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# Influence of stearic acid monolayers upon the procaine adsorption from underlying alkaline aqueous solutions

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**Adsorption of procaine at the air/water interface and its penetration into stearic acid monolayers from aqueous subphase of pH 8 are studied by measuring surface tension of aqueous procaine solutions and by recording surface pressure vs. mean molecular area curves for stearic acid monolayers spread onto procaine solutions of different concentrations. The amount of procaine in the interface is derived by means of Gibbs' equation. Results are compared to those obtained earlier at pH 2 and on unbuffered subphases. With increasing pH an increasing procaine adsorption and procaine penetration is observed. This phenomenon is interpreted in terms of protolytic equilibria in which participate both surfactants procaine and stearic acid.**

## Introduction

Studies performed by using different experimental techniques showed some anesthetics to extend the surface area of monolayer films maintained at constant surface pressure [1,2] and to increase the surface pressure of lipid films maintained at constant area [3–5]. The molecular origin of the effects observed is presumed to be the weakening of the packing of lipids due to the anesthetic molecules inserted [2]. The efficiency of anesthetics in increasing the surface pressure was observed to be directly proportional to their relative anesthetic efficiency [3].

In these experiments frequently lipid monolayer membranes were used [2,4,5]. The reason for using these model system is the correlation between the anesthetic efficiency and the oil/water partition coefficients of anesthetics [5]. Further, the monolayer represents the half of a lipid bilayer, which plays a very important rôle in biomembranes. Monolayers spread at the plane air/water interface are sufficiently stable and suitable for experimental research, providing a convenient structural framework for the experimental study of physico-chemical interactions between the film forming molecules and various subphase components (drugs, electrolytes, soluble proteins etc.). These monolayer studies allow us to obtain direct information, at the molecular level, concerning the conformation and packing of molecules having biological significance in natural biomembranes in conditions near the in vivo ones.

In our previous paper [6] the stearic acid monolayer has been chosen as lipid membrane model, since stearic acid is a classical film forming substance, frequently used in monolayer studies, having one of the simplest structures. The influence of procaine, dissolved in the aqueous subphase, upon the properties of the stearic acid monolayer, spread at the air/water interface, has been studied. These investigations showed procaine to have an expanding effect upon the stearic acid monolayer, suggesting the idea that in the monolayer procaine molecules are inserted between the stearic acid molecules, i.e., in the interface the adsorption of procaine molecules occurs.

The adsorption ($\Gamma_2^0$) of the soluble surfactant 2 dissolved in water (noted as component 1) at the air/water interface obeys Gibbs' equation

$$\Gamma_2^\circ = -\frac{1}{kT}\left(\frac{\partial \sigma_2}{\partial \ln c_2}\right)_T \quad (1)$$

where $\sigma_2$ and $c_2$ stand for the surface tension of the surfactant solution and the molar concentration of surfactant 2, respectively, $k$ and $T$ are Boltzmann's constant and absolute temperature, respectively.

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If the surface of the surfactant solution is covered by a monolayer of the insoluble surfactant 3, interactions may appear between the molecules of surfactants 2 and 3, both at their polar headgroups and hydrocarbon chains. Due to these interactions the adsorption of component 2 may increase and this phenomenon is frequently called monolayer penetration [7–9]. In this case, at the air/water interface a mixed monolayer is formed, containing besides water molecules also molecules of both surfactants 2 and 3. Thermodynamically, the equilibrium between the bulk subphase and this mixed monolayer can be treated by using Gibbs' equation [7,8], which under isothermal conditions and for a constant mean molecular area $A_3$ of the insoluble surfactant 3, has the following form [8]:

$$\Gamma_2' = \frac{1}{kT}\left(\frac{\partial \pi}{\partial \ln c_2}\right)_{T,A_3} \tag{2}$$

where $\Gamma_2'$ stands for the number of soluble surfactant molecules 2 adsorbed per unit area of the 'free' interface, not covered by insoluble surfactant molecules 3, $\pi$ means the surface pressure defined as $\pi = \sigma_0 - \sigma$, $\sigma_0$ representing the surface tension of pure water.

The adsorption of molecules 2 per unit area of the mixed monolayer ($\Gamma_2$) can be calculated from $\Gamma_2'$ by taking into account the actual area necessity of the film forming molecules 3, viz. the partial mean molecular area of 3, denoted as $\bar{A}_3$. One obtains [7,8]:

$$\Gamma_2 = \left(1 - \frac{\bar{A}_3}{A_3}\right)\Gamma_2' \tag{3}$$

Attempts were made to approximate $\bar{A}_3$ by $A_3^0$, i.e., by taking the mean molecular area of 3, at the same $\pi$ but in the absence of the subphase component 2, for $\bar{A}_3$ [7]. Another procedure consists in deriving $\bar{A}_3$ from $A_{23}$ vs. $x_2^M$ curves, where $A_{23}$ stands for the mean molecular area of 2 and 3 in the mixed monolayer and $x_2^M$ for the molar fraction of 2 in the monolayer, calculated by neglecting the number of moles of water ($n_1$) in the monolayer [8]. Since the molecules of surfactant 3 are anchored into the surface layer through their polar headgroups, we propose to take for $\bar{A}_3$ the collapse area $A_{3c}$ of surfactant 3.

In our researches [10], penetration of procaine (P) into stearic acid monolayers has been studied on aqueous subphases of pH 2 and on unbuffered subphases. These studies revealed that at a given procaine concentration ($c_2$) the procaine adsorption increases with increasing pH, due to the protolytic equilibria in which procaine participates. At pH 2 in the solution there is a mixture of single and double protonated cationic species ($PH^+$ and $PH_2^{2+}$), whereas in unbuffered solutions (pH between 5 and 5.6) one has practically only $PH^+$, having a higher surface activity as compared to $PH_2^{2+}$ [6].

Anyway, in the presence of an stearic acid monolayer an enhanced procaine adsorption has been observed. Upon compression of the monolayer the procaine adsorption increases up to a maximum value and then it diminishes. This means that at higher surface densities of the stearic acid molecules the penetrated procaine molecules are gradually expulsed from the monolayer. The expulsed procaine molecules seem to form a subjacent monolayer interacting in vertical direction with the stearic acid monolayer, entailing an important increase of the collapse pressure of the latter.

The extent of the procaine penetration is characterized by the penetration number, defined as the ratio between the number of surfactant molecules 2 and 3 per unit area of the monolayer, i.e.

$$n_p = \Gamma_2/\Gamma_3 = \Gamma_2 A_3 \tag{4}$$

Combining Eqs. 2, 3 and 4 and by taking $A_{3c}$ for $\bar{A}_3$, one obtains:

$$n_p = \frac{A_3 - A_{3c}}{kT}\left(\frac{\partial \pi}{\partial \ln c_2}\right)_{T,A_3} \tag{5}$$

Our studies showed $n_p$ to increase with increasing pH.

In the present paper the adsorption of procaine (P) at the air/water interface is studied in the absence and presence of stearic acid monolayer the subphase pH being equal to 8. At this pH value in the solution besides $PH^+$ cations also neutral procaine molecules occur. Further, the stearic acid monolayer becomes a charged one, consisting of stearate anions, in contrast with pH 2, where the monolayer is formed only of neutral stearic acid molecules, and with (pH 5–5.6) where the neutral stearic acid molecules are the major species, but stearate anions also appear [11].

## Materials and Methods

Stearic acid used was a commercial product of p.a. purity (Schuchardt). Procaine was used as procaine chlorohydrate, commercial product (Hoechst). $KH_2PO_4$ and $Na_2HPO_4 \cdot 2H_2O$ used for the buffer solutions were also of p.a. purity.

Surface tensions of procaine solutions not covered by stearic acid monolayers were measured at 20 °C by using du Noüy's ring method.

Stearic acid were spread at the air/aqueous solution interface at 22 °C, by using benzene as spreading solvent. The surfactant solution was placed onto the subphase by means of a micropipette. It was left 15–30 min for evaporation of benzene and the monolayer obtained was compressed. The equilibrium between the monolayer and the subphase was established rapidly, allowing us to record a $\pi$ vs. $A_3$ curve in 10 to 30 min. Compression speeds between 0.005 and 0.025 $nm^2$/

molecule per min ensured a good reproducibility [6,11]. Compression of the monolayer was performed discontinously by using the Wilhelmy method. In all the cases a number of 8 to 10 compression isotherms have been recorded under identical experimental conditions.

The subphase was redistilled water, containing 0.1 M phosphate buffer and varying amounts of procaine, dissolved ahead of spreading the stearic acid monolayer.

## Results and Discussion

*Adsorption of procaine in absence of stearic acid*

The surface tension of procaine solutions as function of the logarithm of procaine molar concentration ($c_2$) is presented in Fig. 1. Due to the logarithmic concentration scale used in Fig. 1, the procaine adsorption ($\Gamma_2^0$) can easily be obtained according to the Gibbs' equation (Eqn. 1), by means of graphical derivation.

As seen from Fig. 1, at higher $c_2$ values, the $\pi$ vs. log $c_2$ plot becomes linear, indicating the saturation of the interface. The mean molecular area of procaine calculated from the slope of this linear portion is of 0.91 nm$^2$/molecule, much less than found at pH 2 (2.07 nm$^2$) and on unbuffered subphase (1.96 nm$^2$). This large difference might be due to the protolytic equilibria in which procaine participates. In acidic and neutral media only cationic species are present, but at pH 8 also the neutral procaine molecules have an important weight [6], therefore the electrostatic repulsion between the adsorbed molecules becomes less, allowing a closer packing. According to our molecular model calculations [12], the area necessity of procaine in a horizontal, lying down position at the interface is about 1.5 nm$^2$, if the molecules are randomly oriented, or are thought to freely rotate. In the case of closely packed, oriented molecules this area necessity is only 0.64 nm$^2$. This means that, presumably, even at pH 8 the procaine molecules are adsorbed at the air/water interface in a horizontal position.

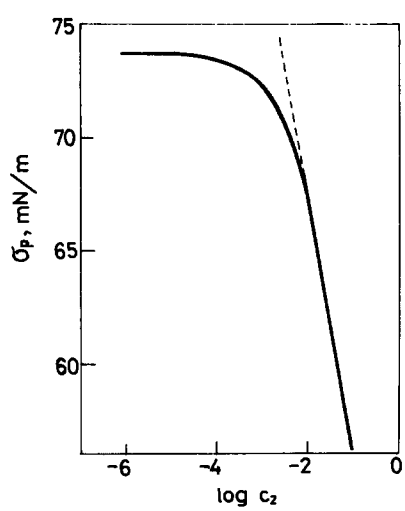


Fig. 1. Surface tension of procaine solutions as function of the logarithm of procaine concentration ($c_2$).

TABLE I

*Procaine adsorption ($\Gamma_2^0 \times 10^{-13}$, molec./cm$^2$) at the air/water interface without stearic acid monolayer, obtained by means of Eqn. 1*

| $c_2$ (M) | $\Gamma_2^0$; subphase | | |
|---|---|---|---|
| | pH 2 | unbuffered | pH 8 |
| $10^{-3}$ | 0.267 | 0.694 | 1.463 |
| $10^{-2}$ | 0.908 | 2.616 | 11.017 |
| $10^{-1}$ | 4.150 | 5.103 | 11.017 |
| 1 | 4.831 | 5.103 | 11.017 |

In Table I the adsorption values ($\Gamma_2^0$), obtained at different pH values are presented as function of $c_2$. As seen from Table I, with increasing pH the concentration $c_2$, at which $\Gamma_2^0$ attains its limiting value, corresponding to the saturation of the interface, diminishes. Meanwhile, with increasing pH even this limiting $\Gamma_2^0$ value gradually increases. Both phenomena indicate that the surface activity of the molecular species increases in the order $PH_2^{2+} < PH^+ < P$.

*Compression isotherms and surface characteristics of stearic acid monolayers*

Compression isotherms, i.e. $\pi$ vs. $A_3$ curves of stearic acid monolayers spread at the air/aqueous procaine solution (pH 8) interface, recorded for several subphase procaine concentrations, are given in Fig. 2. The shape of these curves is very different from those obtained for

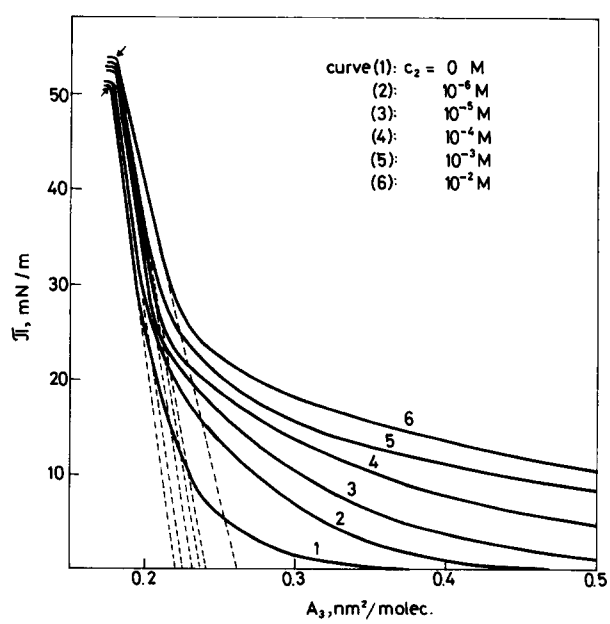


Fig. 2. Compression isotherms of stearic acid monolayers spread onto aqueous procaine solutions (pH 8), at different procaine concentrations ($c_2$).

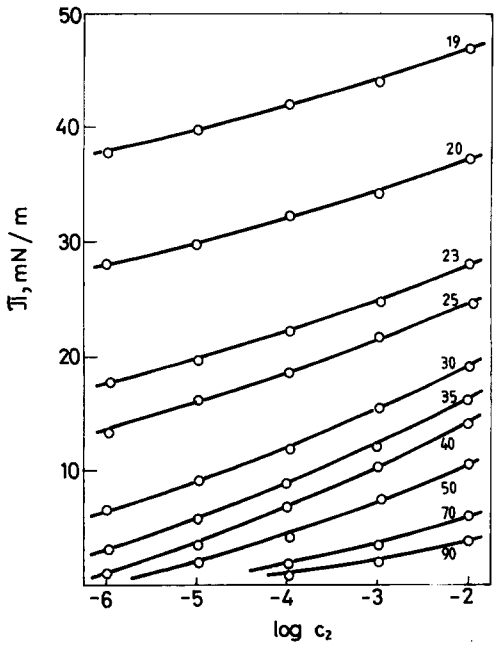


Fig. 3. $\pi$ vs. log $c_2$ curves for constant mean molecular area $A_3$ of stearic acid. Figures near the curves indicate the value of $A_3$ expressed in Å$^2$/molecule.

pH 2 and for unbuffered systems [10]. At pH 8 the stearic acid monolayer is charged, formed by stearate anions and consequently no phase transition is observed and the monolayer remains in the liquid condensed state up to the collapse. As far as the influence of the subphase procaine concentration is concerned, it is similar to that observed at lower pH values, i.e., with increasing $c_2$ the monolayer becomes more expanded and the isotherms exhibit increasing collapse pressure values.

From the isotherms given in Fig. 2 surface characteristics of the stearic acid monolayer have been derived, viz. collapse area $A_{3c}$, corresponding to the sudden slope change at high $\pi$ values, limiting molecular area $A_{30}$, by extrapolating to $\pi = 0$ the linear portion of the isotherms corresponding to large $\pi$ values, collapse pressure $\pi_c$ and surface compressional modulus defined as $C_{s0}^{-1} = -A_{30}(d\pi/dA_3) = A_{30}\pi_c/(A_{30} - A_{3c})$. These characteristics are presented in Table II.

As seen from Table II, $A_{30}$ increases with increasing $c_2$, indicating an expanding effect of the subphase procaine. Reversely, $C_{s0}^{-1}$ decreases with increasing $c_2$, revealing a fluidizing effect of procaine, leading to a larger compressibility of the film. Both effects plead for penetration of the procaine molecules into the stearic acid monolayer. Since $A_{3c}$ remains practically the same, irrespective of the $c_2$ value, one may presume that at higher surface densities of stearic acid, i.e., at high $\pi$ values, the procaine molecules are squeezed out from the monolayer. The collapse pressure increases with increasing $c_2$, i.e., the subphase procaine has a stabilizing effect upon the monolayer, similarily as at lower pH values. Presumably, the procaine molecules expulsed from the monolayer remain in a subjacent layer, continuing to interact with the SA molecules (stearate anions) in the vertical direction, which entails the increase of $\pi_c$.

TABLE III

*The derivative $(\partial\pi/\partial \log c_2)_{T,A_3}$ for mixed stearic acid and procaine monolayers ($\pi$ measured in mN/m, $c_2$ in mol/l) as function of the subphase procaine concentration $c_2$ and mean molecular area $A_3$ of stearic acid*

| $A_3$ (nm$^2$/mol) | Derivative value; $c_2$ (M) | | | |
|---|---|---|---|---|
| | $10^{-5}$ | $10^{-4}$ | $10^{-3}$ | $10^{-2}$ |
| 0.90 | 0 | 0.80 | 1.25 | 1.70 |
| 0.80 | 0 | 1.25 | 1.65 | 1.95 |
| 0.70 | 0 | 1.55 | 2.00 | 2.25 |
| 0.60 | 0.70 | 1.90 | 2.40 | 2.75 |
| 0.50 | 1.50 | 2.50 | 3.10 | 3.40 |
| 0.40 | 2.70 | 3.25 | 3.50 | 3.65 |
| 0.35 | 2.80 | 3.30 | 3.50 | 3.65 |
| 0.30 | 2.65 | 3.10 | 3.25 | 3.45 |
| 0.25 | 2.50 | 2.70 | 2.90 | 3.10 |
| 0.23 | 2.20 | 2.50 | 2.70 | 2.95 |
| 0.20 | 2.00 | 2.25 | 2.50 | 2.70 |
| 0.19 | 1.90 | 2.15 | 2.35 | 2.50 |

*Procaine penetration into the stearic acid monolayer*

Gibbs' equation (Eqn. 2) allows us to estimate the amount of procaine penetrated into the stearic acid monolayer. By deriving from the compression isotherms (Fig. 2) $\pi$ values corresponding to a given constant $A_3$, $\pi$ vs. log $c_2$ curves may be obtained for different $A_3$ values. A set of such curves is presented in Fig. 3. By means of graphical derivation $(\partial\pi/\partial \log c_2)_{T,A_3}$ values can be obtained from these curves. Derivative values are summarized in Table III.

TABLE II

*Surface characteristics of stearic acid monolayers on procaine-containing aqueous subphases at pH 8*

| $c_2$ (M) | 0 | $10^{-6}$ | $10^{-5}$ | $10^{-4}$ | $10^{-3}$ | $10^{-2}$ |
|---|---|---|---|---|---|---|
| $A_{3c}$ (nm$^2$/molecule) | 0.176 | 0.178 | 0.180 | 0.180 | 0.180 | 0.180 |
| $A_{30}$ (nm$^2$/molecule) | 0.220 | 0.226 | 0.232 | 0.236 | 0.240 | 0.260 |
| $\pi_c$ (mN/m) | 51 | 51 | 52 | 52.5 | 53 | 53.5 |
| $C_{s0}^{-1}$ (mN/m) | 255 | 240 | 232 | 221 | 212 | 174 |

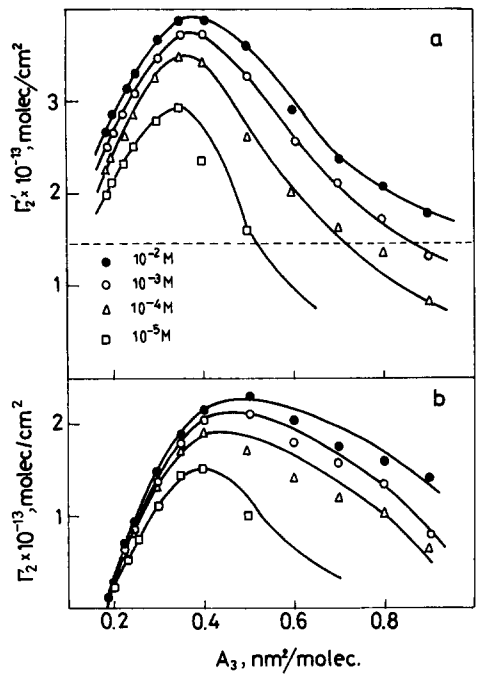


Fig. 4. P adsorption per unit area of the 'free' interface (a) from the stearic acid monolayer and of the mixed (stearic acid and procaine) monolayer (b), as function of $c_2$ and $A_3$. Figures after the symbols of experimental points: subphase procaine concentration ($c_2$). Horizontal dashed lines: procaine adsorption at the air/water interface for $c_2 = 10^{-3}$ M, in absence of stearic acid.

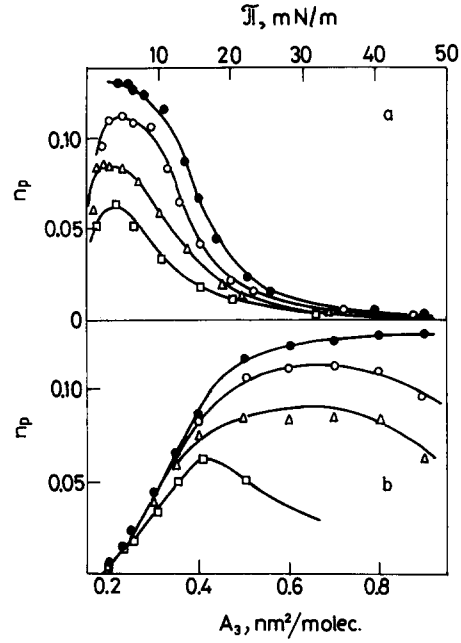


Fig. 5. Penetration numbers calculated by means of Eqn. 5 as function of $\pi$ (a) and of $A_3$ (b). Symbols as in Fig. 4.

The general picture is similar as in the case of amino acid penetration into lecithin monolayers [8] and for procaine penetration into stearic acid monolayers on acidic and unbuffered subphases [10]. The slope of the curves from Fig. 3 increases with decreasing $A_3$, attains a maximum at about 0.35 nm$^2$ and further it decreases anew, indicating that at the compression of the monolayer, first, an enhanced procaine adsorption is observed, followed by an expulsion of the penetrated molecules at low $A_3$ values.

The procaine adsorption per unit area of the 'free' interface of the stearic acid monolayer ($\Gamma_2'$) calculated by means of eqn. 2 is given in Fig. 4a as function of $A_3$ for different $c_2$ values. Horizontal dashed line indicates the procaine adsorption in the absence of the stearic acid monolayer ($\Gamma_2^0$) from a $10^{-3}$ M procaine solution. As can be seen on the figure, $\Gamma_2'$ values depass very much this $\Gamma_2^0$, indicating, also at pH 8, an important interaction between the stearic acid and procaine molecules at the air/water interface, leading to the procaine penetration.

Procaine adsorption per unit area of the stearic acid monolayer ($\Gamma_2$) was calculated by means of Eqn. 3, taking $\bar{A}_3 = A_{3c} = 0.18$ nm$^2$. Results are visualized in Fig. 4b. The maximum of the curves is shifted a little towards higher $A_3$ values, as compared to the $\Gamma_2'$ vs. $A_3$ curves. One observes that for $c_2 = 10^{-3}$ M there is a large $A_3$ interval in which $\Gamma_2$ depasses $\Gamma_2^0$, i.e. the number of procaine molecules per unit area of the mixed interface is larger than this number in the adsorbed procaine monolayer in the absence of stearic acid film, although an important part of the interface is occupied by the polar headgroups of the stearic acid molecules.

Penetration numbers calculated by means of Eqn. 5 are given in both $n_p$ vs. $\pi$ (a) and $n_p$ vs. $A_3$ (b) plots in Fig. 5. As can be seen, $n_p$ exhibits a maximum at a surface pressure of about 5 mN/m. Its maximum value is much higher than the values for subphases of lower pH [10]. The influence of $c_2$ and of the subphase pH upon the maximum $n_p$ values is illustrated in Table IV. The important increase of $n_p$ with increasing pH is a co-operative effect of the protolytic equilibria in which both surfactants participate. On the one hand, the successive deprotonations $PH_2^{2+} \rightarrow PH^+ \rightarrow P$ lead to the formation of molecular species with higher surface activity. On the other hand, deprotonation of the neutral stearic acid molecules and the formation of the stearate anions entail the appearance of important electrostatic

TABLE IV

*Maximum penetration number values of procaine into stearic acid monolayers*

| Subphase | Maximum $n_p$ value; $c_2$ (M) | |
|---|---|---|
| | $10^{-3}$ | $10^{-2}$ |
| pH 2 | 0.040 | 0.051 |
| Unbuffered | 0.059 | 0.089 |
| pH 8 | 0.113 | 0.130 |

attractions between the oppositely charged surfactant ions.

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