Effect of thiol concentration and incubation time on formation of self-assembled monolayers on gold-coated substrates

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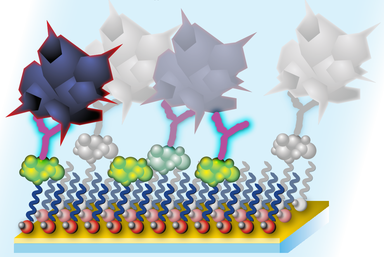
Introduction

Self-assembled thiol monolayers (SAMs) are an essential part of an innovative live virus detection technique: optofluidic nanoplasmonic biosensors[1]. This technique is based on capturing viruses by antibodies immobilized at a biosensor's surface and then using surface plasmon resonance (SPR) to detect the captured viruses.



This is how a SAM can be made. A gold-coated silicon or glass substrate is incubated in a thiol ethanol solution. The SAM formed on the gold film as a result of incubation is then treated (functionalized) with a solution of EDC and NHS in ethanol to ensure the subsequent immobilization of a protein. After a protein layer is created on top of the functionalized SAM, the chip is incubated in an antibody solution, and the assembled gold/SAM/protein/antibody biosensing system is ready for capturing viruses. The goal of our research was to investigate the effects of thiol concentration and incubation time on formation of SAMs on gold-coated silicon or glass substrates. The prepared SAMs were analyzed using FTIR and ellipsometry.

Experimental Methods

In our research, we used 5 different MUA concentrations (0.1 mM, 0.25 mM, 0.5 mM, 1 mM, and 2 mM) of 11-mercaptoundecanoic acid (MUA) in ethanol and two incubation times: 18 hours and 24 hours. In addition to that, two chips were incubated in 0.5 and 1 mM thiol ethanol solutions for 6 hours (see Table 1). Also, one chip was immersed in pure ethanol for 24 hours to serve as a reference.

Glass and silicon chips coated with thin (100 nm) gold films were used as substrates. The chips were cleaned in piranha solution (1 part of hydrogen peroxide to 3 parts of sulfuric acid) for 5 minutes, then bathed in water for 2 minutes, and dried off with nitrogen. Immediately after cleaning, the chips were immersed in MUA solutions of different concentrations. Each chip was placed in a separate jar.

After incubation, the chips were rinsed in ethanol, then in water, bathed in water for 5 minutes, and dried off with nitrogen.

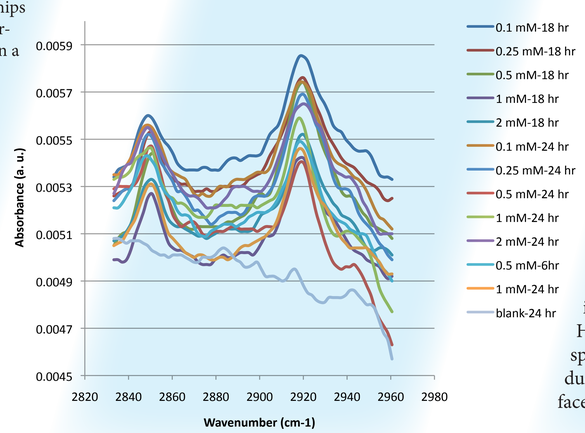
Table 1:

Concentration, mM	Incubation time, hours
0.1	18, 24
0.25	18, 24
0.5	6, 18, 24
1	6, 18, 24
2	18, 24
0	24

Results and Discussion (FTIR)

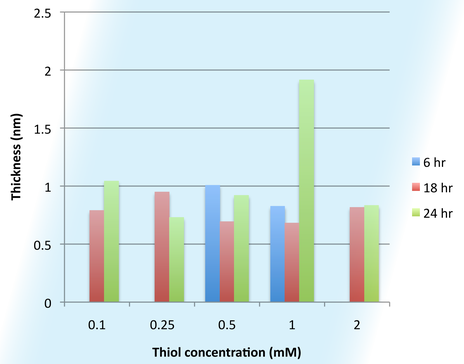
To determine whether MUA was attached to the chip surface we looked for peaks in absorbance located at 2919 and 2850 cm^{-1}, which correspond to two distinct vibrational modes (asymmetric CH2 and symmetric CH2 stretches respectively) of the MUA intramolecular bonds[2].

Although we successfully observed the peaks in the absorbance spectra, their intensity did not show any dependence on the MUA concentration or chip incubation time . The blank chip incubated in ethanol did not show any peaks around 2919 and 2850 cm^{-1}.



Results and Discussion (Ellipsometry)

For the ellipsometry measurements, the SAMs were assumed to be Cauchy layers with the refractive index of 1.45. The optical constants of the gold film were determined using a piranha-cleaned chip. The measurements were taken for three angles of incidence (65, 70, and 75) in the wavelength range 400 – 1000 nm. The measured thicknesses of MUA varied from 700 to 1000 nm with one outlier at 1900 nm (1mM, 24 hrs).



The ellipsometry results did not indicate any noticeable relationship between incubation time and thickness of SAM. However, it was noticed by visually inspecting the chips that the chip that produced the outlier had the best quality surface: no scratches or specks.

Conclusion

The conducted studies showed that the MUA concentrations from 0.1 mM to 2 mM combined with the incubation time from 6hrs to 24 hrs produced equally strong peaks around 2919 and 2850 cm^{-1}. The thickness measurements also did not demonstrate any noticeable relationship between the thickness of a SAM and incubation time or between the thickness of a SAM and thiol concentration. It allows us to conclude that lower concentrations of thiol and shorter incubation times can be used to achieve successful SAM formation on gold.

From the lab to the classroom

Ellipsometry: How It Works?

Targeted audience: 9th grade introductory physics class

Lesson Objectives:

1. Differentiate between polarized and nonpolarized light.
2. Explain how light can be polarized by absorption and by reflection.
3. Differentiate between linear, circular, and elliptical polarization.
4. Explain how a polarizing filter works.
5. Describe what ellipsometry is and what it is used for.

Teaching resources:

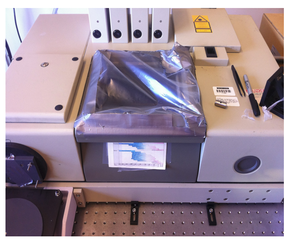
Computer simulations of polarization and Brewster's angle.

Hands-on activities and labs on exploring how polarizing filters work and measuring Brewster's angle.

FTIR

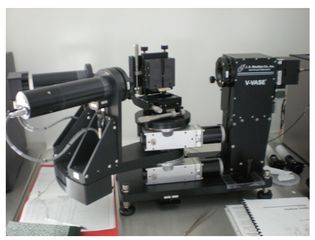
FTIR spectroscopy is a method for determining the structure of a material. FTIR stands for Fourier Transform Infra Red. When the frequency of the incident infrared radiation matches the vibration frequency of an atom, molecule, or a group of atoms bonded together within a molecule (a so-called "functional group"), the infrared radiation is absorbed. The amount of absorbed radiation depends on the strength of the bond. So, if we measure absorbances of different frequencies of infrared radiation from a sample, we can obtain information about the chemical structure of the sample.

An FTIR spectrometer consists of a source of infrared radiation, an interferometer, and a detector. A radiation source produces a broadband beam of infrared radiation. An interferometer converts the infrared beam into an interference signal (interferogram) containing all of the infrared frequencies. The interference signal goes to the sample, where some radiation is absorbed, and then to a detector. The final interferogram is then mathematically transformed (Fourier transformation) into an absorption s pectrum containing the structural "fingerprint" of the sample.



ELLIPSOMETRY

Ellipsometry is a method for determining thicknesses of thin films and the optical constants of materials using polarized light. A beam of light from a lamp is linearly polarized by a polarizer in the input unit and is sent to a sample that is mounted on the sample stage. When the beam is reflected from the sample, it changes its polarization and becomes elliptically polarized. The change in polarization of the beam is measured by the detector. The obtained information is processed with a computer. The computer calculates the values of the physical parameters that we want to determine, such as the thickness of a film or its index of refraction.



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