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Dedicated to Professor Valer Fărcăşan
at his 85th anniversary

ATOMIC FORCE MICROSCOPY STUDIES OF LANGMUIR-BLODGETT FILMS. I. STRUCTURES OF COLLAPSED STEARIC ACID MONOLAYERS

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ABSTRACT. Our previous theoretical studies on the collapse mechanism of stearic acid monolayers at the air/aqueous solution of pH 2 interface have suggested that the collapse mechanism can be described by a nucleation process subsequently followed by a growth of the nuclei. In the present work we will provide more evidence on the collapse mechanism and on collapsed structures of stearic acid monolayers by using atomic force microscopy (AFM) on Langmuir-Blodgett (LB) films of stearic acid transferred from monolayers, at the air/water interface, on hydrophilic substrates. Topographic and phase AFM images have shown several types of collapsed structures identified by us as straight bilayer ridges and buckling areas, three layered collapsed fragments, and multilayered colloidal particles. By using cross section profiles through the topographic images, the said collapsed structures were found in equilibrium with condensed stearic acid monolayer domains. These results are in substantial agreement with our earlier theory on collapse mechanism of Langmuir monolayers and with similar reported data in the literature. Finally, they confirm that AFM provides an accurate analysis on the packing of the molecules in monolayers and in collapsed structures and on the formation of LB film domains at the nanometer scale.

Key words: atomic force microscopy, Langmuir-Blodgett films, stearic acid, collapsed structures.

INTRODUCTION

Stearic acid (SA) is an example of an amphiphilic biocompound that is insoluble in water and self-assembles as a stable Langmuir monolayer at the air/aqueous solution of pH 2 interface. Due to its high surface stability, SA is considered as a model compound suitable for nanolayers research.

Traditionally valuable information on the molecular organization of SA in monolayers is obtained from compression isotherms [1-3], which are given in terms of surface lateral pressure as function of mean molecular area [1-7]. To study the surface properties and to visualize the surface structure of a biocompound nanolayer, a variety of experimental techniques and methods have been developed.

Thus, during the past several decade sophisticated methods have been developed such as fluorescent microscopy [8, 9], Brewster angle microscopy [10], and synchrotron X-ray diffraction [10-13], which provide a more detailed picture of the molecular organization in different biocompound monolayers. However, these methods show a limited lateral resolution of structures existing in the plane of the film.

By transfer of a monolayer to a solid substrate [10-13] a Langmuir-Blodgett (LB) film is obtained and further techniques are available for the LB film investigations. Therefore, absorption-reflection infrared spectroscopy and ellipsometry [2], Fourier transform infrared spectroscopy [13], electron scanning microscopy [14, 15] and surface plasmon resonance [16] have been used to investigate monolayer domains and molecular orientation of various biocompounds but none of these techniques can provide direct information on nano-meter scale structure of LB film surfaces.

Recently, the atomic force microscopy (AFM) was developed and it is used for visualization of various phases which can occur in nanolayers, for their surface structure investigations as well as for size analysis of supramolecular aggregates [17-29]. The AFM is a novel high-resolution surface imaging technique for nano-meter scale size structures with angstrom-scale lateral and normal resolution. AFM operates by measuring the forces acting between a probe (also called tip) attached to a cantilever and the LB film, which is called usually the LB sample.

Further, the AFM coupled with LB film technique offer the possibility of studying the evolution of film structures at collapse as the Langmuir monolayers evolve from two to three dimensions. The study of the mechanism of collapse and collapsed structures [14, 15, 30-38] is also useful to build-up model membranes for complicated biological membranes to investigate the structure, activity and function of biologically relevant biocompounds, under controlled conditions of lateral pressures. LB films formed by amphiphilic molecules, like stearic acid, occupy a unique place at the crossroads of materials science, nanoparticles technology, complex soft materials physics, interfacial physical chemistry of oriented supramolecular structures, colloidal and surface chemistry, physical biology, pharmacology, molecular biophysics and biomedicine.

The goal of our present work is to find more evidence about the collapse mechanism and to visualize the collapsed structures of SA films by using LB technique and AFM investigations.

EXPERIMENTAL SECTION. MATERIALS AND METHODS. CHEMICALS AND LANGMUIR MONOLAYERS

Stearic acid was purchased from Sigma, n-hexane was purchased from Merck, and other chemicals were purchased from Reactivul Bucharest, all of high purity requested for Langmuir monolayer research.

Stearic acid (SA) was dissolved in n-hexane at a concentration of 1mg/ml and spread on acid aqueous solution of 0.01 M HCl of pH 2, in order to avoid ionization of SA molecules, in the teflon trough, at 20 ^{o}C. After a waiting time of

5 to 10 minutes to allow the solvent to be fully evaporated, the Langmuir monolayer of stearic acid was manually compressed at a speed of 10 cm^2/min and the compression isotherm was recorded as described by us in detail elsewhere [1].

The SA monolayer was compressed to its collapse and then transferred to the hydrophilic substrate by vertical dipping method or by horizontal transfer method, as we previously reported [2]. In detail, the transfer of LB films, at incipient collapse of SA monolayer (where its lateral surface pressure is 40.8 mN/m) or from an over compressed SA monolayer at 45 mN/m, was performed on glass by horizontal deposition method. For comparison, the transfer of LB films from over compressed SA monolayers at 45 mN/m was also performed on glass covered with thin aluminium mirror by vertical transfer method at low transfer speed of 0.5 cm/min. The over compressed SA monolayers have reached an advanced collapse state.

The aqueous subphase was prepared with water of 18 Mohm cm obtained by using an Elgastat system and it was adjusted at pH 2 with hydrochloric acid.

LANGMUIR-BLODGETT TECHNIQUE AND LB SAMPLE PREPARATION

The LB films of SA supported on 0.1 mm thick freshly cleaned glass or glass covered with aluminium mirror, were built up using Langmuir-Blodgett technique. Prior to SA film deposition, the glass was well cleaned with chromic acid and rinsed with two-distilled water. The hydrophilic glass support was lifted horizontally from water phase through the SA monolayer compressed at collapse (40.8 mN/m) or compressed over its lateral collapse pressure at 45 mN/m. To protect the LB films from dust, the plates with LB films were covered with 50 ml beakers. This procedure might make the evaporation of water slower as compared to that in an open air environment.

For comparison, the glass substrate covered with thin aluminium mirror was lifted vertically from water phase through the Langmuir nanolayer of SA over compressed at 45 mN/m, when the nanolayer exists in an advanced collapse state. Due to the experimental configuration of this transfer method, the support with LB film was held vertically in the ambient atmosphere at room temperature until the transferred SA film was dried (about 10 minutes).

This work demonstrates that, both models of LB samples, LB vertically and LB horizontally transferred, showed a good transfer quality of the LB films. At least three LB films were prepared for every independent LB sample, under controlled conditions.

Then, tens of AFM scans were made for each LB film, and the images from different LB films showed that the morphological reproducibility is good for one specific LB sample. Even more, LB samples of stearic acid on glass and on aluminium mirror were measured several days after the transfer and occasionally several months up to one year after the transfer of SA nanolayers. The reproducibility of AFM images demonstrated that the transferred SA films have a high stability.

ATOMIC FORCE MICROSCOPY IMAGING AND ANALYSIS

Investigation of the surface morphology and domain structure of the LB films of SA was conducted in tapping mode on a research AFM system described elsewhere [29]. The AFM was calibrated by using freshly cleaved highly oriented pyrolytic graphite (HOPG). Direct visualization of LB film structures was performed with AFM equipped with a 90 µm scanner (x-y), 1.5 µm (z) scanner, with scan control and commercial software.

Non-contact conical shaped tips of silicon nitride coated with aluminium were used for AFM tapping mode. The tip was on a cantilever with the length of 110 µm, width of 40 µm and thickness of 2 µm. The resonant frequency of the cantilever was in the range of 200 - 300 kHz with a spring constant of 17.5 N/m. The manufacturer specification for the terminal tip radius of curvature is less than 10 nm, the full tip cone angle less than 20 degrees and its height in the range of 15 – 20 µm.

The AFM images reported in the present work were two-dimensional (2D) topographies and the three-dimensional (3D) views of 2D-topographies, all performed in tapping mode. For comparison, occasionally, the phase image is also given. All images were processed using the standard procedures for AFM.

The AFM images cover a range of LB film areas from 10 x 10 µm^2 to 2 x 2 µm^2. AFM observations were repeated on different areas of the LB sample. The images were obtained from at least three macroscopically separated areas on each LB sample. Dimensions of the SA domains were measured directly from AFM topographic images and the thickness variations were estimated from cross section profile analysis.

An important property of AFM is its ability to measure the height of observed LB film domains. Therefore, the main criterion to distinguish among various structures, such as monolayer, bilayer, trilayer or other forms, is the height of the observed domains in LB films. The images of the domains have bell-shape forms, as observed in the cross section profiles. Because the lateral dimensions are broadened due to a tip-related effect [39], the size of a domain was determined as the width of its bell shaped form measured at its half height.

RESULTS AND DISCUSSION

COMPRESSION ISOTHERM OF LANGMUIR MONOLAYER OF STEARIC ACID

A Langmuir monolayer (also called nanolayer) is generally obtained by spreading a known number of SA molecules on the air/aqueous solution of pH 2 interface in a Langmuir teflon trough of a precise known area. Teflon barriers placed across the trough serve to vary the area of the SA nanolayer in compression – expansion cycles, and in consequence, the mean molecular area of the SA amphiphilic molecule is precisely determined, and simultaneously, the surface pressure is measured by using a surface tension sensor in the Langmuir technique [1, 2]. The surface pressure (π, expressed in mN/m) is calculated as the difference between the surface tension of the pure subphase against air (σ_0, given in mN/m) and the surface tension (σ, in mN/m) of the air/water interface with Langmuir nanolayer.

By compression of stearic acid monolayer spread at the air/aqueous phase of pH 2 interface, the compression isotherm is obtained (Fig. 1) and it exhibits sharp modifications which illustrate phase transitions under controlled conditions. Previously, we have already demonstrated that SA film exhibits a variety of characteristic phases, like condensed liquid (CL, with characteristic limiting area of stearic acid molecule A'_0), solid (S, with limiting molecular area A_0) and collapsed phases under known experimental conditions [1-3]. The two dimensional (CL – S) phase transition from condensed liquid to solid is observed at a lateral surface pressure of about 26 mN/m.

The collapse surface pressure is the highest surface lateral pressure, marked by an arrow on the isotherm (Fig. 1), to which a Langmuir monolayer can be compressed at the air/water interface without the formation of a detectable collapsed bulk phase [3, 30] of the film forming SA molecules. This collapsed state corresponds to an incipient collapse of the SA monolayer (40.8 mN/m) at the air/water interface.

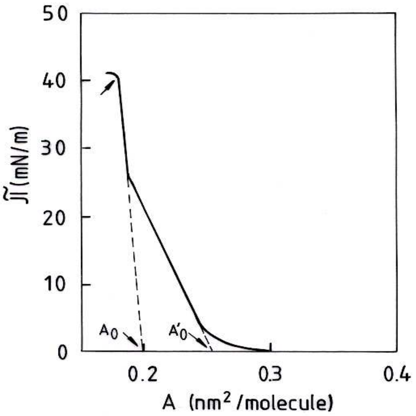

Fig. 1. Compression isotherm of stearic acid (SA) monolayer, surface pressure versus mean molecular area, at the air/aqueous solutions of pH 2 interface, at 20 °C. For symbols see the text. The arrow at high surface pressure indicates the incipient collapse.

To deeply understand the collapsed structures, SA monolayer was over compressed, above its incipient collapse state, to higher surface lateral pressures (45 mN/m). This new state corresponds at an advanced collapse state of SA films, which was further investigated by AFM on LB films of stearic acid transferred on solid substrates by both deposition methods described above under these new conditions.

STEARIC ACID MONOLAYER ORGANIZATION AT THE AIR/WATER INTERFACE

In our earlier studies, the characteristic phases in Langmuir monolayers of SA at the air/water interface have been evidenced by using compression isotherms, surface compressibility measurements and geometric molecular models [1]. At the monolayer incipient collapse (40.8mN/m), the rodlike SA molecules are vertically oriented in the most extended all-trans conformation of their saturated hydrocarbon chains. Some geometric considerations on the film organization may be useful for understanding the structural characteristics at collapse, or when over compressed above its collapse.

The study of molecular models showed that in the most extended conformation of SA molecule the polar COOH group can be readily accommodated beneath the hydrocarbon chain and the height of the entire molecule attains 26.2 Ǻ [1]. This molecular height can be considered as an important parameter relevant to the monolayer's thickness and to the morphology of SA monomolecular film at its monolayer collapse. This height is in good agreement with the value of 2.5 nm determined for stearic acid monolayer by AFM investigations [27].

COLLAPSE MECHANISM OF LANGMUIR MONOLAYERS OF STEARIC ACID

From previous studies on the collapse mechanism of an insoluble Langmuir monolayer at the air/water interface theoretical models were developed [14, 15, 30-35]. The states of collapsing processes [14, 15, 33] for an over-compressed Langmuir monolayer are shown in Fig. 2.

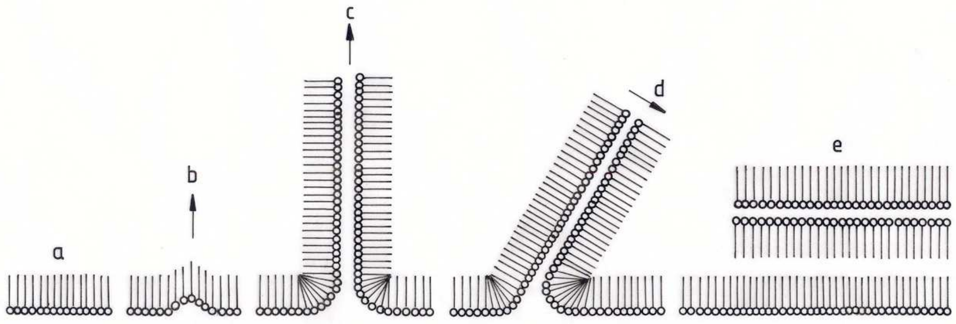

Fig. 2. States of collapsing processes and collapsed structures obtained during the over compression of condensed stearic acid monolayers. Arrows show the movement of molecules in different structures (see the text). The drawings are not at scale.

Experimentally, the incipient collapse is often observed at a very reproducible collapse pressure, corresponding to a metastable equilibrium between the nanolayer and the undetectable freshly collapsed bulk phase [30, 36].

The collapse pressure (π_c in mN/m) value and the corresponding molecular area (A_c) value for SA are easily determined from compression isotherm (see the arrow in Fig. 1). At high surface pressure of about 40.8 mN/m, the Langmuir monolayer is metastable and collapsing processes or relaxation phenomena occur, which may represent the formation of either two-dimensional micelles or islands of close packed surfactant molecules, both entailing formation of nuclei of the new phase and subsequent growth of these nuclei.

With heterogeneous kinetic processes, the Prout-Tompkins equation is frequently applied [37, 38], which was adapted [31] and refined by us [32-34] for the collapse mechanism (Fig. 2) of the insoluble Langmuir monolayers.

Theoretical studies of collapsing processes in SA monolayer [33] showed that by increasing the lateral surface pressure over the incipient collapse pressure of SA monolayer, at incipient collapse SA monolayer existing in a two-dimensional condensed phase (Fig. 2a), a buckling process might evolve in the plane of the monomolecular film (Fig. 2b) which entails film breaking and the formation of bilayers as vertical ridges (Fig. 2c) which can rise to the air phase. These ridges bend (Fig. 2d) and end up in breaking down over the remaining monomolecular film as a collapsed fragment and therefore, trilayers (Fig. 2e) may appear. Further, we will provide more detailed experimental evidence on collapsed structures obtained in SA monolayers by using LB technique and AFM investigations.

ATOMIC FORCE MICROSCOPY OF LB SAMPLES OF STEARIC ACID

We carried out studies at an incipient collapse of SA monolayers (for a collapse pressure of 40.8 mN/m) and at over compressed conditions beyond the monolayer collapse (called advanced collapse for a lateral surface pressure of 45 mN/m), in order to characterize their surface structure by AFM tapping mode and bring light on the collapse mechanism. The sensitivity of AFM measurements is very high and accurate information on the surface morphology at the molecular level is obtained.

For the beginning, the AFM observations have been carried out on LB samples horizontally transferred on glass, at incipient collapse, as shown in Fig. 3, namely 2D-topography (panel A), 3D-topography (B), and cross section profile (C) along the line shown in panel A. The three dimensional image (B) was obtained by image processing of the two-dimensional topography (A).The morphology of the SA film at incipient collapse is typically shown in Fig. 3 (panels A and B). The SA film structure consists of condensed monolayer domains aggregated in irregular and in circular forms with bridges among them. The highest thickness of the SA film remains almost unchanged at 28 ± 2Å as shown in cross section profile (Fig. 3C). This thickness corresponds to the height of SA molecules (25 Å [27]) in their all-trans conformation (26.2 Å [1]) vertically oriented on the LB surface. This value can be considered as an important parameter relevant for the thickness of a SA monolayer relatively well packed at incipient collapse in condensed domains. However, Fig. 3A and Fig. 3B show the presence of some pinholes particularly in the middle of the circular condensed domains and some other defects are visualized around the condensed domains.

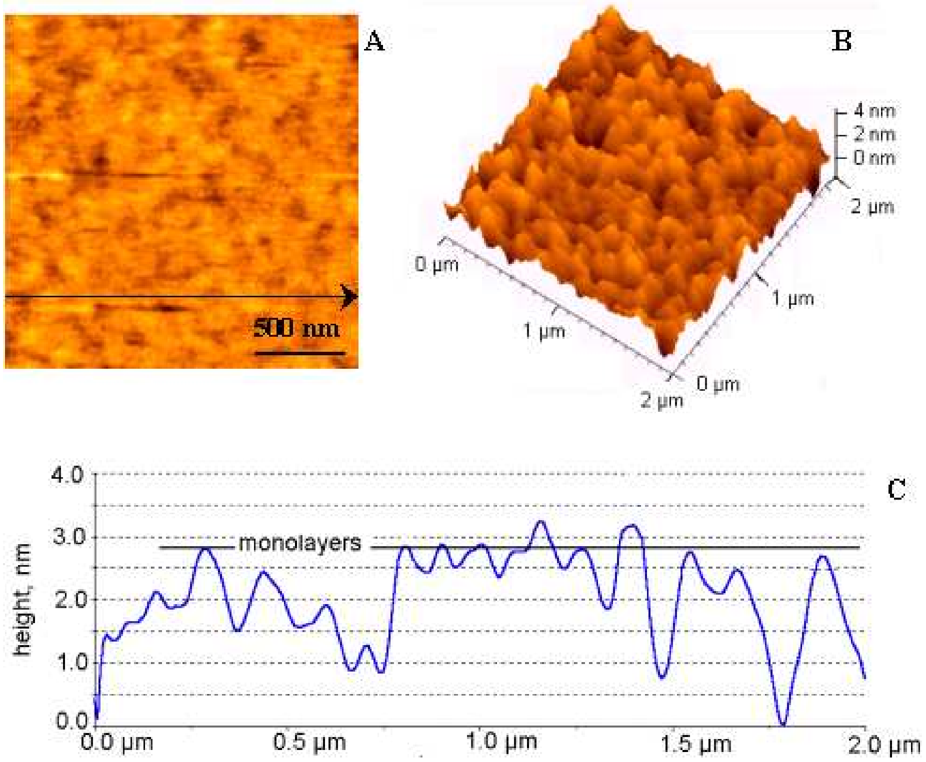

Fig. 3. AFM images, 2D topography (scanned area 2 x 2 µm^2, panel A), 3D topographic view (panel B) and cross section profile (panel C) along the line in panel A for stearic acid LB sample horizontally transferred on glass at incipient collapse (40.8 mN/m) from SA monolayers at the air/aqueous solution of pH 2 interface.

Further, the AFM images are given in Figs. 4 and 5 for an advanced collapse, when the SA monolayer was over compressed at 45 mN/m before the film transfer on glass substrate.

The morphology of the SA film is shown in Fig. 4, 2D-topography (A), 3D-topography (B), and section profile (C) along the line shown in panel A. Apparently, the surface of SA film contains two types of supramolecular aggregates with monolayer and trilayer structures as indicated in Fig. 4C. The trilayer domains are of irregular rounded forms (Fig. 4, panel A and B).

On Fig. 4C, the highest thickness of the SA film is about 77 Å and it represents the thickness of three layered highly ordered domains. The lower height value of 26 ± 2Å is almost constant and it represents monolayer domains as shown in cross section profile (Fig. 4C). The whole film surface is dominated by the three layered structures (see, the line in Fig. 4A) and monolayer domains (see, the marked square area in Fig. 4A) decorated with some large colloidal particles with a thickness of about 200Å (see arrows in Fig. 4B).

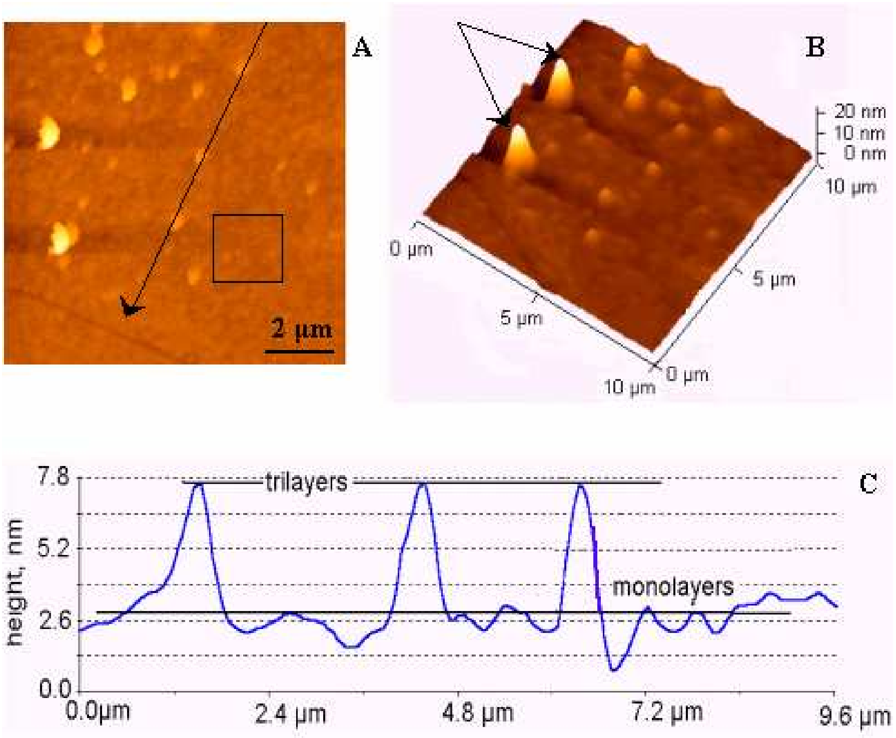

Fig. 4. AFM images, scanned area 10 x 10 µm^2, 2D topography (panel A) and 3D topographic view (B) of stearic acid LB sample horizontally transferred from over compressed monolayer at advanced collapse (45 mN/m) at the air/aqueous solution of pH 2 interface and cross section profile (C) along the oblique line in panel A (see the arrow); the marked area indicates monolayer domains.

Furthermore, to investigate deeply the morphology of monolayer domains at advanced collapse of SA films, enlarged AFM images were obtained by scanning the area marked in Fig. 4A and they are given in Fig.5 (panels A and B) together with the cross section profile (Fig. 5C) along the line shown in Fig. 5A. The section profile shows the height of SA film between 26 Å and 28 Å corresponding to monolayer domains of 240nm average size (Fig. 5C). On the other hand, the nano-structure of the SA monolayer at advanced collapse seems to be quite rough and several large holes (Figs. 5A and 5B) with a similar depth (Fig. 5C) were observed on the surface.

The marked area seen in large-scale AFM image (Fig.4A,10x10µm^2) showed a smooth, high quality surface of monolayer area. However, this smooth surface morphology at a high magnification (Fig. 5A, 2 x 2µm^2) presents larger monolayer domains at advanced collapse than those observed at incipient collapse of SA monolayers (for the same magnification, see Fig. 3A, 2 x 2µm^2). This observation can reflect some real modifications in the LB film morphology due to the increased lateral surface pressure for advanced collapse state of SA monolayers. This might also be overlapped with an AFM artifact, which can be associated with a tip dilation of the lateral dimensions of nano-scale objects.

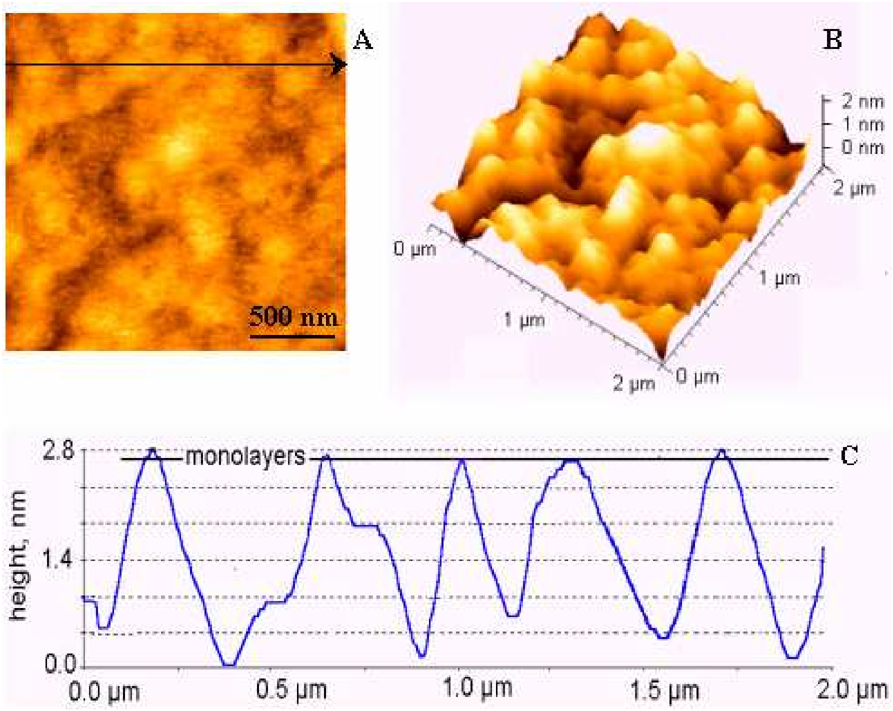

Fig. 5. AFM images of stearic acid LB sample obtained from the almost flat area (scan size 2 x 2 µm^2) which is marked as a square in Fig. 4A; 2D topography (A), 3D topographic view (B) and section profile (C) along the line in panel A.

It is to be mentioned that the AFM tapping mode allows simultaneous acquisition of both topographic data (topographic image) and material-properties data (phase image). The topographic image represents the sample surface structure which is obtained by monitoring the cantilever's oscillation amplitude changes in response to tip to sample spacing, as shown above. Simultaneously, the phase image is produced and can be used to map variations in surface properties such as elasticity, adhesion, and friction. The phase image is obtained by monitoring of the phase lag between the signal that drives the cantilever to oscillate and the cantilever oscillation output signal. Changes in the phase lag reflect changes in the mechanical properties of the sample surface.

To further check the morphology of the LB films of SA, the AFM observations have been carried out on LB films, vertically transferred on glass covered by aluminium mirror, at advanced collapse for a lateral pressure of 45 mN/m. These

AFM observations are given in Fig. 6, 2D-topography (A), phase image (B), section profile (C) along the line shown in panel A, and the 3D-topography (D) which was obtained by image processing of the two-dimensional topography (A).

The images of the LB film of SA (Figs. 6A and 6B) correspond to an advanced collapse state and they show a film structure with an irregular distribution of various domains mostly rounded in shape. The correlation between topographic (Fig. 6A) and phase (Fig. 6B) images suggests that these AFM imaging techniques are complementary and they permit to make observations both on the structural characteristics and on mechanical properties of LB film surfaces, respectively. It is to be noted that there are clear characteristic features in Fig. 6B, comparable with those in Fig. 6A, relevant for modifications of physical and chemical properties of various domains from the LB film surfaces.

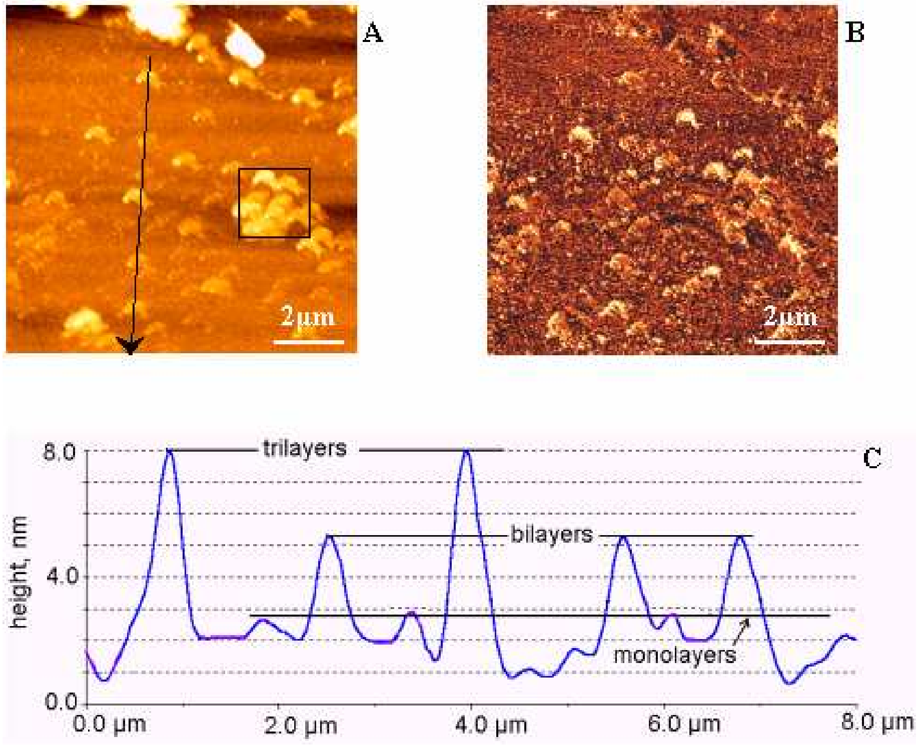

Fig.6. 2D topographic (A) and phase (B) AFM images (10 x 10 µm^2) of LB sample of stearic acid transferred from an over compressed monolayer at 45 mN/m, being in an advanced collapse state, on acid subphase of pH 2. LB sample was deposited by vertical transfer on glass covered by an aluminium thin film; panel C represents the cross section profile along the oblique line (see the arrow) shown in Fig. 6A; the marked area indicates trilayer domains.

The cross section profile (Fig. 6C) indicates the presence of “bilayer” domains with the height of about 53 Å, which is almost two times that of stearic acid monolayer, besides monolayers with the height of about 27 Å and trilayers with the height of 80 Å. These bilayer domains can designate the buckling areas (Fig. 2b) or bilayer ridges (Figs. 2c and 2d) which apparently grow up to a height corresponding to the thickness of a SA bilayer in substantial agreement with AFM observations on collapsed SA film transferred on hydrophobic HOPG [21]. The average size of SA domains is comprised in the range of 300 nm to 500 nm. The dimensions of these domains given in Fig. 6C are visualized also in Fig. 6D.

The marked area in Fig. 6A indicates trilayer domains (Fig. 2e) clustered as it is also evidenced in Fig. 6D. These trilayer domains are larger than in the rest of the scanned area. Fig. 6A shows also the presence of several large colloidal particles with the height in the range of 120 – 200 Å as determined from Fig. 6D (see arrows).

The image analysis of Figs. 3-6 indicates similar structural features of the LB films, formed at the collapse of stearic acid monolayers either horizontally transferred on glass (Figs. 3-5) or vertically transferred on an aluminium mirror (Fig. 6). This is attributed to the high stability of the hydrophobic groups, i.e., methyl groups on the surface of the outermost layer of the LB films in air. Previously, we have pointed out that odd numbered LB films of stearic acid can be deposited at more than 200 layers on hydrophilic glass [2]. The experimental LB film structures visualized by AFM two- and three dimensional topographical images, phase image and cross section profiles can be explained by collapse mechanism proposed previously [15, 32, 33].

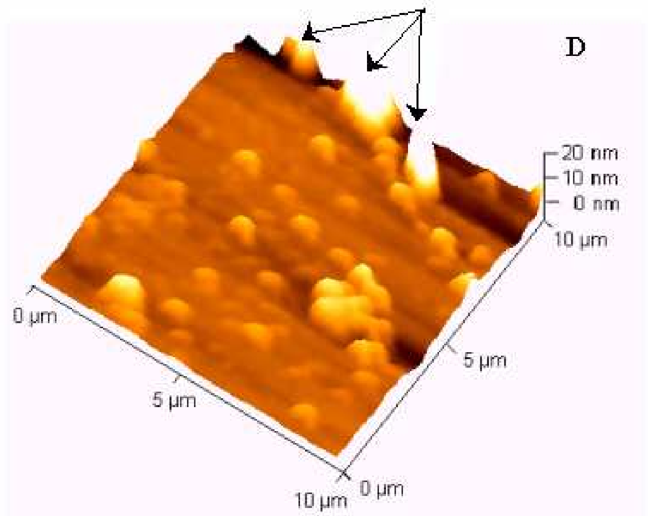

Fig.6D.The 3D view of the 2D topographic AFM image (Fig. 6A) for stearic acid LB sample. Arrows indicate colloidal particles.

It is to be emphasized that the height of a SA molecule in its extended all – trans conformation is about 26.2 Å as it was determined by us previously [1] from geometric models. This calculated length of a SA molecule is close to the value recently found of 25 Å /molecule of SA by AFM investigations [27] and it almost coincides with the height value of 26Å preponderantly found for monolayer domains in cross section profiles shown above.

In present studies, AFM technique has been found suitable to investigate the surface morphology, defects and domain structure of LB films of stearic acid horizontally and vertically transferred on solid substrates from Langmuir monolayers of stearic acid at the air/water interface for two lateral surface pressures characteristic for incipient and advanced collapse of SA monolayers. From AFM observations of the collapsed structures, trilayers and monolayer domains were found within the advanced collapsed SA film. In addition, straight ridges and buckling domains, which show a thickness of double molecular layers [21], and an accumulation of colloidal particles [22, 24, 27] were identified in total agreement with literature data.

CONCLUSIONS

The atomic force microscopy was used to study the surface structure of Langmuir-Blodgett films of stearic acid at the nanometer scale, the transition from monolayers to multilayered collapsed phase and the collapse mechanism. Analysis of topographic and phase images showed differences among various surface morphologies suggesting that there are also modifications of the surface properties of LB films in various supramolecular film arrangements.

LB films of stearic acid horizontally and vertically transferred from air/aqueous solution of pH 2 interface on glass and on glass covered with an aluminium mirror, respectively, showed a similar morphology at advanced collapse. From AFM observations of the collapsed structures and from section profile through topographic images, straight ridges or buckling areas, which show a thickness of double molecular layers, trilayers, colloidal particles and monolayer domains were identified in substantial agreement with our previously published theory on the collapse mechanism of Langmuir monolayers and with reported literature data. These results demonstrate that the surface structure of collapsed LB films of stearic acid is preserved in both vertical LB transfer and in horizontal LB transfer.

This approach might be very promising in the study of biologically relevant molecules since molecular processes like the orientation of molecules, their packing in the surface network, and the collapse of their self-assemblies can occur in biological membranes.

In this work, we were able to demonstrate that the SA films maintained their film stability for one year. This knowledge would be useful in determining the duration and the stability of these nanolayers with possible use in various medical micro-devices coated with fatty acids films and in drug delivery systems.

Further studies are in progress in our laboratories which will bring more detailed information regarding the stabilizing forces in the freshly collapsed and in advanced collapsed fragments within nanolayers.

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