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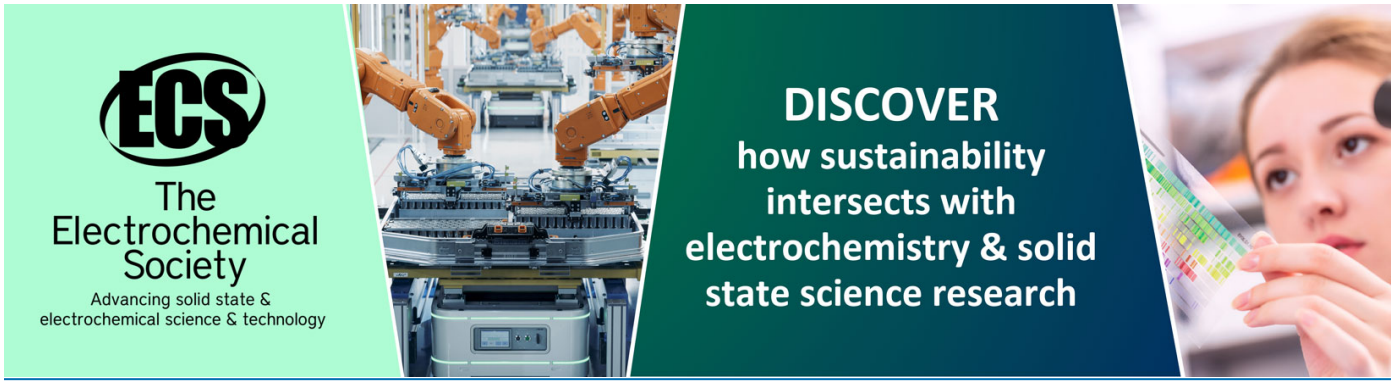


Study on self-assembly of novel hydrophilic silane containing PEG segments and its resistance to protein adsorption

M G Liu[1,*], R Zhao[1] and S Q Liu[2]

[1]Naval Logistics Academy, Tianjin 300450, China
[2]Hengshui University, Hebei 053000, China

[*]E-mail: 2935267lmg@163.com

Abstract. A new type of hydrophilic silane containing PEG (polyethylene glycol) segments was produced in this study. Membranes were produced through self-assembly. Hydrophilic silane was characterized by FTIR, ^{1}H-NMR; The chemical composition and surface morphology of the self-assembled membrane surface were characterized by AFM, CA , SEM; The material's resistance to protein adsorption was studied by UV-Vis and coomassie brilliant blue method [1]. The results showed that the novel hydrophilic silane containing PEG segments can be successfully assembled on silicon substrates; self-assembled membranes and blank silicon substrates in comparison, protein adsorption decreased from 274.8 μg/cm^2 to 145.7 μg/cm^2. This study has important reference significance for the preparation of anti-protein adsorption and bacterial adhension materials.

Keywords: Hydrophilic Silane; Self-assembly; Anti-protein Adsorption

1. Introduction

Polyethylene glycol molecular brushes are non-toxic, non-immunogenic, hydrophilicity and good biocompatibility, it can form a highly hydrophilic layer that suppresses the approach of bacteria, generates repellent penetrating force, and reduces the mobility of polymer segments. It can effectively prevent non-selective adsorption of proteins and reduce bacteria and bacteria microbial bonding [2-10].

Silicone materials are widely used in biomaterials due to a series of excellent characteristics such as physiological inertia and biocompatibility. However, the extremely hydrophobic surface of the silicone material makes it irreversibly adsorb a large amount when it comes in contact with blood plasma protein and cause adverse reactions such as blood coagulation. To this end, modifying the surface of the silicone material to improve its surface biocompatibility has become the focus of current discipline research [11-15].

A new type of hydrophilic silane containing PEG segments was produced through polyethylene glycol and organosilanes reacting, which can combine the advantages of the two materials. The hydrophilic silane was grafted onto the surface of the silicon wafer by self-assembly to form a self-assembled material with excellent performance, and the research was carried out to inhibit protein adsorption.

2. Experimental

2.1. Materials

3-aminopropyltriethoxysilane(chemically pure), 2,4-toluene diisocyanate(chemically pure), polyethylene glycol(chemically pure) were purchased from Wuhan Jiangbei Chemical Reagent Co., Ltd., Wuhan, China; acetone (analytical grade), n-butylamine(analytical grade), bromocresol green indicator(analytical grade) were purchased from Tianjin Kermel Chemical Reagent Co., Ltd., Tianjin, China; bovine serum albumin(chemically pure), coomassie brilliant blue G-250 dye reagen(analytical grade), ethanol (analytical grade) were purchased from Tianjin Xingyue Chemical Co., Ltd., Tianjin, China; sodium dihydrogen phosphate(chemically pure), disodium hydrogen phosphate(chemically pure), sodium chloride(chemically pure), phosphoric acid(chemically pure), concentrated sulfuric acid(analytical grade), 30% hydrogen peroxide(analytical grade), toluene(analytical grade) were purchased from Tianjin Tianli Chemical Co., Ltd., Tianjin, China; silicon wafer (1cm*1cm) was purchased from Xi 'an Qiyue Biotechnology Co., Ltd., China.

2.2. Preparation of novel hydrophilic silane containing PEG segments

The 2,4-toluene diisocyanate, 3-aminopropyltriethyl and acetone (as a solvent) were successively added in a 500ml three-neck flask which was equipped with a stirrer, a condenser, and a dropping funnel. The mixed solution was stirred at 60℃ for 2h. The progress of the reaction was monitored by measuring the -NCO content through the di-n-butylamine method[16]. When the -NCO content was close to the theoretical value, polyethylene glycol was added to the above mixture solution. The mixed solution was stirred at room temperature for 90 min. After the completion of the reaction, the solvent was extracted under reduced pressure, and then the hydrophilic silane containing PEG segments was prepared well. The synthetic reaction of the hydrophilic silane is shown in figure 1.

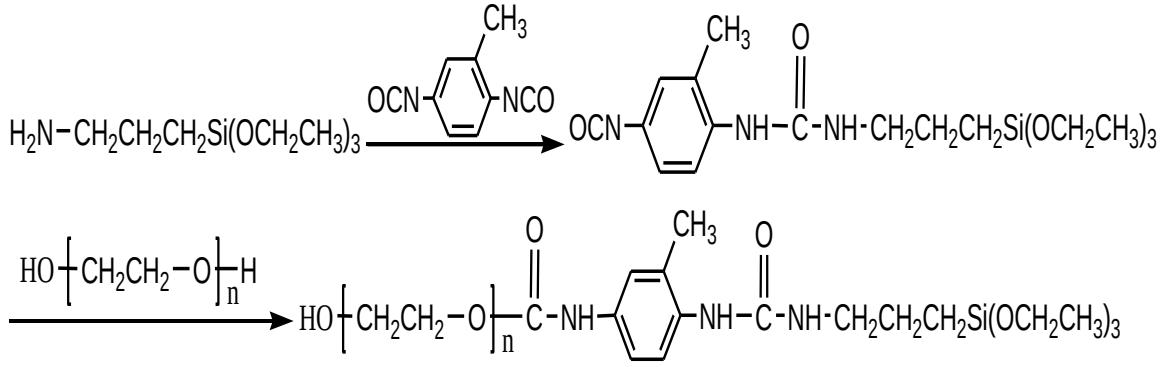


Figure 1. Synthetic reaction of the hydrophilic silane.

2.3. Self-assembly

In this experiment, the preparation of hydrophilic silane self-assembling membrane was based on the role of chemical bonds (including hydrogen bonds). The experimental process was as follows:

(1) The silicon wafer was sequentially washed with toluene, acetone and ultrapure water for 10 minutes, the wafer was immersed in piranha solution at 90 ℃ and was taken out after 30 minutes. The wafer was cleaned with deionized water and acetone for 10 minutes and was blow-dried with high-purity nitrogen.

(2) A certain amount of new hydrophilic silane was dissolved in an appropriate amount of acetone solution, to obtain a solution of hydrophilic silane. The treated silicon substrate was soaked in the above solution for 2h and was placed in an oven at 120°C for 1 h. The self-assembled silicon substrate was ultrasonically cleaned in acetone, ethanol and deionized water for 5 minutes to remove unreacted hydrophilic silane. and the self-assembled silicon substrate was taken out and was blow-dried with high-purity nitrogen. The principle of self-assembly is shown in Figure 2.

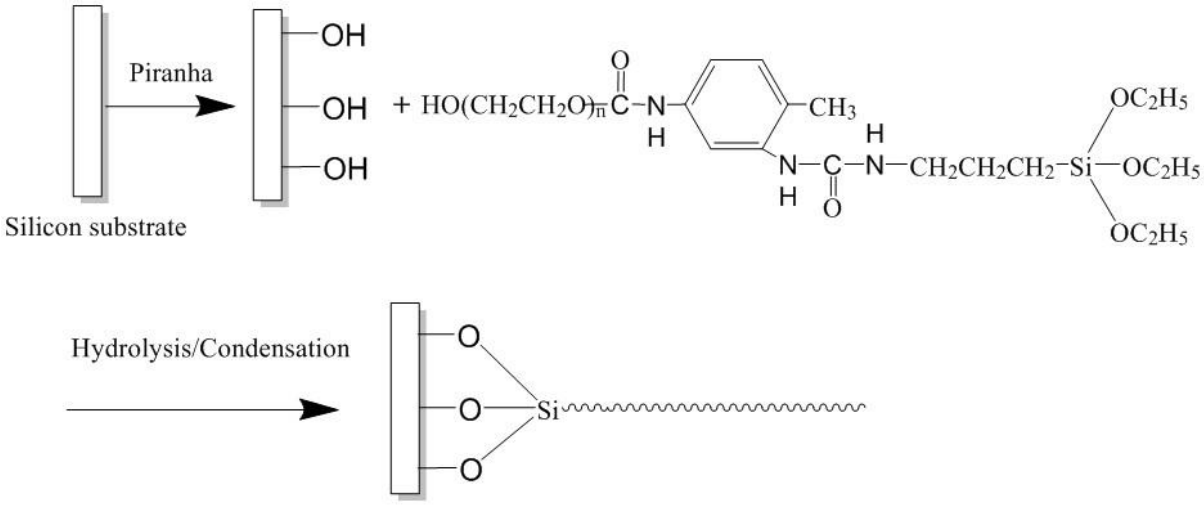


Figure 2. The principle of self-assembly.

2.4. Characterization and measurement

Fourier infrared spectroscopy (FTIR) was measured using a spectrum BX II fourier infrared spectrometer manufactured by PerkinElemer Instrument Company. The coating membrane method and KBr tablet method were used. The test wave number range was 400 ~ 4000cm^{-1} and the resolution were 0.8 cm^{-1}.

Nuclear magnetic resonance analysis: AVANCE Digital 500MHz NMR (German Bruker), $CDCl_3$ as solvent, TMS as chemical shift internal standard.

AFM test: SPI3800-SPA-400 type atomic force microscope manufactured by Seiko Instrument Co., Japan. A rectangular Si_3N_4 probe was used to measure in tap mode, the magnification was 5μm × 5μm.

Contact angle test: JGW-360a contact angle tester produced by Chengde Shenghui Testing Machine Co., Ltd., the test temperature was 25°C. The water drops dropped onto the silane surface by hanging, the angle between the water droplet and the surface of the material was observed through JGW-360a contact angle tester. The obtained angle value was the static water contact angle. Three points on the diagonal line are measured from each sample surface and the average value was taken.

SEM test: Nava Nano SEM 430 manufactured by FEI in the Netherlands. The high vacuum mode with a magnification of 30 to 300,000 times was used.

Ultraviolet spectroscopy (UV-Vis) characterization: The absorbance of protein solution was determined by uv-3000 UV-VIS spectrometer after the sample adsorbed protein. During the measurement, the protein solution which was added with coomassie brilliant blue reagent was poured into a quartz cuvette as the solution to be tested, and the solvent (water or buffer) which was added with coomassie brilliant blue was used as a blank control. The absorbance at 595 nm was read directly on the UV spectrophotometer.

2.5. Anti-protein adsorption test

Comassie brilliant blue G-250 was a dye-binding method for determining protein content. Comassie brilliant blue G-250 was red in the free state and the maximum light absorption was 488nm; when it was combined with protein, it became cyan and the protein-the pigment conjugate had the maximum light absorption at the wavelength of 595nm. Its light absorption value was proportional to the protein content, so it could be used for the quantitative determination of protein.

(1) Configuration of protein solution and preparation of standard curve

a. Preparation of protein solution

The g-bovine serum albumin (BSA) was used to prepare a 1.0mg/mL standard protein solution. Coomassie brilliant blue G-250 dye reagent configuration: a total of 100mg coomassie brilliant blue G-250 was weighed and dissolved in 50mL 95% ethanol, 120 mL of 85% phosphoric acid was added to the above solution and diluted to 1 L with water to obtain coomassie brilliant blue reagent.

b. Determination of standard curve

Taking 7 test tubes, 1 blank and the remaining test tubes were added water and reagents respectively and the bovine serum albumin standard solution were diluted to 0, 0.01 mg/mL, 0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL, 0.1 mg/mL solutions. 1.0mL solution was removed respectively from each test tube accurately and was added into a test tube which contained 4.0mL coomassie brilliant blue G-250 solution. The mixed solution was shake well at room temperature for 5min and was measured the absorbance at 595nm wavelength. The concentration of bovine serum albumin standard solution was taken as the abscissa, the absorbance were taken as the ordinate to obtain the standard curve, as shown in Figure 3.

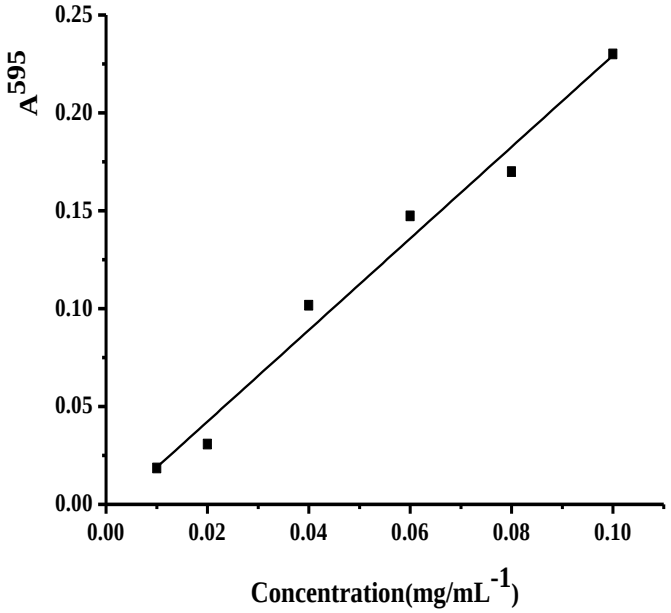


Figure 3. Standard curve for the determination of BSA by the constant method.

Data fitting to the standard curve gave the fitting equation ($R^2 = 0.9784$):

$$y = 2.3394x - 0.0045 \quad (1)$$

In the formula, y: A_{595} was the absorbance of each sample at 595nm; x was the concentration of the protein solution after soaking, $R^2 = 0.9784$ showed that the linear fit was good.

(2) Configuration of buffer solution of PBS (pH = 7.4)

A total of 71.6g Na_2HPO_4 $12H_2O$ was weighed and dissolved in 1000ml water to make a 0.2mol/L Na_2HPO_4 solution; A total of 31.2g $NaH_2PO_4 \cdot 2H_2O$ was weighed and dissolved in 1000ml water to make a 0.2mol/L NaH_2PO_4 solution. 120 mL of 85% phosphoric acid was added to the above solution and diluted to 1 L with water to obtain coomassie brilliant blue reagent.81ml 0.2 mol/L Na_2HPO_4 solution and 19ml 0.2 mol/LNaH_2PO_4 solution were taken and mixed well, and diluted to 1000ml with deionized water, 0.9% (g/100mL) NaCl was added into the above solution and you can configure a buffer solution of 0.02 mol/L PBS (pH = 7.4).

(3) Test for anti-protein adsorption

After the silicon substrate was graft-modified with the hydrophilic silane, it was immersed in a buffer solution of PBS. At room temperature, the surface under an organism-like environment was obtained, and then the modified silicon substrate was immersed in the protein solution for a certain

period of time and was take out. After each sample adsorbed the protein, the absorbance of the protein solution was measured as below.

Taking several test tubes, one blank, and 1.0 mL of protein solution after adsorbing the protein were added to the remaining test tubes, and then 4.0 mL of coomassie brilliant blue reagent were added into each test tube. After the reagent was added 2 to 5 minutes, you can start using the cuvette. The light absorption value A_{595} of each sample at 595nm were measure on a spectrophotometer. The first test tube, that is, 1.0mLH_2O plus 4.0mL Coomassie brilliant blue solution treated as the blank control. The protein solution value at A_{595} after the remaining test tubes adsorbed protein was measured. It can be used to find out the protein content of unknown samples according to the standard curve.

Calculation formula of surface adsorption amount:

$$q = \frac{(c_0 - x) * v}{s} \tag{2}$$

In the formula, q is the surface adsorption amount; c_0 is the initial protein solution concentration; x is the protein solution concentration after soaking; v is the volume of protein solution; s is the adsorption area.

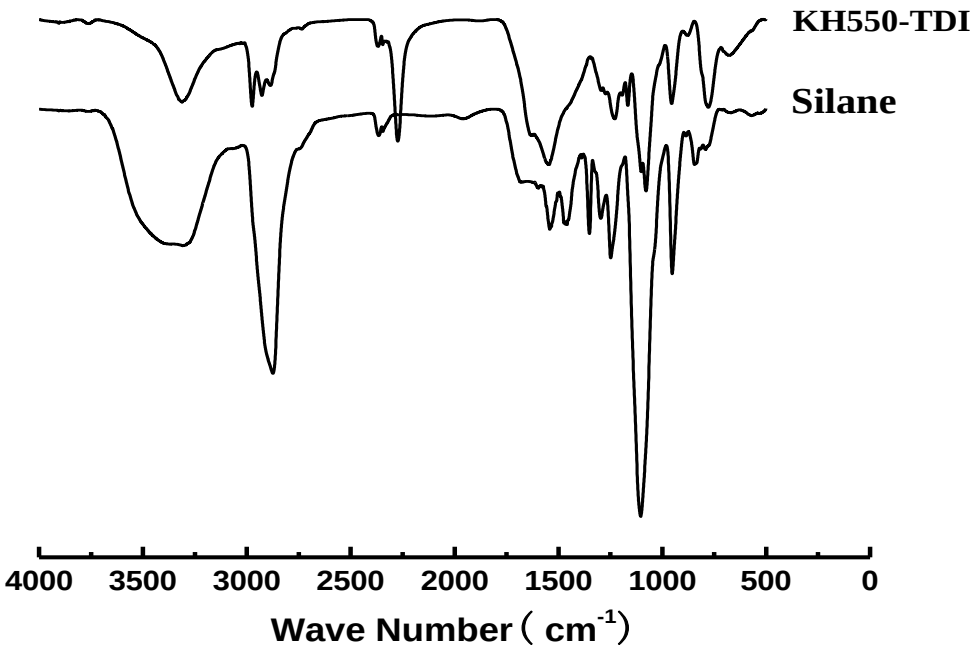


Figure 4. Infrared spectrum of KH550-TDI reaction product and hydrophilic silane.

3. Results and Discussion

3.1. Infrared spectrum analysis of hydrophilic silane

Figure 4 is the infrared spectrum of KH550-TDI reaction product and hydrophilic silane. It could be seen that in the spectrum of KH550-TDI reaction product, strong absorption occurs at 3313, 2974, 2272, 1545, 1078, 778cm^{-1} (as shown in Table 1), which met the characteristics of KH550-TDI reaction product. Hydrophilic silane was the reaction product of KH550-TDI and PEG. In the spectrum, the stretching vibration peak of the isocyanate group at 2272 cm^{-1} disappears, and at the same time, the stretching vibration peak of the hydroxyl group and the amide group merges into a wide absorption peak, indicating that the isocyanate group reacted with some of the hydroxyl groups in the PEG.

Table 1. Infrared peak corresponds to the reaction group.

Frequency, cm^{-1}	Group
3313	the stretching vibration peak of amide group
2974	the stretching vibration peak of methylene group
2272	the stretching vibration peak of isocyanate group
1545	the stretching vibration peak of carbonyl group
1078	the stretching vibration peak of the silicon-oxygen bond
778	the stretching vibration peak of the benzene ring

3.2. Nuclear magnetic resonance spectroscopy analysis of hydrophilic silane

Figure 5 is the ^{1}H-NMR spectrum of hydrophilic silane. As can be seen from figure 5, the chemical shift at δ = 3.7ppm was the characteristic absorption peak of methyl hydrogen on Si-OCH_3; the chemical shift at δ = 3.4 ~ 3.6 ppm was the characteristic absorption peak of hydrogen in ethoxy group in the silane PEG segment; the appearance of these characteristic absorption peaks indicated that the product had a polyethylene glycol segment and a KH550 segment. The results showed that the target product was obtained through the above synthesis reaction.

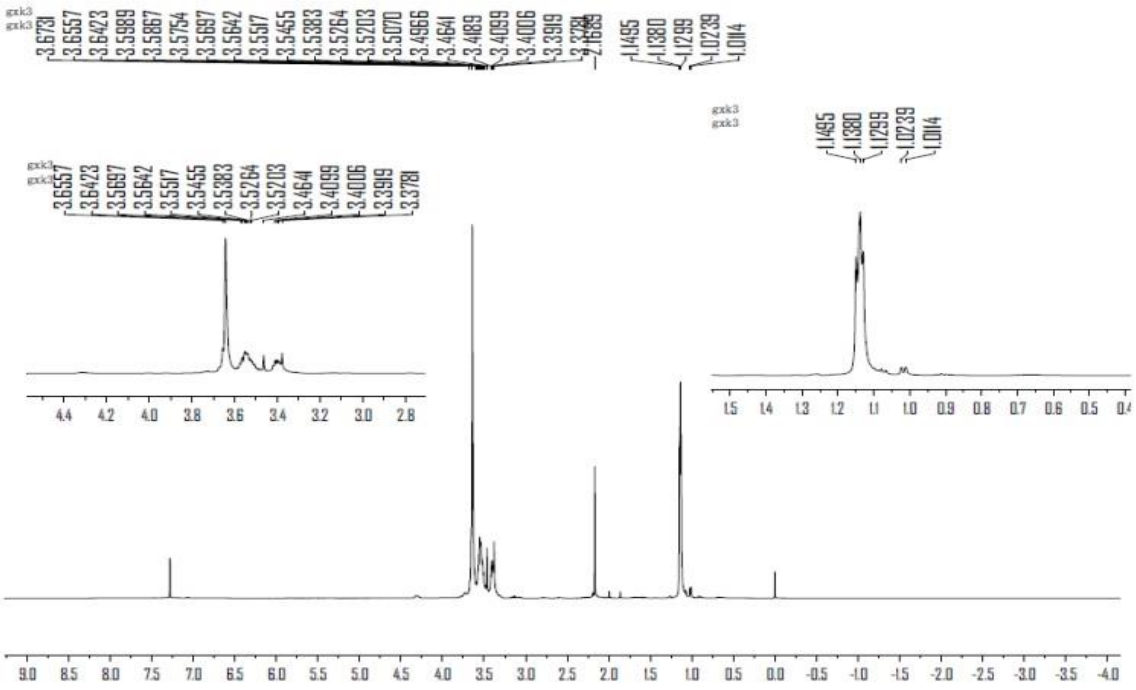


Figure 5. ^{1}H-NMR spectrum of hydrophilic silane.

3.3. Atomic force analysis of silane self-assembled membrane

Figure 6 is an atomic force perspective view of a blank silicon substrate and the figure 7 is an atomic force perspective view of the self-assembled membrane. It could be seen from the figure 6 that the surface of the blank silicon substrate was relatively smooth, and the highest height of the plane was 2 nm and the surface roughness was 0.27 nm. It could be seen from the figure 7 that the surface of the self-assembled membrane was relatively rough, a small peak with a size around 7nm were formed and the height of the plane was 5-10nm and the surface roughness was 0.65nm, which indicating that the self-assembled membrane of hydrophilic silane containing polyethylene glycol segments was successful.

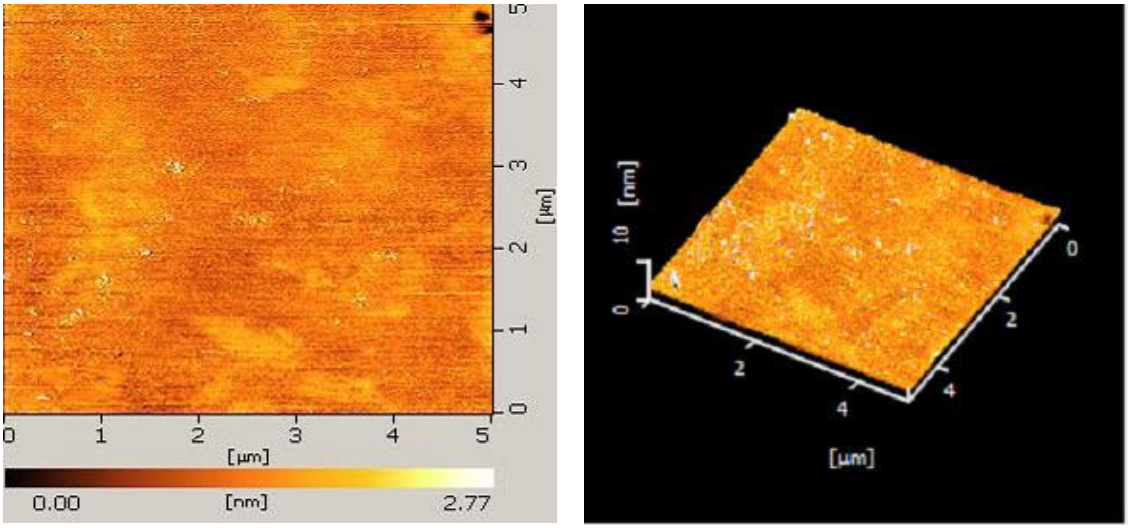


Figure 6. AFM images of blank silicon substrate.

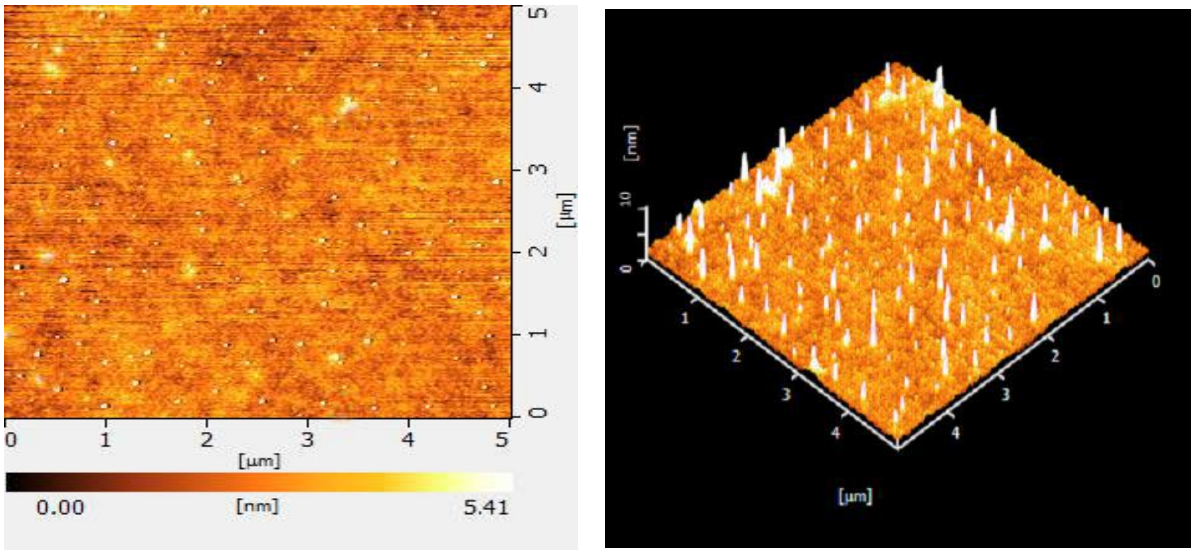


Figure 7. AFM images of silane self-assembled membrane.

3.4. Hydrophilicity analysis of the self-assembled membrane

It could be seen from the figure 8 that the contact angle of water on the surface of blank silicon substrate(X) and the self-assembled membrane(Y) were 45.72 ° and 20.58 °. On the one hand, after the hydrophilic silane was grafted onto the surface of the silicon wafer, the polyethylene glycol segments were arranged in the outside the surface of the self-assembly membrane. Because the intermolecular forces between the polyethylene glycol segments were small, and they had low interfacial energy for water solubility, flexibility and high movement, so that the polyethylene glycol segments can bind a large number of water molecules to reduce the contact angle; On the other hand, the surface roughness had a greater impact on the hydrophilicity of the material surface. The surface of the self-assembly membrane with a large roughness could increase the dispersion area of water molecules, so that the surface of silicon after self-assembly was enhanced in hydrophilicity.

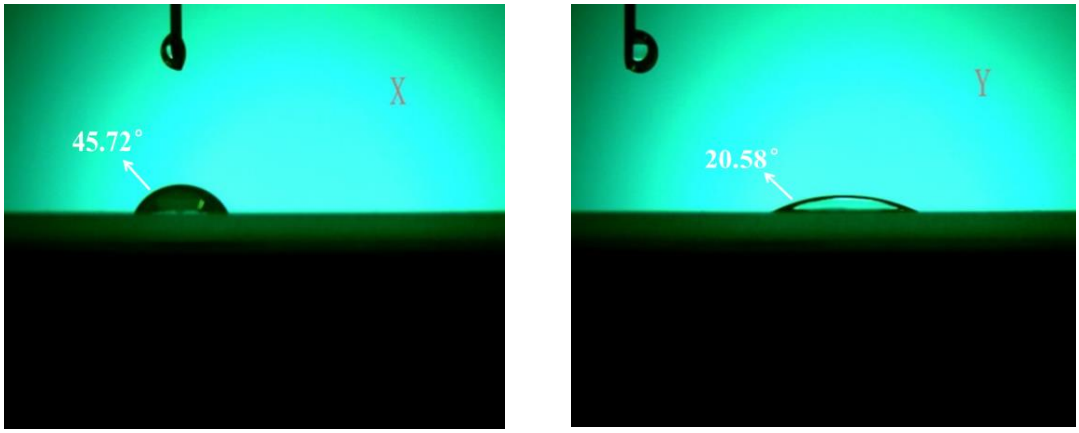
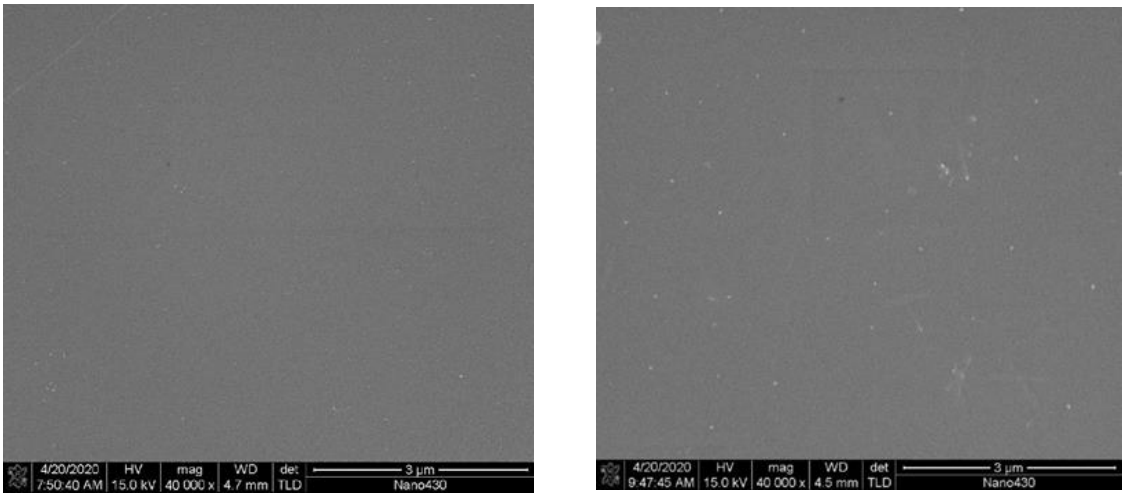


Figure 8. Contact angle of water on the surface of blank Silicon substrate(X) and the self-assembled membrane(Y).

3.5. Scanning electron microscopy analysis of silane self-assembled membranes

In Figure 9, (a) and (b) are SEM photographs of a blank silicon substrate and the self-assembled membrane at a magnification of 40,000 times. From the figure 8, it could be seen that the blank silicon substrate had no substance and the surface was very smooth. Significant changes have occurred in the self-assembled membrane. The regular lattice structure appeared on the surface of the self-assembled membrane. Some of the larger dots might be formed by entanglement of PEG brushes.



(a) (b)

Figure 9. SEM photographs of (a) blank Silicon substrate and (b) silane self-assembled membrane.

3.6. Anti-protein adsorption analysis on the surface of self-assembled membrane

The silicon substrates and self-assembled monolayers were immersed in a 0.06 mg / mL BSA protein solution at room temperature. The absorbance changes of the protein solution at 595 nm were measured after blank silicon substrates and the self-assembled membranes were soaked in BSA protein solution for a certain time. As shown in Figure 10.

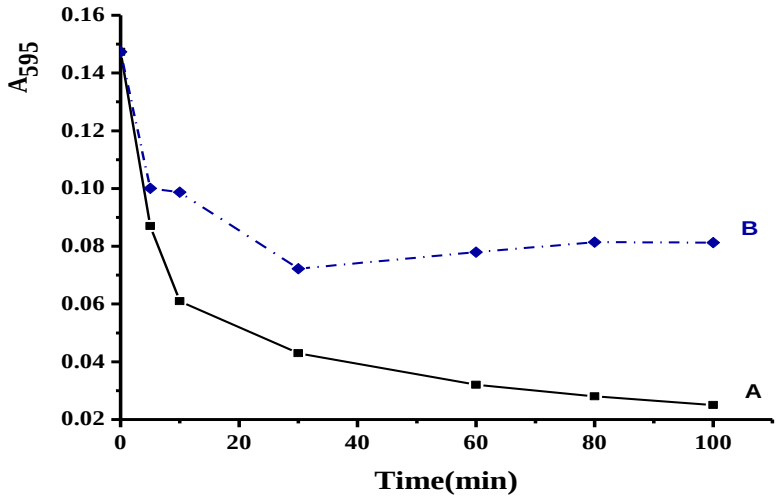


Figure 10. The curve of the absorbance value of A_{595} in BSA of blank silicon substrate (A)and self-assembled membrane (B) with time.

The protein concentration and surface adsorption in the solution were calculated as shown in Figure 11.

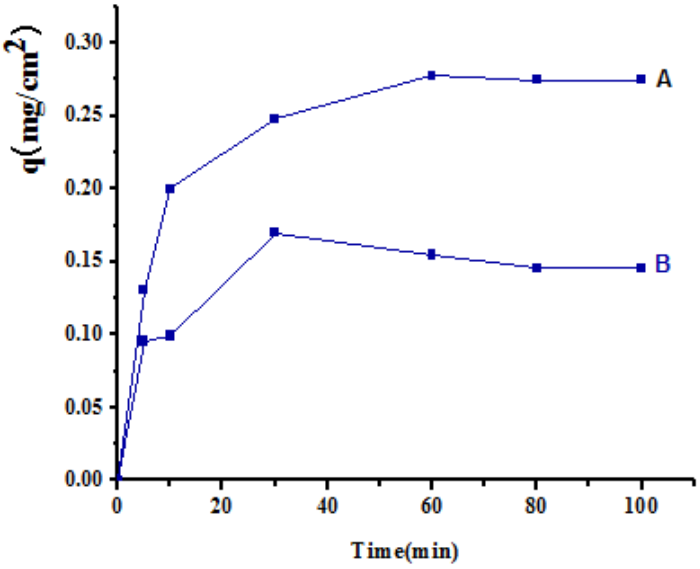


Figure 11. The curve of the surface adsorption amount of blank silicon substrate and self-assembled membrane with time.

In Figure 10, the A and B curves represent the changes in the absorbance of the blank silicon substrate and the self-assembled membrane over time. From the figure 11, it could be seen that within the first 30 minutes of immersion, the absorbance of the blank silicon substrate decreased significantly, and the protein concentration in the solution decrease rapidly, indicated that the blank silicon substrate was prone to adsorb proteins, and the absorbance continues to decrease with time; while the concentration of protein in solution of the self-assembled membrane decreased in the first 30 minutes, but the anti-protein adsorption capacity was better than that of the blank silicon substrate. Within the period of 30min to 100min, the adsorption amount of self-assembled membranes did not increase with time. After calculation, the surface adsorption amount of blank silicon substrates was 274.8μg/cm^2 and the self-assembled membrane was 145.7μg/cm^2, which indicated that the self-assembled membrane had good stability against protein adsorption.

4. Conclusion

A new type of hydrophilic silane containing PEG segments was produced through polyethylene glycol materials and organosilanes reacting, which can combine the advantages of the two materials. The hydrophilic silane was grafted onto the surface of the silicon wafer by self-assembly to form a self-assembled material with excellent performance; The research was carried out to inhibit protein adsorption and the surface adsorption amount of the self-assembled membrane immersed in the protein solution decreased from 274.8 μg/cm^2 to 145.7 μg/cm^2 compared to the blank silicon substrate; This study provides a new method and experimental basis for preparation of biological materials.

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