

pH-Induced Morphological Changes of Carboxymethyl Chitosan Self Assembled Monolayer Films

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ABSTRACT: Self assembled monolayer (SAM) films of carboxymethyl chitosan (CM-CHI) were prepared by immersing mica in CM-CHI aqueous solution at pH = 1.7. The obtained films were further rinsed in baths of water at different pH. The pH induced morphological change of carboximethyl chitosan SAM films was investigated by atomic force microscope using tapping mode. The results showed that the morphology of the films was remarkably affected by the pH of rinse bath. Three obvious morphological changes were discussed and explained by the com-

petition between electrostatic interaction and hydrogen bond. We draw attention to the "Crater" structures in the films and find that the formation and evolution of "Crater" structures have a close relationship with the pH of rinse bath. The formation mechanism of the other two special structures was also discussed. © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 122: 993–998, 2011

Key words: self assembly; electrostatic; carboxymethyl chitosan; polyampholyte; atomic force microscope

INTRODUCTION

The first study of colloid particles adsorbed on the opposite charged substrate was reported by Iler¹ in 1966, but it did not attract much interest.² It was the work reported in 1991 by Decher,³ in which multilayer films were prepared by alternative adsorption of polyelectrolyte and organic molecules, makes a new period of layer-by-layer (LBL) technique. In the last decade, a lot of studies^{3–8} have been done to investigate the self assembly mechanism and the structure controllable of LBL self assembled multilayer films. These studies evidently extended the range of materials used to LBL self assembly since the studies had been more interested in polyelectrolytes first. Now, the materials can be used in LBL self assembly have been expanded from organic to inorganic materials, from polyelectrolyte to biomacromolecule, even some bacteria.^{9–12} Compared with the technique of LB and self assembly based on chemical adsorption, LBL does not need any complex facilities or serious preparation environment. Furthermore, the structure, thickness and functions of the LBL multilayer films are controllable easily, so that LBL can be used in the modification of the surface of electrodes, or even the irregular substrates.¹³

Weak polyelectrolytes can not be totally ionized in solution, which makes it possible to control the surface structure and function of the self assembled multilayer films by adjusting the pH of solution. A lot of LBL self assembly of weak polyelectrolytes studies^{14–19} have focused on the electrostatic interaction of $-\text{COOH}$ and $-\text{NH}_2$. Some biocompatibility polyelectrolytes, such as chitosan and alginate, have been used to prepare microcapsules^{20,21} and multilayer for controllable release and antibody immobilization⁸ or even enzyme catalysis.²² It was noticed that the electrostatic reaction is not strong enough to counteract the effect of the pH changes of environment, so LBL self assembled multilayer structure has to be frozen for avoiding its dissolving by some way.¹⁸ Amidation by heating is a good way to fix the LBL self assembled multilayer films based on $-\text{COOH}$ and $-\text{NH}_2$. However, what we are discussing is that the LBL self assembled multilayer films consists of two different kinds of polyelectrolyte. Could LBL self assembly been made up of only one kind of polyampholyte?

Carboxymethyl chitosan (CM-CHI), as a derivative of chitosan, usually exists in the form of sodium salt, which makes it as a polyanion in aqueous solution. It is believed that the addition of HCl can convert $-\text{COONa}$ of polysaccharides to $-\text{COOH}$.²³ Then, the polysaccharides can not be used for LBL self assembly for it is neutral. However, the excess HCl will provide enough H^+ for the protonation of $-\text{NH}_2$ in the C_2 site of CM-CHI. The most important is that it can obtained nanoporous thin film by heating the CM-CHI self assembled multilayer without

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further phase separation.⁷ Because the adjacent layers of CM-CHI always have one kind of groups that could but have not been ionized or protonated according to the respective self assembly conditions. Either heating to form amide ($-\text{NH}-\text{CO}-$) or chemical cross-linking with glutaraldehyde,²⁴ the structure of multilayer will not be disarranged markedly.

Relatively few publications concern studies on the change in the surface topography of the self assembled monolayer (SAM), and factors which affect the surface properties. It is important to learn the surface structures and properties of the monolayer to build the multilayer films.

The research material presented in this article in the form of complex investigations constitutes an essential extension of the knowledge within this scope.

EXPERIMENTAL SECTION

Materials

CM-CHI ($M_w = 200,000$) was purchased from Bomei biochemical without any further purification. All the inorganic agents used are AR grade. The concentration of polyelectrolyte solution was 1 mg/mL and the pH was adjusted with HCl and NaOH.

Sample preparation

All self assembly processes were carried out on fresh mica (8 mm \times 8 mm). The pH of CM-CHI aqueous solution as polycation in this work is 1.7. All monolayer samples were obtained by immersing the substrate, mica, in CM-CHI aqueous solution with a 1.7 pH value. After the 10 min immersion in CM-CHI acidic aqueous solution, it was moved to three successive rinse baths with light agitation for 20 s, 20 s, and 10 s, respectively. Then, all samples were placed in vacuum oven for 20 min at 30°C.

Characterization

The observation on the surface of CM-CHI SAM film was focused on the morphology changes during its growth process. The morphology characterizations were carried out by atomic force microscope (AFM) of SHIMADZU SPM 9500-J3 of Japan at a temperature of $25 \pm 1^\circ\text{C}$ by tapping mode. The silicon cantilevers used in this work were obtained from ETALON of Russia. The spring constant, curvature radius, and resonant frequency used in this work were 5.8N/m, 10 nm, and 165 kHz, respectively. The scan rate was 0.5 Hz with a scanning density 512 lines/frame. Before the morphology characterization, all the SAMs had been dried 3 h in

vacuum oven at 30°C. All images listed in this paper are the height modes.

RESULTS AND DISCUSSION

Effluences of the rinse bath pH on the morphology of self assembled monolayers

CM-CHI exists in the form of polycation in the low pH ($\leq 2.1 \pm 0.1$) aqueous solution. Below this pH value, the changes of pH value of CM-CHI aqueous solution result in only the changes of linear charge density. And that will induce the corresponding changes of adsorption conformation of CM-CHI molecules. However, after the pH excess 2.1, it is not only means the changes of linear charge density but also the reversal of a positive or negative electric property. The later phenomenon has been confirmed that when pH value near 6.8 ± 0.1 with the adding of HCl the color of CM-CHI aqueous solution (original pH is 8.5) turned to milkiness. Additional HCl even induces the formation of floccules. Nevertheless the further acidification re-dissolved the floccules and the solution became clear gradually, and totally transparent again at the pH about 2.1. It is necessary to choose an appropriate pH value to maintain the original morphology of SAMs according to the effluence of the pH value of rinse bath discussed previously.

Different rinse bath resulted in obvious difference in morphology of SAMs can be seen from Figure 1. The morphology changes mainly show as the following styles: (1) sharp increase of the molecular aggregates [especially in Fig. 1(D)]; (2) the changes of the compaction of SAMs [compare with Fig. 1(B,C)]; (3) the formation of special structure which likes as "crater." Comparing the Figure 1(A) with Figure 1(D), the densities of the molecular aggregates in all the first three photos were approximate equivalent though the pH value of rinse baths higher one by one. This result showed that the formation of the molecular aggregates were independent on the electrostatic interaction of intro/inter molecules as a reversal of electric property of CM-CHI molecules took place at higher pH. A comparison of Figure 1(A) with Figure 1(D) showed that the formation of molecular aggregates could be described as following. Below the level of pH 3.4, the protonation degree of $-\text{NH}_2$ is enough to unfold the coil to the extended conformation by the repulsion interaction of different units of intramolecules. Most molecules tend to adsorb on the substrate in the profile of "flat" or "loop".²⁵ However, some accidental aggregation of partially adsorbed or not adsorbed molecules exposing out of SAMs was unavoidable. The primary driving force of the aggregation may be the hydrogen bonds of inter/intro-molecules. Then the

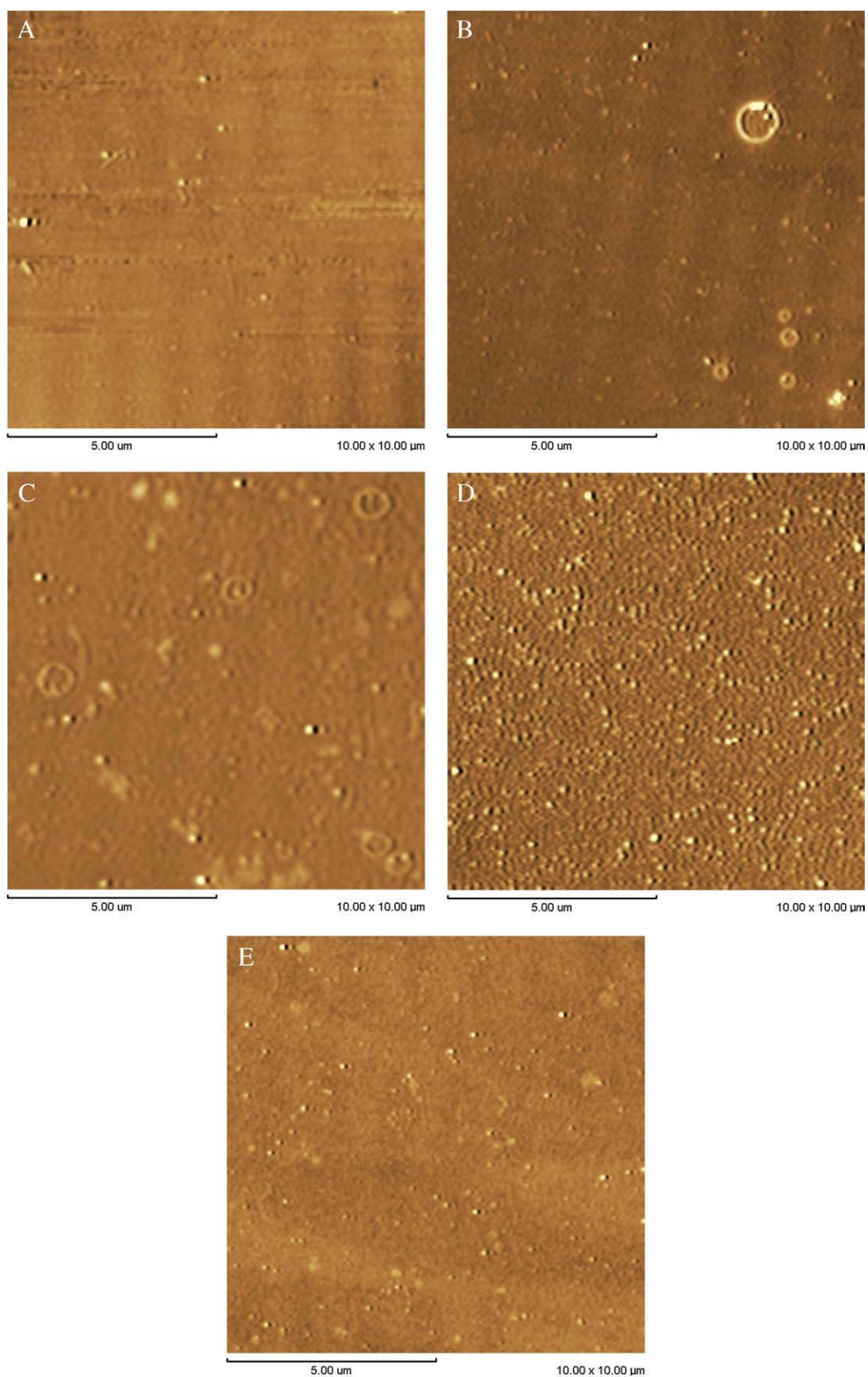


Figure 1 The morphology of SAMs. (A) Self assembled at pH 1.7 aqueous solution and rinsed at the pH 1.7. (B) Self assembled at pH 1.7 aqueous solution and rinsed at the pH 2.3. (C) Self assembled at pH 1.7 aqueous solution and rinsed at the pH 3.2. (D) Self assembled at pH 1.7 aqueous solution and rinsed at the pH 4.3. (E) Self assembled at pH 1.7 aqueous solution and rinsed at the pH 1.7 before the 4.3 rinse bath. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com].

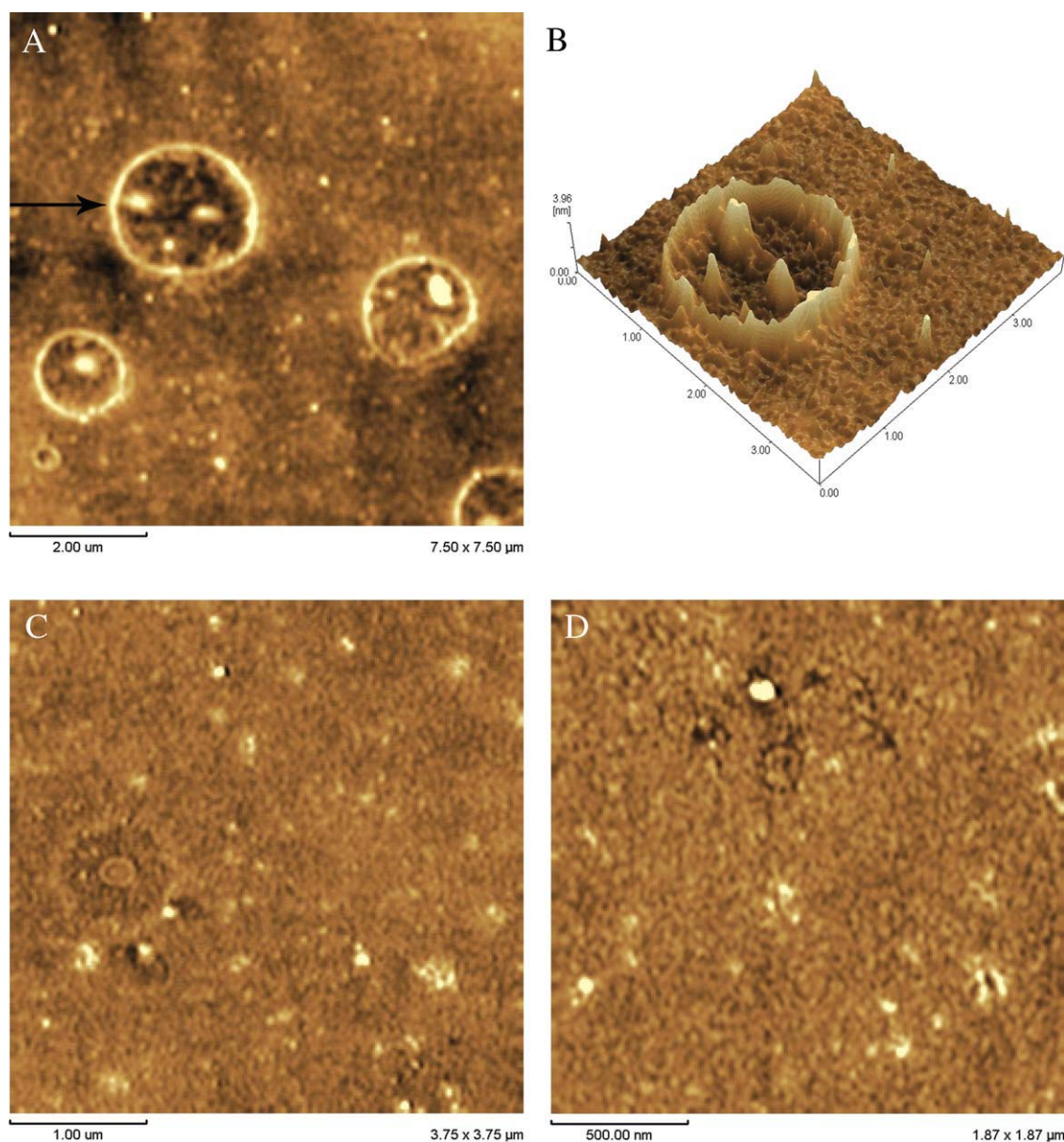


Figure 2 The morphology of SAMs. (A) Self assembled at pH 1.7 aqueous solution and rinsed at the pH 3.1. (B) Self assembled at pH 1.7 aqueous solution and rinsed at the pH 3.1, enlarged picture of the arrow area in Figure 2(A). (C) Self assembled at pH 1.7 aqueous solutions and rinsed at the pH 3.1, and immersion in 7.0 aqueous solutions for 24 h. (D) Self assembled at pH 1.7 aqueous solutions and rinsed at the pH 3.1, and immersion in 7.0 aqueous solutions for 24 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com].

further heating processing froze the aggregates as irregular spherical particles. Higher rinse bath pH reduced the degree of protonation of CM-CHI molecules, which resulted in the probability of aggregation increase obviously. The number of molecular aggregates increased significantly as shown in Figure 1(D). According on the discussion above, the compaction changes of SAMs can be considered as the consequence of the competition of hydrogen bond reaction and electrostatic repulsion interaction of the polyelectrolyte molecules. Comparing with Figure 1(A,E), the further rinse bath of 4.3 pH decreased the degree of protonation of molecules adsorbed on mica,

of course not too weak to entirely desorbs from mica, which provides the chance of aggregation for the adjacent molecules. Thus, the apparent morphology of Figure 1(E) was more compact than that of Figure 1(A). The same result can be seen in Figure 1(C). But there were some differences between Figure 1(C,E) since the compaction changes of the later should be bases on the induced phase separation of the frozen conformation of molecules adsorbed on the substrate.⁶ It may be the reason why the compaction in the Figure 1(C,E) was so different. The formation and evolution of the special structure "crater" will be discussed thoroughly in the next section.

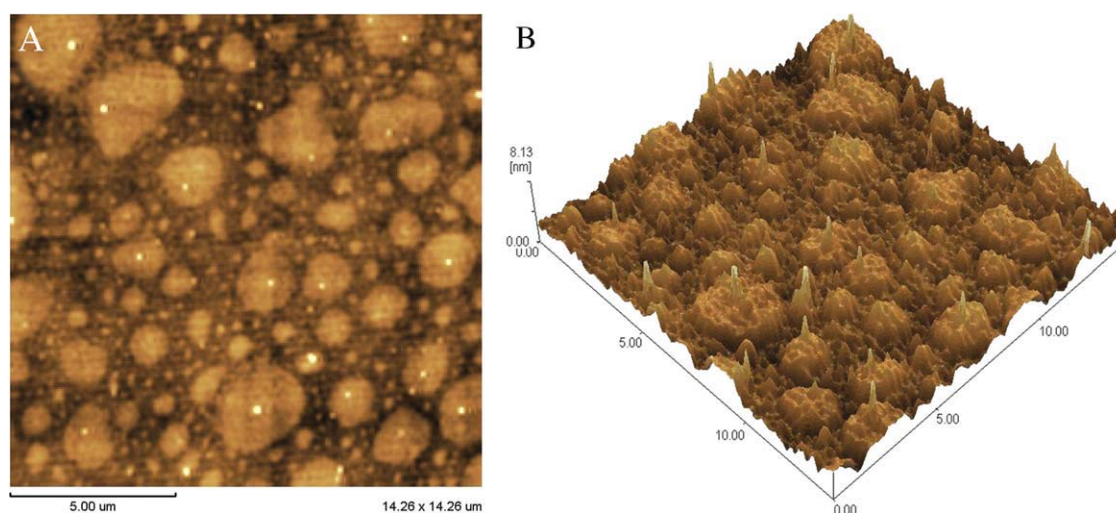


Figure 3 The “protruding flat” morphology of SAM obtained by same pH value of self assembly and rinse bath of water (Self assembled at pH 0.37 aqueous solution and rinsed at the pH 0.37). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com].

The formation and evolution of “crater” structure

To investigate the formation and evolution of the special structure “crater,” a series of samples were prepared in the same pH value of polyelectrolyte aqueous solution at different pH value of rinse bath. The characterization of morphology showed that the “crater” structure can be captured when the pH of rinse bath ranged from 2.2 to 3.4. Profile analysis showed the diameter of “crater” distributes from hundreds nanometer to several microns. Figure 2(A) showed the morphology of “crater” obtained self assembly at pH 1.7 and rinse bath at pH 3.1. Figure 2(B) was the corresponding 3D view of the “crater” structure emphasized by arrow in Figure 2(A). Profile analysis indicated that the protruding cylindrical edge was more than 3.8 nm above the base level.

Except three prominences, with different height and size, there was no evident difference in height between the interior and exterior of the “crater.” Moreover, the interior of the “crater” is obviously more sparse than that of the exterior [as seen in Fig. 2(A)]. Figure 2(C,D) were the morphology of the same sample after a 24 h immersion in aqueous solutions at pH value 7.0. Profile analysis showed that the average height of the interior and exterior of the “crater” was approximately in the same level after the further immersion process. And the surface of the whole sample tended to be sparse evidently after the further immersion process.

It is believed that the “crater” is the consequence of the polyelectrolyte molecules aggregated with hydrogen bonds interaction. The material source of

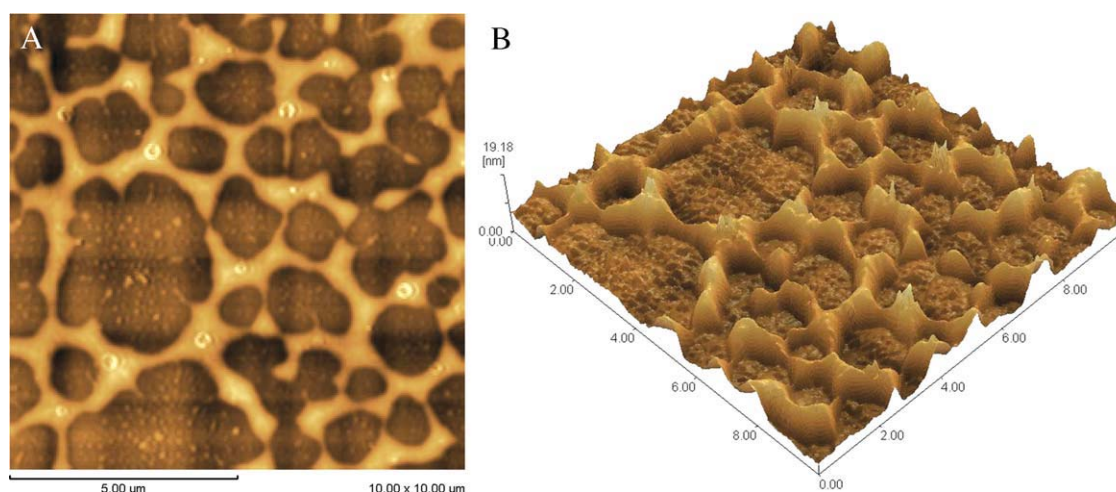


Figure 4 The “sunken flat” morphology of SAM obtained by different pH value of self assembly and rinse bath of water (Self assembled at pH 1.7 aqueous solution and rinsed at the pH 3.4). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com].

the "foundation" may be the molecules adsorbed on the substrate by electrostatic interaction, and the "wall" may be the molecules which should have been washed away after the rinse bath process. However, these molecules aggregated and developed the "foundation molecules" since the hydrogen bonds interaction was stronger than the electrostatic repulsion reaction in this situation. Another feasibility of its formation by phase separation or conformation transformation was neglected at first because the height of the interior and exterior of the crater was approximate. It means that there were not enough materials to construct the "wall." The further immersion in the aqueous solution with a pH value of 7.0, which was high enough to convert the electric property of the polyelectrolyte molecules, induces the "wall" dissolving with the increase of electrostatic repulsion of negative charges of different polyelectrolyte molecules segments. Most of all is that the SAM should be integral even in the aqueous solution except the obvious phase separation. And this will ensure the further adsorption of polyanions implement successfully.

Two "flat" morphologies captured in characterization

Another two special morphologies were obtained as seen in Figures 3 and 4, Figure B is the corresponding 3D view of Figure A). The sample shown in Figure 3 was obtained by self assembly and rinsed in the same pH value (0.4). Profile analysis indicated that the average height of the protruding flat was approximately 2.5 nm higher than that of base level, and the diameter ranges from hundreds nanometer to microns. Contrast with the former, the morphology of the sample shown in Figure 4, which was self assembled and rinsed in the pH value of 1.7 and 3.4, respectively, exist as the form of sunken flat. Profile analysis showed that the sunken height was lower than 7 nm, and the diameter was hundreds nanometer. The difference of those two structures in height was reasonable since the pH value of self assembly pH was different evidently. A lot of relevant studies have showed that the thickness of SAM films changed evidently with the linear charge density from several angstroms to nanometers.^{14,15} Lower pH resulted in a more extended conformation of CM-CHI molecules, and this induces a lower thickness of the SAM film (as seen in Fig. 3). When the pH value of the rinse bath of water was high enough to result in the equilibrium of repulsion reaction with hydrogen interaction of adjacent polyelectrolyte molecules, the aggregation of the adjacent molecules will be indispensable (as seen in Fig. 4).

CONCLUSIONS

The SAM prepared by the same self assembly pH and rinsed in baths of water at different pH. The pH induced morphological change of carboxymethyl chitosan SAM films was investigated by AFM using tapping mode. A kind of morphology structure likes as "crater" was captured. Both the formation of molecular aggregates and "crater" structure were explained by the electrostatic-hydrogen bond interaction model. The pH value of rinse bath water to induce the formation of special structure "crater" ranges from 2.2 to 3.4. After a 24 h immersion in aqueous solution at 7.0 pH, the "crater" tends to be dissolved. The phase separation rather than desorption of SAM in same condition provides the feasibility of further adsorption step of CM-CHI as polyanion.

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