

Polysaccharides as a template for silicate generated by sol–gel processes

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Abstract

The polysaccharides, as established previously (Yu.A. Shchipunov, *J. Colloid Interface Sci.* 268 (2003) 68; Yu.A. Shchipunov, T.Yu. Karpenko, *Langmuir* 20 (2004) 3882), can manipulate the formation of hybrid silica nanocomposites by sol–gel processes. Here atomic force microscopy was applied to show whether carbohydrate macromolecules serve as a template for silicate. Mica was used as a substrate to adsorb polysaccharide. It was found that its surface is not neutral to the sol–gel processes, providing the silica precipitation. To hinder it, the mica was protected by a monomolecular film of arachidic acid with the help of a Langmuir–Blodgett technique. Hydrophobically modified cationic hydroxyethylcellulose was adsorbed from a diluted aqueous solution. It was demonstrated that the carbohydrate macromolecules located on the hydrophobic surface did promote silica precipitation, serving as a template.

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1. Introduction

A new type of hybrid polysaccharide–silica materials was fabricated by sol–gel processing when a novel precursor, tetrakis-(2-hydroxyethyl) orthosilicate (THEOS), was applied in [1–3]. This was possible to carry out owing to the advantage of THEOS over the currently used tetramethoxy- and tetraethoxysilane. This precursor is completely water-soluble, which favored its compatibility with biopolymers. Furthermore, evolved ethylene glycol instead of alcohols in the course of THEOS hydrolysis is also favorable for biocompatibility [4]. In turn the polysaccharides influenced the sol–gel processes and manipulated them. First of all, a strong accelerating effect was established, which is caused, as suggested in [1,3], by catalysis of the processing. Furthermore, the polysaccharides regulated the structure of hybrid materials. The structure-driven effect was related to the precipita-

tion of silica on carbohydrate macromolecules. This resulted in the formation of a silica shell that looked like a fibril. The fibrils thus formed were self-organized into a three-dimensional network. Its density depended on the polysaccharide type and concentration as well as on the charge density of carbohydrate macromolecules [3]. The processing catalysis and silica templating were explained by the formation of hydrogen bonds with hydroxyl groups of polysaccharides [3].

These experimental observations are of importance from the point of view that the regulating role of biopolymers in silica synthesis in living nature has been discussed in many publications (see, for example, reviews in [5–7]). It is believed that glycoproteins, proteoglycans, and polysaccharides are the main nucleating and templating agents in living cells [6,8]. The catalytic and templating effects of proteins were confirmed in vitro in Refs. [9–11]. The first experimental results concerning polysaccharides were described in Refs. [1–3]. They led to the inference that silica nucleated on carbohydrate macromolecules, generating a shell around them. This means that the polysaccharides really provide the regulating role in the living cells, controlling the biominer-

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alization processes. A direct confirmation of silica precipitation on carbohydrate macromolecules has not been presented yet.

The aim of this article was to examine if the silica generated by the sol–gel processes does actually precipitate on carbohydrate macromolecules. To reach the stated aim, atomic force microscopy (AFM) was applied.

2. Materials and methods

2.1. Materials

The silica precursor tetrakis(2-hydroxyethyl) orthosilicate (THEOS) was synthesized from tetraethoxysilane (ABCOR, Germany) as detailed in [12]. Arachidic acid was purchased from Wako Pure Chemical Industries Ltd. cationic hydroxyethylcellulose (cat-HEC) and hydrophobically modified derivative (HM-cat-HEC) were obtained from Hoechst (Germany). They both contained the cationic groups presented as a glycidyl trimethylammonium chloride ($-\text{CH}_2-\text{CHOH}-\text{CH}_2-\text{N}^+(\text{CH}_3)_3\text{Cl}^-$) and HM-cat-HEC, as well as the hydrocarbon radicals ($-\text{C}_{12}\text{H}_{23}$). The structural formulas are seen in Fig. 1. The molecular weight of both polysaccharides was 950.000 kDa; cat-HEC contained 0.16 charged groups per monomer residue, HM-cat-HEC 0.36 cationic groups and 0.014 hydrocarbon chains per monomer residue. The polysaccharides were used as received. Deionized water was prepared with the help of Milli-Q (Millipore Co.).

2.2. Hydrogel preparation

The sol–gel processes were performed in accordance with the procedure developed in [1,2]. An aqueous solution of

cat-HEC was initially prepared and left for a day. Once an appropriate weighed amount of the precursor was added into the polysaccharide solution, it was thoroughly stirred and set aside at ambient temperature at least for 1 week. The gel could settle within a few minutes and, therefore, a week was an optimal period of time to complete the hydrogel formation.

2.3. Sample preparation for the AFM observations

Freshly cleaved muscovite mica was used as the solid support for all samples. Its surface was covered by an arachidic acid monolayer with the help of the Langmuir–Blodgett technique. The covered mica surface was gently rinsed with water and then left in the desiccator under high vacuum for a night. To adsorb HM-cat-HEC on the acid monolayer, the prepared mica was immersed into an aqueous solution containing 10^{-4} wt% of polysaccharide for 5 min. When it was taken out of the solution, the mica surface was gently rinsed with water, blown with nitrogen, and placed into the desiccator under high vacuum.

2.4. Langmuir–Blodgett (LB) technique

A NL-LB140S-MWC trough from the Nippon Laser and Electronics Lab (Nagoya, Japan) was used to transfer a monolayer to a mica surface. The rectangle trough was filled with water at a temperature of 25°C . The pressure/area isotherms were recorded with the help of a Wilhelmy quartz plate. The volume of arachidic acid solution in chloroform needed to form a monolayer was spread on the water surface by means of a Hamilton microsyringe. The attainment of an equilibrium state after the evaporation of the organic solvent was determined by measuring the surface pressure. The prepared monolayer was transferred by dipping a freshly

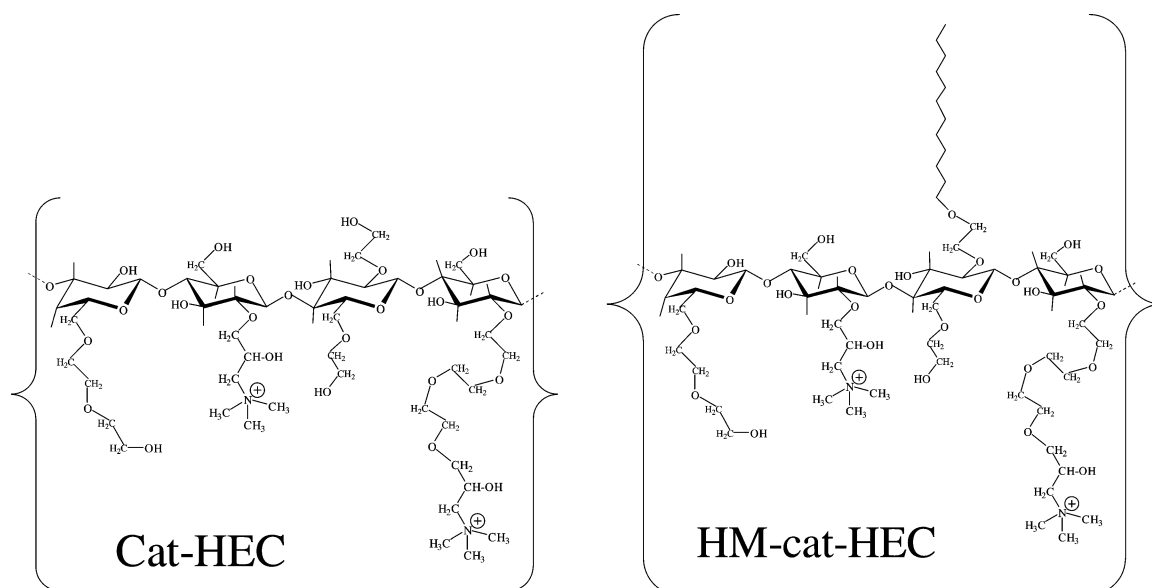


Fig. 1. Structural formulas of examined polysaccharides.

cleaved mica sheet into the trough at a rate of ca. 1 cm/min. The transfer procedure was performed at a constant surface pressure of 30 mN/m to achieve the formation of a uniformly packed monolayer over the entire mica surface.

2.5. Atomic force microscopy (AFM)

AFM images were taken in air at ambient temperature with the help of a Nanoscope III (Digital Instruments, Santa Barbara, CA). It was equipped with a microfabricated silicone cantilever. The tapping mode was best suited to visualize the transferred monolayer, adsorbed polysaccharide, and precipitated silicate on the mica surface.

2.6. Scanning electron microscopy (SEM)

Micrographs were taken with a FE-SEM Leo 1530 electron microscope. A polysaccharide–silica hydrogel was frozen by the liquid nitrogen and then it was cleared to have a freshly prepared surface for observation. This was covered by an evaporated platinum layer.

3. Results

3.1. Sol–gel processes in the solution bulk

The sol–gel processes were triggered when THEOS was mixed with an aqueous solution of cat-HEC. It was unnecessary to add a catalyst or increase the temperature. The processes took place at the neutral pH of aqueous solution and even reduced temperature, that is, under conditions where the sol–gel transition is not observed in the case of common precursors (see, e.g., [13,14]). What is also important is that the polysaccharide sharply accelerated the kinetics of sol–gel processes. Jellification could be observed within a few minutes after mixing with the precursor in an aqueous solution, whereas it happened after at least a few hours when acid was used to catalyze the processes. The accelerating effect was accounted for by the catalytic effect of biopolymers on the sol–gel processes [1,3].

A fabricated jelled material may be assigned to hydrogels because the main solvent is water. Ethylene glycol is also generated in the course of precursor hydrolysis. Its amount depended on the THEOS concentration introduced into the reaction mixture. It usually did not exceed 10 wt%.

The jellification of the aqueous solution due to the sol–gel process resulted in the formation of a monolithic hydrogel. The maturation processes occurring with time could cause shrinkage of the gel phase, which was accompanied by slow solution separation. These processes, called syneresis, were usually observed in the case of uncharged polysaccharides [3]. The amount of separated solution did not exceed 20 wt%. When cat-HEC was used, syneresis did not take place. This behavior is typical of systems containing cationic polysaccharides [3]. A difference was found when THEOS

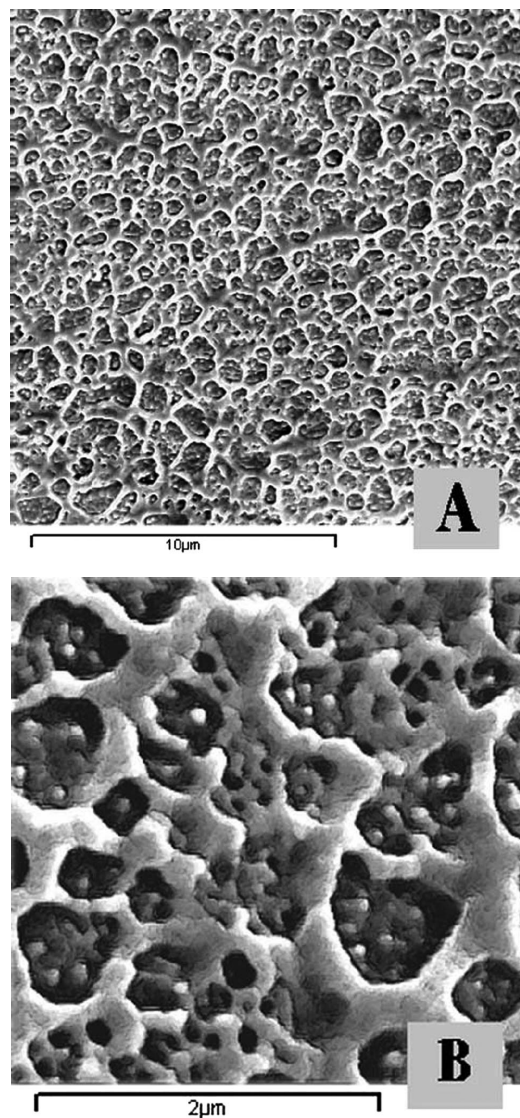


Fig. 2. SEM micrographs taken with smaller (A) and larger (B) magnifications of hydrogel synthesized in an aqueous solution of cat-HEC. The concentration of polysaccharide was 1.8 wt% and THEOS 10 wt%.

was introduced into a solution with HM-cat-HEC. This resulted in precipitation of the reaction products soon after the mixing. The main difference of HM-cat-HEC from cat-HEC is caused by the presence of hydrocarbon chains. Therefore, it may be inferred that its increased hydrophobicity is responsible for the precipitating effect.

The internal structure of hydrogel synthesized in solution with cat-HEC is obvious from Fig. 2. There are two SEM pictures taken with various magnifications. The hydrogel was prepared by the admixing of 10 wt% of THEOS into an aqueous solution containing cat-HEC (1.8 wt% after the mixing). As one can see in Fig. 2A, the main morphological element of gel structure is a network from crossed filaments. They are made up of silicate (Fig. 2B). The silica network formation provided the jellification of the solution. The cat-HEC falls into the group of polysaccharides that are incapable of forming hydrogel [15].

The observed structure of hybrid polysaccharide-silica materials in Fig. 2 raises a question about the arrangement of macromolecules and silicate in them. We may conceive two opportunities that are determined whether the organic and inorganic components interact with each other or not.

- (i) *Noninteracting components.* This means that polysaccharide is neutral to the products of silane hydrolysis and silicate generated in the course of sol–gel processes. The carbohydrate macromolecules could be entrapped into the silica matrix, as happens, for example, where enzymes are immobilized by means of the sol–gel technique [16]. Furthermore, polysaccharides and silicate can form interpenetrating networks in the jelled material, as suggested by Gill and Ballesteros [4,17].
- (ii) *Interacting components.* This occasion was considered in [1,3]. The suggestion was that the polysaccharides form hydrogen bonds with silanols produced in the course of the precursor hydrolysis. The interactions provide the silica nucleation on carbohydrate macromolecules that serve as a template for silicate.

To decide which mechanism is valid, we have applied AFM. As the substrate, mica was used. Our intention was to adsorb polysaccharide macromolecules initially on the substrate surface and then treat it with a solution of the precursor. If the silicate had interacted and precipitated on carbohydrate macromolecules, it could easily be ascertained on the condition that the mica is inert to the silicate generated in situ.

3.2. Sol–gel processes near the mica surface

Our test experiment demonstrated that the mica could not be referred to materials the surface of which is neutral to the products of sol–gel processes. Fig. 3 presents an image of a mica surface placed in an aqueous solution with 10 wt% of THEOS for 5 min. This was a freshly prepared solution in which a catalyst to trigger the sol–gel processes was not added. However, one may see a silica precipitate on the mica surface. It is worth mentioning that the solution itself was unchanged; that is, the sol–gel transition did not take place in the bulk. The silicate was generated only in the close vicinity of the mica surface. This might be explained by the presence of hydroxyl groups that can exact a catalytic effect on the sol–gel transition.

Our following step was to protect the mica surface by covering it by a monolayer of arachidic acid. This was done with the help of the LB technique. The transferred acid molecules contacted the substrate surface by their carboxyl group, whereas the hydrocarbon chain was directed outward. This made the mica surface hydrophobic. Since the silicate does not interact and precipitate on hydrocarbons, this should exclude the passing of sol–gel processes near the substrate surface.

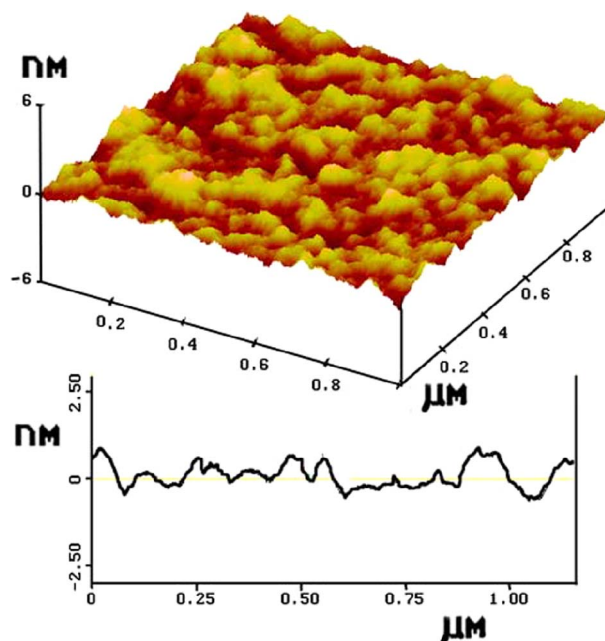


Fig. 3. AFM image (top) of the mica surface with silicate precipitated after 5 min of exposure in 10 wt% aqueous solution of THEOS and cross-sectional profile (bottom) traced over the surface.

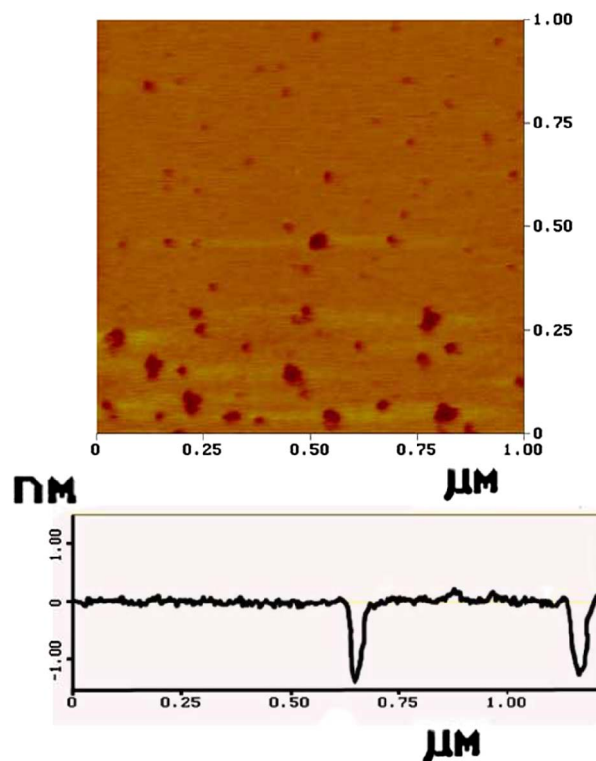


Fig. 4. AFM image (top) of the mica surface covered by a monomolecular arachidic acid film and cross-sectional profile (bottom) traced over it.

An AFM image of the mica surface covered by a monolayer of arachidic acid is given in Fig. 4. One may see dark spots. Their depth, as follows from the given cross-sectional profile, corresponds to the acid monolayer thickness. This

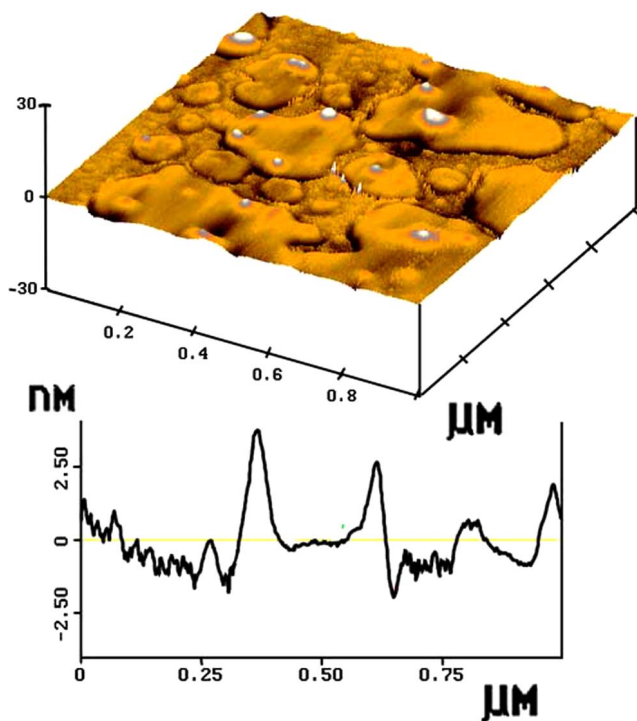


Fig. 5. AFM image (top) of the mica surface covered by an acid film after 5 min of exposure in 10 wt% aqueous solution of THEOS. Cross-sectional profile traced over the surface is presented at the bottom.

means that the spots present gaps in the monolayer. They are rather numerous. It was impossible to obtain a continuous coating and so a part of the mica surface was not protected by the acid monolayer. Therefore, once the mica with the monolayer had been immersed in the precursor solution for 5 min, we found parts of the surface covered by precipitated silicate. This is obvious from the AFM image in Fig. 5.

It should be pointed out that there are notable differences in the textures of silica precipitates shown in Figs. 3 and 5. In the latter case, it looks like isolated fusions in which one can resolve peaks remembering alps. They are well seen in the cross-sectional profile represented in the bottom. It may be suggested that the peaks are in the place of defects existing in the monolayer. They were initial centers for silica nucleation. When these polar gaps in the monolayer had been filled by a sufficient amount of silicate, it started spreading along the hydrophobic surface. This is the cause for the precipitate in Fig. 5 to look like fusing.

The mica covered first by an acid monolayer was used to adsorb carbohydrate macromolecules. We found from a preliminary experiment that hydrophobically modified cat-HEC was best suited to be adsorbed strongly enough on the hydrophobic surface. It is believed that the hydrocarbon chains of polysaccharide could penetrate into the monolayer, serving as anchors for the rest of a macromolecule. Cat-HEC was less strongly adsorbed on the hydrophobic surface. Therefore, experiments were performed with HM-cat-HEC.

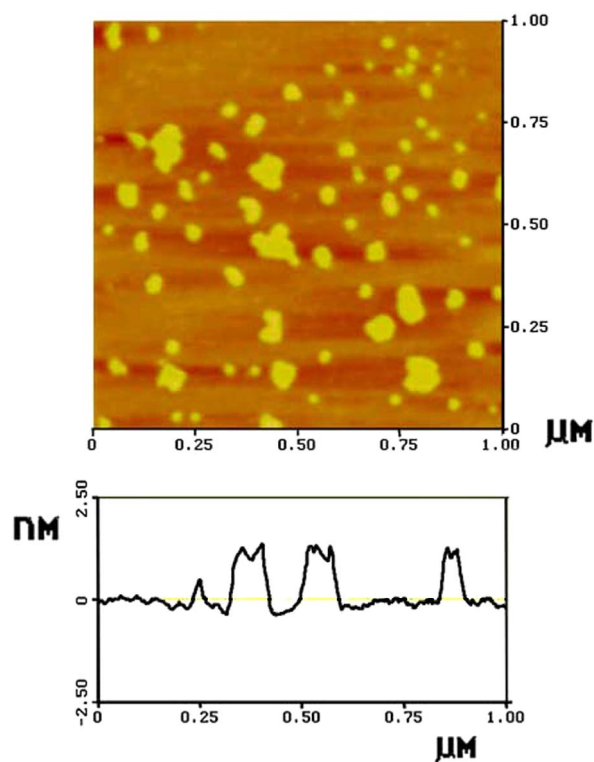


Fig. 6. AFM image (top) of the mica surface covered by a monomolecular arachidic acid film with adsorbed HM-cat-HEC and cross-sectional profile (bottom) traced over it.

An AFM image of a sample prepared by HM-cat-HEC adsorption on the mica initially covered by an arachidic acid monolayer is presented in Fig. 6. There are isolated light-colored spots. They represent coiled macromolecules of polysaccharide. It seems that there are both isolated and aggregated carbohydrate macromolecules.

It is pertinent to note that gaps usually were not observed in the monolayer with adsorbed HM-cat-HEC. Their absence may be attributable to the preferential adsorption of carbohydrate macromolecules at these points. The reason is that the gaps provide an access of macromolecules to the polar mica surface uncovered by acid molecules, where the polysaccharide is adsorbed more strongly than at the hydrophobic surface of monolayer. The presence of polar centers formed by carbohydrate macromolecules gives hope that no others will trigger the nucleation of silicate.

The experimental results are shown in Fig. 7. There are AFM images of surfaces of two samples that were treated in a precursor solution for different time. Fig. 7A demonstrates a result of treatment over 5 s. One may find polysaccharide macromolecules, as in Fig. 6, but their height is increased (see the cross-sectional profile below). This conclusion follows from the comparison with a horizontal dotted line marking off the maximum height of adsorbed macromolecules found before the treatment (Fig. 6). The increased molecular dimension implies the appearance of silica precipitate that was generated during the short treatment in the precursor solution.

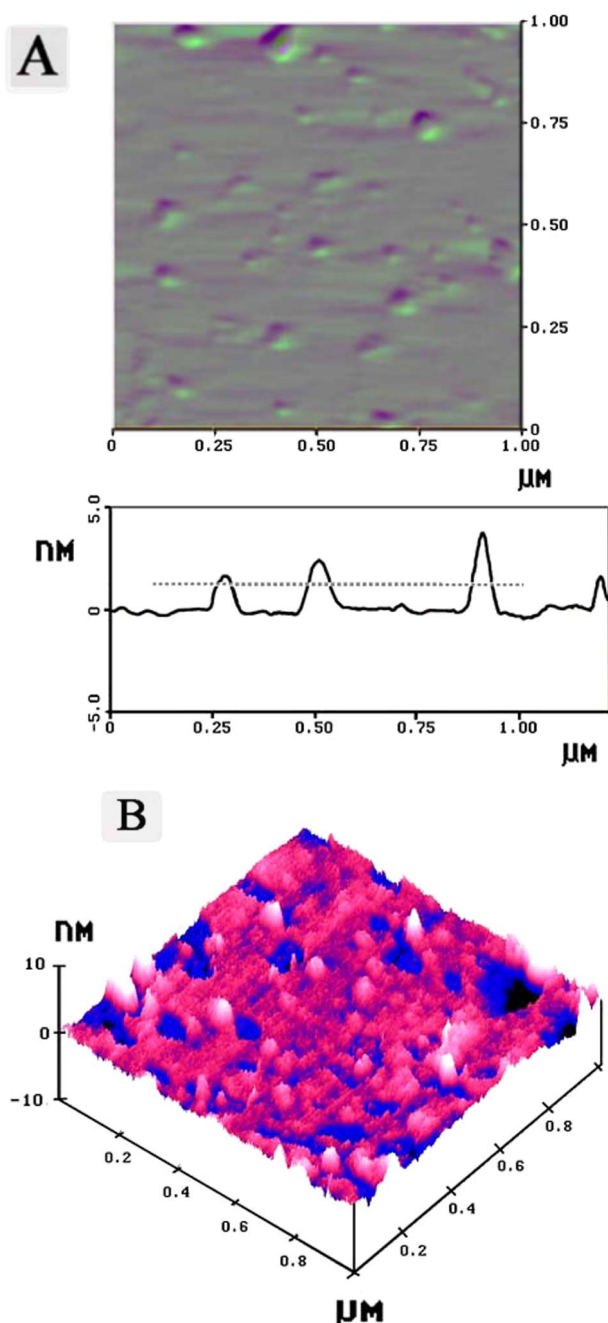


Fig. 7. AFM images of the mica surface covered by a monomolecular arachidic acid film with adsorbed HM-cat-HEC after 5 s (A) and 60 s (B) of an exposure in 10 wt% aqueous solution of THEOS. A cross-sectional profile traced over the image in (A) is presented at the bottom. The dotted line demonstrates the maximum height of carbohydrate macromolecules in Fig. 6.

A result of the prolonged treatment (60 s) is seen in Fig. 7B. It is impossible to distinguish isolated carbohydrate macromolecules in the AFM image. They are coated by silica precipitate. It seems plausible that silicate, when covering a macromolecule, started spreading over the surface and merging with neighboring silica precipitates. As a result, a structure resembling a network was formed. In our opinion, the AFM images in Figs. 7A and 7B point to the

fact that the silicate was really nucleated and then precipitated on the polysaccharide macromolecules. This means that they act as a template.

4. Discussion

The experimental systems described above are summarized as schematic drawings in Fig. 8. The upper row portrays the successive steps leading to the experimental system, in which the hydrophobic surface incorporates hydrophilic centers formed by macromolecules of polysaccharide. To achieve this a monolayer of fatty acid oriented to the mica surface by the functional groups was first placed by the LB technique and then the hydrophobically modified cat-HEC was adsorbed onto it from aqueous solution. The carbohydrate macromolecules filled the gaps in the acid monolayer, while the hydrocarbon chains additionally strengthen their linkage with the surface through penetration into the monolayer. Therefore, the adsorbed polysaccharide sets up sole centers for further silica nucleation.

The lower row in Fig. 8 serves to represent the same samples after treatment in the precursor solution. The silicate precipitated on various surfaces differed notably in its morphology. This is obvious from comparison of AFM images in Figs. 3, 5, and 7. The precipitation was not accompanied by the jellification of the bulk of the solution. Silica was generated by means of the sol-gel processes only near the mica surface. This allows suggesting that both the bare mica and polysaccharide promote sol-gel processing.

It is worth mentioning that there is a rather good resemblance in the morphology of silicate in Figs. 2 and 7B. The former presents the SEM picture of a hydrogel, i.e., the morphology of a material formed in three-dimensional space; the latter, the AFM image of a surface where the processes were located in a two-dimensional region. The observed resemblance should be taken as for the suggestion offered in [1,3] that the network structure formed in the hydrogel was a result of silica precipitation on carbohydrate macromolecules.

It should be pointed out that the results presented in Figs. 2 and 7B were obtained with cat-HEC and HM-cat-HEC, respectively. The polysaccharides differed, as noted above, in their behavior in the solutions. The latter did not provide their jellification after the addition of THEOS, which was related to its increased hydrophobicity. When HM-cat-HEC was adsorbed on the acid monolayer, the hydrocarbon chains integrated into the monolayer were covered by the hydrophilic part of the carbohydrate macromolecules, which was pointed toward the aqueous solution. Therefore, one may expect that the adsorbed HM-cat-HEC and cat-HEC should not differ from each other in the influence on the sol-gel processes proceeding in the adjacent aqueous layers. This offers an explanation for why the images in Figs. 2 and 7B may be correlated.

Thus, the experimental data obtained by the AFM technique enabled us to demonstrate independently that the

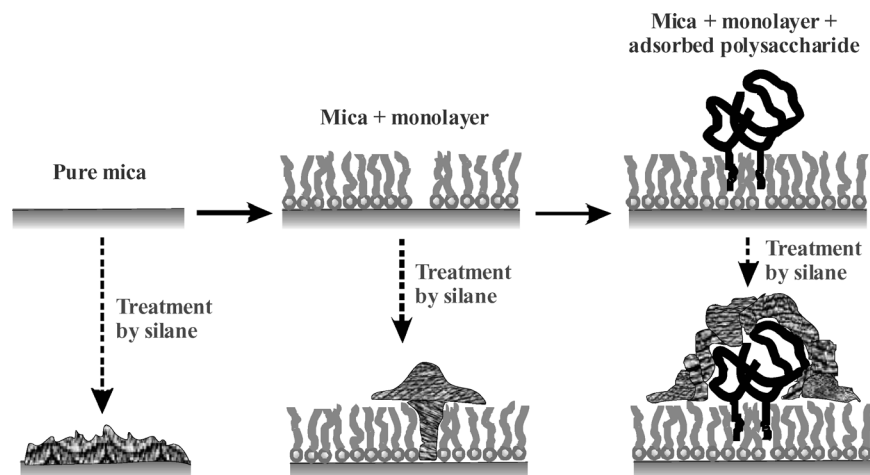


Fig. 8. A schematic representation of experimental steps for the coating of a mica surface by a LB monomolecular film of arachidic acid and the adsorption of HM-cat-HEC on it (upper row) as well as the subsequent silica precipitates on these surfaces, formed after an exposure in the THEOS solution (bottom row). Details are discussed in the text.

macromolecules of polysaccharides serve as the nucleating centers for the silanols generated by the sol–gel processes in the THEOS solution. This creates a base for the subsequent precipitation of silicate and formation of inorganic shell around a carbohydrate macromolecule. The neighboring silica shells can merge with each other owing to further silica precipitation, which gives cross-linked filaments as seen in Figs. 2 and 7B.

Acknowledgments

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