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INTERFACIAL AND WETTING BEHAVIOR OF CATIONIC, ANIONIC AND NONIONIC SURFACTANTS IN THE ABSENCE AND PRESENCE OF LYSOZYME

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Abstract. Herein, we discuss various physicochemical properties of cationic (CTAB), anionic (SDBS) and nonionic (TX-100) surfactants in the absence and presence of lysozyme, at different temperatures by using tensiometery. The surface excess (Γ_{max}) decreases with the increase in temperature for all three kinds of surfactants in the absence and presence of lysozyme, but the most prominent decrease is to be observed for SDBS as compared to CTAB and TX-100 in the presence of lysozyme. The minimum area per molecule (A_{min}) follows the opposite trend as expected. In addition, contact angle analysis was also done to observe the wettability of poly(methyl methacrylate) (PMMA) surface by these surfactants in the absence and presence of lysozyme.

Keywords: surface tension, surfactants, lysozyme, surface excess, wettability.

1. Introduction

Lysozyme (Lys) is an antimicrobial and small monomeric globular protein widely distributed in various biological fluids [1]. It is constituted by 129 amino acid residues with a specific pattern that contain 6 tryptophan (Trp), 3 tyrosine (Tyr) and 4 disulfide bonds, and two dominant fluorophores (Trp 62 and Trp108) are also arranged close to the substrate binding site that plays an important role in binding with a substrate or an inhibitor and in stabilizing the structure, as shown by high-resolution crystal structure [2]. Owing to the physiological and pharmaceutical functions, such as anti-inflammatory, anti-viral, immune modulatory, anti-histaminic and anti-tumor activities, it has been extensively used in the pharmaceutical and food fields [3-5].

Surfactants have much accountability to lower the interfacial tension and form various kinds of aggregates in solutions like supramolecular structures such as micelles and bilayers, because of their amphiphilic nature [6]. Solubilization of membrane proteins, protein solubilization in reverse micelles, surface fouling and cleaning, and stabilization of food colloids are influential applications of protein-surfactant interactions. There are different types of intermolecular forces results in the relatively complex interactions between surfactants and proteins because of the charged groups and hydrophobic portions of both, *i.e.* ionic surfactants and proteins. The electrostatic forces may involve interaction of ionic head groups of surfactants to the oppositely charged group on the protein surface, while non-polar tail groups of surfactants may bind to non-polar sites on the protein surface through hydrophobic forces and such type of interactions dependent on the nature of proteins as well as the surfactant. At lower surfactant concentration, former forces are responsible while at higher concentration that latter ones are responsible, and with the increase in surfactant concentration, the protein-surfactant complex becomes initially more hydrophobic than the protein itself and then decreases in hydrophobicity.

The increase in wettability of a solid surface (PMMA) has become an important task for widespread applications and this can be enhanced with the help of different single chain surfactants [7, 8], mixed surfactant systems [9-11] and additives [12, 13]. Furthermore, the adsorption of proteins to the solid surfaces plays an important role in controlling cell interactions with surfaces. However, very few studies of proteins adsorption with complex proteins mixtures and surfactants on surfaces have been done and due to this, the phenomenon is not well understood [14]. Herein, the

wetting properties of PMMA by different surfactants (CTAB, SDBS and TX-100) in the absence and presence of lysozyme exhibit some interesting results. Liu *et al*. [15] proposed methyl methacrylate/*N,N*-dimethylacrylamide (MMA/DMA) copolymers for use as hydrogels. Hydrogels are three-dimensional polymeric networks and have found widespread applications in the biomedical industries, including drug delivery agents, prosthetic devices and contact lenses [16, 17].

The literature on the individual effect of cationic/anionic/non-ionic surfactants on water soluble proteins [18-21] is available, but the comparative studies of these kinds of surfactants with the water soluble proteins are scared. In this regard herein, we are investigating the comparative effect of different kinds of surfactants, *i.e*. cetyltrimethylammonium bromide (CTAB), sodiumdodecylbenzene sulphonate (SDBS) and *t*-octyl phenoxypolyethoxyethanol (n = 9–10, TX-100) in the absence and presence of the lysozyme by using the tensiometery at different temperatures. The contact angle analysis was also done to show the wettability of poly(methyl methacrylate) (PMMA) in the absence and presence of lysozyme by these three different kinds of surfactants on PMMA at room temperature. The aim of the present work is to investigate the interfacial and wetting properties of different kinds of surfactants in absence and presence of lysozyme, and their thermodynamics.

2. Experimental

2.1. Materials

Lysozyme (≥ 98 %, Sigma, USA), CTAB (≥ 98 %, Spectrochem PVT. LTD., Mumbai), SDBS (≥ 99 %, Sigma Aldrich, USA), and TX-100 (≥ 98 %, SRL, Mumbai) were used as received, without further purification. Their aqueous stock solutions of specific concentration were prepared in doubly distilled water. The specific conductance of doubly distilled water is 1.82 μS/cm, *i.e*. measured by the Eutech conductivity bridge having a cell constant of 1.02.

2.2. Surface Tension Measurements

Surface tension g was measured by Delta-Pi Langmuir microtensiometer (Kibron, Helsinki, Finland) based on the Wilhelmy method and utilizing a small diameter (0.51 mm) special alloy wire. The temperature of the measurement cell was controlled by a Grant GD120 water thermostat with a temperature stability of ±0.02 K. The wire used in the measurement was cleaned by red hot burning from butane gas through a blazer. The value γ for each set of experiment was measured by successive addition of concentrated solution of the mixture in pure water and in lysozyme solution of known concentration at definite temperature. In order to determine the values of critical micelle concentration (*cmc*), two linear fits were used for each of the isotherms. The first line was fitted to the interval of concentration characterized by a linear decrease of the surface tension and the second one to the region of concentration with nearly constant surface tension. The *cmc* was determined from the break point of the surface tension g *vs*. log$C$ curves and accuracy on the individual surface tension reading is approximately ±0.2 m·Nm^{-1}.

2.3. Contact Angle Measurements

The contact angle measurements were made by using Pheonix 150 (SEO, Korea) [22] to quantify the changes in the wetting properties of pure lysozyme and in the presence of SDBS, CTAB, and TX-100 *via* drop shape analysis method on PMMA, at room temperature. 6 μl solutions were used to create a droplet on the PMMA surface. The data analysis was carried out by using surface ware 7 ver.10.11 software. The images were captured at a rate of 25 frames/second from a total of 100 frames.

3. Results and Discussion

The physicochemical and thermodynamic properties, of different kind of surfactants in the absence and presence of lysozyme are discussed in the following terms:

3.1. Critical Micellar Concentration

As shown in Table 1, the *cmc* value increases with the rise of temperature in case of pure cationic (*i.e*., CTAB) and anionic (*i.e*., SDBS) surfactants as well as in the presence of lysozyme, while *cmc* value decreases in case of non-ionic surfactant (*i.e*., TX-100). For ionic surfactants the increase is higher in case of SDBS as compared to CTAB and slight increase is also observed in both cases in the presence of lysozyme (Table 1). In case of non-ionic surfactant (*i.e*., TX-100), there is a very slight change to be observed. It is worth mentioning that *cmc* of ionic amphiphiles first decreases at low temperatures and increases at high temperatures [23], while in case of non-ionic surfactants, the *cmc* decreases with increasing the temperature [24]. In addition for ionic systems, continuous increase in *cmc* with temperature is also reported in some cases [25, 26].

3.2. Interfacial Properties

An effective measure of the adsorption at the air/water interface is measured by the surface excess, Γ_{max} (mol·m^{-2}), calculated by the Gibbs adsorption equation [27]:

$$\Gamma_{\max} = -\frac{1}{2.303nRT}\left(\frac{\partial g}{\partial \log C}\right) \quad (1)$$

and the minimum area per molecule, $A_{\min}$ (Å^2) by the following equation [27]:

$$A_{\min} = \frac{10^{20}}{N_A \cdot \Gamma_{\max}} \quad (2)$$

where R, T, and N_A are gas constant, temperature (in Kelvin), and Avogadro's number, respectively; n is introduced to allow for simultaneous adsorption of cation and anion, its value is taken 2 for ionic surfactants while 1 for non-ionic surfactant and ($\partial\gamma/\partial \log C$) represents the slope of the plot between γ *vs.* log$C$.

The values of $\Gamma_{\max}$ and $A_{\min}$ are reported in Table 1. $\Gamma_{\max}$ value decreases with the increase in temperature, where as $A_{\min}$ values increase. Thus, two factors complete each other in case of all three kinds of surfactants are pure, as well as in the presence of lysozyme, that suggests these systems involve both electrostatic and hydrophobic interaction [28]. In case of ionic surfactants, the value of $\Gamma_{\max}$ decreases more in case of SDBS as compared to CTAB that suggests the complex formation between SDBS and lysozyme is more favourable as compared to CTAB and lysozyme. At the same time $A_{\min}$ increases opposite to $\Gamma_{\max}$.

The surface pressure at *cmc*, π_{cmc}, was calculated from Eq. (3) [29]:

$$p_{cmc} = g_0 - g_{cmc} \quad (3)$$

where γ_0 and γ_{cmc} refer to surface tension of the solvent system that is aqueous solution of lysozyme in the present study and surface tension of the solution at *cmc* value. Its value is maximum in case of TX-100, whereas in case of ionic surfactants, the values are higher for SDBS as compared to CTAB, because of its more hydrophobic nature.

The values of pC_{20}, which are given by Eq. (4) (C_{20} being the concentration required to reduce the surface tension of solvent by 20 m·Nm^{-1} [27]), increase with the increase in α_1.

$$pC_{20} = -\log_{10} C_{20} \quad (4)$$

The greater the value of pC_{20}, the lower is the concentration needed to reduce the value by 20 mN·m^{-1}. This result reveals that the system is more surface active.

Table 1

Various interfacial properties (*cmc*, $\Gamma_{\max}$, $A_{\min}$, π_{cmc} and pC_{20}) for different surfactants in the absence and presence of lysozyme at different temperatures

Temperature, K	*cmc/cmc**, mM	$\Gamma_{\max}\cdot10^7$, mol/m^2	$A_{\min}$, Å^2	π_{cmc}	pC_{20}
SDBS					
298	1.38/1.23	25.97	63.93	37.0	3.34
308	1.45	20.57	80.73	38.1	3.29
318	1.57	16.65	99.76	41.3	3.79
SDBS+ Lysozyme					
298	1.50/1.29	15.38	107.98	39.0	3.42
308	1.53	13.72	121.05	38.1	3.38
318	1.60	12.23	135.81	41.8	3.48
CTAB					
298	0.92/0.89	25.00	66.42	34.9	3.57
308	0.93	23.27	71.35	36.2	3.66
318	0.94	21.47	77.33	35.2	3.62
CTAB+ Lysozyme					
298	0.98/0.91	24.55	67.63	33.4	3.22
308	1.06	22.24	74.67	33.3	3.32
318	1.09	20.52	80.92	35.7	3.26
TX-100					
298	0.30/0.28	36.02	46.11	44.99	0.04
308	0.28	33.80	49.14	40.24	0.03
318	0.27	30.24	54.92	44.37	0.02
TX-100+Lysozyme					
298	0.29/0.30	39.34	42.20	42.37	0.05
308	0.27	33.68	49.31	43.10	0.04
318	0.26	30.17	55.04	41.58	0.03

Table 2

Various thermodynamic parameters (ΔG_m^0, ΔG_{ads}^0, ΔH_m^0, ΔH_{ads}^0, ΔS_m^0, and ΔS_{ads}^0) for different surfactants in the absence and presence of lysozyme

Temperature, K	ΔG_m^0, kJ·mol^{-1}	ΔS_m^0, J·mol^{-1}·K^{-1}	ΔH_m^0, kJ·mol^{-1}	ΔG_{ads}^0, kJ·mol^{-1}	ΔS_{ads}^0, kJ·mol^{-1}·K^{-1}	ΔH_{ads}^0, kJ·mol^{-1}
SDBS						
298	-37.58	86.83	-11.70	-37.72	92.09	-10.28
308	-38.55		-11.80	-38.73		-10.37
318	-39.31		-11.70	-39.56		-10.28
SDBS+ Lysozyme						
298	-16.11	45.53	-2.54	-16.36	49.93	-1.48
308	-16.60		-2.58	-16.88		-1.45
318	-17.02		-2.54	-17.36		-1.48
CTAB						
298	-17.30	56.35	-0.51	-17.44	57.57	-0.28
308	-17.87		-0.52	-18.03		-0.30
318	-18.43		-0.51	-18.50		-0.28
CTAB+ Lysozyme						
298	-17.16	42.45	-4.51	-17.30	44.36	-4.08
308	-17.53		-4.45	-17.67		-4.01
318	-18.01		-4.51	-18.19		-4.08
TX-100						
298	-20.18	5.38	-18.58	-20.35	5.45	-18.68
308	-20.95		-19.29	-21.07		-19.39
318	-21.78		-20.07	-21.93		-20.20
TX-100+ Lysozyme						
298	-20.14	5.52	-18.49	-20.25	5.63	-18.57
308	-20.91		-19.21	-21.04		-19.31
318	-21.78		-20.03	-21.92		-20.13

3.3. Thermodynamic Properties of Micellization and Adsorption

Several thermodynamic parameters were calculated at the air/water interface as well as in the micelles by using the different thermodynamic equations. These thermodynamics parameters suitably represent the systems feasibility.

The standard Gibb's free energy of micellization, ΔG_m^0, can be evaluated from Eq. (5):

$$\Delta G_m^0 = RT \cdot \ln X_{cmc} \quad (5)$$

where X_{cmc} is the value of *cmc* in mole fraction units.

All the values of ΔG_m^0 are negative, as shown in Table 2, indicating that the process of micelle formation is spontaneous and general trend shows that ΔG_m^0 values for pure surfactant is more negative as compared to those in the presence of lysozyme. With the rise in temperature the values become more negative in all the cases. The results reveal that the micellization process is more favorable for SDBS in the presence of lysozyme as compared to CTAB and TX-100.

Standard entropy of micellization (ΔS_m^0) was calculated from the temperature dependence of standard Gibb's free energy of micellization using the relation (6) [30, 31]:

$$\Delta S_m^0 = -\frac{\partial(\Delta G_m^0)}{\partial T} \quad (6)$$

and standard enthalpy of micellization (ΔH_m^0) was obtained from the Gibb's-Helmholtz equation:

$$\Delta H_m^0 = \Delta G_m^0 + T\Delta S_m^0 \quad (7)$$

For the studied systems the ΔS_m^0 values (Table 2) are positive, suggesting that the value is maximum for pure SDBS compared to CTAB while in case of TX-100, its value is much lower as compared to ionic surfactants. In addition, an exothermic ΔH_m^0 suggests that micellization is favored by the enthalpy change, the same as entropy effect. The ΔH_m^0 values (see Table 2) explain different trends in all three surfactants. In case of SDBS the value tends towards the increase in the presence of lysozyme, while for CTAB the value is significantly decreased in the presence of lysozyme. In case of TX-100

there is almost no change in the value of ΔH_m^0 in the presence of lysozyme.

The molar free energy at the maximum adsorption attained at *cmc*, $G_{\min}$, is calculated using Eq. (8) [32]:

$$G_{\min} = g_{cmc} A_{\min} N_A \quad (8)$$

G_{min} is the minimum free energy of the given surface with fully adsorbed amphiphile molecules. Lower the value of the free energy, more stable is the surface formed. The value of $G_{\min}$ is minimum for TX-100 and maximum for CTAB at all temperatures in the absence of lysozyme, while in its presence there is almost no change in its value for TX-100, slight increase is observed for CTAB and a significant increase in case of SDBS (Fig. 1).

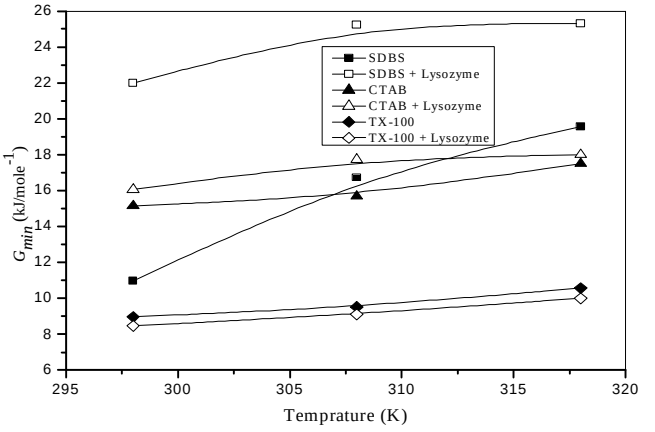

Fig. 1. Variation of $G_{\min}$ with the temperature for different surfactants in the absence and presence of lysozyme

The standard Gibb's energy of adsorption, ΔG_{ads}^0, is evaluated by using Eq. (9) [33]:

$$\Delta G_{ads}^0 = \Delta G_m^0 - \frac{p_{cmc}}{\Gamma_{\max}} \quad (9)$$

where $\pi_{cmc} = \gamma_0 - \gamma_{cmc}$ is the surface pressure at the *cmc*; γ_0 and γ_{cmc} are the surface tensions of pure solvent and of the amphiphilic solutions at the *cmc*, respectively. The values of ΔG_{ads}^0 follow the same trend as of ΔG_m^0 in all cases but the are slight more negative.

The values of ΔH_{ads}^0 and ΔS_{ads}^0 (Table 2) are evaluated from Eqs. (10) and (11), as before from relationships corresponding to Eqs. (6) and (7):

$$\Delta S_{ads}^0 = -\frac{\partial(\Delta G_{ads}^0)}{\partial T} \quad (10)$$

$$\Delta H_{ads}^0 = \Delta G_{ads}^0 + T\Delta S_{ads}^0 \quad (11)$$

The values of ΔS_{ads}^0 are also positive but slightly greater than ΔS_m^0 values in all cases, that reflects the greater freedom of motion for the hydrocarbon portion of the surfactants at the planar air/aqueous solution interface as compared to the interior surface of the micelle. In the presence of lysozyme, the ΔS_{ads}^0 values decrease in case of ionic surfactants, and the decrease is more prominent in case of SDBS as compared to CTAB. For TX-100 the value is slightly increased. The ΔH_{ads}^0 values are also negative in all cases and slightly lower in magnitude as compared to ΔH_m^0 but the trend is the same as for ΔH_m^0.

3.4. Structural Effects on Micellization and Adsorption

The work involved ($\Delta G_{mic}^0 - \Delta G_{ads}^0$) in transferring the surfactants molecule from a monolayer and to the micelle in the absence and presence of lysozyme at zero surface pressure was calculated as prescribed by Rosen [34] and is listed in Table 3. As shown in Table 3, the "work of transfer" (*i.e.*, the ease of adsorption to form a monolayer at zero surface pressure relative to the ease of micellization) shows almost no change except in the case of SDBS in the presence of lysozyme with the change in temperature from 298 to 318 K. In addition, the positive values of $\Delta G_{mic}^0 - \Delta G_{ads}^0$ suggest the greater positive entropy change upon adsorption than micellization [35].

3.5. Wetting Properties

To explore the wetting behaviour of PMMA by CTAB, SDBS and TX-100 with and without lysozyme, the values of contact angle below and above the *cmc*, and at the *cmc* were observed (Fig. 2).

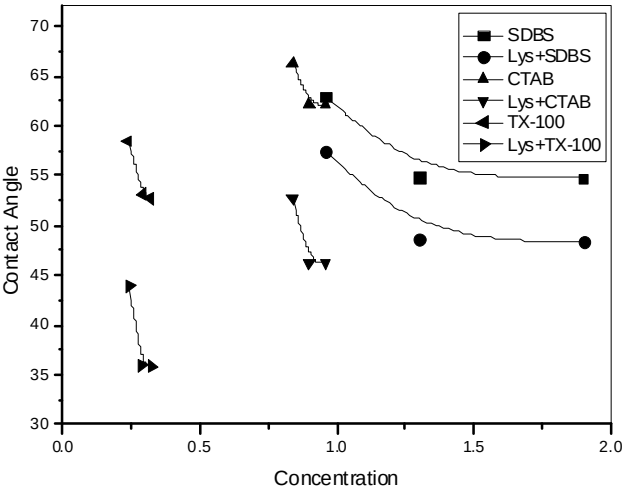

Fig. 2. Plot of concentration *vs.* contact angle for different kinds of surfactants in the absence and presence of lysozyme

Table 3

Structural effects on micellization and adsorption for different surfactants in the absence and presence of lysozyme

Temperature, K	$(\Delta G_m^0 - \Delta G_{ads}^0)$, kJ·mol^{-1}	$T(\Delta S_m^0 - \Delta S_{ads}^0)$, kJ·mol^{-1}· K^{-1}	$(\Delta H_m^0 - \Delta H_{ads}^0)$, kJ·mol^{-1}
SDBS			
298	0.14	-1.57	-1.42
308	0.18	-1.62	-1.43
318	0.25	-1.67	-1.42
SDBS+ Lysozyme			
298	0.25	-1.31	-1.06
308	0.28	-1.36	-1.07
318	0.34	-1.40	-1.06
CTAB			
298	0.14	-0.36	-0.22
308	0.16	-0.37	-0.22
318	0.16	-0.39	-0.22
CTAB+ Lysozyme			
298	0.14	-0.57	-0.43
308	0.14	-0.59	-0.44
318	0.18	-0.61	-0.43
TX-100			
298	0.13	-0.021	0.10
308	0.12	-0.022	0.10
318	0.15	-0.023	0.13
TX-100+Lysozyme			
298	0.11	-0.030	0.08
308	0.13	-0.031	0.10
318	0.14	-0.032	0.10

From Fig. 2 it can be seen that in the absence of lysozyme, the value of contact angle is maximum for CTAB, followed by SDBS and the minimum value was observed for TX-100 on PMMA. Furthermore, the contact angle values are less in SDBS as compared to CTAB because of more hydrobhobic nature of SDBS as compared to CTAB. The data also reveal that the solid-liquid interaction was maximum for TX-100 because of polyoxyethylenes groups. The adsorption of such type of surfactants initially proceeds *via* hydrogen bonding between their ethylene oxide groups and an adsorbent surface [36, 37] that results in minimum contact angle values for TX-100. In addition in the presence of lysozyme the contact angle value (63.80 for pure lysozyme on PMMA) decreases for all three surfactants because of hydrophobic-hydrophobic interactions, but the decrease is maximum for TX-100. Therefore, it was suggested that the wetting of PMMA in the presence of lysozyme was found to be more with TX-100 at the air-water interface, while in case of CTAB and SDBS (in the presence of lysozyme) the effect is reversed as in the absence of lysozyme due to strong complex formation between CTAB-lysozyme and the SDBS-lysozyme. This strong complex is formed actually because of the negative charge on lysozyme (aspartate) that strongly binds with CTAB due to the positive nature.

4. Conclusions

It has been shown from the above discussion that the presence of lysozyme alters the *cmc* of all three kinds of surfactants in such a manner that the *cmc* value increases with the rise of temperature in case of pure cationic (*i.e.*, CTAB) and anionic (*i.e.*, SDBS) surfactants, as well as in the presence of lysozyme. The *cmc* value decreases in case of non-ionic surfactant (*i.e.*, TX-100). If ionic surfactants are used, the value of Γ_{max} decreases more in case of SDBS as compared to CTAB that suggests the complex formation between SDBS and lysozyme is more favourable as compared to CTAB and lysozyme. The value A_{min} increases in the trend opposite to Γ_{max}. The G_{min} value is less in case of TX-100, which reveals that TX-100 is adsorbed strongly on the surface in the presence of lysozyme. The results concerning contact angle suggested that the wettability of PMMA is more with the non-ionic surfactant (TX-100) in the presence of lysozyme than that with ionic surfactants (SDBS/CTAB).

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МІЖФАЗНІ ТА ЗМОЧУВАЛЬНІ ВЛАСТИВОСТІ КАТІОННИХ, АНІОННИХ І НЕЙОННИХ ПАР У ПРИСУТНОСТІ І ВІДСУТНОСТІ ЛІЗОЦИМУ

Анотація. *З використанням тензіометрії досліджені фізико-хімічні властивості катіонних (CTAB), аніонних (SDBS) і нейоногенних (TX-100) ПАР в присутності і відсутності лізоциму за різних температур. Встановлено, що величина надлишку поверхні (Γ_{max}) зменшується із збільшенням температури для всіх трьох видів ПАР в присутності і відсутності лізоциму, але найбільш виражене зниження спостерігається на SDBS у порівнянні зі CTAB і TX-100 у присутності лізоциму. Як і очікувалось, величина мінімальної площі на одну молекулу (A_{min}) має протилежну тенденцію. Проведено аналіз для визначення крайового кута змочування поверхні полі(метил метакрилату) досліджуваними ПАР в присутності і відсутності лізоциму.*

Ключові слова: *поверхневий натяг, поверхнево-активні речовини, лізоцим, надлишок поверхні, змочування.*