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# Tribological efficacy and stability of phospholipid-based membrane lubricants in varying *p*H chemical conditions

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In this study, the authors examine the influence of joint chemical environment by measuring changes in the tribological properties (friction coefficient and charge density) of contacting surfaces of normal and degenerated cartilage samples in bath solutions of varying *p*H (2.0–9.0). Bovine articular cartilage samples (n = 54) were subjected to several surface measurements, including interfacial energy, contact angle, and friction coefficient, at varying *p*H. The samples were delipidized and then subjected to the same measurement protocols. Our results reveal that the interfacial energy and charge density, which have been shown to be related to friction coefficient, decrease with *p*H in the acidic range and approach constant values at physiological (or synovial fluid) *p*H of 7.4 and beyond it, i.e., toward basic *p*H domain. The authors conclude that this rather complex response explains the long-term efficacy with respect to ageing and associated *p*H changes, of the phospholipid layers that facilitate the almost frictionless, hydration–lubrication involving contact in the mammalian musculoskeletal system. © *2016 American Vacuum Society*. [http://dx.doi.org/10.1116/1.4939246]

## I. INTRODUCTION

Articular cartilage plays an important role in joint mobility by reducing static contact stresses and facilitating lubrication and wear resistance. The lubrication provided by articular cartilage is almost frictionless[1] and has been attributed to the presence of a nanothick lipid-filled surface amorphous layer (SAL)[2] and the pressurized fluid that weeps out from its fluid-saturated gel-like matrix during physiological function.[3,4] This surface amorphous layer is a multibilayered porous membrane that provides a very low sliding friction and comprises mostly of polyanionic ($-PO_4^-/-PO_4^-$) cartilage/cartilage surfaces that is surrounded by electrolyte with charged macromolecules (proteoglycans aggregate, phospholipids spheres, and hyaluronan).[5]

The importance of the structure of multibilayered porous membranes to the functions and well-being of mammalian joints, particularly in articular cartilage, and other areas of the musculoskeletal system, such as alveoli, pleura, and peritoneal cavities, has been recognized and highlighted by many researchers.[6–9] It has been argued that infant distress syndrome is attributable to the breakdown of the saturated phospholipids of the chest cavity,[10] while articular cartilage degeneration is known to be accompanied by loss of the surface active phospholipids (SAPL) overlay that facilitates its lubrication function. One of the prominent risk factors associated with joint tissue degeneration is ageing, where a significant population above the age of 60 years suffer from osteoarthritis,[11] a condition which is often preceded and often initiated by loss of the lipid layer of articular cartilage,[12,13] with subsequent loss of superficial proteoglycans (PGs) and disruption of surface collagen network.

With regards to articular cartilage,[14] it was reported that age affects cartilage composition, while another study[15] demonstrated that there is a strong relationship between *p*H, age, and friction coefficient. This is partly because as

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articular cartilage ages, the amount of superficial chondrocytes, responsible for synthesis of SAL, decreases.[16] Thus, the joint function degenerates as the lubrication efficiency of the SAPL layer is altered.[17,18] In addition, it is also arguable that changes in the joint environment during degeneration play a significant role in decreasing the lubrication efficiency of the SAPL layer.[17,18]

Variations in the acid–base (*p*H) equilibrium of the synovial fluid have strong influence on the electrical charge of articular cartilage and its frictional properties. The change in electrical charge is due to the binding of hydrogen or hydroxyl ions on the functional groups of PGs, collagen, and phospholipids (PLs). The electrical charges of the nonsoluble and solid matrix components of articular cartilage contain *p*H-sensitive functional groups, including PLs ($-NH_3^+$ and $-PO_4^-$), collagen ($-NH_3^+$ and $-COO^-$), and PGs ($-COO^-$ and $-SO_3^-$) (Fig. 1). The aggregate of PGs, collagen, and phospholipids isoelectric point (IEP) corresponds to the negatively charged carboxylate group ($-COO^-$), sulfate group ($-SO_3^-$), phosphate ($-PO_4^-$), and positively charged amino groups ($-NH_3^+$). The reported values of the IEP for bovine articular cartilage (AC) is in the *p*H range ~2.5–3.5 (Refs. 18–21) and 4.1 in other studies.[22–24]

The plots (Fig. 1) show that there exists a stable *p*H range, between 6 and 8, with the charged macromolecules and synovial fluid in the presence of phospholipids, proteoglycans aggregate, PGs, and hyaluronate, giving a support for frictionless lubrication on the articular surface. The pKa of their sulfate groups are about 2, and that of their functional groups are carboxylate groups in the range 3.5–5, phosphate group is between 2 and 3, and the amino groups' pKa are greater than 9.

At *p*H of ~7.4, which is the physiological *p*H (or synovial fluid), phospholipids of articular cartilage can be considered as being negatively charged and collagen being electrically neutral.[19,20] The pKa of the different functional groups involved in articular cartilage lubrication are shown as a function of *p*H (Fig. 1). In articular cartilage, the most functional groups of *PGs, collagen, and PLs*, e.g., ($-PO_4^-$), ($-COO^-$), and ($-SO_3^-$) are negatively charged at *p*H 7 ± 1.

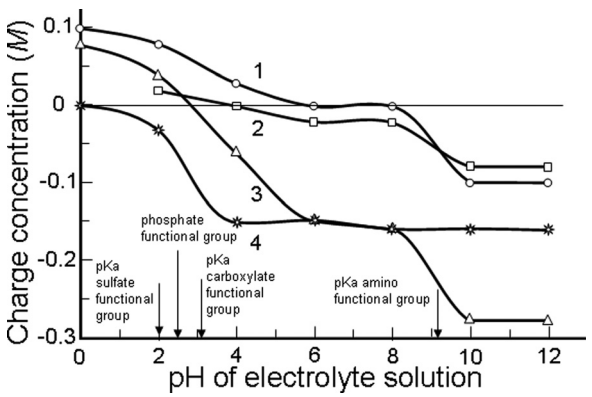


FIG. 1. Electrical charges of the nonsoluble and solid matrix components of articular cartilage: collagen (curve 1), phospholipids (this study curve 2), proteoglycan aggregates (curve 4), and synovial fluid (curve 3) vs *p*H of electrolyte. For comparison, data for curves 1, 3, and 4 were obtained from the literature (Ref. 21).

In this paper, we attempt to establish the nature of the relationship between changes in *p*H and the tribological characteristics of the amphoteric cartilage surface, including interfacial energy, charge density, and friction coefficient. To meet this objective, a model of phospholipid membranes was created, and the interfacial energy measured, since the interfacial energy, charge density, and the friction coefficient of the joint mechanism are interrelated.

## II. MATERIALS AND METHODS

### A. Materials

In the experiment, we used phosphatidylserine (PS) and sphingomyelin (SM) (both 99% pure, Fluka AG, Switzerland) as a synthetic phospholipid substance to make model membrane (used in the form of liposomes) for determination of interfacial energy. The solution used to form the model membrane contained 20 mg/ml of a lipid in *n*-decane. The lipid was dissolved in chloroform to prevent oxidation; the solvent was evaporated in an argon atmosphere, and the residue was dissolved in *n*-decane, which had been additionally purified by distillation. Buffer solutions covering were prepared according to Britton *et al.*[25] They were used by adding 0.2 M sodium hydroxide to 100 ml of a solution of the composition: 0.04 M acetic acid (80%, produced by POCh), 0.04 M phosphoric acid (POCh), and 0.04 M boric acid (POCh). A suitable *p*H of the buffer was adjusted using sodium hydroxide solution (at 291.15 K). The aqueous solution of 0.1 M KCl (*p*H 6.90) used for the measurements of interfacial energy of the membranes was prepared using triply distilled water and KCl (POCh). The *p*H of the electrolyte was carefully controlled during the measurements.

Articular cartilage specimens (n = 54) were collected from bovine knees aged 15–20 months. Osteochondral plugs, 5 and 10 mm in diameter, were harvested from lateral and medial femoral condyles using a circular stainless steel cutter. The cartilage disks were cut into 3-mm plugs with underlying bone. Two types of samples were tested: untreated bovine cartilage and bovine cartilage treated with a Folch reagent[26] (a 2:1 v/v mixture of chloroform and methanol), and a lipid-rinsing solution to remove the lipids from the surface of the cartilage. After preparation, the specimens were stored at −20 °C in saline of 0.155 M NaCl (*p*H = 6.9), and fully defrosted prior to testing. The cartilage disks were then glued onto stainless steel surfaces with upper cartilage holder, 5 mm diameter, and lower cartilage holder, 10 mm diameter, and friction tests were conducted in the universal buffer solution.

A radiometer *p*H-meter with an electrode (Schott-BlueLine 16 *p*H type) was used in the experiment. This instrument was calibrated according to the International Union of Pure and Applied Chemistry recommendations.[25] The apparatus and the microelectrophoretic method used are described in the literature.[18,27] The value of the interfacial energy $\gamma$ was measured in 8–12 replicates with about seven instrumental readings of the lipid spherical cap. The results of $\gamma$ as a function of *p*H are given in Fig. 2.

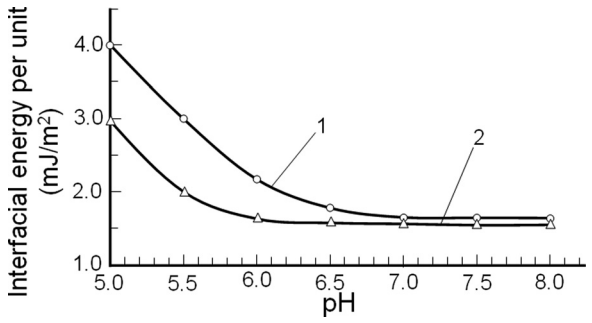


FIG. 2. Variation of interfacial energy of model membranes (used in the form of liposomes) formed by SM (1), PS (2) as a function of *p*H over the range 5.0–8.0. Interfacial energy (%) standard deviation (SD) 5–10.

### B. Delipidization procedure

The cartilage samples were delipidized using a Folch reagent (2:1 v/v mixture of chloroform and methanol) to gradually remove the PL bilayers from the cartilage surface.[26] The samples were immersed in the reagent mixture for 2 min, at the same meniscus. After PL extraction, the sample was placed in saline solution for 1 h to remove the residue of the solvent and promote rehydration. The wettability and friction coefficient of each sample surface before and after delipidization was then measured. Other authors have used isopropanol[28] and an enzymatic procedure with phospholipase A.[29,30] The delipidization procedure removed most of the PL although some amount of a hydrophobic proteolipid remained as a minor component.[9,30]

### C. Interfacial energy measurement

The interfacial energy $\gamma$ of the lipid bilayers was determined by measuring the radius of curvature $r$ of $a$ convex surface formed when a pressure difference $\Delta p$ is applied to its sides. This was based on Young's and Laplace's equation

$$2\gamma = r\Delta p, \tag{1}$$

where the components of Eq. (1) are as defined above.

The dependence of interfacial energy on the *p*H of electrolyte solution is defined by the equation below[29]

$$\gamma = \gamma_{\max} + 2sRT\ln\left(\sqrt{\frac{K_a}{K_b}} + 1\right) - sRT\ln\left[\left(\frac{K_a}{a_{H^+}} + 1\right)\left(\frac{a_{H^+}}{K_b} + 1\right)\right], \tag{2}$$

where $K_a$ and $K_b$ are acid and base equilibrium constants, respectively, $s$ (mol $m^{-2}$) is the surface concentration of phospholipids, $a_{H^+}$ is the hydrogen ion ($H^+$) concentration, $R$ is the gas constant, $T$ is temperature, and $\gamma_{\max}$ is the maximum interfacial energy of the lipid membrane.

### D. Contact angle measurements

The contact angle was measured using a KSV CAM100 computerized tensiometer. A drop of the 0.155 M saline solution was deposited on the air-dried cartilage surface. The contact angle measurements of the normal (not depleted), partial, and completely depleted cartilage samples were carried out under dry-air atmosphere at 295 ± 2 K and a relative humidity ~50 ± 5%, between 40 and 100 min of the sample drying time (Fig. 4).[31] The contact angle test, on the normal and partially delipidized cartilage samples, was repeated at least five times.

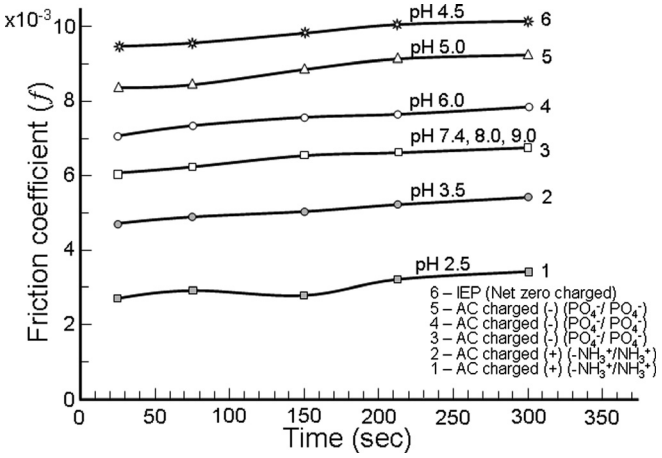


FIG. 3. Friction coefficient ($f$) for cartilage/cartilage tribopair tested using the Britton–Robinson universal buffer solutions at different *p*H values (2.5–9.0), under 15 N loading and 1 mm/s sliding velocity for 300 s. Friction coefficient (%) (SD) 10–15.

### E. Friction test in universal buffer solutions (*p*H 2.0–9.0)

Prior to the friction tests, the lubricants were prepared using the Britton–Robinson universal buffer solution[25] and its *p*H values were measured. The friction coefficient measurements of cartilage/cartilage tribopair were performed with *p*H values between 2.0 and 9.0. The tested samples were equilibrated with each buffer under load (15 N) for 5 min, and the friction coefficient ($f$) as a function of *p*H are presented in Fig. 3. A total of six tests were conducted using fresh samples for each experimental setup with at least four repetitions per specimen pair, from which the mean and standard deviation were calculated. The measurements were performed using a sliding pin-on-disk tribotester T-11 manufactured by the National Institute for Sustainable Technologies Research, Radom, Poland. The tests were conducted at room temperature, at a speed of 1 mm/s during 15 min, and under a load of 15 N (1.2 MPa) which correspond to the physiological lubrication condition.[27]

## III. RESULTS AND DISCUSSION

### A. Interfacial energy of phospholipid bilayer

This paper explains how phospholipid-based membranes maintain fixed electrical charges under varying *p*H conditions. The interfacial energy of contacting surfaces of lipid membranes was measured at varying *p*H of electrolyte solutions made from these lipids. Two distinct regions can be

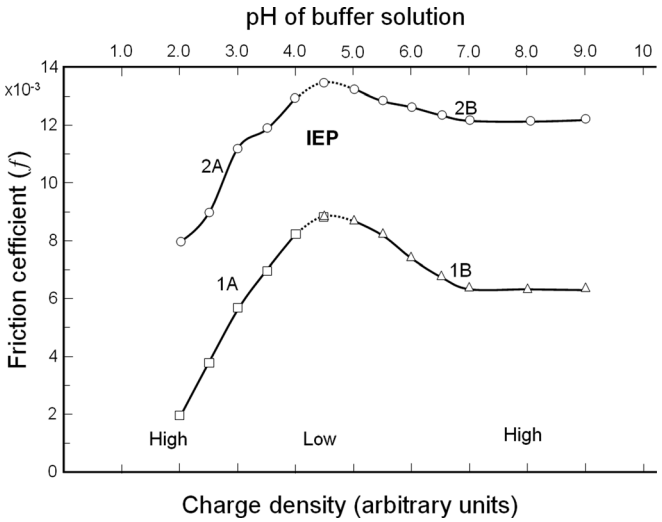


FIG. 4. Plot of charge density at the buffer solution *p*H against friction coefficient (*f*) of bovine cartilage. Curve 1A, 1B for normal cartilage, wettability of 103°; curves 2A and 2B for cartilage with partial surface lipid depletion, wettability of 83°. Friction coefficient (%) (SD) 10–15.

identified in the graph (Fig. 2): the acidic zone and the basic/alkaline zone.

The interfacial energy decreases in the acidic *p*H range (5.0–6.0), as a result of the $-PO_4H$ functional group losing proton ($-PO_4H > -PO_4^-$). Here, all the lipid membranes display unstable behavior, with the rate of decay of interfacial energy between membranes formed with SM and PS exhibiting highest rate of instability. The transition region lies in the *p*H range of 6.0–6.8, while the basic zone has a *p*H range of 6.5–8.0. The basic zone is a region of stability where all the model lipid membranes appear to be stable, corresponding to the safe *p*H range observed in biological systems ($pH \sim 7.4$). The surface of articular cartilage is covered by SAPL membranes, which exhibits similar behavior as the model membranes used in this study. The dependence of interfacial energy of the lipid membranes formed by the two model phospholipids (SM and PS) on *p*H of electrolyte solution is apparent from Fig. 2. Phospholipids, which are amphoteric molecules containing functional $-NH_2$ (and $-PO_4H$) groups, are affected by the solution's *p*H. The acidity constants of the phosphate group ($-PO_4H$) expressed by $pK_a$ are: 2.59 for SM and 2.42 for PE.[32] When the *p*H of the solution is increased from *p*H 5.0 to 8.0, the phosphate group begin to lose their charges (protons), i.e., ($-PO_4H \rightarrow -PO_4^-$), leaving the surface negatively charged, leading to a decrease in the interfacial energy.

The decrease in interfacial energy with *p*H change is strongly related to the increase in the polar part of the surface energy $(-PO_4)^-$. The interfacial energy has seemingly reached the inflection point ($\gamma \sim 1.6$ mJ/m) at $pH \sim 6.5$ and remains fixed for all the *p*H values tested afterward. This suggests that the minimum surface energy of the phospholipid $(-PO_4)^-$ group has been reached over a wide and safe *p*H range of 7.0–8.0, covering the biological $pH \sim 7.4$ condition.

### B. Solution *p*H versus friction of cartilage/cartilage surfaces

The friction between cartilage/cartilage surfaces carrying the same sign of charges was investigated using a friction tester. We observed that the friction coefficient was strongly dependent on the interfacial interaction between the two cartilage surfaces (Fig. 3).

The tests for curves 1 and 2 were conducted before the IEP using pairs of positively charged cartilage surfaces ($-NH_3^+/-NH_3^+$). Tests for curves 3, 4, and 5 were carried out after the IEP using pairs of negatively charged cartilage surfaces ($-PO_4^-/-PO_4^-$), while curve 6 was obtained at $pH \sim 4.5$ using cartilage surfaces at IEP with no net electrical charges [$NH_3^+ (CH_2)_n PO_4^-$].

The effect of interfacial interaction on the cartilage friction can be observed from the behavior between two cartilage/cartilage surfaces carrying charges. Figure 3 shows the *p*H profiles of the coefficient of friction for two kinds of cartilage surfaces, positively and negatively charged in aqueous buffer solutions. Polyanion ($-PO_4^-/-PO_4^-$) cartilage/cartilage surfaces and the polycation ($-NH_3^+/-NH_3^+$) cartilage/cartilage surfaces slid over each other and showed differences in the friction coefficient at the acidic and basic *p*H range. The friction of the polycation surface of cartilage is about three times higher than that of polyanions surface.

### C. Cartilage surface charge density

The dependence of friction between two cartilage surfaces on the network charge density is demonstrated in Fig. 4. Charge density modulation of cartilage surface was made by varying the buffer solution *p*H, resulting in different charge densities for cartilage, part A ($-NH_3^+$) and part B ($-PO_4^-$). The variations of friction coefficient of cartilage as a function of the charge density exhibit a nonlinear characteristic for these amphoteric charged surfaces. As shown in Fig. 4, the friction coefficient increased (curves 1A and 2A) and decreased (curves 1B and 2B), with the decrease and increase in the charge density, respectively. The increase in charge density enhances electrostatic repulsion between two cartilage surfaces, which favors the formation of a thicker water layer between the surfaces and thus decreases the friction.[33,34] From the above experimental results, it is demonstrated that the cartilage friction is largely dependent on the electrostatic interaction between two cartilage surfaces.[23,24] The change in friction between two cartilage surfaces carrying positive and negative charges can be attributed to the proton transfer reactions. The increase in friction (curves 1A and 2A) can be expressed by the reaction progressing on the cartilage surface: ($-NH_3^+ \rightarrow -NH_2$) and the decrease in friction (curves 1B and 2B) as a result of the $-PO_4H$ functional group loses proton ($-PO_4H \rightarrow -PO_4^-$).

Maximum friction was observed at a *p*H when cartilage surface was at the IEP. The isoelectric point is the *p*H, at which a phospholipid molecule carries no net electrical charge, e.g.,

$$H_2N(CH_2)_nPO_4H \rightleftharpoons H_3N^+(CH_2)_nPO_4^-.$$

The effect of the charge density was studied by sliding normal (AC/AC, wettability 103°) and modified surface (AC/AC, wettability 83°). The friction between two normal surfaces (AC/AC) with high charge density showed the lowest value (Fig. 4, curves 1A and 1B). With the reduced number of phospholipid bilayers, i.e., the decrease in negative charge density, cartilage exhibited much higher friction (Fig. 4, curves 2A and 2B). The above results demonstrate that the friction of cartilage surfaces is largely dependent upon the charge density of the cartilage. By varying the charge density of cartilage, the friction coefficient can be varied by about 1 order of magnitude in the examined experimental range. The modulation of charge density of the cartilage surface was achieved via phospholipids depletion process, which alters the swelling ability of cartilage. It should be noted that the degree of swelling of cartilage is high at low *p*H (2–4.5) or for a positively charged surface and no much change in swelling after IEP, or for a negatively charged surface.[24] Due to the electrostatic repulsion between charged surfaces ($-NH_3^+/-NH_3^+$) at low *p*H and high degree of swelling, a low friction coefficient is observed.[18,24] The degeneration of articular cartilage is also often combined with changes in the *p*H of the synovial fluid: rheumatoid arthritis 7.4–8.1 and osteoarthritis 7.4–7.6 with concomitant effect on the state of cartilage surface wettability.[18] Wettability contact angles of arthritic cartilage surfaces are 56°–80°, and the normal cartilage wettability is 105°. The fractional characteristics reveal that friction increased with *p*H of synovial fluid of rheumatoid and osteoarthritis joints.[5] The coefficient of friction measured depends largely on the electrostatics, as readily combined with protons' accepting and donating properties, presumably, with "proton wave" overall action, supporting the electrostatic hydration repulsion under departure from acid–base equilibrium state.[35–37]

## IV. CONCLUSIONS

In this study, we examined the influence of *p*H on phospholipid bilayer properties such as interfacial energy, charge density, and friction of the amphoteric cartilage surface. The surface interfacial energy of model PLs bilayer stabilizes at *p*H between 6.5 and 8.0, being negatively charged. We also showed that the superficial PL bilayer covering articular cartilage surface has a primary function of creating a hydrophilic charged surface.

## ACKNOWLEDGMENT

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