Boundary Lubricants with Exceptionally Low Friction Coefficients Based on 2D Close-Packed Phosphatidylcholine Liposomes

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Liposomes are widely used in pharmaceutical applications, primarily as drug delivery vehicles, as well as in gene therapy and for diagnostic imaging.[1–3] Here we report that certain phosphatidylcholine liposomes, when adsorbed onto sliding surfaces in a 2-dimensional close-packed array, may act as exceptionally efficient boundary lubricants at physiologically high pressures[4] under water. We created small unilamellar vesicles (SUVs) of hydrogenated soy phosphatidylcholine (HSPC) lipids which self-assemble in close-packed layers on solid surfaces to reduce the coefficient μ of sliding friction between them down to values $\mu \approx 10^{-4} - 2 \times 10^{-5}$, at pressures up to at least ca. 12 MPa (ca. 120 atmospheres). Such low values of the friction at these high pressures have not been attained with any boundary lubricants. This remarkably low friction is attributed to lubrication by the highly-hydrated phosphocholine head-groups exposed at the vesicle walls, stabilized against high pressures by the close-packing and by the rigidity of the gel-phase liposomes.

A dispersion of HSPC-SUV with a unimodal size distribution (diameter 65 nm) was prepared as described below (Experimental); freshly cleaved mica surfaces were incubated in the dispersion, then rinsed and mounted in a surface force balance[5] (SFB) under water.

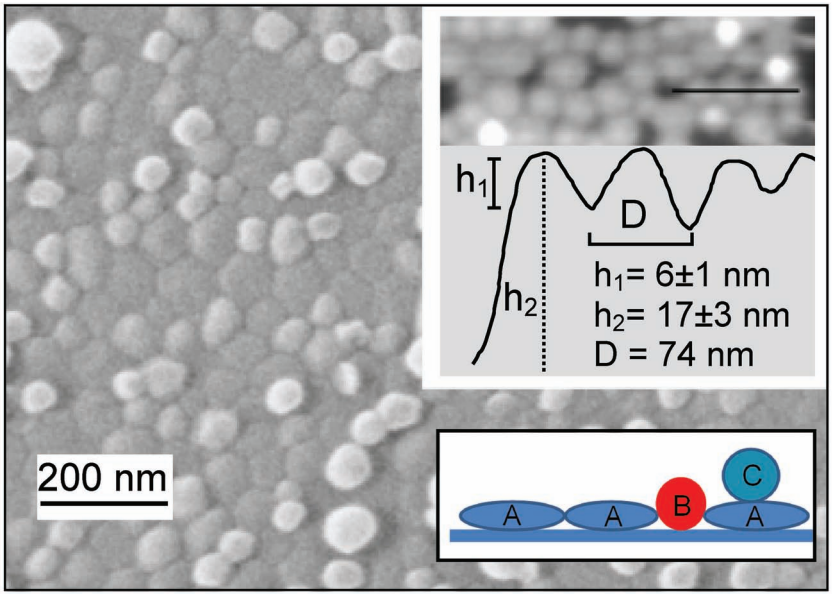

Figure 1. Cryo-SEM image of the HSPC-SUV adsorbed on freshly cleaved mica. The lower inset schematically interprets this in terms of liposomes that have flattened (A), those with less available space for flattening (B) and those (C) on top of the close-packed surface-attached layer that were not removed by the washing. The top right inset shows an AFM profile of the same close-packed layer; holes in the originally close-packed layer arise through removal of liposomes by the AFM tip.

Similar liposome-coated mica surfaces were imaged using atomic force microscopy (AFM) and cryo-scanning-electron-microscopy (cryo-SEM), as shown in **Figure 1**. The cryo-SEM image shows a honeycomb pattern characteristic of flattened close-packed spheres, overlaid by a loose, sparse layer of individual liposomes, which were not fully removed by the rinsing following the incubation. The AFM image (inset) indicates that the liposomes are flattened by the adsorption from their unperturbed dispersion diameter (ca. 65 nm) to ca. 20 nm; a very similar value for the thickness of the adsorbed liposome layers, 21±2 nm, was obtained by hydrodynamic measurements.

Normal and shear forces, $F_n(D)$ and $F_s(v_s, D)$ respectively, between the interacting, liposome-coated mica surfaces as a function of their closest separation D and sliding velocity v_s, were determined in the SFB[5,6] and are shown in **Figure 2**. At large separations $F_n(D)$ decays exponentially with D, with a decay length in the range 70±20 nm, characteristic of electrostatic double layer interactions. On closer approach, a sharper increase in the repulsion with decreasing D is attributed to

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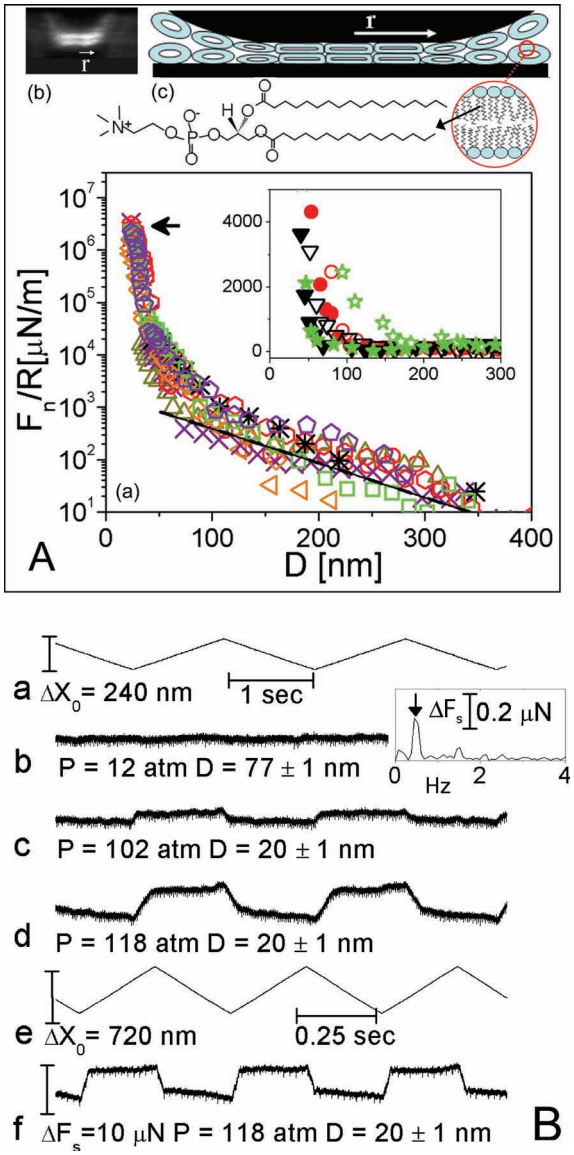

Figure 2. A) (a) SFB-measured[5] normalized force $F_n(D)/R$ first-approach profiles between interacting HSPC-SUV coated mica surfaces across water. Water had 18.2 MΩ cm resistance with total organic content levels of 1–4 ppb; pH = 5.8. The black line is the far-field force variation due to electrostatic double layers predicted by the DLVO model[30], in line with literature profiles[17,30] across no-salt-added water. The inset compares first approach (empty symbols) and second approach (corresponding full symbols) profiles from given contact positions. (b): The flattened interference fringes shown correspond to a pressure of 10 ± 1 MPa (arrow in (a)); $r = (2.2 \pm 0.3)\times10^{-5}$ m. They provide a direct section through the contact zone (schematically shown in (c)); the mean pressure $P = F_n/A$, where the contact area $A = \pi r^2$. B) Typical shear (or friction) force F_s versus time traces taken directly from the SFB. Trace a shows the applied back-and-forth lateral motion Δx_0, while traces b–d show typical responses at different compressions (and corresponding D values; the frequency analysis of trace b shows that at this pressure the shear force is within the noise level of the signal, i.e. smaller than can be measured with the SFB[8]. Traces e and f are respectively the applied lateral motion and transmitted shear force traces, for a 3-fold larger lateral motion amplitude and a 12-fold larger sliding velocity relative to trace d, revealing a similar magnitude of F_s for the same pressure, showing the measured shear forces are independent of the sliding amplitude.

steric interactions between the opposing liposome layers, due initially to the squeezing-out of the 'loose' vesicles on top of the surface-attached layers (Figure 1), and subsequently to the distortion of the adsorbed liposome layers themselves. At the highest pressures accessed in our study the surfaces approach to a 'hard-wall' repulsion at a separation $D_{hw} = 21\pm2$ nm, corresponding to 4 bilayers arising from the two flattened liposome layers. At these compressions the surfaces of the originally curved mica surfaces become flattened, as seen in the shape of the optical fringes, Figure 2A(b). The onset of steric repulsion on a second and subsequent approach at any given contact point, at $D \approx 40$–50 nm, is significantly closer in than on first approach, inset to Figure 2A(a), suggesting that the loose liposomes on top of the adsorbed layer are irreversibly squeezed out of the contact region on compression and shear.

The shear or frictional forces F_s transmitted between the surfaces as they were made to slide past each other were determined at different compressions (mean pressures $P = (F_n/A)$ up to ca. 12 MPa, where A is the measured contact area), different sliding amplitudes Δx_0 and sliding velocities v_s ($5–2 \times 10^3$ nm s^{-1}), and were recorded directly as a series of shear-force F_s versus time traces.[5] Representative traces are shown in Figure 2B, and reveal unambiguously that the transmitted frictional forces remain very low up to the highest pressures probed, P = ca. 12 MPa. On a second and subsequent compression run at a given contact point, the frictional forces were systematically smaller for a given pressure than on a first approach. Within the noise, F_s values at all pressures, shear amplitudes and shear velocities studied were constant throughout a given trace (up to at least several hundred cycles), indicating the stability of the lubricating layers over the range of our parameters.

The F_s versus F_n results are summarized in **Figure 3**, revealing friction coefficients $\mu = (F_s/F_n)$ in the range $\mu = (2 \times 10^{-3} – 5 \times 10^{-4})$ at pressures up to ca. 6 MPa on a first compression, with systematically lower μ on a second and subsequent compressions at a given contact point, as shown by the solid symbols in the main Figure 3A (and inset). We attribute this change to the squeeze-out of the loose liposome over-layer following the first compression, corresponding to the change in $F_n(D)$ following the first approach. At higher loads the shear forces reveal extremely low friction coefficients, down to $\mu = 2 \times 10^{-5}$, as shown by the curves in Figure 3A (inset), up to the highest mean pressures attained in this study, P = ca. 12 MPa. The dependence of F_s on v_s is shown in Figure 3B for different high pressures, indicating, within the scatter, little variation in friction over nearly 3 orders-of-magnitude in sliding velocities ($5–2 \times 10^3$ nm s^{-1}).

These findings demonstrate that close-packed phosphatidylcholine liposome layers, under water, may reduce the coefficient of sliding friction μ between surfaces, at pressures up to over 100 atm, to levels $\mu \approx (10^{-4} – 2 \times 10^{-5})$. This is lower than has hitherto been attained by any boundary lubricant system at such high pressures in aqueous media, including the best naturally-occurring lubrication, which is that between healthy sliding articular cartilage surfaces in major mammalian joints,[4,7] and has clear implications for lubrication in aqueous systems. This is of particular relevance in the light of the possible role conjectured for surface–active phospholipids in the very efficient lubrication of synovial joints and other

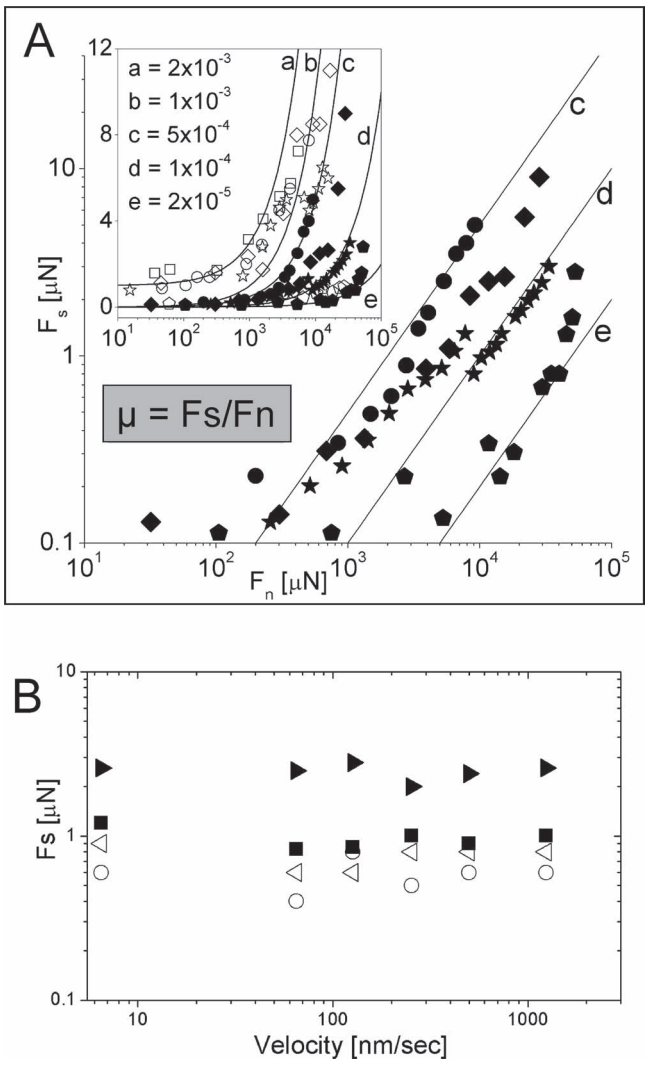

Figure 3. A) Friction forces F_s versus applied loads F_n between two HSPC-SUV-coated mica surfaces, based on traces such as in figure 3. The inset shows the F_s versus F_n variation on a first approach (empty symbols) and on second or subsequent approaches (corresponding filled symbols). Curves a–e show the variation $F_s = \mu F_n$, with friction coefficient μ values as indicated in the inset. Pentagonal symbols (⬟, ⬠, for which the lowest friction is indicated) are from an experiment where excess liposomes were washed for 5 min following incubation in the liposome solution, in contrast to 1 min washing for other symbols. B) Friction forces F_s variation with sliding velocity v_s for different compressions (○ 74 atm; ◁ 94 atm; ■ 107 atm; ▶ 118 atm) showing little variation within the scatter over nearly 3 decades in v_s.

biological tissues.[8,9] Earlier work[10] has examined liposome layers using a friction-force microscope (though the sharp tip with radius smaller than the liposomes may lead to ploughing effects), and liposomes added to soft (cartilage) surfaces using a tribometer,[11] as well as lipid bilayers.[12,13] Recently friction coefficients down to 4×10^{-4} at pressures up to 7.5 MPa were observed between covalently grafted polyzwitterionic brushes.[6] Values of ca. 2×10^{-3} or lower have also been measured between other boundary lubricants, including neutral polymer brushes in organic solvents, polyelectrolyte brushes in aqueous media, surfactant monolayers and lipid bilayers, as well as gel surfaces and charged surfaces across hydrated ions.[14–21] All these, however, were at mean contact pressures of only up to 0.3 MPa or less, lower by 40-fold or more than that attained in the present study.

The extreme reduction of friction between the close-packed liposome layers is attributed primarily to the hydration layers surrounding the phosphocholine groups at the outer surface of the opposing vesicle layers as they slide past each other. Such phosphocholine groups (Figure 2A) are highly hydrated, with up to 15 water molecules reported–depending on the method of measurement–in the primary hydration shell for the case of liposomes in the gel state,[22–25] i.e., at temperatures $T < T_m$, the gel-to-liquid-crystalline transition temperature (as applies for the HSPC liposomes in our study). These hydration water molecules are tenaciously attached, yet rapidly-relaxing,[16] thereby providing a ball-bearing like effect,[16] which has been termed 'hydration lubrication'. This arises because the water of hydration can sustain a large pressure without being squeezed out from between the surfaces, while at the same time the hydration shells relax rapidly, ensuring a fluid like response on shear as long as the shear rate is less than the relaxation rate.[16] In contrast to earlier studies[14,16,17,21] where hydration lubrication was implicated, the hydrated phosphocholine layers exposed by the liposomes in our system display an extreme stability to pressure, attributed to three factors: The closed vesicular nature of the liposomes and their close-packing on the surfaces, which is driven by attraction between their exposed dipolar phosphocholine groups and the negatively charged mica surface,[26] as shown directly by separate experiments (Experimental); secondly, the uniformity of the vesicle layer arising from their size distribution; and finally, in particular the rigidity of the liposomes arising from their being in the gel-phase. These three factors, together, in particular, with the hydration lubrication mechanism arising from the exposed, highly-hydrated close-packed phosphocholine groups, contribute to the uniquely efficient lubrication we measure relative to earlier studies. The contact region during the sliding may consist of fused adjacent liposomes leading to a continuous lamellar phase, or of closed, flattened vesicles. The latter scenario (tentatively shown in the cartoon in Figure 2A(c)) is consistent with a number of observations: The large range of steric forces, $D \approx 40$–50 nm, on second and subsequent approaches at a given contact point, which is also consistent with the independently determined hydrodynamic thickness of 21±2 nm per layer following compression and shear (whereas fusion to a lamellar phase would lead to much shorter steric range, ca. 20 nm); the variation of $F_n(D)$, yielding a bending rigidity which remains comparable to that of gel-phase liposomes on a second and subsequent approach (see Experimental Section); and the fact that when liposomes in their less-rigid liquid-crystalline phase (below) are compressed, they do undergo rupture and "squeeze-out" from between the surfaces.

Other measurements, summarized in **Table 1**, support our attribution of the low friction to the hydration-lubrication mechanism. When the highly-hydrated outer phosphocholine layers are screened by using poly(ethylene glycol)(PEG)-coated-HSPC-SUV[1] layers as the boundary layers, the friction is very much higher than with the pure HSPC component: This is consistent

Table 1. Sliding friction coefficient $\mu = (F_s/F_n)$ between two liposome-coated mica surfaces sliding past each other (on second and subsequent approaches) up to maximal pressures P_{max}.

Liposome	P_{max} (MPa)	μ
HSPC-HUV	11.8 ± 0.5	0.0004 – 0.00002
POPC-SUV	1.2 ± 0.3[a)]	0.065 ± 0.035
PEG-HSPC-SUV	1.0 ± 0.2[a)]	0.1075 ± 0.084

[a)]At higher pressures the frictional force was higher than the maximal shear force that could be applied in the SFB, so that no sliding between the surfaces occurred.

with the idea that it is the hydrated PC headgroups that provide the lubrication, and coating them by PEG – which is known to be only-weakly hydrated and a relatively poor lubricant[27] – at the outer liposome surfaces greatly increases the friction. The role of liposome rigidity is emphasized by measuring the friction between mica surfaces each coated with a layer of similarly-sized SUVs of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), which, unlike the HSPC-SUVs, are in the liquid-crystalline phase at room temperature (T_m(POPC) = –3 °C). We found that such layers provided poor lubrication (and at pressures larger than ca. 1 MPa the friction was larger than could be measured in the SFB, Table 1). Normal force profiles between surfaces coated with POPC-SUVs (not shown), indicated that at higher pressures these more fluid liposome layers collapsed and were partly squeezed out from between the surfaces, attributed to their being in the liquid-crystalline-phase (rather than the more rigid gel phase HSPC-SUVs). Importantly, the efficient lubricity of HSPC liposomes described here extends also to physiological salt concentrations: A recent study (Goldberg et al., to be published) shows that such liposomes result in high pressure friction coefficients as low as 6×10^{-4} at salt concentrations up to 0.15 M, and we are also extending this work to non-atomically-smooth surfaces.

In summary, we have shown that certain phosphatidylcholine liposomes may form close-packed boundary layers on surfaces under water that lead to a striking reduction in the sliding friction, resulting in friction coefficients down to 2×10^{-5} at pressures up to more than 100 atm. This is attributed to hydration-lubrication by the highly-hydrated phosphocholine groups exposed at the surface of the liposomes, together with the very robust nature of the adsorbed layers of vesicles, arising from their closed structure, their uniformity and close packing on the surface, and particularly their rigidity by virtue of being in the gel phase.

Experimental Section

Surface Force Balance: The SFB and its protocols for measuring normal and shear forces have been described in detail.[5] Our results are based on six independent experiments (different pairs of mica surfaces), each with multiple contact points. All measurements were carried out at 23.5 ± 0.5 °C.

Liposomes and characterization: Multilamellar vesicles (MLVs) were prepared by hydrating HSPC at 62 °C (above the gel-to-liquid crystalline phase transition temperature, T_m, of HSPC, 53 °C).[1] MLVs were downsized to form SUVs, ~65 nm in diameter, by stepwise extrusion through polycarbonate membranes starting with a 400-nm and ending with 50-nm-pore-size membrane, using a Lipex 100 mL extruder system (Northern Lipids, Vancouver, Canada). The HSPC-SUV liposomes were characterized for size distribution by dynamic light scattering and further by cryo-SEM (Figure 1) as described in ref. [28].

Hydrodynamic Measurements: These were carried out in the SFB by measuring the forces $F_n(D)$ arising via hydrodynamic interactions when one surface is oscillated at large separations D, and yield the hydrodynamic layer thickness, as described for macromolecular layers,[29] and also in ref. [28].

Measurement of HSPC-SUV Adhesion to mica Surface: An HSPC-SUV-coated mica surface and an opposing bare mica surface adhere under water, as measured in the SFB. A surface energy $\gamma_{HSPC/mica} = -14 \pm 4$ mJ m^{-2} was evaluated from the Johnson-Kendall-Roberts relation[30] $F_{pulloff} = 3\pi R\gamma_{HSPC/mica}$, where R is the mean radius of curvature of the uncompressed surfaces and $F_{pulloff}$ is the SFB-determined force to separate the surfaces.

Liposome Bending Rigidity: The Young's modulus E = [stress (σ)/strain (ε)] of the liposomes was estimated from the $F_n(D)$ versus D profiles [21]. From the relation[31] for the bending rigidity or modulus $K_C = Eh^3/[12(1-\nu^2)]$, where h is the liposome wall thickness (taken as 5nm for the HSPC bilayer) and ν is the Poisson's ratio (taken as 0.5), these Young's moduli yield estimates[28] for $K_{C,HSPC}$ and for $K_{C,POPC}$, the bending rigidity of the HSPC and POPC liposomes, consistent with the values expected for these respective gel-phase and liquid-crystalline-phase vesicles.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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