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Research Article

The Amphoteric Nature of Phospholipid Bilayer Verified by the Surface Energy

Zenon Pawlak[1], Kehinde Q Yusuf[2] , Isaac O Afara[3], Adekunle Oloyede[4]

[1]Tribochemistry Consulting, Salt Lake City, Utah 84117, USA and University of Economy, Biotribology Laboratory, 85-229 Bydgoszcz, Poland

[2]College of Medical Rehabilitation Sciences, Department of Prosthetics and Orthotics, Taibah University, PO Box 344, Madinah, Kingdom of Saudi Arabia

[3]Department of Applied Physics, University of Eastern Finland, Kuopio, Finland

[4]School of Chemistry, Physical and Mechanical Engineering, Science and Engineering Faculty, Queensland University of Technology, GPO Box 2434, Brisbane, QLD 4001, Australia

***Corresponding Author:** Zenon Pawlak Tribochemistry Consulting, Salt Lake City, Utah 84117, USA and University of Economy, Biotribology Laboratory, 85-229 Bydgoszcz, Poland.

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Abstract

Background The surface energy the phospholipidic spherical membrane vs. pH, see **Fig. 4A** helped us to understand amphoteric character of cartilage surface (introduced together first time in literature) [4].

Methods The samples of articular cartilage were taken from the knees of an aged one to two-years-old ox. Friction coefficient (cartilage/cartilage) pair and the interfacial energy (γ) of a spherical lipid bilayer curves vs pH have “a bell-shaped curve” expressing amphoteric cartilage nature by the isoelectric point (IEP).

Results It was found that the lowest energy values cover working conditions at pH 7.3 (±) 1.0 of natural joints, **see Fig.4 A, B.** The obtained “a bell-shaped curve” for (cartilage/cartilage) pair friction and spherical PLs bilayer is a novelty in this paper.

Conclusions The observations have led to the conclusion that friction coefficient (cartilage/cartilage) pair and surface energy of a spherical lipid bilayer curves vs pH have (IEP) and expressing amphoteric surfaces character. The surface energy corresponds well with friction coefficient phospholipid amphoteric bilayer surface.

Keywords: charged amphoteric surface; friction (cartilage/cartilage) pair; surface energy of spherical lipid bilayer; isoelectric point (IEP); a bell-shaped curve

Introduction

In chemical analysis cartilage surface, phosphatidylcholine was found to be the major phospholipid class, Fig 1. The surface amorphous layer (SAL) of cartilage constituting three phospholipids: phosphatidylcholine 41%, phosphatidylethanolamine 27% and sphingomyelin 32 % [1]. Phospholipids are amphoteric molecules that contain both acidic ($-PO_4H$) and basic ($-NH_2$) functional groups [**2**]. The pH at which the molecule charge is zero or a neutral is known as the molecule's isoelectric point, IEP. A neutral molecule is named as a zwitterion. Zwitterions, formerly called dipolar ions, are ions with both a negative and positive charges.

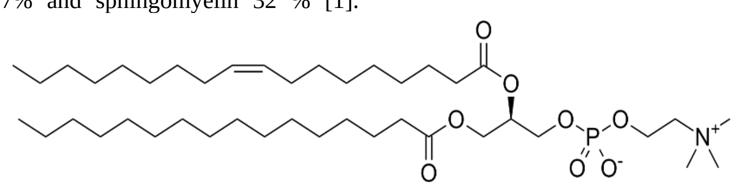

Figure 1: *Structural formula of phosphatidylcholine (Palmitoyl-oleyl-sn-phosphatidylcholine)*

The phosphatidylcholine shown in Fig. 1 has quaternary ammonium ion (QA), positively charged [$(CH_3)_3N^+$-] possesses strong adsorption ability to most biological surfaces negatively charged or hydrophilic [**2, 3**].

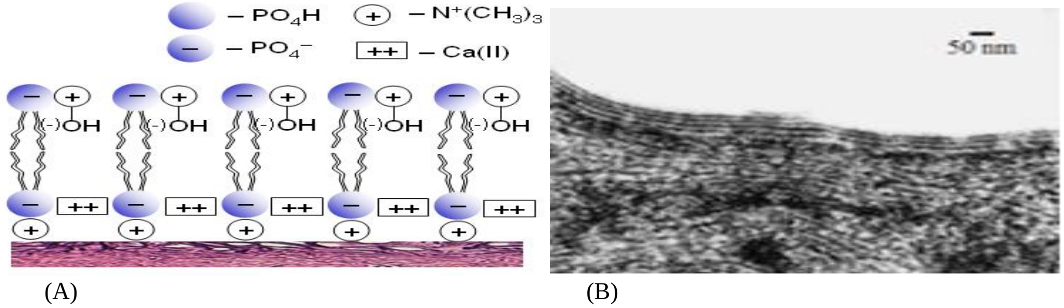

Figure 2: *(A) Hydrophilic cartilage model with the negatively charged surface for pH 7.3 with strong adsorption (-N+(CH3)3 – (proteoglycan) and strong cohesion (-PO4-) (Ca2+)(-PO4-) (B) An image made by electron microscopy of human knee cartilage with phospholipid bilayers adsorbed on the surface [3, 4].*

Phospholipids adsorbed to negatively-charged proteoglycan matrix form phospholipid (membrane) negatively charged surface ($-PO_4^-$) and hydrophilic. Molecular model for adsorption of a cationic quaternary ammonium (QA) ion to the hydrophilic surface and cohesion barrier imparted by phosphate-to-phosphate bonding by Ca (II) cation, Fig. 2(A). Strong adsorption and strong cohesion are necessary for a surfactant to provide good lubricant, Fig. 2 (B). In synovial fluid at pH 7.3, the concentration of hydroxide ions is high enough to deactivate charge of quaternary ammonium ion (QA), with association constant, $K_{ass} \sim 5 \times 10^5$ [4, 7].

$[(CH_3)_3N^+-] + (^-OH) = (CH_3)_3N^+ (^-OH)$

The interfacial energy (or surface energy) parameter of spherical lipid bilayer showed that low-friction coefficient of natural joints [4] at pH 7.3 ± 1 ran in lowest energy range, see Fig. 4. Results from cartilage study are consistent with the results from the study surface of multilayer of weak polyelectrolyte (hyaluronic acid / poly (L-lysine) [**6**].

In this paper, we study (cartilage/cartilage) pair friction and spherical lipid bilayer surface energy vs pH expecting to determine cartilage amphoteric character and isoelectric point (IEP). The observations have led to the conclusion that friction coefficient (cartilage/cartilage) pair and surface energy of a spherical lipid bilayer curves vs pH have (IEP) and expressing amphoteric surfaces character. The both curves have "bell-shaped" were not introduced in literature together and this is novelty of this paper.

Methods and Materials

The samples of articular cartilage were taken from the knees of an aged, one to two-years-old ox. Osteochondral plugs, which diameters were 5 and 10 mm long, were collected from lateral and medial femoral condyles with the use of a circular stainless-steel cutter. The articular cartilage discs were then cut in such a way to form 3-mm plugs with the underlying bone. The samples were stored at 253 K in 0.15 M NaCl solution (pH = 6.9) and, before testing, they were fully defrosted. To prepare the buffer solutions, 0.2 M sodium hydroxide was added to 100 mL of a solution made of 0.04 M acids: acetic (80% of the solution), phosphoric and boric acids. A sodium hydroxide solution was used at 22ºC to adjust to a suitable buffer pH [4]. The electrolyte pH was controlled using a pH-meter in the process of the measurements.

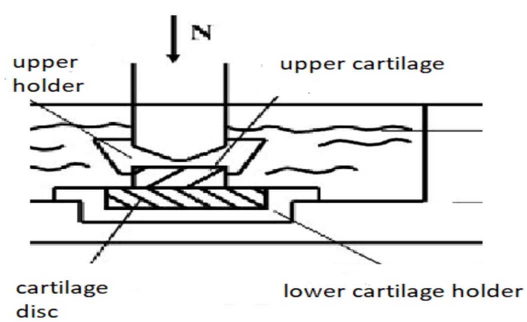

Figure 3: *Schematic diagram of the friction test apparatus*

Measurement the surface energy of the spherical lipid bilayer model

The methodology of using the surface energy apparatus and the apparatus itself, have been described in [4, 8]. The method which was applied for determining the surface energy was founded on the Young and Laplace's (Y-L) standard formula (1).

$$2\gamma = R\Delta p \qquad (1)$$

The surface energy (γ) of the phospholipid bilayer was calculated using the results of the measured curvature radius (R), when the surface was convex, surface shaped while a pressure difference (Δp), was applied to both sides [8, 9]. The interfacial energy **(γ)** as seen in a "bell-shaped curve" of spherical lipid bilayer formed from phospholipids (PLs) vs. pH, Fig. 4 with the isoelectric point, IEP, 4.0 ± 0.2 is based on our experimental results supported by literature data [4, 5, 7 - 9]. The instrument and method applied for finding the interfacial energy are presented elsewhere in literature [8, 9]. The results of interfacial energy as a function of pH are shown in Fig. 4. Surface energy values usually have the units of mJ/m^2. Some sources quote the critical surface tension in units of mN/m, which is equivalent to surface energy.

Friction test

The apparatus was designed to provide a reciprocating sliding motion between two samples of cartilage immersed in a buffer solution (Fig. 2). The friction coefficient (*f*) was measured using of the sliding friction tester pin-on-disc tribotester T-11 manufactured by NISTR, Poland. The discs of articular cartilage were glued to the holders, using adhesive glue. Finally, friction tests were carried out and diagram of friction coefficient as a function of test time was generated. The tribotester measured the friction between two samples of articular cartilage which were equilibrated with each buffer under a load for 5 minutes, at room temperature The speed of sliding discs was very low (1mm/s for 10 min), and the load was 15 N (1.2 MPa), which is equivalent to the natural physiological conditions. A total of five tests were carried out using fresh samples for each experimental set-up with at least three repetitions per specimen pair, from which the mean and standard deviation were calculated.

Results and Discussion

He determined physicochemical parameters (isoelectric point, (IEP) and surface energy (γ) mJ/m^2) have values: phosphatidylcholine (IEP = 4.12, (γ) = 3.53), phosphatidylethanolamine (4.18, 4.06), phosphatidylserine (3.80, 2.93) and sphingomyelin (4.01, 4.42). Using own and literature experimental data [4, 5, 7, 8, 9] the optimal „bell-shaped curve" **Fig. 4A** was obtained. The model of phospholipidic spherical membrane „bell-shaped curve", **Fig. 4A** with the isoelectric point IEP at pH ~ 4.1 represents amphoteric character of PLs membrane. Changes describing the surface energy correspond well with changes of wettability and friction coefficient amphoteric bi-layer surface [4]. The surface energy of the model membrane of spherical lipid bilayers has „bell-shaped curve" shoving amphoteric character and lowest energy at a pH 6.5 to 9.0. The friction coefficient of the (cartilage/cartilage) pair vs pH showing the similar curve run to the surface energy for spherical bilayer, Fig.4 (A, B).

At low pH range changes of surface energy Fig. 4A and friction (cartilage/cartilage) pair Fig 4 (B) corresponds well to the proton transfer **(a)** ($-NH_3^+ \rightarrow -NH_2$) and after IEP at a higher pH range of 4.2 to 6.5 changes of interfacial energy corresponds well to the phosphate proton transfer **(b)** ($-PO_4H \rightarrow -PO_4^-$).

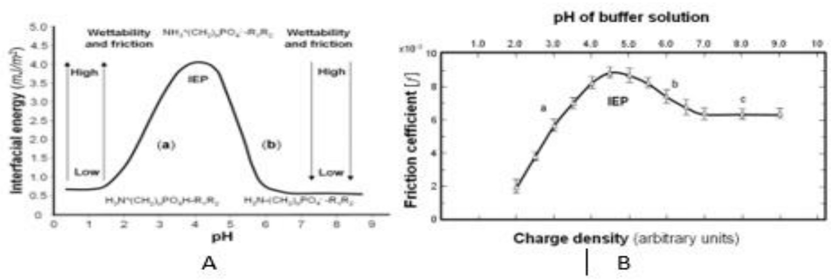

Figure: 4 (A) *The surface energy spherical PLs membrane optimal curve vs buffer pH and (B) a typical plot of friction test between (cartilage/cartilage) pair vs buffer pH*

Figure 4 (B) represents *(f)* vs pH for the (cartilage/cartilage) pair. It is evident that *(f)* increases with pH and reached maximum at the isoelectric point ~ 4.2 with asymmetric curve. Study of cartilage surface deformation (softening) vs pH showed increased surface deformation from pH 1 to pH 4.5 range [**10**]. Observed softening is associated with lower friction; see curve B (a) and this is interpretation of curve asymmetry.

In Fig. 4 (A) the surface energy is plotted against the pH at which a phospholipid molecule carries no net electrical charge, IEP.

$$\text{IEP}: \quad H_2N(CH_2)_n PO_4H\text{-}R_1R_2 \;\rightleftharpoons\; H_3N^+(CH_2)_n PO_4^-\text{-}R_1R_2 \qquad (2)$$

$$(\mathbf{a})\ \text{pH } 0.2 \text{ to } 4.2\ (-NH_3^+ \rightarrow -NH_2 \qquad (\mathbf{b})\ (\text{pH } 4.2 \text{ to } 6.5)\ (-PO_4H \rightarrow -PO_4^-) \qquad (3)$$

Phospholipid amphoteric molecules containing both positive and negative charges depending on the functional groups are affected by the solution's pH. At a low pH, PLs amino group occurs in the protonated ($-NH_3^+$) form (eq. phosphatidylethanolamine) and the phosphate group ($-PO_4H$) occurs in its molecular form; a situation that is characterized by a low surface energy. As the pH of the solution is increased, the amino groups begin to lose their proton ($-NH_3^+ \rightarrow -NH_2$), eq. 3a, this resulting in an increase in the surface energy with a magnitude approaching a maximum. This maximum would occur, as shown in Fig. 4 at the isoelectric point, IEP which corresponds to the pH at which PLs or surface carriers have no net electrical charge, or when the negative and positive charges become equal (see eq. 1) and the ($-PO_4H$) functional group begin to lose their proton ($-PO_4H \rightarrow -PO_4^-$), eq. 3b, this resulting in a decrease in the surface energy with a magnitude approaching a minimum surface energy. The IEP value can be used to indicate the global basic or acidic character of a zwitterion molecule, and compounds with IEP > 7 can be considered basic, and those with IEP < 7 can be considered acidic. The IEP of most biological amphoteric molecules is in the pH range of 3.5 to 7 [11].

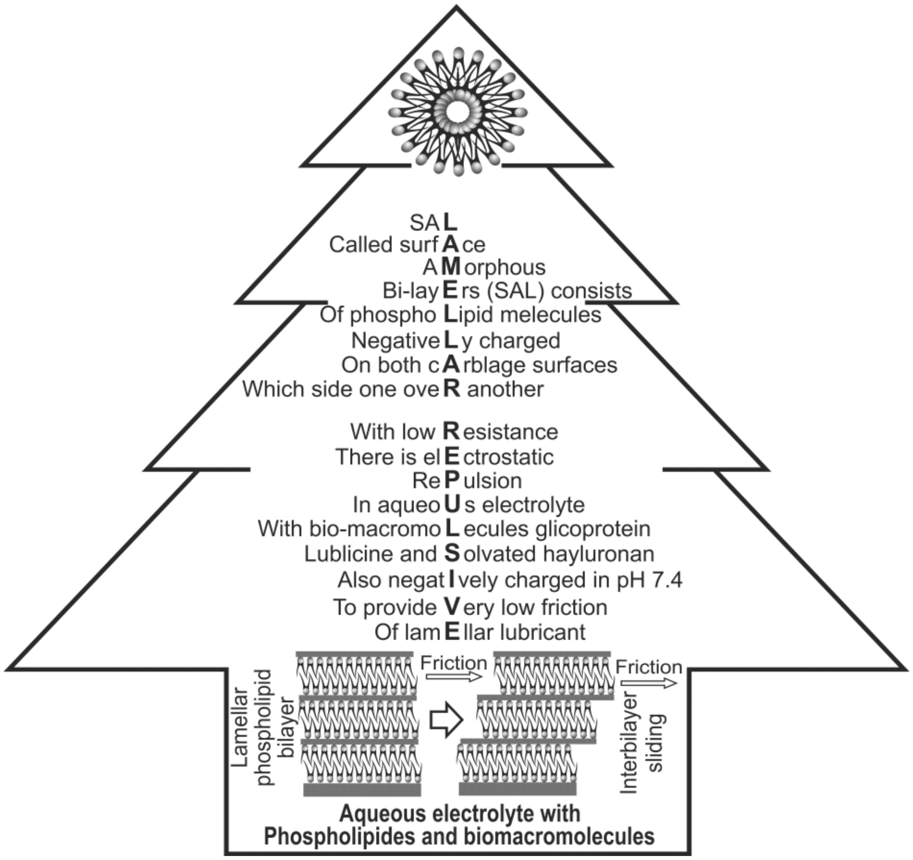

Figure 5. (A) "*Biotribiological tree" is structured by surface amorphous layer and lubricated by boundary lamellar-repulsive-slippage hydration mechanism*

A "Biotribochemical tree" shown in Fig. 5 summarizes our understanding of some of the essential processes occurring on (cartilage/cartilage) pair surface with participation of the phospholipid bilayers and synovial fluid [2, 12, 13]. Fig. 5 is based on lamellar –repulsive slippage mechanism supported by amorphous multilayer (SAL) formed on the articular hydrophilic surface. SAL membrane is providing boundary lamellar-repulsive hydration lubrication [4, 14]. The lamellar-repulsive mechanism is supported by phospholipid lamellar phases and charged macromolecules from synovial fluid between charged cartilage surfaces. The low friction results as a consequence of a short-range hydration repulsion (nanometer-scale) between the interface of negatively charged (-PO_4^-) cartilage surfaces and contribution of macromolecules in the synovial fluid and most importantly a lamellar slippage of bilayers, Fig.5 bottom. The acid-base properties of the phospholipid bilayer significantly influence their wettability and surface friction properties [4].

The natural cartilage surface	The phospholipidic spherical membrane
- unsymmetrical "bell-shaped" curve[a)], Fig. 4B - the isoelectric point (IEP) at 4.2 ± 0.2 - at pH 7.3 the lowest surface energy value - negatively charge surface - "bell-shaped curve" expressing amphoteric cartilage nature by the isoelectric point (IEP).	- symmetric "bell-shaped" curve, Fig. 4A - the isoelectric point (IEP) at pH 4.0 ± 0.2 - at pH 7.3 the lowest coefficient value - negatively charge surface - "bell-shaped curve" expressing amphoteric surface nature by the isoelectric point (IEP).

a) Surface cartilage deformation (softening) at low pH below IEP. Observed softening is associated with lower friction

Table 1: *Comparison cartilage surface membrane with the phospholipidic spherical membrane*

Conclusions

The surface energy the phospholipidic spherical membrane vs pH has the "bell-shape curve" is expressing amphoteric character of phospholipid bilayer. Positive surface charge at low pH 0.5 to (IEP ~ 4) and negative surface charge from (IEP) to pH 6.5 has the lowest energy over a wide range of pH (6.5 to 9.0). It was found that the lowest energy values cover working conditions at pH 7.3 (±) 1.0 of natural joints. The observations have led to the conclusion that friction coefficient (cartilage/cartilage) pair and surface energy of a spherical lipid bilayer curves vs pH are similar and expressing amphoteric nature by (IEP) with the highest friction and energy value on their curves. Determination the surface energy of spherical lipid bilayers helped us to understand better mechanism of lubrication in natural joints. The surface energy of spherical lipid bilayer is expressed by "bell-shaped" model curve has amphoteric character and lowest surface energy at a pH 7.3 ± 1 evidently supporting lubrication mechanism of the natural joints (see Table 1).

Conflict of interest

The author(s) declare that they have no conflicting interests.

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