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Protein-fatty Acid Interaction in Spread Monolayers

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Abstract — The pressure-area isotherms at air-water interface of pure protein (bovine serum albumin, β-lactoglobulin, α-lactalbumin) and pure fatty acid (stearic acid and arachidic acid) and their mixtures of different weight fractions have been studied at pH 3.0, 7.0 and 12.0 respectively using Langmuir balance method. The surface pressures π of protein monolayer at different surface areas A of the pure proteins at different pH-values of the subphase are fitted in a virial equation expressed in linear form so that virial coefficients of different proteins have been evaluated at fixed pH. From the plot of second virial co-efficient Bs against several pH values, the isoelectric pH of BSA adsorbed at air-water interface is observed to be 7.0. From the analysis of π - A curves for the fatty acid and pure protein of several binary surface compositions, π - A curves for their mixtures have been obtained. These experimental values have been compared with their ideal values using additivity rule. Such analysis indicates that fatty acid and protein undergo binding interaction in monolayer phase at a given value of in many cases.

Keywords : *Spread monolayer, protein-fatty acid, lipid protein interaction, virial coefficient, air-water interface.*

INTRODUCTION

Protein-lipid interaction is important in a number of fields such as biochemistry, food technology, detergent industry, pharmaceutical sciences etc. Albumin in blood serum binds lipids for transportation in different regions of the cellular system [1]. In cell membrane, different proteins are in complex interaction with lipids containing various

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types of fatty acids [2–8]. In dietary systems, the biological surfactants bile salts of various types interact with lecithin, cholesterol and proteins to form stable colloidal or miceller systems of bile following complex metabolic pathways. These bound surfactants are also responsible for digestion of food in the living system. Mixed monolayer systems of protein-surfactant mixtures at air-water interface are interesting model for lipid-protein interaction existing in cell membrane [9–10.]

The interaction of lipids with proteins by the monolayer technique has been studied in the past by many workers [11–23]. Nino et al. [24–27] and others [28] have studied protein-surfactant, protein-lipid interaction in mixed films at air-water interface using Langmuir balance, Brewster-Angle Microscopy (BAM) etc. Vollhardt and Fainerman [29] have reviewed the penetration of the soluble ampliphile (surfactant, protein) into two-dimensionally aggregated lipid monolayers. In a number of publications, Sugihara et al. [30–32] reported on the miscibility of mixed monolayers of different systems containing free bile acids, conjugate bile acids and cholesterol in terms of the relations of average molecular area, with surface pressure, surface potential and surface dipole moment. They have concluded that bile acids cannot mix with cholesterol. Bile acids themselves forms homogeneous mixed monolayers but the system deviates from ideal mixing.

Research in this area is also being pursued actively in recent years. Work has been focused on various aspects such as structure of lipid monolayers [33], hydrolysis of phospholipid and lipo-polysaccharide monolayers [34,35] and intermolecular forces in lipid monolayers [36]. Mixed monolayers of lipids and interaction of mixed lipids in monolayers has been studied [37–39]. Mircheva et al. [40] reported the proteolysis of alpha gliadin monolayer spread at the air/water interface. Baszkin [41] has reviewed the molecular recognition behavior in spread protein monolayer film at air/water interface. The influence of lipid molecules in modifying protein-membrane and protein-small molecule interactions have also been emphasized [42,43]. Finally a number of recent reports on biomolecular interactions in monolayers studied the interaction of lipids with cyclic neuropeptide [44], surfactant protein [45] and modified proteins [46,47].

In the present paper, the surface pressure π has been measured for pure proteins and fatty acid-protein mixtures as function of area A per different fractions of fatty acid - protein mixture. The results will be analysed in the light of interaction of protein molecules with each other or with different fatty acids at the interface.

Materials and methods :

Pure bovine serum albumin (lot no. H6F-9390), β-lactoglobulin (lot no. 76H7125), stearic acid (lot no. 95F7205) and arachidic acid (lot no. 115F0428) were obtained

from Sigma Chemical Company, USA. All other reagents like NaCl, NaOH, HCl used were of analytical grade. All values of pH in the subphase were unbuffered and its ionic strength was maintained by the addition of NaCl. Double distilled water was used throughout the experiment.

The experiments were performed using a computer attached Langmuir film balance based on Wilhelmy plate method as described in our previous papers [48,49]. The spreading solvent for protein was water and that for fatty acid was chloroform. Protein and fatty acid were always made in the concentration range 1 mg/ml and total volume of aliquot for each experiment was 25 μl.

The mixed film of surfactant and protein was prepared in the following procedure : Required amount of protein solution was first spread on the subphase of the experimental system and allowed 30 minutes for spreading and rearrangement of protein in the monolayer. Thereafter, to make desired weight fraction of protein (Wp) and surfactant (Ws) respectively required amount of surfactant solution was dissolved in chloroform and the experiment was performed after another 15 minutes to rearrange the mixed monolayer film and evaporating the chloroform.

RESULTS AND DISCUSSION :

We have already shown that the fatty acids in the insoluble monolayer [48] in contact with aqueous subphase at pH 3.0 remain in completely unionized state whereas at pH 12.0 the fatty acids in the monolayer are extensively ionized. In the intermediate value of pH equal to 7.0, the dissociation of the fatty acid at the interface is only partial. We shall now be interested to study the binary mixture of a fatty acid (stearic or arachidic acid) and a globular protein (BSA, β-lactoglobulin, α-lactalbumin) which were spread on the fluid interface together.

It has been shown earlier [48] that values of π for fatty acids in the monolayer phase may be taken to be equal to $\pi_k + \pi_s + \pi_e$ and their individual contributions may be calculated at pH 3.0 and pH 12.0 when fatty acid is nonionic and ionic states respectively. π_k stands for kinetic contribution of pressure and π_c represents surface pressure due to electrical repulsion in charged monolayer. π_s is the cohesive pressure between the spread molecules in the monolayer phase for the neutral and charged monolayers.

It is well-known that globular proteins are able to spread on the aqueous subphase [11]. The pressure-area curves of pure proteins, bovine serum albunin (BSA), β-lactoglobulin (β-lg) and α-lactalbumin (α-lac) have been presented in Fig. 1 at pH 7.0 and ionic strength 0.10 respectively. The pH values of all these proteins

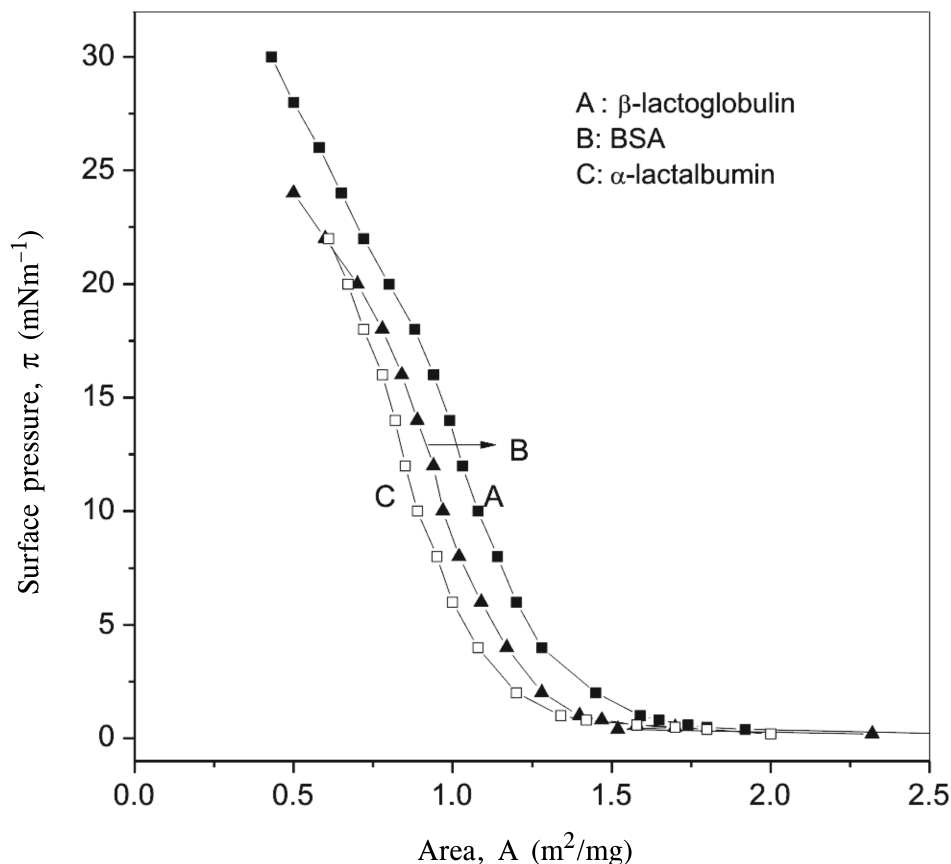

Fig. 1. Surface pressure (π) – area (A) curves for different proteins at air/water interface at pH 7.0.

are higher than their respective values of isoelectric points at the bulk phase.

Analogous to the three dimensional virial equation of state for gases and polymer solutions [50], following expressions have been reported for two dimensional insoluble films of amphiphiles and proteins [51,52].

$$\pi A_t = A_s + B_s \pi + C_s \pi^2 \quad (1)$$

Here As, Bs and Cs represent first, second and third virial coefficients of the surface virial equation (1). Values of these coefficients for a protein depends on molecular weight, molecular size charge and electrostatic potential, extent of unfolding of protein in the spread film and association of protein occurring in the insoluble film. W_2 is

the constant weight of a protein spread on the total surface area At which may be varied for different values of π. M_2 is the molecular weight of the proteins which for BSA, β-lactoglobulin and α-lactalbumin are 66,000, 18,400 and 14,400 (subunit) respectively previously obtained by different physicochemical and surface pressure techniques. Values of the first virial coefficient A equal to W_2RT/M_2 for different proteins [53] have been included in Table 1. Equation (1) can be written as

$$(1/ \ [\pi A_t - A_s] = B_s + C_s \pi \quad (2)$$

The plot of $(1/\pi[\pi A_t - A_s]$ vs π appears to be linear (not shown) in wide range of π so that second and third viral coefficients for different proteins can be obtained from the slopes and intercepts of these plots. These values compared in Table 1 in appropriate units involve all types of interactions mentioned earlier. In Fig. 2, second virial coefficients of BSA have been ploted against pH value of subphase. Values of B_s decrease from pH 3.0 to pH 7.0 and then increases sharply at pH 12.0. This observation indicates that the virial coefficient, B contains effect of excluded area of the molecules, its charge, counterion binding unfolding of protein, and multiple protein-protein interaction which are actually changing with the pH value of subphase. The shift of isoelectric pH minimum value of BSA at absorbed air-water interphase is observed from bulk isoelectric pH 4.8 to 7.0. Similar shift of isoelectric pH of BSA at different solid-liquid interfaces have been observed [53,54].

In order to establish mutual miscibility of the protein and the surfactant in the two dimensional phase, monolayer properties of the mixed films of proteins and surfactants have been investigated by many workers [30–32, 55–57]. Reciprocal of measured values of A may be equal to total amount of these two spread components per unit area of the surface. In Tables 2, 3 and 4, at given value of π, values of

TABLE 1.

Virial coefficients in equation (1) for proteins

System	pH	A_s	B_s	C_s
BSA	3.0	9.11	379	–8.95
BSA	5.0	9.11	362	–8.24
BSA	7.0	9.11	304	–7.93
BSA	12.0	9.11	478	–19.6
β-lg	7.0	34.4	362	–8.49
α-lac	7.0	43.0	292	–6.54

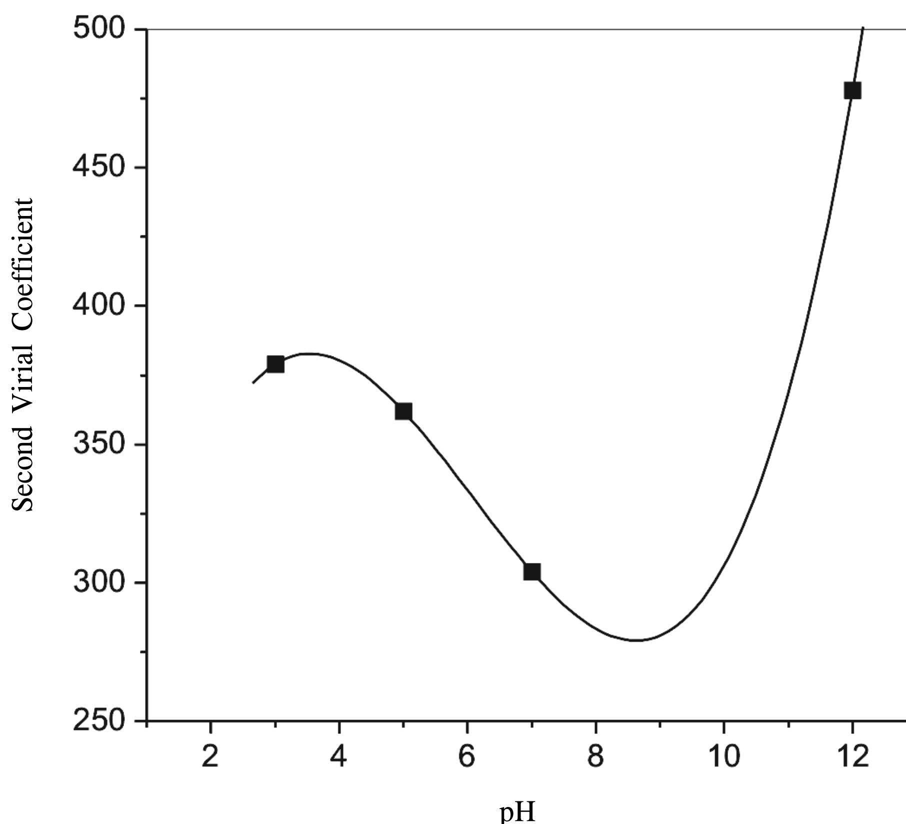

Fig. 2. Second virial coefficient vs pH for BSA monolayer at air/water interface.

different weight fractions of the mixture W_p or W_s are presented. W_p and W_s are weight fractions of protein or surfactant present in the interfacial phase which may be known experimentally $W_p + W_s = 1$. In Tables 2, 3 and 4 Γ_{mix} for mixtures at arachidic acid - lactoglobulin, stearic acid - BSA, stearic acid - lactoglobulin mixtures respectively have been presented for various weight ratios Ws at different values of pH at constant temperature 25°C and ionic strength 0.10 at fixed values of π.

If the protein and fatty molecules do not interact with each other in the spread film, then the ideal value $(\Gamma_{max})_i$ for the mixture at a fixed value of π will be given by the equation,

$$(\Gamma_{mix})_i = \Gamma_p W_p + \Gamma_s W_s \tag{3}$$

Value of W_p can be calculated from known values of W_s given in the Tables 2 to 5. Γ_p and Γ_s values are calculated from reciprocal values of A for pure protein (W_p = 1) and pure fatty acid (W_s = 1) in Tables 2, 3 and 4 and Figs. 3 and 4 respectively. The ideal values of $(\Gamma_{mix})_i$ for various protein - fatty acid mixtures calculated from equation (3) are also presented in Tables 2, 3 and 4. In Table 3,

TABLE 2.

Values of Γ_{mix} and $(\Gamma_{mix})_i$ for arachidic acid – α-lactalbumin mixture

Subphase : 25°C, μ = 0.10, pH – 7.0

II mNm^{-1}	W_s	$\Gamma_{mix} \times 10^7$ kgm^{-2}	$(\Gamma_{mix})_i \times 10^7$ kgm^{-2}
6	0	10.7	–
	0.3	10.9	12.1*
	0.5	12.0	13.0*
	0.7	13.0	14.0*
	1.0	15.4	–
10	0	11.9	–
	0.3	12.1	13.5*
	0.5	13.3	14.3*
	0.7	14.3	15.3*
	1.0	16.7	–
16	0	13.7	–
	0.3	14.0	15.2*
	0.5	15.2	16.2*
	0.7	16.2	17.1*
	1.0	18.6	–
20	0	15.1	–
	0.3	15.3	16.6*
	0.5	16.7	17.6*
	0.7	17.7	18.6*
	1.0	20.1	–

TABLE 3.

Values of Γ_{mix} and $(\Gamma_{mix})_i$ for stearic acid – protein mixture at $\pi = 10 mNm^{-1}$
Subphase : 25°C, $\mu = 0.10$

System mixture	W_s	$\Gamma_{mix} \times 10^7$ kgm^{-2}	$(\Gamma_{mix})_i \times 10^7$ kgm^{-2}
BSA – St Ac	0	8.9	–
(pH - 3.0)	0.3	11.0	10.8
	0.5	11.0	12.0*
	0.7	12.9	13.2
	1.0	15.0	–
BSA – St Ac	0	9.7	–
(pH - 12.0)	0.3	11.5	11.6
	0.5	13.2	12.9
	0.7	13.8	14.3*
	1.0	16.2	–
BSA – St Ac	0	10.3	–
(pH - 7.0)	0.3	11.3	11.7
	0.5	11.8	12.7*
	0.7	13.5	13.6
	1.0	15.0	–
β-lg – St Ac	0	9.3	–
(pH - 7.0)	0.3	10.7	11.2
	0.5	11.8	12.4
	0.7	13.0	13.8
	1.0	15.7	–
α-lac – St Ac	0	11.2	–
(pH - 7.0)	0.3	11.9	12.1
	0.5	12.6	12.7
	0.7	12.5	13.2*
	1.0	14.1	–

TABLE 4.

Values of Γ_{mix} and $(\Gamma_{mix})_i$ for arachidic acid – protein mixture at π = 10mN/m

Subphase : 25°C, π = 0.10

System mixture	W_s	$\Gamma_{mix} \times 10^7$ kgm^{-2}	$(\Gamma_{mix})_i \times 10^7$ kgm^{-2}
BSA – Ara-Ac	0	10.9	–
(pH – 7.0)	0.3	12.5	12.2
	0.5	13.6	13.0*
	0.7	14.7	13.9*
	1.0	16.3	–
α-lg– Ara-Ac	0	10.2	–
(pH – 7.0)	0.3	12.0	11.9
	0.5	13.2	13.7*
	0.7	14.3	13.4*
	1.0	16.1	–

values of Γ_{max} for mixture of α-lactalbumin and arachidic acid have been shown against W_s for π equal to 6, 10, 16 and 20 mN.

In Figs. 3 and 4 plots of values of A - π are shown for fatty acid - protein mixtures for different weight fractions W_s of the mixed systems. From extensive data given in Tables 2 to 4 we note that only in some systems marked with asterisk, $(\Gamma_{mix})_i$ is significantly higher than Γ_{mix} presumably due to protein fatty acid interaction in the monolayer phase. In many other systems (not marked with asterisk) $(\Gamma_{mix})_i$ and Γ_{mix} are observed to be quite close to each other. The protein fatty acid interaction in these limited cases may be taken to be negligible and the mixtures may be taken to behave as ideal system in the limits of experimental error.

We find from the data presented in Tables 2, 3 and 4 that fatty acids and proteins forming the mixture in many cases do not interact each other and thus behave as ideal mixture obeying ideal equation 2. But for many systems, the results (presented with asterisk in these tables) deviate from ideality and for all such systems, Γ_{mix} is less than $(\Gamma_{mix})_i$. We like to propose that such reduction of the value of Γ_{mix} from ideal value occurs due to the binding of fatty acid to protein in the monolayer phase.

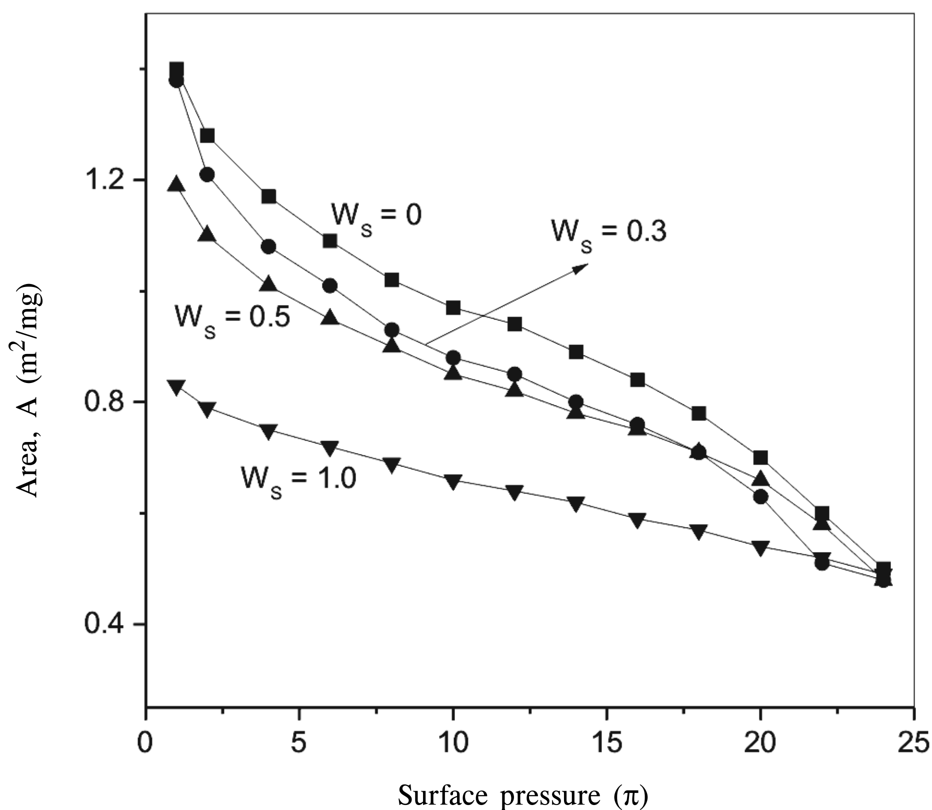

Fig. 3. π-A Isotherm of BSA-stearic acid mixture at different values of W_s. Data are shown for W_s equal to 0, 0.3, 0.5 and 1.0.

Values of the difference $(\Gamma_{mix})_i$ - Γ_{mix} represented by $\Delta\Gamma_{mix}$ for different systems are presented in Table 5.

We like to point out also that $\Delta\Gamma_{mix}$ represents the parameter for interaction between a protein and the surfactant in the mixture containing Γ_p Kg of protein and Γ_s Kg of surfactant per square meter of the surface mixed in a given weight fraction ratio W_s. We may imagine that $\Delta\Gamma_{mix}$ Kg of the fatty acid remains bound to Γ_p Kg of protein per square meter so that $(\Gamma_{mix})_i$, is equal to $\Gamma_{mix} + \Delta\Gamma_{mix}$. Thus $\frac{\Gamma_p}{M_p}$ moles of protein in the monolayer phase are imagined to bind $\frac{\Delta\Gamma_{mix}}{M_s}$ moles of fatty acid

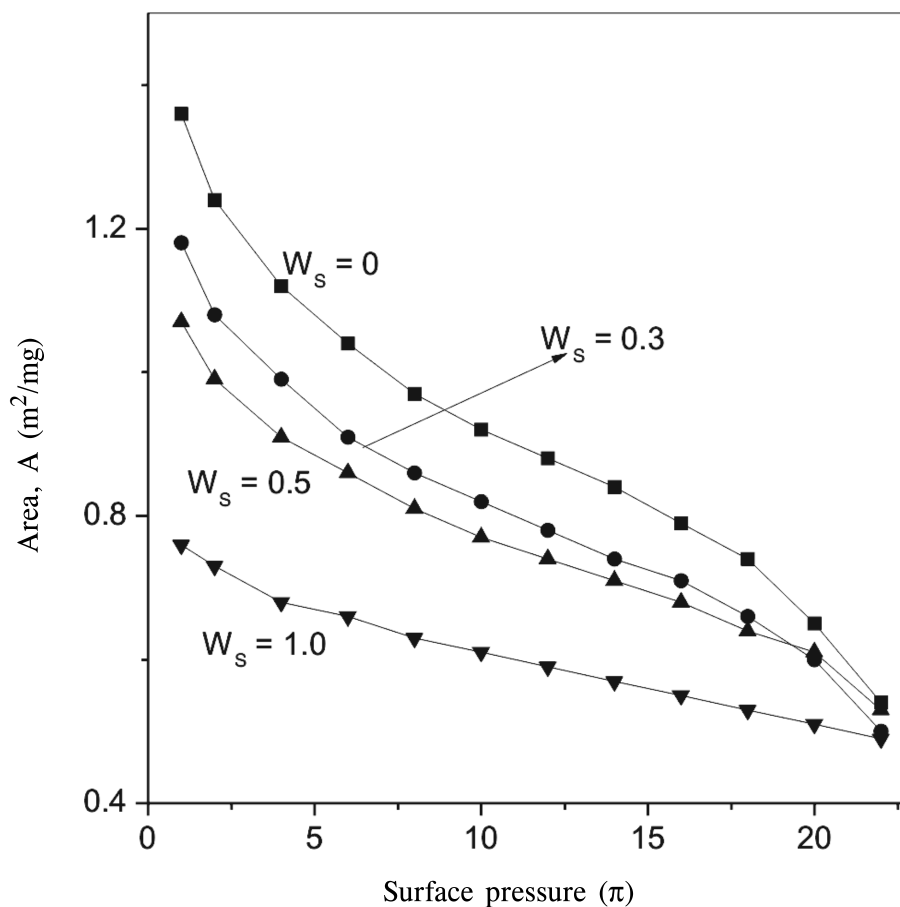

Fig. 4. π-A Isotherm of BSA-arachidonic acid mixture at different values of W_s. Data are shown for W_s equal to 0, 0.3, 0.5 and 1.0.

in the spread film. Moles of the surfactant (Γ'_s) bound per mole of protein can be calculated from equation (4).

$$\Gamma'_s = \frac{\Delta\Gamma_{mix}}{\Gamma_p} \cdot \frac{M_p}{M_s} \tag{4}$$

Here M_p and M_s stand for molecular weight of protein and surfactant respectively. Values of Γ_s are presented in Table 5.

TABLE 5.

Binding parameters for protein - surfactant in spread monolayer

Subphase : 25°C, μ = 0.10, pH – 7.0

System of mixture	π (mN/m)	W_s	$\Delta\Gamma_{mix} \times 10^7$ (kg/m^2)	$\Gamma_s = \frac{\Delta\Gamma_{mix}}{\Gamma_p} \frac{Mp}{Ms}$ (moles of surfactant/ moles of protein)
BSA – St Ac (pH - 3.0)	10	0.50	1.0	44
BSA – St Ac (pH - 12.0)	10	0.70	0.50	30
BSA – St Ac (pH - 7.0)	10	0.50	0.90	37
β-lg – St Ac	10	0.30	0.48	5
(pH - 7.0)		0.50	0.59	7
		0.70	0.78	13
α-lg – St Ac (pH – 7.0)	10	0.70	0.69	10
β-lg – Ara Ac (pH - 7.0)	10	0.50	0.52	8
α-lac – Ara Ac	6	0.30	1.19	12
(pH – 7.0)		0.50	0.98	8
		0.70	1.01	7
	10	0.30	1.38	8
		0.50	0.99	7
		0.70	0.96	11
	16	0.30	1.19	6
		0.50	0.91	6
		0.70	0.96	9
	20	0.30	1.10	6
		0.50	0.91	5
		0.70	0.89	8

From Table 5, we note with considerable interest that binding of free stearic acid to BSA is significantly high compared to low binding of other free fatty acids to other proteins. It is known that cellular membrane binds significant amount of free fatty acids like binding of stearic acid to BSA. This process leads to certain important aspect of biological transport phenomena. The residues of fatty acids attached to membrane lipids may not attach this free fatty acid but in interaction with each other forming lamellar micelles. Our present results obtained in-vitro protein-free acid system may be relevant in the lipid-protein binding process in membrane for vivo system.

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