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pH Effect of the Sphingomyelin Membrane Interfacial Tension

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Abstract The effect of pH on the interfacial tension of a sphingomyelin membrane in aqueous solution has been studied. Three models describing H^+ and OH^- ion adsorption on the bilayer lipid surface are presented. In models I and II, the membrane surface is continuous, with uniformly distributed functional groups as centers of H^+ and OH^- ion adsorption. In model III, the membrane surface is composed of lipid molecules, with and without adsorbed H^+ and OH^- ions. The contribution of each individual lipid molecule to the overall interfacial tension of the bilayer was assumed to be additive in models I and II. In model III, the Gibbs isotherm was used to describe adsorption of H^+ and OH^- ions at the bilayer surface. Theoretical equations are derived to describe the interfacial tension as a function of pH for all three models. Maximum interfacial tension was observed experimentally at the isoelectric point.

Keywords Sphingomyelin · Interfacial tension · Effect of pH on interfacial tension · Adsorption equilibrium

Introduction

The majority of membrane lipids are glycerophospholipids, such as phosphatidylcholine (PC) (Fig. 1). Phosphosphingolipids, such as sphingomyelin (SM), are also found in membranes (Fig. 1).

PC and SM are amphiphiles that contain a hydrophilic phosphorylcholine head group and two long hydrophobic, hydrocarbon chains. The conformation and charge distribution of these two phospholipids are similar; however, they are chemically different (Ohvo-Rekila et al. 2002). PC has a glycerol backbone linked to two fatty acids via ester bonds, while SM has a long-chain sphingoid base with an amide-linked acyl chain. Therefore, the interfacial region of SM is substantially more polar than the interfacial region of PC. Another important chemical difference between the two choline-based phospholipids is that the SM interfacial region contains both hydrogen bond donating and accepting groups, while the corresponding region in PC contains only hydrogen bond accepting groups (Chiu et al. 2003; Ohvo-Rekila et al. 2002).

In biological membranes, the length and number of saturated bonds in the hydrocarbon chains of SM and PC are different. Naturally occurring PCs typically contain two hydrocarbon chains of similar length. The acyl chain on the first glycerol carbon is usually saturated, while the acyl chain on the second glycerol carbon is normally unsaturated, typically containing one to six double bonds in cis configuration (Barenholz and Thompson 1980, 1999). In contrast, naturally occurring SMs contain more saturated hydrocarbon chains (Niemela et al. 2006).

Sphingolipids are essential for eukaryotic life (Barenholz 2004; Merrill et al. 1997; Vesper et al. 1999; Weis 1999). SM is not found equally distributed in different cellular membrane types. More than half of the SM in cells is found in the plasma membrane (Ishitsuka et al. 2004; Filippov et al. 2006). The primary role of sphingolipids is as structural components of biological membranes, but they also play an essential part in organizing the lateral domain structure of membranes and act as second messengers. SMs are also found in serum lipoproteins. Recently, it has been

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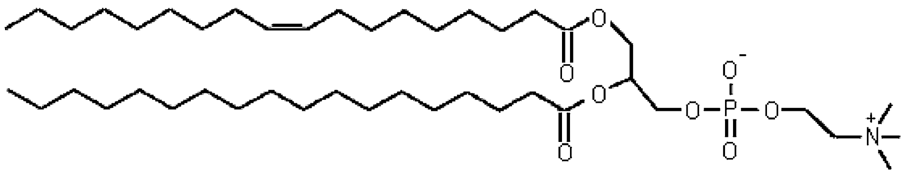
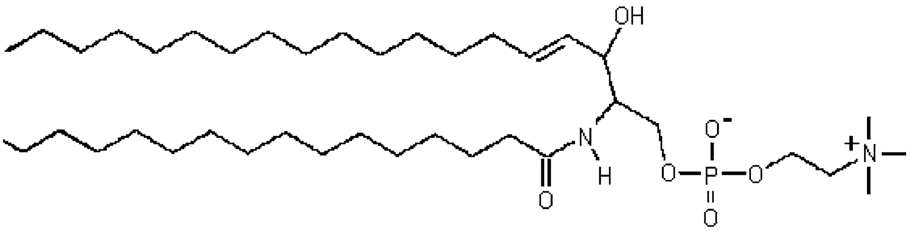


Phosphatidylcholine (PC, lecithin)



Sphingomyelin (SM)

Fig. 1 Structure of PC and SM

suggested that dietary SM could also be involved in protection against diseases, such as arteriosclerosis and colon cancer (Koval and Pagano 1991).

The effect of pH on the interfacial tension of bilayer lipid membranes formed from PC, phosphatidylserine (PS) and phosphatidylethanolamine (PE) has been described previously (Petelska and Figaszewski 2000, 2002a, b). The work presented here is a continuation of these studies using membranes composed of SM.

Theory

From the aqueous side of the membrane, the SM layer has uniformly distributed $-PO^{(-)}$, $-\overset{(+)}{N}(CH_3)_3$, $-CO^{(-)}$ and $-\overset{(+)}{N}H_2$ groups. The SM membrane surface can be modeled in three ways (Fig. 2). In model I, the membrane surface is continuous, with uniformly distributed functional groups, where H^+ and OH^- ions are adsorbed equally, regardless of the type of functional group.

In model II, the acid–base equilibria between the $-PO^{(-)}$ group and the H^+ ion, the $-\overset{(+)}{N}(CH_3)_3$ group and the OH^- ion and the $-CO^{(-)}$ and $-\overset{(+)}{N}H_2$ groups with the H^+ and OH^- ions are not equivalent.

In model III, Gibbs equation was used to account for the volume of the adsorbed ions. The dependence of lipid membrane interfacial tension on pH has been described previously using a simplified description based on Gibbs isotherm but only near the membrane isoelectric point (Petelska and Figaszewski 2000).

Model I

In model I (Fig. 2a), the pH dependence of lipid membrane interfacial tension is described in terms of acid–base equilibria. Uniformly distributed active centers at which

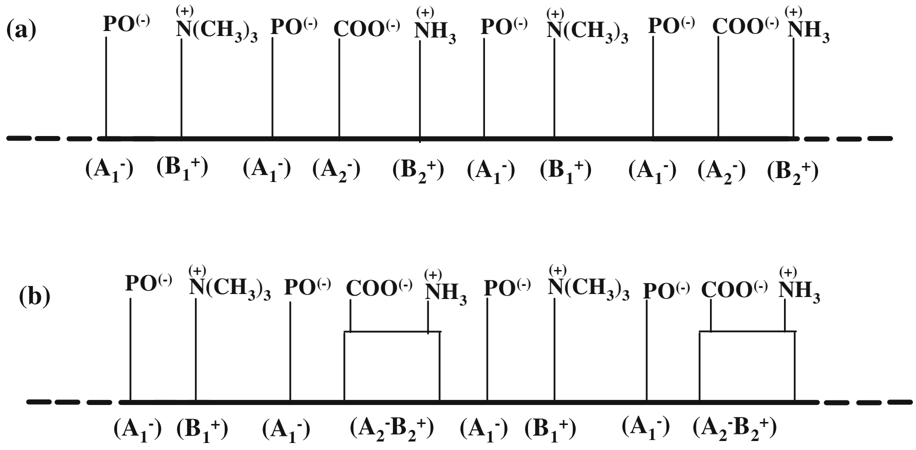


Fig. 2 Representation of SM membrane surface interactions with H^+ and OH^- ions, model I (**a**) and model II (**b**)

H^+ and OH^- ions can be adsorbed are present on the side of the membrane facing the aqueous solution. They are schematically described with Eqs. 1–4:

$$\underset{(A_1^-)}{-PO^{(-)}} + H^{(+)} \Leftrightarrow \underset{(A_1H)}{-PO^{(-)} - H^{(+)}} \tag{1}$$

$$\underset{(B_1^+)}{-\overset{(+)}{N}(CH_3)_3} + OH^{(-)} \Leftrightarrow \underset{(B_1OH)}{-\overset{(+)}{N}(CH_3)_3 - OH^{(-)}} \tag{2}$$

$$\underset{(A_2^-)}{-CO^{(-)}} + H^{(+)} \Leftrightarrow \underset{(A_2H)}{-CO^{(-)} - H^{(+)}} \tag{3}$$

$$\underset{(B_2^+)}{-\overset{(+)}{N}H_2} + OH^{(-)} \Leftrightarrow \underset{(B_2OH)}{-\overset{(+)}{N}H_2 - OH^{(-)}} \tag{4}$$

Eight types of active centers—A_1^-, A_1H, B_1^+, B_1OH, A_2^-, A_2H, B_2^+ and B_2OH—are found on the membrane surface. The adsorption equilibria are described by Eqs. 1–4.

The surface concentration of the lipid is equal to the number of lipid molecules divided by the membrane surface area. The surface concentrations of the active center ions $a_{A_1^-}, a_{A_1H}, a_{B_1^-}, a_{B_1OH}, a_{A_2^-}$, a_{A_2H}, $a_{B_2^+}$, a_{B_2OH}, H^+ (a_{H^+}) and OH^- (a_{OH^-}) are determined by acid–base equilibria (Eqs. 5–8).

$$K_{A_1} = \frac{a_{A_1H}}{a_{A_1^-} \cdot a_{H^+}} \tag{5}$$

$$K_{B_1} = \frac{a_{B_1OH}}{a_{B_1^+} \cdot a_{OH^-}} \tag{6}$$

$$K_{A_2} = \frac{a_{A_2H}}{a_{A_2^-} \cdot a_{H^+}} \tag{7}$$

$$K_{B_2} = \frac{a_{B_2OH}}{a_{B_2^+} \cdot a_{OH^-}} \tag{8}$$

The lipid surface concentration (s) is described by Eqs. 9–12.

$$a_{A_1H} + a_{A_1^-} = s \tag{9}$$

$$a_{B_1OH} + a_{B_1^+} = s \tag{10}$$

$$a_{A_2H} + a_{A_2^-} = s \tag{11}$$

$$a_{B_2OH} + a_{B_2^+} = s \tag{12}$$

where $a_{A_1^-}, a_{A_1H}, a_{B_1^+}, a_{B_1OH}, a_{A_2^-}, a_{A_2H}, a_{B_2^+}, a_{B_2OH}$ (mol m^{-2}) are the surface concentration values of membrane components.

The total interfacial tension can be calculated assuming that the contribution of each active center to the overall interfacial tension is additive (Eq. 13).

$$\gamma = \gamma_{A_1^-} + \gamma_{A_1H} + \gamma_{B_1^+} + \gamma_{B_1OH} + \gamma_{A_2^-} + \gamma_{A_2H} + \gamma_{B_2^+} + \gamma_{B_2OH} \tag{13}$$

The expressions describing the contributions of individual forms of the SM molecule to the interfacial tension are as follows:

$$\gamma_{A_1^-} = \gamma^0_{A_1^-} \cdot \frac{a_{A_1^-}}{s} \tag{14}$$

$$\gamma_{A_1H} = \gamma^0_{A_1H} \cdot \frac{a_{A_1H}}{s} \tag{15}$$

$$\gamma_{B_1^+} = \gamma^0_{B_1^+} \cdot \frac{a_{B_1^+}}{s} \tag{16}$$

$$\gamma_{B_1OH} = \gamma^0_{B_1OH} \cdot \frac{a_{B_1OH}}{s} \tag{17}$$

$$\gamma_{A_2^-} = \gamma^0_{A_2^-} \cdot \frac{a_{A_2^-}}{s} \tag{18}$$

$$\gamma_{A_2H} = \gamma^0_{A_2H} \cdot \frac{a_{A_2H}}{s} \tag{19}$$

$$\gamma_{B_2^+} = \gamma^0_{B_2^+} \cdot \frac{a_{B_2^+}}{s} \tag{20}$$

$$\gamma_{B_2OH} = \gamma^0_{B_2OH} \cdot \frac{a_{B_2OH}}{s} \tag{21}$$

Equations 5–21 can be combined into Eq. 22, which describes the pH dependence of the lipid membrane interfacial tension.

$$\begin{aligned}\gamma = {} & \gamma^0_{A_1^-}\frac{1}{K_{A_1}a_{H^+}+1} + \gamma^0_{A_1H}\frac{K_{A_1}a_{H^+}}{K_{A_1}a_{H^+}+1} + \gamma^0_{B_1^+}\frac{1}{K_{B_1}a_{OH^-}+1} \\ & + \gamma^0_{B_1OH}\frac{K_{B_1}a_{OH^-}}{K_{B_1}a_{OH^-}+1} + \\ & \gamma^0_{A_2^-}\frac{1}{K_{A_2}a_{H^+}+1} + \gamma^0_{A_2H}\frac{K_{A_2}a_{H^+}}{K_{A_2}a_{H^+}+1} + \gamma^0_{B_2^+}\frac{1}{K_{B_2}a_{OH^-}+1} \\ & + \gamma^0_{B_2OH}\frac{K_{B_2}a_{OH^-}}{K_{B_2^+}a_{OH^-}+1}\end{aligned} \tag{22}$$

where γ (N m^{-1}) is the measured interfacial tension of the lipid membrane and $\gamma^0_{A_1^-}, \gamma^0_{A_1H}, \gamma^0_{B_1^+}$, $\gamma^0_{B_1OH}$, $\gamma^0_{A_2^-}, \gamma^0_{A_2H}, \gamma^0_{B_2^+}$, $\gamma^0_{B_2OH}$ (N m^{-1}) are the specific interfacial tension values of membrane components.

Model II

In model II (Fig. 2b), the acid–base equilibria between the $-PO^{(-)}$ group and H^+ ions, the $-\overset{(+)}{N}(CH_3)_3$ group and OH^- ions and the $-CO^{(-)}$ and $-\overset{(+)}{N}H_2$ groups with H^+ and OH^- ions are not equivalent. Eight active centers (A_1^-, A_1H, B_1^+, B_1OH, $A_2^-B_2^+$, $A_2^-B_2OH$, $A_2HB_2^+$, A_2HB_2OH) can be distinguished at the membrane surface.

The adsorption equilibria are described by Eqs. 23–27.

$$A_1^- + H^+ \Leftrightarrow A_1H \tag{23}$$

$$B_1^+ + OH^- \Leftrightarrow B_1OH \tag{24}$$

$$A_2^-B_2^+ + OH^- \Leftrightarrow A_2^-B_2OH \tag{25}$$

$$A_2^-B_2^+ + H^+ \Leftrightarrow A_2HB_2^+ \tag{26}$$

$$A_2^-B_2OH + H^+ \Leftrightarrow A_2HB_2OH \tag{27}$$

The surface concentration of the lipid is equal to the number of lipid molecules divided by the membrane surface area. The surface concentrations of the active center $a_{\mathrm{A}_1^-}$, $a_{\mathrm{A}_1\mathrm{OH}}$, $a_{\mathrm{B}_1^+}$, $a_{\mathrm{B}_2^+\mathrm{A}_2^-}$, $a_{\mathrm{B}_1\mathrm{OH}}$, $a_{\mathrm{B}_2\mathrm{OHA}_2^-}$, $a_{\mathrm{B}_2^+\mathrm{A}_2\mathrm{H}}$, $a_{\mathrm{B}_2\mathrm{OHA}_2\mathrm{H}}$, H^+ (a_{H^+}) and OH^- (a_{OH^-}) ions are determined by acid–base equilibria (Eqs. 28–32).

$$K_1 = \frac{a_{\mathrm{A}_1\mathrm{H}}}{a_{\mathrm{A}_1^-} \cdot a_{\mathrm{H}^+}} \tag{28}$$

$$K_2 = \frac{a_{\mathrm{B}_1\mathrm{OH}}}{a_{\mathrm{B}_1^+} \cdot a_{\mathrm{OH}^-}} \tag{29}$$

$$K_3 = \frac{a_{\mathrm{A}_2^-\mathrm{B}_2\mathrm{OH}}}{a_{\mathrm{A}_2^-\mathrm{B}_2^+} \cdot a_{\mathrm{OH}^-}} \tag{30}$$

$$K_4 = \frac{a_{\mathrm{A}_2\mathrm{HB}_2^+}}{a_{\mathrm{A}_2^-\mathrm{B}_2^+} \cdot a_{\mathrm{H}^+}} \tag{31}$$

$$K_5 = \frac{a_{\mathrm{A}_2\mathrm{HB}_2\mathrm{OH}}}{a_{\mathrm{A}_2^-\mathrm{B}_2\mathrm{OH}} \cdot a_{\mathrm{H}^+}} \tag{32}$$

The lipid surface concentration (s) is described by Eqs. 33–35.

$$a_{\mathrm{A}_1\mathrm{H}} + a_{\mathrm{A}_1^-} = s \tag{33}$$

$$a_{\mathrm{B}_1\mathrm{OH}} + a_{\mathrm{B}_1^+} = s \tag{34}$$

$$a_{\mathrm{A}_2^-\mathrm{B}_2^+} + a_{\mathrm{A}_2^-\mathrm{B}_2\mathrm{OH}} + a_{\mathrm{A}_2\mathrm{HB}_2^+} + a_{\mathrm{A}_2\mathrm{HB}_2\mathrm{OH}} = s \tag{35}$$

where $a_{\mathrm{A}_1^-}, a_{\mathrm{A}_1\mathrm{H}}, a_{\mathrm{B}_1^+}, a_{\mathrm{B}_1\mathrm{OH}}, a_{\mathrm{A}_2^-\mathrm{B}_2^+}, a_{\mathrm{A}_2^-\mathrm{B}_2\mathrm{OH}}, a_{\mathrm{A}_2\mathrm{HB}_2^+}$, $a_{\mathrm{A}_2\mathrm{HB}_2\mathrm{OH}}$ (mol m^{-2}) are the surface concentration values of membrane components.

Assuming that contributions of the individual forms to the interfacial tension are additive, Eq. 36 can be written.

$$\gamma = \gamma_{\mathrm{A}_1^-} + \gamma_{\mathrm{A}_1\mathrm{H}} + \gamma_{\mathrm{B}_1^+} + \gamma_{\mathrm{B}_1\mathrm{OH}} + \gamma_{\mathrm{A}_2^-\mathrm{B}_2^+} + \gamma_{\mathrm{A}_2^-\mathrm{B}_2\mathrm{OH}} + \gamma_{\mathrm{A}_2\mathrm{HB}_2^+} + \gamma_{\mathrm{A}_2\mathrm{HB}_2\mathrm{OH}} \tag{36}$$

The expressions describing interfacial tension values of the individual forms of the SM membrane considered can then be written (Eqs. 37–44).

$$\gamma_{\mathrm{A}_1^-} = \gamma^0_{\mathrm{A}_1^-} \cdot \frac{a_{\mathrm{A}_1^-}}{s} \tag{37}$$

$$\gamma_{\mathrm{A}_1\mathrm{H}} = \gamma^0_{\mathrm{A}_1\mathrm{H}} \cdot \frac{a_{\mathrm{A}_1\mathrm{H}}}{s} \tag{38}$$

$$\gamma_{\mathrm{B}_1^+} = \gamma^0_{\mathrm{B}_1^+} \cdot \frac{a_{\mathrm{B}_1^+}}{s} \tag{39}$$

$$\gamma_{\mathrm{B}_1\mathrm{OH}} = \gamma^0_{\mathrm{B}_1\mathrm{OH}} \cdot \frac{a_{\mathrm{B}_1\mathrm{OH}}}{s} \tag{40}$$

$$\gamma_{\mathrm{A}_2^-\mathrm{B}_2^+} = \gamma^0_{\mathrm{A}_2^-\mathrm{B}_2^+} \cdot \frac{a_{\mathrm{A}_2^-\mathrm{B}_2^+}}{s} \tag{41}$$

$$\gamma_{\mathrm{A}_2^-\mathrm{B}_2\mathrm{OH}} = \gamma^0_{\mathrm{A}_2^-\mathrm{B}_2\mathrm{OH}} \cdot \frac{a_{\mathrm{A}_2^-\mathrm{B}_2\mathrm{OH}}}{s} \tag{42}$$

$$\gamma_{\mathrm{A}_2\mathrm{HB}_2^+} = \gamma^0_{\mathrm{A}_2\mathrm{HB}_2^+} \cdot \frac{a_{\mathrm{A}_2\mathrm{HB}_2^+}}{s} \tag{43}$$

$$\gamma_{\mathrm{A}_2\mathrm{HB}_2\mathrm{OH}} = \gamma^0_{\mathrm{A}_2\mathrm{HB}_2\mathrm{OH}} \cdot \frac{a_{\mathrm{A}_2\mathrm{HB}_2\mathrm{OH}}}{s} \tag{44}$$

Equations 28–44 can be combined into Eq. 45, which describes the pH dependence of the lipid membrane interfacial tension.

$$\gamma = -m_1 a^2_{\mathrm{H}^+}\gamma - m_2 a_{\mathrm{H}^+}\gamma - m_3 a^2_{\mathrm{OH}^-}\gamma - m_4 a_{\mathrm{OH}^-}\gamma + m_5 a^2_{\mathrm{H}^+} + m_6 a_{\mathrm{H}^+} + m_7 a^2_{\mathrm{OH}^-} + m_8 a_{\mathrm{OH}^-} + b \tag{45}$$

where

$$m_1 = \frac{K_1K_4}{M}$$

$$m_2 = \frac{K_1K_2K_4K_\mathrm{w} + K_1 + K_1K_3K_5K_\mathrm{w} + K_4}{M}$$

$$m_3 = \frac{K_2K_3}{M}$$

$$m_4 = \frac{K_1K_2K_3K_\mathrm{w} + K_2 + K_2K_3K_5K_\mathrm{w} + K_3}{M}$$

$$m_5 = \frac{K_1K_4(\gamma^0_{\mathrm{A}_1\mathrm{H}} + \gamma^0_{\mathrm{B}_1^+} + \gamma^0_{\mathrm{B}_2^+\mathrm{A}_2\mathrm{H}})}{M}$$

$$m_6 = \frac{K_4\left(\gamma^0_{\mathrm{A}_1^-} + \gamma^0_{\mathrm{B}_1^+} + \gamma^0_{\mathrm{B}_2^+\mathrm{A}_2\mathrm{H}}\right) + K_1\left(\gamma^0_{\mathrm{A}_1\mathrm{H}} + \gamma^0_{\mathrm{B}_1^+} + \gamma^0_{\mathrm{B}_2^+\mathrm{A}_2^-}\right)}{M} + \\ + \frac{K_1K_2K_4K_\mathrm{w}\left(\gamma^0_{\mathrm{A}_1\mathrm{H}} + \gamma^0_{\mathrm{B}_1\mathrm{OH}} + \gamma^0_{\mathrm{B}_2^+\mathrm{A}_2\mathrm{H}}\right) + K_1K_3K_5K_\mathrm{w}\left(\gamma^0_{\mathrm{A}_1\mathrm{H}} + \gamma^0_{\mathrm{B}_1^+} + \gamma^0_{\mathrm{B}_2\mathrm{OHA}_2\mathrm{H}}\right)}{M}$$

$$m_7 = \frac{K_2K_3\left(\gamma^0_{\mathrm{A}_1^-} + \gamma^0_{\mathrm{B}_1\mathrm{OH}} + \gamma^0_{\mathrm{B}_2\mathrm{OHA}_2^-}\right)}{M}$$

$$m_8 = \frac{K_3\left(\gamma^0_{\mathrm{A}_1^-} + \gamma^0_{\mathrm{B}_1^+} + \gamma^0_{\mathrm{B}_2\mathrm{OHA}_2^-}\right) + K_2\left(\gamma^0_{\mathrm{A}_1^-} + \gamma^0_{\mathrm{B}_1\mathrm{OH}} + \gamma^0_{\mathrm{B}_2^+\mathrm{A}_2^-}\right)}{M} + \frac{K_2K_3K_5K_\mathrm{w}\left(\gamma^0_{\mathrm{A}_1^-} + \gamma^0_{\mathrm{B}_1\mathrm{OH}} + \gamma^0_{\mathrm{B}_2\mathrm{OHA}_2^-}\right) + K_1K_2K_3K_\mathrm{w}\left(\gamma^0_{\mathrm{A}_1\mathrm{H}} + \gamma^0_{\mathrm{B}_1\mathrm{OH}} + \gamma^0_{\mathrm{B}_2\mathrm{OHA}_2^-}\right)}{M} + \frac{K_1K_2K_3K_5K_\mathrm{w}\gamma^0_{\mathrm{B}_1\mathrm{OH}}}{M}$$

$$b = \frac{K_1K_2K_3K_5K_\mathrm{w}^2\left(\gamma^0_{\mathrm{A}_1\mathrm{H}} + \gamma^0_{\mathrm{B}_2\mathrm{OHA}_2\mathrm{H}}\right) + K_1K_2K_\mathrm{w}\left(\gamma^0_{\mathrm{B}_1\mathrm{OH}} + \gamma^0_{\mathrm{B}_2^+\mathrm{A}_2^-}\right) + K_1K_3K_\mathrm{w}\left(\gamma^0_{\mathrm{A}_1\mathrm{H}} + \gamma^0_{\mathrm{B}_1^+}\right)}{M} + \frac{K_3K_5K_\mathrm{w}\left(\gamma^0_{\mathrm{A}_1^-} + \gamma^0_{\mathrm{B}_1^+} + \gamma^0_{\mathrm{B}_2\mathrm{OHA}_2\mathrm{H}}\right) + K_2K_4K_\mathrm{w}\left(\gamma^0_{\mathrm{A}_1^-} + \gamma^0_{\mathrm{B}_2^+\mathrm{A}_2\mathrm{H}} + \gamma^0_{\mathrm{B}_1\mathrm{OH}}\right) + \gamma^0_{\mathrm{A}_1^-} + \gamma^0_{\mathrm{B}_1^+} + \gamma^0_{\mathrm{B}_2^+\mathrm{A}_2^-}}{M}$$

$$M = 1 + K_1K_2K_\mathrm{w} + K_1K_2K_3K_5K_\mathrm{w}^2 + K_1K_3K_\mathrm{w} + K_2K_4K_\mathrm{w} + K_3K_5K_\mathrm{w}$$

where γ (N m^{-1}) is the measured interfacial tension of the lipid membrane, K_w is the ionic product of water and $\gamma^0_{\mathrm{A}_1^-}$, $\gamma^0_{\mathrm{A}_1\mathrm{H}}, \gamma^0_{\mathrm{B}_1^+}, \gamma^0_{\mathrm{B}_1\mathrm{OH}}, \gamma^0_{\mathrm{A}_2^-\mathrm{B}_2^+}, \gamma^0_{\mathrm{A}_2^-\mathrm{B}_2\mathrm{OH}}, \gamma^0_{\mathrm{A}_2\mathrm{HB}_2^+}, \gamma^0_{\mathrm{A}_2\mathrm{HB}_2\mathrm{OH}}$ (N m^{-1}) are the specific interfacial tensions of the membrane components.

Theoretical interfacial tension values were calculated using Eq. 46.

$$\gamma = \frac{m_5a^2_{\mathrm{H}^+} + m_6a_{\mathrm{H}^+} + m_7a^2_{\mathrm{OH}^-} + m_8a_{\mathrm{OH}^-} + b}{1 - m_1a^2_{\mathrm{H}^+} - m_2a_{\mathrm{H}^+} - m_3a^2_{\mathrm{OH}^-} - m_4a_{\mathrm{OH}^-}} \quad (46)$$

Model III

In previous studies (Petelska and Figaszewski 2003a, b), it was assumed that there is a surface excess of H$^+$ and OH$^-$ ions. This assumption is commonly used when describing adsorption phenomena (Gawłowski et al. 1995; Scharifker and Zelenay 1988), but it is correct only in the case where adsorption is strong and the concentration of the adsorbed ion in solution is low. In the present case, the surface excess definition derived from Gibbs equation (Bikerman 1958) should be strictly respected.

In model III, the adsorption equilibria are described by Eqs. 1–8. The acid–base equilibria are described by Eqs. 9–11, where the lipid surface concentration is denoted by s.

The Gibbs equation can be written as in Eq. 47.

$$d\gamma = -\sum \Gamma_i d\bar{\mu}_i \quad (47)$$

If H$^+$ and OH$^-$ ions are adsorbed at the lipid surface, then the Gibbs equation can be written as in Eq. 48.

$$d\gamma = -\Gamma_{\mathrm{A}_1\mathrm{H}}d\bar{\mu}_{\mathrm{H}^+} - \Gamma_{\mathrm{A}_2\mathrm{H}}d\bar{\mu}_{\mathrm{H}^+} - \Gamma_{\mathrm{B}_1\mathrm{OH}}d\bar{\mu}_{\mathrm{OH}^-} - \Gamma_{\mathrm{B}_2\mathrm{OH}}d\bar{\mu}_{\mathrm{OH}^-} \quad (48)$$

The equations describing surface excess of the H$^+$ and OH$^-$ ions in terms of the definition resulting from the Gibbs equation can be written as in Eqs. 49 and 50.

$$\Gamma_{\mathrm{OH}^-} = a_{\mathrm{B}_1\mathrm{OH}} + a_{\mathrm{B}_2\mathrm{OH}} - V_{\mathrm{H}^+}(a_{\mathrm{A}_1\mathrm{H}} + a_{\mathrm{A}_2\mathrm{H}})a_{\mathrm{OH}^-} - V_{\mathrm{OH}^-}(a_{\mathrm{B}_1\mathrm{OH}} + a_{\mathrm{B}_2\mathrm{OH}})a_{\mathrm{OH}^-} \quad (49)$$

$$\Gamma_{\mathrm{H}^+} = a_{\mathrm{A}_1\mathrm{H}} + a_{\mathrm{A}_2\mathrm{H}} - V_{\mathrm{H}^+}(a_{\mathrm{A}_1\mathrm{H}} + a_{\mathrm{A}_2\mathrm{H}})a_{\mathrm{H}^+} - V_{\mathrm{OH}^-}(a_{\mathrm{B}_1\mathrm{OH}} + a_{\mathrm{B}_2\mathrm{OH}})a_{\mathrm{H}^+} \quad (50)$$

where V_{H^+}(m^3 mol^{-1}) is the H$^+$ ion volume in the adsorption layer and V_{OH^-} (m^3 mol^{-1}) is the OH$^-$ ion volume in the adsorption layer.

Substitution of Eqs. 49 and 50 into the Gibbs equation yields Eq. 51.

$$\begin{aligned} d\gamma = {} & -RTa_{\mathrm{A}_1\mathrm{H}}\frac{da_{\mathrm{H}^+}}{a_{\mathrm{H}^+}} - RTa_{\mathrm{A}_2\mathrm{H}}\frac{da_{\mathrm{H}^+}}{a_{\mathrm{H}^+}} + RTV_{\mathrm{H}^+}a_{\mathrm{A}_1\mathrm{H}}da_{\mathrm{H}^+} + RTV_{\mathrm{H}^+}a_{\mathrm{A}_2\mathrm{H}}da_{\mathrm{H}^+} \\ & + RTV_{\mathrm{OH}^-}a_{\mathrm{B}_1\mathrm{OH}}da_{\mathrm{H}^+} + RTV_{\mathrm{OH}^-}a_{\mathrm{B}_2\mathrm{OH}}da_{\mathrm{H}^+} - RTa_{\mathrm{B}_1\mathrm{OH}}\frac{da_{\mathrm{OH}^-}}{a_{\mathrm{OH}^-}} - RTa_{\mathrm{B}_2\mathrm{OH}}\frac{da_{\mathrm{OH}^-}}{a_{\mathrm{OH}^-}} \\ & + RTV_{\mathrm{H}^+}a_{\mathrm{A}_1\mathrm{H}}da_{\mathrm{OH}^-} + RTV_{\mathrm{H}^+}a_{\mathrm{A}_2\mathrm{H}}da_{\mathrm{OH}^-} + RTV_{\mathrm{OH}^-}a_{\mathrm{B}_1\mathrm{OH}}da_{\mathrm{OH}^-} + RTV_{\mathrm{OH}^-}a_{\mathrm{B}_2\mathrm{OH}}da_{\mathrm{OH}^-} \end{aligned} \quad (51)$$

Equations 5–12 are then used to determine a_{A_1H}, a_{A_2H} and a_{B_1OH}, a_{B_2OH} (Eqs. 52–55).

$$a_{A_1H} = \frac{K_{A_1} s a_{H^+}}{1 + K_{A_1} a_{H^+}} \tag{52}$$

$$a_{B_1OH} = \frac{K_{B_1} s a_{OH^-}}{1 + K_{B_1} a_{OH^-}} \tag{53}$$

$$a_{A_2H} = \frac{K_{A_2} s a_{H^+}}{1 + K_{A_2} a_{H^+}} \tag{54}$$

$$a_{B_2OH} = \frac{K_{B_2} s a_{OH^-}}{1 + K_{B_2} a_{OH^-}} \tag{55}$$

Substitution of Eqs. 52–55 into Eq. 51 eliminates a_{A_1H}, a_{A_2H} and a_{B_1OH}, a_{B_2OH}. Equation 51 is integrated to yield Eq. 56, assuming that $\gamma = \gamma_{\max}$, $a_{H^+} = a_{H^+}^{\max}$ and $a_{OH^-} = a_{OH^-}^{\max}$.

convex surface formed by applying a pressure difference, Δp, on its sides. The method used was based on Young and Laplace's equation (Adamson 1960):

$$2\gamma = R\Delta p$$

Measurements

Interfacial Tension Measurement

The apparatus and the measurement method were described previously (Petelska and Figaszewski 1998, 2000, 2002a, b, 2003a, b). The lipid membranes were formed by the Mueller-Rudin method (Mueller et al. 1963).

The interfacial tension was measured on freshly created lipid bilayer membrane 12–15 times for each pH electrolyte solution.

$$\begin{aligned}
\gamma = {} & \gamma_{\max} - sRT\left(1 + \frac{V_{H^+}}{K_{A_1}} - V_{H^+} K_w K_{A_1}\right) \ln \frac{1 + K_{A_1} a_{H^+}}{1 + K_{A_1} a_{H^+}^{\max}} - sRT\left(1 + \frac{V_{H^+}}{K_{A_2}} - V_{H^+} K_w K_{A_2}\right) \ln \frac{1 + K_{A_2} a_{H^+}}{1 + K_{A_2} a_{H^+}^{\max}} \\
& - sRT\left(1 + \frac{V_{OH^-}}{K_{B_1}} - V_{OH^-} K_w K_{B_1}\right) \ln \frac{1 + K_{B_1} a_{OH^-}}{1 + K_{B_1} a_{OH^-}^{\max}} - sRT\left(1 + \frac{V_{OH^-}}{K_{B_2}} - V_{OH^-} K_w K_{B_2}\right) \ln \frac{1 + K_{B_2} a_{OH^-}}{1 + K_{B_2} a_{OH^-}^{\max}} \\
& - sRTV_{H^+} K_w K_{A_1} \ln \frac{a_{H^+}}{a_{H^+}^{\max}} - sRTV_{H^+} K_w K_{A_2} \ln \frac{a_{H^+}}{a_{H^+}^{\max}} - sRTV_{OH^-} K_w K_{B_1} \ln \frac{a_{OH^-}}{a_{OH^-}^{\max}} \\
& - sRTV_{OH^-} K_w K_{B_2} \ln \frac{a_{OH^-}}{a_{OH^-}^{\max}} + sRTV_{H^+}\left(a_{H^+} - a_{H^+}^{\max}\right) + sRTV_{OH^-}\left(a_{OH^-} - a_{OH^-}^{\max}\right)
\end{aligned} \tag{56}$$

Materials and Methods

SM from chicken egg yolk (98%; Fluka, Neu-Ulm, Germany) was used in the experiment.

Buffers of 2–12 pH range were prepared according to Britton and Robinson (1931) and used as the electrolyte. They were prepared by adding 0.2 M sodium hydroxide to 100 ml of solution having the following composition: 0.04 M 80% acetic acid, 0.04 M phosphoric acid and 0.04 M boric acid from (all from Polish Chemical Reagents, Gliwice, Poland). A suitable pH of the buffer was established depending on the amount of added sodium hydroxide. The initial pH of the prepared buffer was 1.81. It changed to, e.g., 3.29 after 20 cm^3 of NaOH (Polish Chemical Reagents) were added or to 6.80 if 50 cm^3 were added. Britton and Robinson (1931) buffer was used in the experiments because this solution is applied in biochemical experiments as the standard buffer, due to its wide pH range pH (2–12) and composition which does not influence the biological membrane.

The interfacial tension, γ, of the lipid bilayer was determined by measuring the curvature radius, R, of the

The solution used to form the model membrane contained 20 mg/ml of SM in solution (*n*-decan:buthanol 20:1).

Isoelectric Point Determination

In the measurements, the bilayer lipid membranes were also used in the form of liposomes for determining the isoelectric point of a lipid membrane. However, it was difficult to determine the acid–base equilibria because lecithin is insoluble in water; the needed values were determined using liposomes. These can be formed owing to the fact that most phospholipids undergo spontaneous aggregation in water or in aqueous electrolyte solutions if shaken or subjected to ultrasound. Bubbles of spherical shape, from less than 0.1 μm to a fraction of millimeter, are then formed (Jahnson et al. 1971; Zietkiewicz and Slomski 1984). They were formed as follows (Lasic 1995): 10 mg of SM (98%, Fluka) were dissolved in 1–2 cm^3 of chloroform and the solvent was evaporated in an atmosphere of argon until 25–50 μm^3 of lipid film remained in the beaker. Then, 15 cm^3 of 0.9% NaCl were added and the beaker was placed in a water bath (at about 280.15 K). The head of a UD-20 ultrasound generator was then immersed in the solution and the solution was subjected to ultrasound five times for 1.5 min each time. Liposomes of 10–20 nm were obtained (Huang 1963).

Area Occupied by SM Molecule Determination

In order to determine the dependence of interfacial tension of pH of the electrolyte solution using theoretical equations 22, 45 and 56, it is necessary to know the area occupied by the SM molecule. This value was determined used the Langmuir method. Surface tension measurements were carried out in a Langmuir trough equipped with a 9000 Nima Tensiometer (Nima Technology, Coventry, UK) at the water/air interface at 22°C. The dependence of surface tension on monolayer surface area was plotted directly by the ST9002 software (Nima Technology). The measuring procedure was described elsewhere (Petelska et al. 2008; Petelska and Figaszewski 2009). The Teflon trough was filled with subphase, triply distilled water. Known amounts of SM dissolved in 1-chloropropane were placed at the surface using a chromatography syringe. Each sample was left for 10 min to allow the solvent to evaporate before compressing. After the SM monolayer had been prepared, it was compressed by means of a glass barrier, measuring the surface tension and the accessible surface area. The surface tension measurements were made with a very thin glass plate, which reduces the wetting angle to zero. The trough was under a Plexi cover to protect the lipid monolayer against dust and air movements that would perturb the measurements. Data from recorded plots were used to calculate the surface pressure of the monolayer π as a function of surface area per molecule A: $\pi = \gamma - \gamma_0 = f(A)$, where γ_0 is the surface tension of the surface covered with lipids and γ is the surface tension of the pure air/water interface (72.45 mN/m).

Results and Discussion

The effect of pH on SM lipid membrane interfacial tension was determined at room temperature at pH 2–12 (Fig. 3). The maximal interfacial tension was 4.42 mN m^{-1} at pH 4.01. This interfacial tension value is similar to interfacial tension values previously obtained for membranes composed exclusively of PC (3.53 mN m^{-1}, pH 4.12) (Petelska and Figaszewski 2000), PS (2.94 mN m^{-1}, pH 3.80) (Petelska and Figaszewski 2002a) and PE (4.06 mN m^{-1}, pH 4.18 (Petelska and Figaszewski 2002b).

In order to calculate the membrane isoelectric point, it is necessary to determine acid–base equilibrium constants. Liposomes were used to determine the equilibrium constants as described previously (Petelska and Figaszewski 2000). The use of liposomes ensures a uniform distribution of acidic and basic groups in spite of the poor solubility of SM in water.

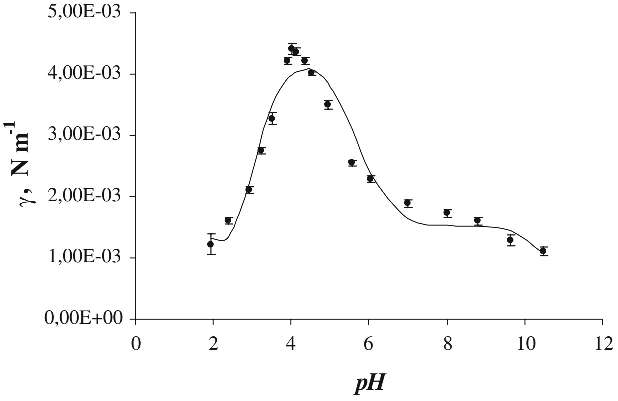


Fig. 3 Effect of pH on the SM membrane interfacial tension, model I. Experimental values (*points*) are compared with calculated values from model I (*solid line*)

Acid–base equilibrium constants were determined by titrating SM liposomes with hydrochloric acid and sodium hydroxide. The pK_{A} of SM was determined to be 2.590 and the pK_{B} was 5.308. Four acid–base equilibrium constants were needed for the model calculations, but only two could be determined from the titration measurements. In a previous study (Petelska and Figaszewski 2000), acid–base equilibrium constants were determined for $-\mathrm{PO}^{(-)}$ ($K_{\mathrm{A}_1} = 10^{2.581}$) and $-\overset{(+)}{\mathrm{N}}(\mathrm{CH}_3)_3$ ($K_{\mathrm{B}_1} = 10^{5.687}$). Assuming that the K_{A} value determined by titration is the mean of K_{A_1} and K_{A_2}, the K_{A_2} value was calculated to be $10^{2.599}$. Similarly, K_{B_2} was calculated to be $10^{4.929}$.

Experimental and theoretical values calculated using Eq. 22 from model I are shown in Fig. 3. In model I, the SM membrane total interfacial tension value is the sum of the component interfacial tensions (A_1^-, $\mathrm{A}_1\mathrm{H}$, B_1^+, $\mathrm{B}_1\mathrm{OH}$, A_2^-, $\mathrm{A}_2\mathrm{H}$, B_2^+ and $\mathrm{B}_2\mathrm{OH}$). K_{A_1}, K_{A_2}, K_{B_1} and K_{B_2} were substituted into Eq. 22 to calculate the interfacial tension values at different pH values. Interfacial tension values for individual components of the SM membrane were determined by linear regression using Excel 2007 (Microsoft, Redmond, WA). $\gamma^0_{\mathrm{A}_1^-}$, $\gamma^0_{\mathrm{A}_1\mathrm{H}}$, $\gamma^0_{\mathrm{B}_1^+}$, $\gamma^0_{\mathrm{B}_1\mathrm{OH}}$, $\gamma^0_{A_2^-}$, $\gamma^0_{\mathrm{A}_2\mathrm{H}}$, $\gamma^0_{\mathrm{B}_2^+}$ and $\gamma^0_{\mathrm{B}_2\mathrm{OH}}$ were -1.23×10^{-2}, 1.30×10^{-1}, -4.79×10^{-3}, -7.62×10^{-3}, 1.76×10^{-3}, -1.43×10^{-1}, -8.43×10^{-1} and 1.99×10^{-3} N m^{-1}, respectively. When the interfacial tension values are negative, it can be assumed that the form does not exist.

Coverage of the lipid membrane surface by H$^+$ and OH$^-$ ions as a function of pH is presented in Fig. 4. The coverage curves at acidic pH values (lines 1 and 2) overlap. Therefore, the SM membrane surface is not covered by H$^+$ and OH$^-$ ions when the pH is near the isoelectric point (i.e., pH 4).

The SM membrane interfacial tensions at different pH values were also calculated using Eq. 45 from model II (Fig. 5). In this model, the total interfacial tension value is the sum of the individual components (i.e., A_1^- A_1H, B_1^+, B_1OH, $A_2^-B_2^+$, $A_2^-B_2OH$, $A_2HB_2^+$, A_2HB_2OH). Determination of the interfacial tension of the individual components was difficult because the association constant values K_3, K_4 and K_5 were unknown ($K_1 = K_{A_1}$ and $K_2 = K_{B_1}$). For this reason, the individual equation coefficients (m_1, m_2, m_3, m_4, m_5, m_6, m_7, m_8 and b) were obtained from linear regression calculations in Excel 2007.

Interfacial tension of the membrane formed from SM was also calculated using Eq. 45. The calculated values are plotted in Fig. 5 versus pH of electrolyte solution; points and the theoretical values, which were derived according to model II and may be represented by a continuous line, present the experimental values. It is seen in Eq. 45 that the total interfacial tension values of the SM membrane are the sum of its individual components, i.e., A_1^- A_1H, B_1^+, B_1OH, $A_2^-B_2^+$, $A_2^-B_2OH$, $A_2HB_2^+$, A_2HB_2OH. However, determination of interfacial tension of the individual components was difficult because the association constant values K_3, K_4, K_5 were unknown (the association constant $K_1 = K_{A_1}$ and $K_2 = K_{B_1}$). For this reason, the individual equation coefficients ($m_1, m_2, m_3, m_4, m_5, m_6, m_7, m_8$ and b) were obtained using the linear regression method of the Excel 2007 program.

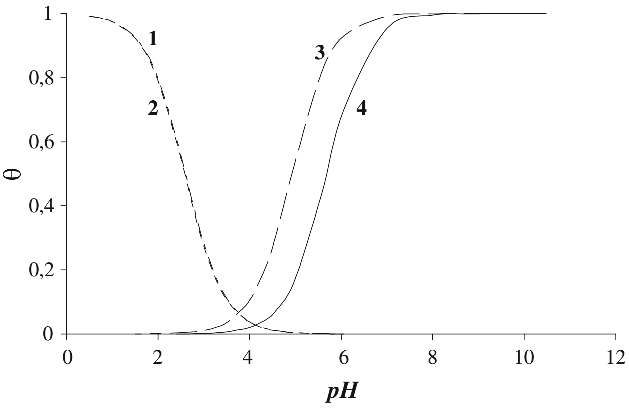


Fig. 4 Lipid membrane surface coverage. Calculated θ values for A_1H (*1*), B_1OH (*2*), A_2H (*3*) and B_2OH (*4*) groups are plotted as a function of pH

In model III, Gibbs isotherm was used to describe the acid–base equilibria between the functional groups of the SM molecules in the membrane and the H^+ and OH^- ions in solution. Experimental and theoretical values calculated using Eq. 56 from model III are shown in Fig. 6.

The surface area of an SM molecule is required in order to calculate the interfacial tension values. The area occupied by an SM molecule was determined to be 45 $Å^2$ using the Langmuir method (Petelska et al. 2008). Other literature values range from 40 to 55 $Å^2$ (Chiu et al. 2003; Shaikh et al. 2001).

Model III yielded the best agreement between the experimental and calculated values. Model II calculated values were closer to the experimental values than model I.

Table 1 summarizes the physicochemical parameters for membranes composed from four phospholipids: SM, PC (Petelska and Figaszewski 2000, 2003b), PS (Petelska and Figaszewski 2002a, 2003a) and PE (Petelska and Figaszewski 2002b). A number of conclusions can be drawn from Table 1. The isoelectric point of all four phospholipids is around pH 4, similar to that observed for biological membranes (Nalecz and Wojtczak 1982). Furthermore, the calculated interfacial tension values for the four phospholipids are consistent with other reported values (Coster and Simons 1968; Tieleman and Berendsen 1996; Tien 1974) and range from 0.2 to 6.0 mN m $^{-1}$. Finally, the surface area occupied by a glycerophospholipid molecule (PC, PS or PE) is almost the same, while the surface area occupied by a phosphosphingolipid molecule of SM is

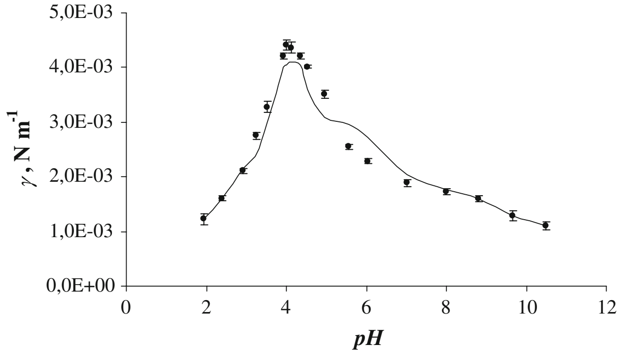


Fig. 5 Effect of pH on the SM membrane interfacial tension, model II. Experimental values (*points*) are compared with calculated values from model II (*solid line*)

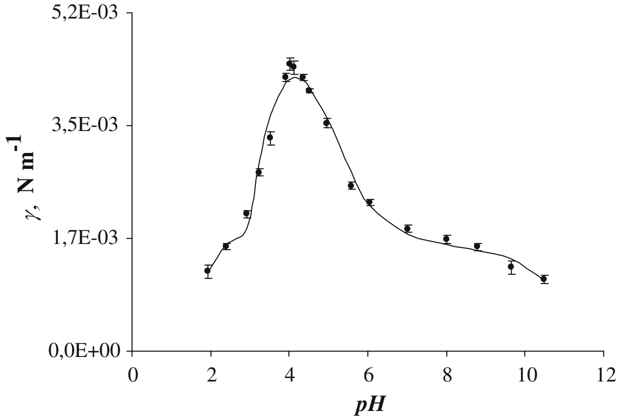


Fig. 6 Effect of pH on the SM membrane interfacial tension, model III. Experimental values (*points*) are compared with calculated values from model III (*solid line*)

Table 1 Physicochemical parameters for SM, PC, PS and PE

Parameter	SM	PC	PS	PE
Interfacial tension (mN m^{-1})	4.42	3.53	2.94	4.06
Isoelectric point (pI)	4.01	4.12	3.80	4.18
Acid–base equilibria				
pK_A	2.590	2.581	3.360	2.420
pK_B	5.308	5.687	9.550	5.980
Surface concentration at pI (mol m^{-2})	3.69×10^{-6}	1.96×10^{-6}	2.42×10^{-6}	2.23×10^{-6}
Surface area ($Å^2$ mol^{-1})	45	85	68.5	74.6
Surface energy (J mol^{-1})	2,100	1,800	1,824	1,854

smaller as a result of the differences in the hydrophobic layer of the membranes.

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