World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:6, No:10, 2012

The study of the Interaction between Catanionic Surface Micelle SDS-CTAB and Insulin at Air/Water Interface

B. Tah, P. Pal, M. Mahato, R. Sarkar, G. B. Talapatra

***Abstract*—**Herein, we report the different types of surface morphology due to the interaction between the pure protein Insulin (INS) and catanionic surfactant mixture of Sodium Dodecyl Sulfate (SDS) and Cetyl Trimethyl Ammonium Bromide (CTAB) at air/water interface obtained by the Langmuir-Blodgett (LB) technique. We characterized the aggregations by Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and Fourier transform infrared spectroscopy (FTIR) in LB films. We found that the INS adsorption increased in presence of catanionic surfactant at air/water interface. The presence of small amount of surfactant induces two-stage growth kinetics due to the pure protein absorption and protein-catanionic surface micelle interaction. The protein remains in native state in presence of small amount of surfactant mixture. Smaller amount of surfactant mixture with INS is producing surface micelle type structure. This may be considered for drug delivery system. On the other hand, INS becomes unfolded and fibrillated in presence of higher amount of surfactant mixture. In both the cases, the protein was successfully immobilized on a glass substrate by the LB technique. These results may find applications in the fundamental science of the physical chemistry of surfactant systems, as well as in the preparation of drug-delivery system.

***Keywords*—**Air/water interface; Catanionic micelle; Insulin; Langmuir-Blodgett film

I. Introduction

INSULIN (INS) is a very demandable protein nowadays. The number of people with diabetics has grown very rapidly and the number is expected to increase in the coming years [1-4]. INS is a small protein [5] consisting of 51 amino acid residues [6, 7]. Each monomer of INS has two peptide chains: chain-A consist of two anionic side groups and no cationic ones, whereas chain-B contains four positively and two negatively charged side groups. Two chains are covalently linked by two disulfide bridges [8-10]. According to the crystallographic study, the chain-A is enclosed between -NH_2 and -COOH terminals of chain-B [6, 7, 11-13]. In both the chains, there are hydrophobic cores. INS interacts with receptor as monomer, yet its most interesting characteristics are the ability to form different structures, including dimers, tetramers, and hexamers. This hormone synthesized polypeptide stored at pancreas as hexameric form [14, 15].

INS is membrane-binding protein, plays a fundamental roll to balance the amount of glucose in bloodstream by mechanism initiated by its binding to specific INS receptors in plasma membranes and initiate glucose transport through cell membrane [16, 17]. It has been used to treat diabetes mellitus since 1922 by the direct injection process that has many problems involving not only painful injections but also the patient's quality of life [18]. To make a substitute way of the INS delivery, many researchers were involved in past decade [19-22]. At present, the most attention has been paid to make a route to oral delivery of INS, however that has a disadvantage, i.e., in gastrointestinal path the peptidase converts the INS molecules into fragments. According to the previous literatures, cyclodextrins and other enhancers have been mixed with INS to enhance the absorption of INS in cell membrane [19, 20, 23-25]. The knowledge about the interactions between cell membrane, different drugs, and protein components can give us a new path of oral delivery of INS. Therefore, a thorough understanding of these interactions is one of the driving forces to study this system.

As a step to understand these interactions, we have used Langmuir monolayer of catanionic surfactant mixtures to mimic the biological membrane and INS as the protein component. The bimolecular interactions in lipid monolayer at the air/water interface can be studied and subsequently the monolayer can be transferred using Langmuir-Blodgett technique, onto a solid substrate. The transferred monolayer can then be use for further characterization as well as for applications [26-30]. This technique is very much informative about the structural changes at the molecular level of protein/enzymes, such as denaturation, folding, unfolding, and aggregation etc. [27, 30-33]. Lypophilic as well as electrostatic interactions govern this structural change. Protein expression also depends on structural change. The incorporation of biomolecules, such as enzymes into the surfactant monolayer provides a great impact into the field of biosensors and biological applications [34]. The stabilization of protein formulation by the use of surfactant has been studied earlier [35-40].

Many authors have been studied the INS-surfactant interactions [6, 41, 42]. According to the previous literature [43], a small amount (normally used in pharmacological applications) of SDS/CTAB does not show any cytotoxic effects and the possibility remains still open to use these catanionic aggregates in nano-biotechnology and for the delivery of drugs.

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Our recent studies show that SDS/CTAB catanionic binary system forms different types of self-assembled structures with different ratios [44]. However, the catanionic vesicle in aqueous environment and surface micelle at air/water interface are formed in a particular ratio of SDS/CTAB (35/65) [44]. These aggregated structures can be immobilized by LB technique [44]. Here we choose SDS/CTAB catanionic binary system as template monolayer at air/water interface.

Apart from studying the INS catanionic surfactant interactions by π-A isotherms and π-t adsorption curves of pure and mixed Langmuir monolayers spread at the air/water interface varying the subphase conditions, the objective of the present work is to verify the influence of catanionic binary system on the structure and aggregation of INS. This is a very important fact, as INS has a tendency to aggregate, which results in the loss of its biological activity. Moreover, after transferring the monolayer to solid supports, different scanning probes microscopic (FE-SEM, AFM) techniques were employed to visualize and characterize the LB film of INS in the presence and absence of catanionic surfactant. In addition, FTIR Spectroscopy was applied to characterize the secondary structure of the protein.

II. Experimental Section

A. Materials

The Human insulin (Actrapid) was purchased from Abbott India Limited, Mumbai, India. The anionic surfactant Sodium Dodecyl Sulfate (SDS) and cationic surfactant Cetyl Trimethyl Ammonium Bromide (CTAB) were purchased from Merck and Himedia, (Mumbai, India) respectively. The Chloroform (UV Grade) and methanol were purchased from Spectrochem and Sisco Research Laboratory, (Mumbai, India) respectively.

B. Methods

i) Study of surface activity by surface pressure (π)–time (t) kinetics measurement

To study the adsorption behavior of protein at different conditions, we have done π–t kinetics in two circumstances. In the first case, the kinetics measurements of pure protein with different concentrations (0.008, 0.015, 0.031, and 0.109 mM) have been done. In the second case, the fixed volume (10 mL) of aqueous solution of INS having concentration (0.031mM) was injected into the subphase of volume 750 mL (typical dimension 200 mm × 100 mm × 37.5 mm). Whereas the different amount of catanionic surfactant mixture (SDS/CTAB) having concentration 0.7 mM and volume ratio (35/65) was spread on the interface, to attain the initial surface pressures of 2, 5 and 10 mN/m, respectively. The concentration of catanionic mixture was adjusted prior to protein addition. To avoid the isoelectric point of INS (nearly 5.4) [45], the subphase water was maintained at pH 7 (which is nearly the pH of blood) by using phosphate buffer solution.

The computerized LB trough used was Teflon-bar-barrier type (model LB2007DC, Apex Instruments Co. India) enclosed in a plexiglass box to reduce film contamination and equipped with a Wilhelmy type balance, to accuracy of (0.01 mN/m. The trough width and length were 200 and 600 mm, respectively. The water with pH = 5.5 and resistivity = 18.2 MΩ-cm was prepared using a Milli-Q apparatus via an ELIX system from Millipore (Billerica, MA). All the experiments were performed at temperature 28±1°C unless otherwise mentioned. At least three independent runs were performed to check the reproducibility.

ii) Pressure-area (π-A) isotherm measurement

For preparation of pure INS monolayer, a known amount of an aqueous solution of INS of concentration of 0.03 mM was spread on the water subphase by a micro syringe. After waiting for 10 min, the monolayer was slowly compressed with a compression speed of 1 $Å^2$/(molecule min).

For the preparation of the pure catanionic surfactant (SDS/CTAB) monolayer, SDS with a concentration of 0.7 mM was prepared in 1:1 chloroform/methanol solvent, and a solution of CTAB with a concentration of 0.7 mM was prepared in chloroform solvent. Finally, the solutions of SDS and CTAB were mixed in specific volume ratio i.e. 35/65. The concentration 0.7 mM is far below than the critical micelle concentration (CMC) [46, 47]. The mixture solution was spread on the water subphase. After a delay of 10 min to allow the solvent to evaporate, the monolayer was slowly compressed to the desired pressures with a compression speed of 1 $Å^2$/(molecule min).

For the preparation of surfactant-INS monolayer, first, we spread mixed catanionic surfactant (concentration 0.7 mM) of different amount to attain the initial surface pressure of 2 mN/m and 5 mN/m, and then INS solution was spread on this surfactant-containing surface. After a delay of 10 min to allow the solvent to evaporate, the monolayer was slowly compressed to the desired pressures with a compression speed of 1 $Å^2$/(molecule min).

iii) Process of Substrate Cleaning

All the substrates (glass and silicon wafer) were cleaned in a liquid soap ultrasonic bath followed by repeated rinsing with Millipore water. They were then immersed in acetone in an ultrasonic bath. Finally, they were cleaned, using Millipore water in the ultrasonic bath. A uniform layer of water onto the slide confirmed the hydrophilicity of the slide [48].

iv)Preparation of LB Film

The monolayer prepared at the air/water interface, were transferred on hydrophilic glass cover slips, through up stroke with a speed of 5 mm/min at constant surface pressure, where the growth rate became minimized. These cover slips were previously immersed in the subphase [49].

v) FE-SEM and AFM Characterizations

High-resolution field emission scanning electron microscope (FE-SEM, model No.: JEOL JSM-6700 F) with use range: 0.5-30 KV with a lateral resolution in the range of 1.2 to 2.2 nm was employed to extract the surface morphology of all transferred LB films and on fine hydrophilic glass substrate.

For study the surface morphology of the LB films, AFM (AFM, VECCO diCP-II Model No AP-0100) imaging was used.

The tapping mode was used in air to minimize any kind of force exerted on the samples from the scanning tip. Thin phosphorus doped silicon cantilever (with no coating on the front side and 50 ± 10 nm aluminum coating at the backside) of resistivity 1-10 Ω-cm was used for scanning. The thickness of the cantilever ranges form 3.5-4.5 μm with a length of 115-135 μm as well as width of 30-40 μm. The processed images were subsequently analyzed for diameter, height, and surface roughness by Proscan 1.8 - Image analysis 2.1. The line profiles were used to calculate surface roughness. The height profile showed the variation between highest peak and lowest valley along the line.

vi) FTIR Spectra

FTIR spectra of LB film of pure INS and mixed surfactants-INS on silicon wafers were recorded at room temperature by Magna-IR (Model No 750 spectrometer, series II), Nicolet, USA. In all the cases, the data were averaged over 100 scans. The resolution of the instrument was 4 cm^{-1}.

III. Results and Discussion

A. Growth kinetics of pure INS at bare surface and at the presence of catanionic mixture (SDS/CTAB) at the surface

We have studied the adsorption behavior of INS at bare air/water interface by measuring the surface pressure (π) versus time (t) at various concentrations of injected INS (C_{INS}). Figure-1A shows such results. The adsorption growth kinetics of INS shows a nature of sigmoid curve.

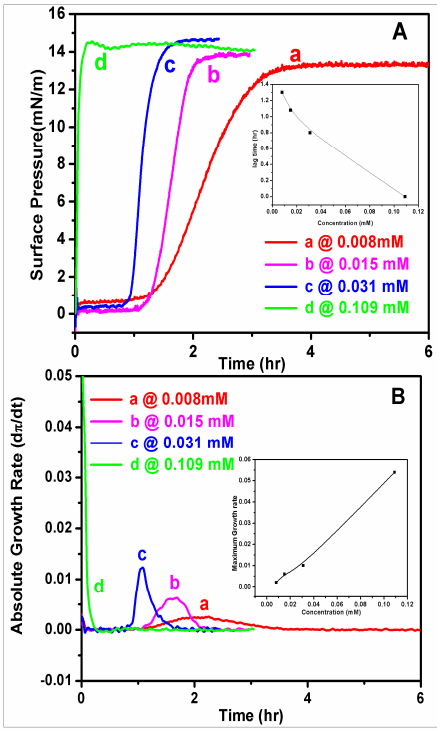

Fig. 1 Curves of Panel-A represent the growth kinetics of pure INS at different C_{INS}. Inset figure represents the lag time vs. C_{INS} curve. Curves of Panel-B represent the absolute growth rate with time at different C_{INS}. Inset figure represents the maximum growth rate vs. C_{INS} curve

It is well known that sigmoid curve consist of 4 phases i.e. i) lag phase, ii) log/exponential phase, iii) retarded phase and iv) saturation phase. For the adsorption of INS at bare liquid surface, there is initial lag times (τ_{lag}), where π remains nearly zero. This is a significant characteristic of protein/enzyme adsorption at the air/water interface, where the interface is lacking sufficient quantity of protein/enzyme for noticeable change in π [50]. The τ_{lag} represents the time required to attain the minimum monolayer coverage for an effective and measurable surface pressure [50]. During the lag phase, the INS molecules appeared at the air/water interface, are insignificant in number, thus on the average they are well apart, hence there is very little or no interaction between neighboring molecules. In course of time, the number of INS molecules at the interface increases and eventually come closer to each other within their interaction radius. As a result, the surface pressure starts increasing after the period of τ_{lag}.

In the exponential phase after the lag period, the surface pressure increases rapidly to cover the vacant spaces at the air/water interface. Here the surface pressure grows exponentially and eventually the rate of growth reaches maximum, where the gradient of the curve is the steepest. Beyond this point the rate of growth decreases and the surface pressure increases slowly with time (retarded phase) and approaches a constant value (saturation phase) (Figure-1A). When C_{INS} is very small (0.008 mM), the surface pressure saturates at ~13mN/m within 3.5 hr (Curve-a in Figure-1A). Further increase of C_{INS} (0.015, 0.031, and 0.109 mM), the adsorption become faster, and less time are needed to attained saturation (Curves b–d in Figure-1A). The adsorption of INS is very fast for C_{INS} = 0.109 mM. The nature of the variation of τ_{lag} with C_{INS} presented in inset of Figure-1A shows that τ_{lag} decreases with the increase of C_{INS}. This type of phenomena was also reported earlier [34].

After the lag period, the surface pressure increases very rapidly and achieves the saturation pressure (π_{sat}) at ~14-15 mN/m in each condition. π_{sat} is the pressure after which the protein cannot increase the surface pressure any more [51]. The fundamental processes associated with protein/enzyme adsorption at air/water interface are diffusion from the bulk to the interface and subsequent exposure of their hydrophobic moieties towards the aqueous medium. After adsorption, they may undergo rearrangement/relaxations and denaturation by thermodynamic forces at the air/water interface in various time scales [34, 52].

To know the change in growth rate with concentration of insulin, we have also plotted the absolute growth rate with time. Absolute growth rate means the rate of change in surface pressure with time (dπ/dt) [53]. In Figure-1B, the peaks of each curve i.e. inflection point [53] indicate the maximum growth rate. The inset of Figure-1B show that the maximum growth rate decreases in one hand, and the time taken to achieve the maximum growth rate increases on the other hand, with the decrement of concentration of insulin. To analyze the kinetics of INS growth mechanism, only the exponential phase of the data of Figure-1A were fitted in single exponential association equation (Equation-1).

$$\pi_t = \pi_0 + A\left[1 - \exp\left(-\frac{t}{t_1}\right)\right] \quad (1)$$

In this equation π_t and π_0 are the surface pressure at time t=t and t=0 respectively. A is the relative contribution and t_1 is the time constant of the growth mechanism of protein at the air/water interface [50].

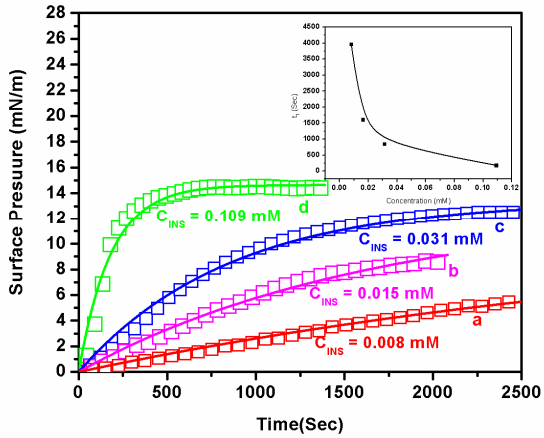

Fig. 2 Curves (a, b, c, and d) represent the growth kinetics of pure INS at different INS concentrations (0.008, 0.015, 0.031, and 0.109 mM respectively) after subtracting the initial lag period. The solid lines represent the fitted curves of raw data according to the Equation-1. The inset shows the variation of time constant (t_1) with concentration of insulin

The results are shown in Figure-2. The single exponential curve fit concludes that, this growth mechanism is a single step first-order reaction [51]. In each condition the fitted R^2 values are nearly 0.99 showing acceptable fit. The curve of inset Figure-2 shows, the growth rate increases or the time constant decreases with the increase of concentration of injected INS. This result relates the observation in Figure-1A.

The adsorption behavior of protein form the subphase (at pH 7) to the air/liquid interface was evaluated earlier by the variation of surface pressure of template monolayer [34, 51]. The affinity of a protein molecule for a template monolayer surface depends on several factors that include both the nature of the monolayer surface and the surface of the protein, which first contacts the monolayer. Figure-3 shows the growth kinetics of INS in presence of catanionic surfactant mixtures at the interface. Here the catanionic mixture, SDS/CTAB of the volume ratio 35/65 was spread at interface, to achieve the initial surface pressures (π_{ini}) of 2, 5, and 10mN/m. We also tried at initial surface pressure at physiological pressure (30 mN/m) [54], but there was no such inclusion of protein and the pressure remains unchanged. We are interested in this particular volume ratio (35/65) of SDS/CTAB binary system since we observed in our earlier work that this ratio of catanionic mixture could form surface micelle at air/water interface [44].

The growth kinetics in Figure-3 is found to be different from that of Figure-2. This dissimilarity arises due to the interaction between protein and surfactant.

Since the INS molecule contains both charged and uncharged sides, interaction of INS with catanionic monolayer involves the electrostatic and hydrophobic interactions [55]. The surfactant covers hydrophobic sites, where aggregation and surface adsorption may occur, or it may act as an artificial chaperonin that catalyzes the refolding of protein [39]. These phenomena increase the saturation pressure to ~18 mN/m. To minimize the free energy of the native INS, surfactant molecules may bind with native INS to a greater degree, rather than to form a denatured state [38] or the surfactant stabilizes the partially unfolded protein [44]. This phenomenon might be responsible for the shape of the π–t curves as shown in Figure-3. Moreover, in presence of template monolayer of catanionic surfactant mixture, the lag time become smaller and the absorption rate become faster. The presence of catanionic mixtures at the interface induces some attractive force on the INS molecules, reduces the lag time. For this experiment, with C_{INS}=0.031mM, the observation shows that at the bare surface, the lag time is ~48 min, whereas in the case of surfactant at the interface, the lag time is reduced to a few seconds only.

The curve-a in Figure-3 shows that there is two-stage growth of INS in presence of catanionic mixture at air/water interface, at π_{ini} = 2mN/m. The initial stage is apparently similar to the growth of the pure INS towards the bare surface but the rate of growth is high. In Figure-3, region 'a' of cartoon figure represents a domain of native INS where the maximum surface pressure reaches ~14mN/m, similar to the previous observation in Figure-1A. Moreover, the surface pressure stays at 14mN/m for next 7 min and then again, rises up with time and achieves the maximum of 17mN/m with slower growth rate. The growth kinetics at the first stage is governed by the diffusion process [51], whereas the next stage is responsible due to the interaction between the protein and binary catanionic surfactant system.

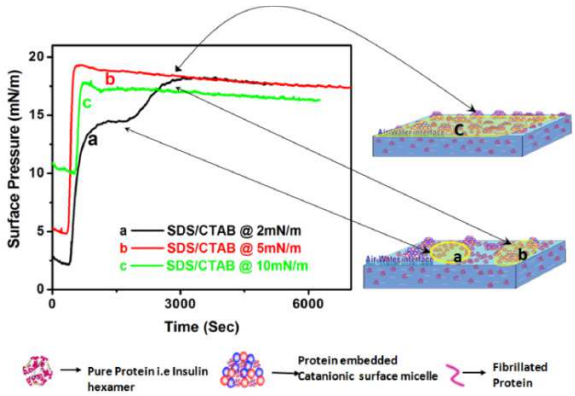

Fig. 3 Growth Kinetics of INS at the presence of catanionic mixture (SDS/CTAB) on the air/water interface at π_{ini}= 2 mN/m (curve-a), 5 mN/m (curve-b), 10 mN/m (curve-c). Cartoon figure region-a shows the domain of native INS, region-b shows a domain of encapsulated INS into catanionic surface micelle, region-c shows a domain consist of protein embedded surface micelle and fibrillar INS

During the second stage growth process, from t=30 to 51 min, the surfactant molecules are surrounded by the protein molecules.

At low surfactant concentration at the surface, i.e. for low π_{ini}, the initial binding of surfactant probably involves the polar heads of surfactant and charged side groups of opposite sign on the protein molecules, although the nonpolar tails of surfactant may also in contact with the protein molecule [6]. Finally, the INS is encapsulated by catanionic surfactant and forms circular type of aggregates. Cartoon figure region 'b' represents a domain of encapsulated INS in catanionic surface micelle.

In curves b and c of Figure-3, no two stage growths are observed. In these cases (π_{ini}=5 and 10 mN/m), the electrostatic interactions between two opposite ionic surfactant and surfactant-protein molecules play dominant role and the surface pressure cannot stay at the saturation pressure of pure INS i.e. (14mN/m). In these conditions, the change in rate of growth cannot be distinguished. According to the previous literature [42], the large number of ionic surfactant is responsible for the denaturation of protein. At the higher π_{ini} of SDS/CTAB, there are sufficient amount of anionic/cationic surfactant molecules. The anionic surfactant (SDS) can bind to cationic sites of INS (i.e. His-5 and 10, Arg-22 and Lys-29) in one hand and the cationic surfactant (CTAB) can bind with anionic sites of INS molecules (i.e. Glu-13 and 21) on the other hand 6. Region 'c' of cartoon figure represents a domain consisting protein encapsulated surface micelle and fibrillar protein. Due to the interaction between surfactant and protein, protein become denatured and unfolded, and finally forms fibrillar structure as evidenced from the FESEM picture (discussed in section 3.3). Beyond a particular concentration of surfactant, the protein unfolding does not occur and excess surfactant simply leads to a surface micelle formation due to the electrostatic interaction between the oppositely charged head groups of anionic and cationic surfactant molecules [56].

B. Pressure–area (π-A) isotherm study for pure INS and INS-catanionic surfactant mixed system

Figure-4 displays the π-A compression isotherms of a Langmuir monolayer of pure INS (curve-a), pure catanionic surfactant mixtures (curve-b) and INS-surfactant mixed system (curve-c). In this experiment, the subphase is pure water of pH 7. For pure INS and INS-surfactant system, area per molecule is calculated in terms of INS monomer and on the other hand, for catanionic surfactant system the area per molecule is calculated in terms of the average molecular weight of catanionic binary system. The diameter of the hexameric INS from a pdb file (3AIY) [57] is nearly 5 nm (inset Figure-4); thus the area of the INS monomer (inset Figure-4) is 3.3 nm2. Moreover, Mushik *et al* gave a unique model about the orientation of INS molecule at air/water interface [58], where the hydrophobic chain-A of INS resides at air/water interface and the chain-B should be oriented towards the adjacent gas phase [58]. The chain-A has 21 residues and the area per amino acid at the air/water interface is 20Å^2/residue [59], so the excepted area of INS could be (21Å×20Å = 420Å^2 = 4.2 nm^2)/INS molecule, in a closely packed monolayer, very similar to the value calculated from the pdb file.

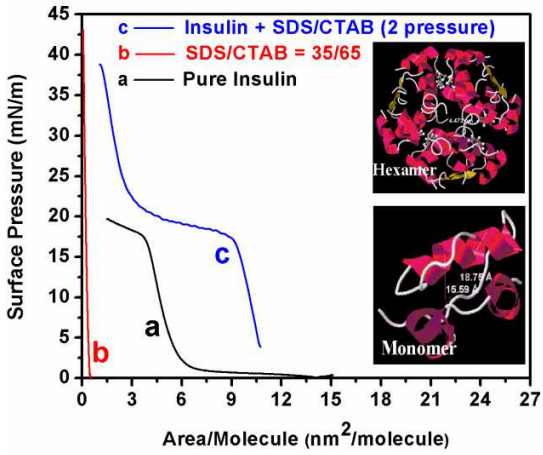

Fig. 4 Represents the π-A isotherm of pure INS (curve a), catanionic surfactant (SDS/CTAB) (curve b) and INS at the presence of SDS/CTAB at pressure 2 mN/m (curve c). Insets represent crystal structure of hexameric and monomeric INS

Curve-a of Figure-4 represents the π–A compression isotherm of INS, virtually reproduces previously reported results found elsewhere [57, 60, 61]. At low monolayer density, the surface pressure is nearly equal to zero. Here the individual molecule is separated by a distance and thermodynamically it is considered as 2D gas state. With further compression, the monolayer leads to the appearance of a gas condensed (G-C) phase transition at ~7-8 nm^2. Another phase transition is observed at area ~4 nm^2. Here the closely packed monolayer of INS is formed. The area/monomer value in this stiff condensed region (~4 nm^2) is in support of our estimated value (~3.3 nm^2) from the crystal structure [57] and from previous literature [59]. The detail study about the isotherm of catanionic surfactant system (curve b) was reported and discussed in our previous work [41]. In the surfactant mixed INS system (curve-c), the area/monomer in condensed phase is shifted from pure INS (~4 nm^2) to ~9 nm^2. It seems that the INS molecules are being unfolded due to the presence of catanionic surface micelle at air/water interface.

C. The SEM and AFM images of Langmuir monolayer film

To observe the surface morphology of the transferred Langmuir monolayer films at different conditions we have done the SEM and AFM imaging. We have transferred the monolayer on hydrophilic glass substrates for this observation. Every LB films were lifted at the saturation pressure. SEM images were recorded for the LB films of pure INS at bare surface and at the presence of the monolayer of catanionic surfactant spread in an amount to achieve the initial surface pressures of 2mN/m and 10mN/m.

Panel-A of Figure-5 represents the FESEM image of LB film of pure INS, shows that the granules type domain structure (nearly 10-15nm in size) of INS molecules. This establishes that the protein is not denatured and remains intact in their native aggregated state.

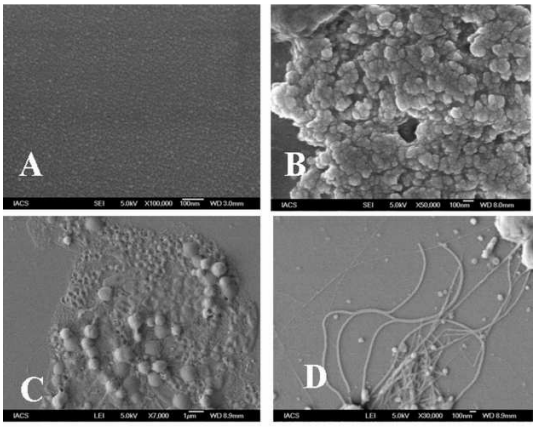

Fig. 5 FESEM images of the LB films of pure INS (Panel A), in presence of catanionic surfactant layer at π_{ini} = 2 mN/m (Panel B) and at π_{ini} = 10 mN/m (Panel C and D). Panel D is of higher magnification

Panel-B of Figure 5 represents the FESEM image of the LB film of INS in the presence of catanionic surfactant at π_{ini} = 2mN/m at the surface. This figure clearly shows near about 100-200 nm sizes particles which are much bigger than the earlier observation.

It may be arises due to the encapsulation of INS molecules by catainionic surfactant. During this phenomenon, the electrostatics interaction may play an important role to form encapsulated INS molecules by catanionic surfactant. The two-stage growth kinetics observed in of Figure-3 (curve-a) is related with this phenomenon.

Panel-C of Figure-5 represents the FESEM image of the LB film of INS in presence of catanionic layer at π_{ini} = 10mN/m at the surface. In this condition, there are sufficient numbers of cationic and anionic surfactant molecules to interact with the polar sites of INS to denature it [54]. The image shows some circular aggregates along with the fibrillated INS.

Panel-D, shows the fibrillated structure of INS when π_{ini} = 10 mN/m. In this condition, the excess number of catanionic surfactants form surface micelles of various sizes (500nm to 1.5µm) due to the electrostatic interactions between the oppositely charged head groups [44]. This conclusion also establishes the reasons for not showing any distinct growth kinetics in the Figure-3 (curve b, c).

Figure-6 shows the AFM image of LB films of INS in presence of catanionic surfactant mixture at π_{ini} = 2 and 10 mN/m at surface. Panel-A of Figure-6 is the AFM image of INS at low π_{ini} (2mN/m) shows the particle-type surface morphology having the diameter nearly 200nm, arises due to the encapsulation of INS molecules by the catanionic surfactant.

Similar observation is also found from FESEM image. The small amount of surfactant, mostly interacts with the INS molecule, encapsulate the protein. Whereas the Panel-B represents the AFM image at π_{ini} =10 mN/m, shows fibrillar structure, justify once again that at high concentration of surfactant denaturation of protein occurs. The above image can also be related to FESEM observation.

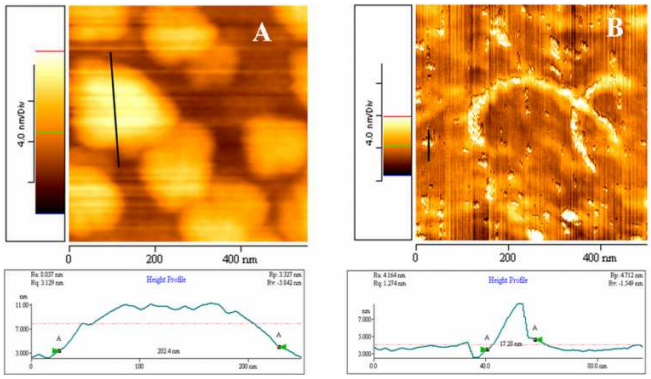

Fig. 6 Panel A and Panel B represent the AFM images of the LB films of INS in presence of catanionic surfactant layer at π_{ini} =2 mN/m and 10 mN/m respectively.

D.FTIR study of Langmuir monolayer film

Figure-7 shows the FTIR spectra of the amide regions of pure INS. The LB film of INS lifted on the hydrophilic silicon substrate at bare interface. The peak at 1560 cm^{-1} in amide II region is due to $-NH_2$ bending moment [62] and the peak at 1650 cm^{-1} in the amide I region is due to α helix in protein. [62] These peaks once again conclude that at LB film INS remain in its native state. Whereas the inset Figure shows the FTIR spectra (only the amide I region) of LB films lifted on the hydrophilic silicon substrate of INS at bare interface (curve-a) and in presence of catanionic surfactant mixture kept at π_{ini} = 2 mN/m (curve–b) and 10mN/m (curve–c) surface pressure at air-water interface. The film was lifted after the surface pressures achieve the saturation pressure (~14 mN/m). It is generally agreed that the peak at 1649 cm^{-1} corresponds to α-helix [63].

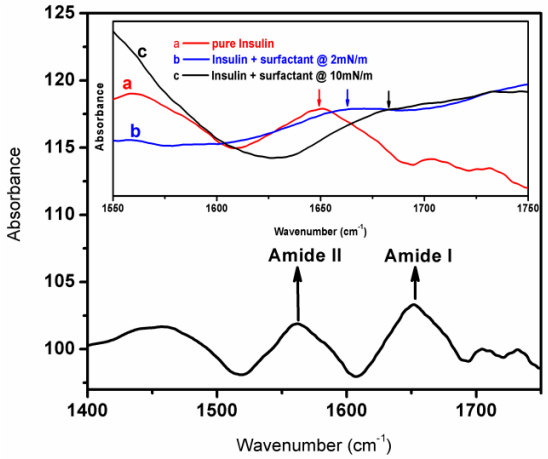

Fig. 7 represents the FTIR spectra of pure INS in amide bands region. The inset shows the amide I band region of LB films of pure INS (curve-a) and in presence of surfactant at π_{ini} =2mN/m (curve-b) as well as at π_{ini} = 10 mN/m (curve-c)

The presence of peak position at ~1650 cm^{-1} in curve-a conclude that it arises due to the α-helix part and suggest that the INS is not denatured after transferring it onto the silicon wafer. On the other hand, the peak positions in the curve–b (π_{ini} = 2mN/m) and curve-c (π_{ini} = 10mN/m) are 1659 cm^{-1} and

1679 cm^{-1} respectively. According to the previous literatures the peaks at 1659 cm^{-1} and 1680 cm^{-1} correspond to the 3_{10}-helix and β-sheet part respectively [64]. From the above spectra we can conclude that, the small amount of surfactant at air/water interface do not denature the INS molecules. In this condition, a part of α-helix is converted to 3_{10}-helix, i.e. the amino acids are arranged in the right-handed helical structure. However, in presence of higher concentration of surfactant at air/water interface, the INS molecules are denatured by unfolding it.

IV. Conclusions

Our study on INS with SDS/CTAB at air/water interface shows different types of morphologies for different amount of catanionic surfactant mixtures. Moreover, the adsorption rate of INS at air/water interface is also increased due to the presence of catanionic surfactant mixtures. It is evident that there are two mechanisms responsible for the growth of surface pressure in presence of small amount of catanionic surfactant. The protein remains in native state in presence of small amount of surfactant mixture. Large amount of SDS/CTAB denature the protein molecules. Smaller amount of surfactant mixture with INS shows surface micelle type structure. This may be considered for drug delivery system. Still, detailed studies are required to understand the dynamic process of the growth of aggregates and different types of interactions, responsible for different morphologies and these are in progress in our laboratory.

Acknowledgement

We thank DST, Government of India (Project No.-SR/S2/CMP-0079/2010 (G)) for partial financial support. M. M. thanks CSIR, Government of India for providing the CSIR-NET fellowship.

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