₄. Such a trend indicates that the mass of the brush layer sensed by the crystal is enhanced with the addition of salts. Compared with previous works^{13,41} in which the solvation state of surface-immobilized polymer was examined, the variation in the mass observed in the present study is very limited. The impact of ions on PMPC brushes as a function of concentration can be demonstrated better by the dissipation factor (ΔD), shown in Figure 2c and d. The increased dissipation factor, as for most salts, suggests that the brush layer becomes more viscoelastic at high ionic strength.

At low concentration, the difference in mass and dissipation measured in solutions of different salts is modest for PMPC. However, at higher salt concentrations, the mass and energy dissipation were observed to increase following the general trend $\text{Ca}^{2+} > \text{Li}^+ > \text{Na}^+ > \text{K}^+$. The increased mass and energy dissipation observed on the PMPC-coated crystal in ionic media could be attributed to either the association of increased amounts of water with the PMPC brushes or the binding of ions to PMPC molecules. Because the hydration state of PMPC brushes is independent of salt concentration, as indicated by ellipsometric results, binding of ions to the polymer molecules is a more likely explanation for the QCM results.

Panels b and d in Figure 2 show QCM data acquired in KCl, KBr, KI, and K₂SO₄ solutions. For all three halide ions, the changes in mass are very limited at low salt concentration. However, for the 100 and 300 mM solutions, there are clear differences, and the change in frequency follows the trend $\text{KCl} < \text{KBr} < \text{KI}$. The same trend is observed for the change in dissipation, ΔD . Hence, for monovalent anions, both the mass and the energy dissipation increase as the concentration of salt

increases. This suggests that an increase in the concentration of salt leads to an increase in the amount of mass associated with the PMPC brush layer, causing more energy dissipation during each cycle.

The stereochemistry of PMPC is probed by a comparison between the two divalent ions used in the QCM experiments. The decreased solvation experienced by the brushes in K_2SO_4 solutions is likely to be due to the SO_4^{2-} ions bridging neighboring quaternary ammonium salts. On the other hand, the large Ca^{2+} ions released in $CaCl_2$ solution bind less closely to the negatively charged phosphate moiety of the PC headgroup and therefore remain in solution screening electrostatic interactions in the same way as the $2Cl^-$ ions released in solution.

Nanofriction Measurements. Quantitative nanofriction measurements were made on PMPC brushes in aqueous media using a gold-coated AFM cantilever. The nanofriction data could be fitted using an equation that treats the friction force as the sum of a load-dependent molecular plowing term and an area-dependent shear term that results from adhesion between the probe and the counter surface⁴²

$$F_F = \mu(F_N + F_a) + \pi \left(\frac{R}{K} \right)^{2/3} \tau (F_N + F_a)^{2/3} \quad (4)$$

where F_F is the friction force, μ is the coefficient of friction, F_N is the load applied perpendicular to the brushes, F_a is the pull-off force, R is the probe radius, K is the elastic modulus, and τ is the surface shear strength. A linear friction–load relationship was observed for PMPC brushes in aqueous ionic media, which is consistent with that of previous FFM studies^{26,40} using gold-coated cantilevers. This indicates that the contact mechanics of the gold-coated AFM tip on PMPC brushes in aqueous solutions is dominated by molecular plowing effects with the area-dependent adhesion contribution to friction (which varies with two-thirds power of the load) being negligible. The coefficient of friction was then determined from the gradient of the friction–load plot.

Both the ellipsometry result and previous force measurements⁴⁰ confirm that the contact area between the AFM probe and a PMPC layer is independent of salt concentration; therefore, any observed change in friction force could be attributed to the effect of salt on either the gold-coated AFM probe, the PMPC molecules, or a combination of both. For examining the effect of salt on the gold surface and the impact of the changed viscosity of salt solutions on friction measurements, a series of control experiments were made for a gold-coated cantilever against a gold-coated substrate. The data showed that the salt concentration used in the present study has a negligible effect on friction between the two gold surfaces, confirming that changes in μ collected on PMPC brushes are solely related to the changes within the brush layer.

The coefficients of friction of PMPC brushes were measured in salt solutions with concentrations in the range of 1–300 mM. A selection of salts, including LiCl, NaCl, KCl, and $CaCl_2$, was used to evaluate the effect of cation size and valency on the nanotribological properties of PMPC brushes. Figure 3a shows the resulting coefficients of friction. It can be seen that, in general, an increase in salt concentration causes a decrease in the value of μ : as the salt concentration increases, PMPC brushes become more lubricious. There are reduced entanglements between PMPC molecules, which results in decreased energy dissipation during plowing by the AFM probe. The

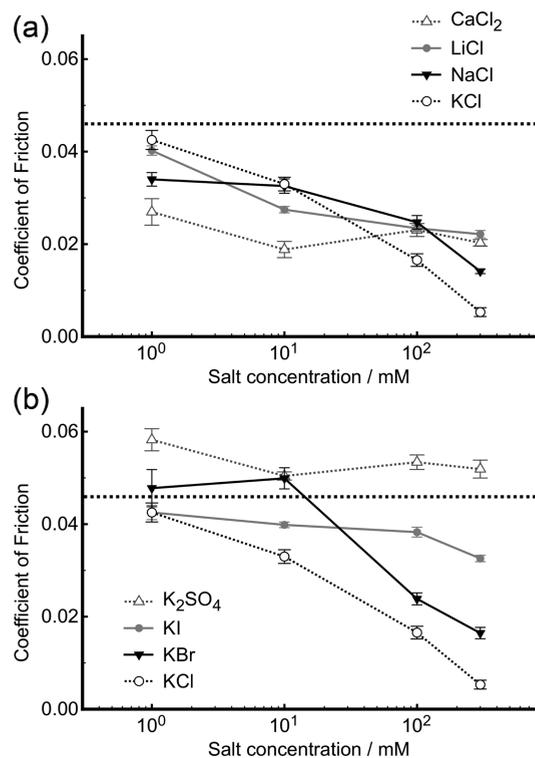


Figure 3. Mean coefficients of friction, μ , obtained for PMPC brushes immersed in aqueous solutions as a function of the salt concentration. The dotted line represents the coefficient of friction acquired in pure water. Error bars represent the standard (statistical) error of the mean of five measurements.

smallest coefficient of friction was measured for PMPC brushes in a 300 mM KCl solution, approximately one-tenth of the mean value determined in pure water. This trend is consistent with previous force measurements on the normal direction that show reduced adhesion between the AFM tip and PMPC brushes with increasing salt concentration.⁴⁰

For KCl, the coefficient of friction decreased with the logarithm of the salt concentration. For NaCl, a continuous decrease in the value of μ was also observed. Although the coefficient of friction was slightly smaller in NaCl than in KCl at a concentration of 1 mM, it was greater at the two largest concentrations (100 and 300 mM) studied. In LiCl solutions, the extent of change in μ with increasing salt concentration was less marked: the value of μ was similar to that measured in KCl at concentrations of 1 and 10 mM but was greater than that in KCl at higher concentrations. Finally, unlike the above-mentioned monovalent salts, $CaCl_2$ had a noticeable impact on the frictional properties of PMPC brushes at low concentration (1 mM). However, the coefficient of friction of the PMPC brush layer became relatively constant with further addition of $CaCl_2$.

In general, it can be seen that addition of salts causes the coefficient of friction of PMPC brushes to decrease. Broadly speaking, within the salt concentration range examined, the effect of KCl on the frictional properties of PMPC brushes is the most substantial, followed by NaCl, and then LiCl. Such a sequence, $\mu(K^+) < \mu(Na^+) < \mu(Li^+)$, follows the Hofmeister series that is an empirical classification of ions according to their effect on protein precipitation.⁴³

Another series of salts, including KCl, KBr, KI, and K_2SO_4 , was selected to evaluate the effect of anions on PMPC brushes.

Figure 3b presents the mean coefficients of friction acquired on PMPC brushes immersed in solutions of these salts. It can be seen that, for the three halide salts, there is in general a reduction in the value of μ as the concentration of salt increases. However, the magnitude of the change is more modest for KI than is the case for KBr and KCl. Only at a concentration of 100 mM does the difference between the coefficients of friction in pure water and in KI solution start to become significant. The small effect that KI has on PMPC was also reported by Mahon and Zhu.¹⁹ At concentrations of 100 and 300 mM, the coefficients of friction follow the order $\mu(\text{Cl}^-) < \mu(\text{Br}^-) < \mu(\text{I}^-)$. At lower concentrations, there is a much more modest difference between the value of μ in pure water and in salt solution, and the trend is less clear.

Rather different behavior was observed in aqueous solutions containing the sulfate anion. Across the full range of concentrations studied, K_2SO_4 yielded similar coefficients of friction, and there was no evidence of concentration dependence in the friction behavior observed; detailed analysis will be presented in the discussion below.

It is notable that the friction coefficients acquired in the present study are one or two orders greater than those obtained from previous studies where a surface force balance or tribometer were used.^{21,44} It is important to note that there are significant differences between the experimental systems used here and in those earlier studies: gold-coated AFM cantilevers were used in contact with PMPC brushes in this work, whereas earlier studies concerned either bilayer/bilayer or brush/brush contacts. Furthermore, bilayers in their fluid state are intrinsically less lubricious than those in the gel state even when they bear a similar charge. The PC groups present along PMPC chains are analogous to bilayers in the fluid state.^{44,45}

DISCUSSION

Effect of Salt Concentration on Brush Thickness. To interpret the seemingly contradictory result that addition of salt causes increased surface mass and decreased coefficient of friction without a change in brush thickness, we begin by rationalizing the effect of ions on the phosphorylcholine (PC) group, the primary functional group of PMPC. Even though conventionally it was thought that the influence of ions on the properties of macromolecules was caused at least in part by “making” or “breaking” bulk water structure, recent studies have confirmed that direct interactions between ions and macromolecules are equally important.⁴⁶

Electrolyte solutions strongly influence the physicochemical properties of PC-contained lipid membranes.^{47–52} Previous studies suggest that the addition of salts could cause local changes in the rates of lateral diffusion of phospholipids, the headgroup tilt, and the membrane thickness upon the association of ions.⁵³ Recent work by Vácha and co-workers reported that anions could pair with the positively charged lipid choline group, whereas cations can create contacts with the negatively charged phosphate groups and oxygen in the carbonyl groups.⁵⁴ When immersing a single-component biological membrane (1,2-dilauroyl-*sn*-glycero-3-phosphocholine, DLPC) in monovalent salt solutions, it was found that the swelling of the lipid multilayer increased monotonically with increasing salt concentration.^{47,55} Such changes in swelling behavior of DLPC membranes were explained by the fact that both the electrostatic repulsion and the van der Waals attraction between lipids are screened progressively with

increasing salt concentration, which results in a swelled multilayer of DLPC. A similar conclusion was also made in separate work by Aroti and colleagues.⁴⁹

However, as for PMPC brushes, it has been proposed that the addition of salts does not cause the loss of hydration lubrication, which is due to the unique structured water molecules taken around PC groups. Literature suggests that there are up to 25 molecules surrounding each PMPC repeat unit, and more importantly, these repeat monomer units do not disturb the hydrogen bonding network between water molecules.^{56,57} The hypothesis that interfacial water molecules near the PC-based self-assembled monolayer (SAM) surface do not form hydrogen bonds with the PC moiety and that they have reorientational dynamics similar to those of bulk water molecules has been supported by other works.⁵⁸ It is believed that water molecules in the hydration layer act as very efficient lubricants while ions bind on polymer chains, and at the same time, the hydration water molecules are able to rapidly exchange with other hydration or free water molecules and are thus able to behave fluidly when sheared at rates lower than these exchange rates.^{21,23}

In the present work, the thickness of hydrated PMPC brushes was found to be consistent in all salt solutions, which indicates that the degree of interaction between water molecules and PMPC chains is independent of ionic strength and the type of salt. Such a finding is consistent with most of the previous studies where the dimensions of PMPC chains either in free form or immobilized on a surface were measured as a function of salt concentration.^{14–18} Similar results were reported in other PC-contained systems. When exposed to salt solutions of various concentrations, the structure of a PC-surfactant monolayer adsorbed on the surface of water was examined using neutron reflectometry.⁵⁷ It was found that salt has little effect on the structure of the PC-surfactant monolayer, including the distributions for the head and alkyl chain and the relative location of each. Furthermore, for PC-based bilayer systems, no significant changes of the bilayer structure were observed when changing the salt concentration to less than 1 M, whereas substantial alterations with chain order, membrane thickness, and membrane rigidity were observed.^{51,55} The influence of segmental dipole orientation on the solution properties of PMPC molecules was examined by dynamic light scattering in a recent work.⁵⁹ It was suggested that PMPC is a swollen excluded-volume chain without significant presence of dipolar correlations, as confirmed by the lack of sensitivity to the ionic strength of the solution.

It is likely that, due to the unique structure water molecules adopt around PMPC brushes, binding of ions to the PC group does not affect the hydration state of PMPC molecules and hence the dimensions of the PMPC chains. However, the binding of ions to PC groups does have an impact on the intra- and intermolecular forces of PMPC brushes. For example, the second virial coefficient A_2 , used to describe the excluded volume effect as a consequence of repulsive intermolecular interaction, was found to increase with the addition of salt.¹⁷ This explanation could be applied to describe the discrepancy between the results reported previously: no salt effect on PMPC was found with systems measuring structure or hydration state of PMPC chains, whereas a notable impact of salt on PMPC was found when using techniques that measure the interactions between PMPC molecules.

Effect of Specific Ions. Although numerous studies,^{50–52,54} both experimental and theoretical, have been performed to

rationalize the effect of ions on phospholipid membranes, no clear consensus regarding the general mechanism has been established. It is widely accepted that, (i) for all ions in the alkali group, lithium shows the strongest affinity to the carbonyl group, binding of sodium is noticeable, and potassium has a very weak binding ability, and (ii) halide anions follow the Hofmeister series in that iodide > bromide > chloride. The binding of potassium ions to PC membranes was found to be much weaker compared to that of Na^+ .⁶⁰ Molecular dynamics simulations showed that iodide exhibits a genuine affinity for the PC-contained membrane due to its pairing with the choline group and its propensity for the nonpolar region of the acyl chains.⁵⁴

In the present work, it is easy to understand the QCM result in which lithium and iodide cause the largest changes in both frequency and dissipation signal. Because no other solvent was introduced at each concentration, changes can only be attributed to the binding of ions. Given that lithium and iodide have the strongest affinity to the PC group, followed by sodium and potassium, or bromide and chloride, the QCM result is consistent with previous works examining the effect of salt on PC-contained membranes.

The effects of specific cations and anions on PMPC observed here are consistent with the hypothesis^{61,62} that the effectiveness of the anions in reducing the dipole potential was $\text{I}^- > \text{Br}^- > \text{Cl}^- > \text{SO}_4^{2-}$. Such an order could be modeled by a partitioning of ions between the membrane and the aqueous phase, which is predominantly controlled by the Gibbs energy of hydration. Cations were also found to be capable of reducing the dipole potential, although much less efficiently than that of anions. The effects of the cations was found to follow trivalent > divalent > monovalent.

Impact of Ions on Nanotribological Properties. In the nanofriction measurements, gold-coated AFM cantilevers were used to minimize the electrostatic interaction between tip and polymer, so that the response of the PMPC brush layer to external stimuli can be measured. Therefore, the frictional result presented in Figure 3 is attributed to the impact of ions on PMPC molecules in the brush layer only. It is probable that the friction force sensed by the AFM cantilever while plowing through a PMPC brush layer is a combination of intra- and intermolecular electrostatic repulsion and van der Waals attractions between surface-anchored PMPC molecules, as depicted schematically in Figure 4. Unlike other polymer brush systems in which the hydration state of polymeric chains determines their nanotribological performance, the hydration state is independent of the salt concentration in the PMPC brush system. It is likely that, with the addition of salts, more ions bind to the PC groups and both intra- and intermolecular electrostatic repulsion become greater, and hence, there is a reduced degree of entanglement between PMPC chains. Therefore, the coefficient of friction is reduced with the addition of salts to the aqueous solution.

It has been shown previously that the frictional and adhesion forces on PMPC brushes decrease by increasing the concentration of sodium chloride, whereas the hydration state of the brush layer, reflected quantitatively by the force–displacement curves, remains constant.⁴⁰ In a separate work, the tribological properties of solid-supported PC-based bilayers were measured in different solutions, from pure water to saline buffer, by Rieu and co-workers.⁴⁴ The authors concluded that the adsorption of ions to the zwitterionic bilayers has a substantial effect on the mechanical and tribological properties

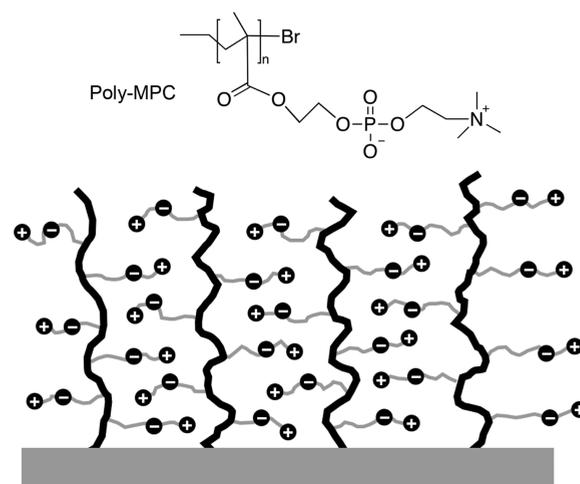


Figure 4. Schematic diagram (not to scale) illustrating the types of lateral interactions between surface-anchored PMPC chains that may explain the data presented here.

of bilayers: for example, a greater resistance to normal indentation due to an increase in bilayer cohesion and better lubrication due to an increase in bilayer–bilayer repulsion. Such a result is consistent with the explanation proposed in the present work.

Because the hydration state of PMPC brushes and the interaction between the AFM tip and brush layer do not play a role in the measured coefficient of friction, μ could be attributed as a combination of two factors: (i) intra- and intermolecular interactions between PMPC chains are screened, and (ii) the binding of ions causes PMPC chains to become stiffer, as demonstrated in previous work.⁶³ Although binding of ions to PC groups could screen the intra- and intermolecular interactions (networks of attractive noncovalent interactions) between PMPC molecules, it could also increase the rigidity of the PC group, which was confirmed by the decrease of bilayer elasticity and shift of the main phase transition temperature.^{51,53} Therefore, it is concluded that, although increasing ionic strength causes weaker interactions within the PMPC chains, which would effectively lead to a lower frictional coefficient, the different level of binding affinity of specific ions to PC groups causes the PMPC molecules to become more rigid. As a consequence, the tribological performance of PMPC brushes is dependent on the degree of ion binding. For example, the ions that have a strong tendency for binding, such as Li^+ and I^- , caused the most notable changes in QCM measurements; however, PMPC chains are more rigid and less lubricious than those in K^+ and Cl^- , where ions do not bind strongly to the PC groups. Although a stiffer chain could result in a more extended conformation, the densely packed PMPC brush layer, prepared by ATRP, warrants that the chains cannot stretch any further: hence, the brush thickness cannot easily increase.

Effect of Divalent Ions. Previous work on PC-based lipid systems suggests that divalent cations have a much greater binding affinity to a phospholipid headgroup than monovalent ions with an association constant on the order of $\sim 10 \text{ M}^{-1}$.^{50,51} The binding constant is reported to be 40 M^{-1} for Ca^{2+} , which indicates that one Ca^{2+} ion will be bound per ~ 3 free lipids in a 10 mM salt solution. The binding affinity of cations to PC membranes depends on several parameters; relevant to the current system are the ion–dipole interactions, whose strength

is determined by the valence of the cation.⁵⁰ It has been found that Ca^{2+} causes significant swelling of a PC-based lipid bilayer at low concentrations, but this gradually reduces as the salt concentration increases due to electrostatic screening. Significant swelling of multibilayers was found with a 0.09 M calcium chloride solution at which concentration the effect of Na^+ is negligible.

Knowledge extracted from the literature concerning bilayer systems is that the interaction between the PC group and ions is dependent on both concentration and type of ion. Similar behavior of Ca^{2+} ions was found in the present study: they cause the greatest changes of mass and energy dissipation in QCM measurements and promote PMPC brushes to be very lubricious at concentrations as low as 1 mM—an impact that was made by monovalent ions at 100 mM. However, the coefficient of friction remains similar in calcium chloride solutions from 1 up to 300 mM.

The effect of SO_4^{2-} on PMPC molecules is opposite to the other ions examined in this work: its interaction with the PC group is strongly unfavorable.⁴⁸ This is confirmed by the experimental result collected in the current work: both mass and energy dissipation decrease in QCM measurements, which indicates that fewer water molecules are associated with PMPC brushes. It is in agreement with the nanotribological result that the frictional coefficient of PMPC brushes increased slightly with the addition of K_2SO_4 as the hydration state of polymer brushes has a strong correlation to friction. Although SO_4^{2-} ions are not able to interact with PC groups, they could break the hydrogen bonding network of water molecules that are next to PC groups due to their kosmotropic nature.⁶⁴

CONCLUSIONS

To summarize, it is proposed that, due to the high degree of hydration of phosphorylcholine (PC) groups combined with the unique structure water molecules have surrounding PC groups, the binding of ions to PC groups does not change the hydration state nor the structure of PMPC brushes at all concentration ranges examined in the current study. However, the addition of ions could change the balance of intra- and intermolecular forces and stiffness of PMPC molecules. The different levels of binding affinity of ions to PC groups were regarded as the main explanation for the ionic-specific effect observed in the QCM results. A combination of the screening effect of repulsive electrostatic forces and the stiffness of the PC groups contributes to the difference between frictional coefficients for all monovalent salts. The divalent cation, Ca^{2+} , has a stronger affinity to PC groups than monovalent ions and hence improves the lubrication performance of PMPC brushes at a concentration as low as 1 mM. The interaction between the PC group and SO_4^{2-} is not favorable, and the addition of SO_4^{2-} can affect the hydrogen bonding network of water molecules next to the PC group, which effectively causes an increase in the friction coefficient.

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Notes

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