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PREPARATION OF CELLULOSE WHISKERS MONOLAYERS ON WAFER SILICON SURFACE BY LANGMUIR–BLODGETT TECHNIQUE

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A detailed description of the preparation of cellulose whiskers monolayer on the silicon wafer surface by Langmuir–Blodgett technique is presented. The Langmuir–Blodgett transfer is realized both by continued compression and sinusoidal compression-expansion conditions of dioctadecyldimethyl ammonium bromide–cellulsoe nanocristals bilayer. The AFM analyze of the cellulose nanocrystals monolayer has shown, that the cellulose whiskers monolayer is more compacted, when the transfer is realized in sinusoidal compression-expansion condition.

Keywords: cellulose nanocrystals, plant cell wall, Langmuir–Blodgett transfer, Atomic Force Microscopy (AFM), surface pressure.

Introduction. It is well known that plant cell walls have a very complicated structure and chemical composition (Fig. 1) [1]. After the growth and the extension of plant cells, large structural and chemical modifications contribute to give the functional specificity of the cell walls. The secondary wall is of a high rigidity. It contains a large proportion of cellulose microfibrils of very crystalline structure forming a compact network. These microfibrils form concentric layers, where they are parallel between them [2]. The complex network surrounding the microfibrils is made of different amorphous polymers (hemicelluloses, lignins and proteins according to the species) [3].

These architectures still raise multiple questions such as, for example, the role of hemicelluloses and lignins in the cohesion between the successive layers of cellulose. Since it is presently not possible to understand directly the role of each macromolecule in the structure and properties of the cell walls, a number of works have been carried out to develop model surfaces of cellulose by spin-coating or Langmuir–Blodgett techniques, in particular starting from derivatives of cellulose (trimethylsilylcellulose) [4], or starting from chemically solubilized cellulose in N-methyl-morpholine-N-oxide [5] or in lithium chloride in dimethylacetamide [6]. The majority of these surfaces were used to study their adhesion properties, the adsorption of polyelectrolytes and surfactants, the interaction forces between cellulose surfaces or between cellulose and other macromolecules (lignins, xylans)

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or their reactivity towards enzymes [5–10]. Thus, the preparation of cellulose model surface is very important for the quantitative study of intermolecular interaction forces in plant cell wall.

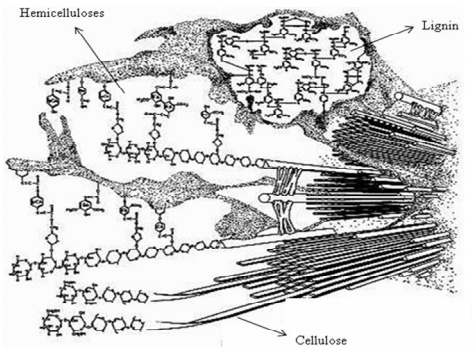

Fig. 1. Plant cell wall structure and composition.

The aim of the present paper is to describe the preparation and the characterization of monolayers of tunicin cellulose whiskers obtained from aqueous diluted suspensions by the Langmuir–Blodgett (LB) technique. In order to establish a relation between the dioctadecyldimethyl ammonium bromide (DODA)–cellulsoe nanocristals (CNs) bilayer surface pressure and the cellulose monolayer structure (whiskers orientation, density) the Langmuir–Blodgett transfer was realized in continued compression and in sinusoidal compression-expansion conditions. The density and orientation of cellulose whiskers in the monolayers were characterized by Atomic Force Microscopy (AFM).

Materials. Fresh specimens of tunic (*Microscosmus fulcatus*) from Mediterranean were purchased on the local market (France). DODA-Br was purchased from Merck and Silicon wafer disks ((100) orientation, diameter of 76 *mm* and thickness of 380 μm) were obtained from ITME-Neyco S.A. (France). Other organic solvents and chemicals were of analytical grade. Deionized water ("Millipore", Molsheim, France, R >18 $M\Omega/cm$) was used as subphase.

Experimental Part.

Langmuir–Blodgett Technique. The adsorption of amphiphilic molecules at the surface of a liquid can be so strong that a compact monomolecular film, abbreviated as monolayer, is formed (Fig. 2, a).

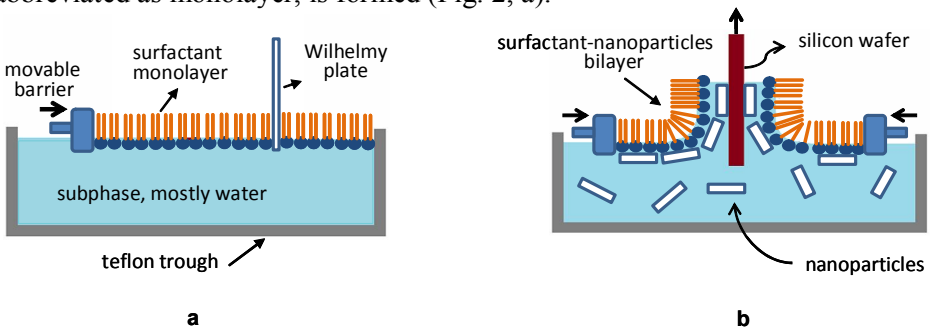

Fig. 2. Langmuir trough with a monolayer indicated by surfactant molecule (a) and LB transfer of nanoparticles-surfactants bilayer on to solid substrates (b).

There are amphiphiles, which practically do not dissolve in the liquid. This leads to insoluble monolayers. In this case the surface excess is equal to the added amount of material divided by the surface area. The most important tool for studying insoluble monolayers is a film balance [11], also called a Langmuir trough (Fig. 2, a).

The modern version of a film balance consists of a temperature-controlled trough, which contains the liquid. The liquid is called "subphase". Usually water is

used as subphase. The surfactant molecules are dissolved in a solvent (often chloroform), which is volatile and not miscible with the subphase. Drops of the solution are placed on the liquid surface and after evaporation of the solvent a surfactant film remains. This process is called "spreading". Via movable barriers the film balance allows to manipulate the density of molecules on the liquid surface by compression or expansion of the film. When compressing the film the area per molecule decreases, when expanding the film, it increases. If the barrier could move freely, it would drift in the direction of the liquid with higher surface tension. In this way the system can reduce its entire free energy. We can imagine that this movement is caused by a film pressure (π), also called "lateral pressure". The film pressure is defined as the difference between the surface tension of the bare subphase (γ_0) and the surface tension of the subphase covered by amphiphiles (γ):

$$\pi \equiv \gamma_0 - \gamma.$$

The film pressure is usually measured by the Wilhelmy plate method. Usually the Wilhelmy plate is a piece of absorbent paper hanging into the water subphase (Fig. 2, b). The force acting on it is proportional to the surface tension. If we compress a surfactant film on water we observe that the surface tension decreases and the surface pressure increases. If the amphiphile is significantly soluble in the liquid, we cannot use a Langmuir trough any longer because amphiphiles would diffuse via the liquid phase to both sides of the barrier.

Preparation of Cellulose Nanocrystals. Cellulose nanocrystals were extracted from the tunic of *Microscosmus fulcatus*. Small fragments of the external wall of tunicate were first treated with a solution of KOH (5% wt/vol.) overnight. The mantles were then washed and submitted to three successive bleaching treatments according to Wise et al. [12]. The bleached fragments were disintegrated in water with a warring blender. The homogeneous suspensions obtained from tunicin fibers were submitted to overnight acid hydrolysis with 65% (wt/wt) H_2SO_4 solution at room temperature and under continuous stirring. The suspensions were washed with water until neutrality and dialyzed against a concentrated solution of PEG (35000 *g*/*mol*) in order to reduce their volume. The resulting concentrated suspensions of nanocrystals from tunicin were stored at $4°C$. The cellulose nanocrystal solution with appropriate concentration was sonicated with a Branson Sonifier for a few minutes before use. The average crystal dimensions were estimated with Image from TEM (or AFM) images of diluted nanocrystals suspensions ($19 \times 9 \times 1000\ nm^3$) [13].

Preparation of the Silicon Substrates. Silicon wafers with (100) surface orientation and an oxide thickness of 20 Å were cut to the desired dimensions (ca. squares of 1.5 to 1 cm^2) and cleaned with $H_2SO_4 : H_2O_2 = 7 : 3$ bath for 30 *min* at $60°C$ before a continuous rinsing with purified water [13].

Langmuir–Blodgett Transfer. Stable layers of cellulose whiskers formed at the air-water interface in the presence of a cationic amphiphilic molecule (DODA) were transferred onto silicon wafers by the Langmuir–Blodgett procedure (Fig. 2, b). The pressure-area isotherms of the Langmuir films were determined on a LB trough (KSV Technology, minitrough 75×330 *mm* equipped with a Wilhelmy type film balance, Finland) [13]. The DODA-Br chloroform solution (20 μL, 1 *mg*/*mL*) was spread on the surface of the cellulose nanocrystal water suspension

with a microsyringe. The increase of the surface concentration of molecules at the interface was evidenced by the increase of the surface pressure during the compression of the film (Fig. 2, b). The transfer of the layer on a silicon wafer substrate was performed by the vertical deposition method at a controlled speed. The transferred layer was washed with chloroform and diluted NaOH in order to remove adsorbed DODA on the whisker film surface.

Characterization of Cellulose Model Surfaces. AFM imaging was performed using Nanoscope IIIa atomic force microscope from "Digital Instruments" (Santa Barbara, CA). The AFM was placed on an active vibration isolation table, so as the eventual external vibration did not hinder the imaging process. A scanner calibrated following the standard procedures provided by "Digital Instruments" with a maximum scan area of 120 μm^2 was used. Experiments were made at constant room temperature around 20°*C*. The samples were mounted onto a stainless steel disk using a sticky slab. The images were made in tapping mode. Commercial 225 μm long cantilevers from "Veeco Instruments" (France) with a resonant frequency around 190 *kHz* were used. Scanning rate of 1 *Hz* or 0.5 *Hz* depending on the image size and a resolution of 512×512 were used. During scanning, proportional and integral gains were increased to the value just below the feedback started to oscillate. Images were processed only by flattening to remove background.

Results and Discussion. In Fig. 3 are presented the isotherms of DODA monolayer and DODA–CNs bilayer obtained at ambient temperature. In both cases during the compression the surface pressures of the adsorption layers increase progressively, which are relied with reduction of the surface area occupied by surfactant molecules on the liquid-air interface. As it can be seen from Fig. 3, for the surface area between 80 and 130 *Mma* (Å^2) the surface pressure of DODA monolayer is slightly higher than of the DODA–CNs bilayer. It can be explained by DODA molecules mobility differences in DODA monolayer and in DODA–CNs bilayer. On the water-air interface DODA molecules without CNs are more mobile, and the spreading of the DODA molecules on the surface takes place more easily. Starting the surface area about 80 *Mma* (Å^2) the surface pressure increases more quickly than DODA–CNs bilayer pressure. It is an expected result and we believe that when the molecule surface area becomes smaller than 80 *Mma* (Å^2) the surface pressure is determined preferentially by interactions of cellulose nanocristals in the adsorption bilayer. This observation is in a very good agreement with our results presented on Fig. 4.

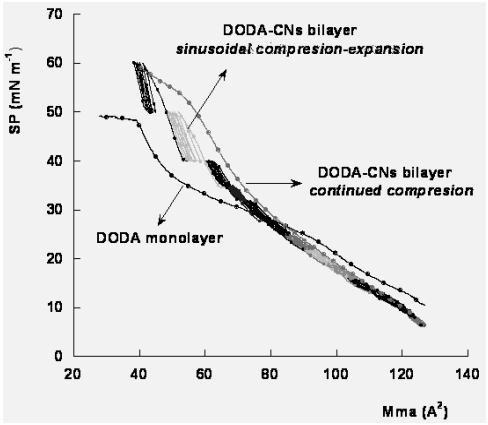

Fig. 3. SP–*Mma* isotherms of DODA monolayer and DODA–CNs bilayer on water-air interface at ambient temperature. DODA–CNs bilayer was compressed in continued compression (progressive line) and sinusoidal compression-expansion (periodically changing lines) conditions.

On Fig. 4 is presented the SP–*Mma* plot of the DODA–CNs bilayer obtained in sinusoidal compression-expansion conditions at ambient temperature. The sinusoidal compression-expansion velocity of movable barrier was 2 *mm*/*s*. The sinusoidal compression-expansion of the DODA–CNs bilayer was carried out for the surface pressure between 40 and 50 *mN*/*m*. As it is shown on Fig. 4 during the sinusoidal compression-expansion of the bilayer the surface area occupied par adsorbed molecules (nanoparticles) decreases, while the surface pressure remains constant. Sinusoidal compression-expansion of the DODA–CNs bilayer was realized also for other surfaces pressures (Fig. 3). It is interesting to note that for the surface areas values between 40 and 80 *Mma* (Å^2) the plot of DODA–CNs bilayer deplaces to the DODA monolayer plot (Fig. 3). This result can be explained by increases of the density of CNs in the DODA–CNs bilayer.

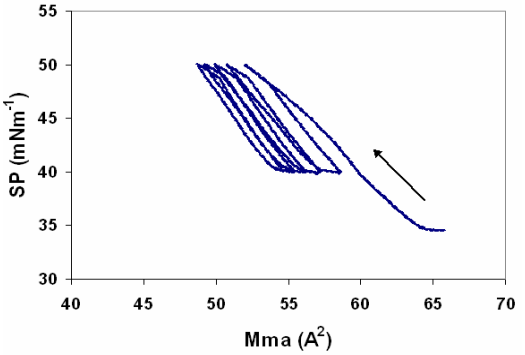

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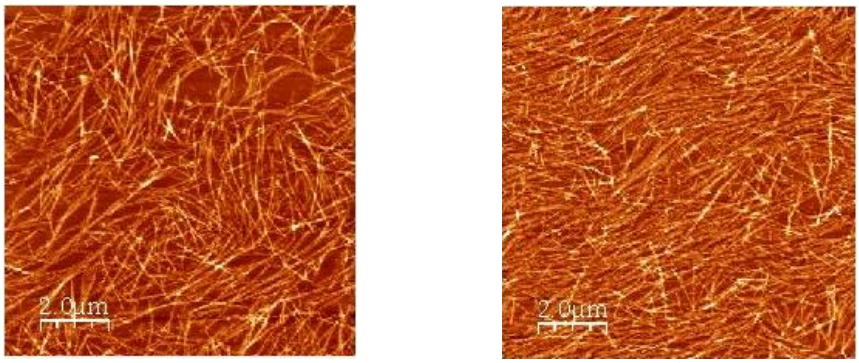
Fig. 4. SP–*Mma* isotherms of the DODA–CNs bilayer on water-air interface at ambient temperature. The DODA–CNs bilayer was compressed in sinusoidal compression-expansion conditions. The sinusoidal compression-expansion velocity of movable barrier was 2 *mm*/*s*.

On Fig. 5 are presented the AFM images of cellulose nanocristals monolayer prepared on silicon wafer surface by LB technique. The DODA–CNs bilayer was transfered at surface pressure equal to 50 *mN*/*m* in continued compression (a) and sinusoidal compression-expansion (b) conditions.



a b

Fig. 5. AFM images of cellulose whiskers monolayers on the silicon wafer surfaces obtained by LB transfer realized in continued compression (a) and sinusoidal compression-expansion conditions (b).

From the images presented in Fig. 5 we see that the cellulose monolayer is more compacted in the case of Langmuir transfer realized in sinusoidal compression-expansion condition.

Conclusion. The shape of the SP–*Mma* ($Å^2$) isotherms of DODA–CNs bilayer mostly depends on the conditions of compression of the bilayer. In the case of the sinusoidal compression-expansion of the bilayer the surface area occupied par adsorbed molecules (nanoparticles) decreases, while the surface pressure remains constant. This result can be explained by increases of the density of CNs in the DODA–CNs bilayer. The structure of the cellulose monolayer prepared by Langmuir–Blodgett method deeply depends on the DODA–CNs bilayer compression conditions before the transfer. In the case of sinusoidal compression-expansion condition the cellulose monolayer is more compacted.

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