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2012 Adv. Nat. Sci: Nanosci. Nanotechnol. 3 045008

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Quantitative analysis of COOH-terminated alkanethiol SAMs on gold nanoparticle surfaces

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Received 13 June 2012

Accepted for publication 11 August 2012

Published 5 October 2012

Online at stacks.iop.org/ANSN/3/045008

Abstract

Surface-functionalization of a self-assembled monolayer (SAM) can be achieved by introducing functional molecules at the terminal. To immobilize biomolecules on a gold substrate, COOH-terminated alkanethiol SAMs are preferably employed. Thiol molecules adsorption on gold surface was performed using thioglycolic acid (TGA, HS-CH₂-COOH) monomers and a self-assembled technique.

Characterization by Fourier transform infrared (FTIR) spectroscopy revealed gold–sulfur (Au–S) bonding through confirming the presence and disappearance of thiol molecules on the Au surface before and after the sample's immersion in the TGA solution. Moreover, FTIR spectra also proved the presence of carboxyl molecules (C=O; OH) at the free end on the gold nanoparticle (AuNP) surface. Quantitative analysis of the carboxyl molecules interacted with methylene blue (MB) ones, and then identification by UV-Vis absorption spectroscopy showed that the average density of the carboxyl molecules on the free end of the alkanethiol SAM was about 3.9×10^{14} molecules per cm².

Keywords: Au–S bond, self-assembled monolayer (SAM), thioglycolic acid (TGA), carboxyl molecular density

Classification number: 4.02

1. Introduction

A well organized bio-interface has attracted much attention for applications to biochips. In order to fabricate a highly reproducible and highly efficient biochip, it is important to control the density of biomolecules on the solid substrate. Moreover, it is essential that the biological probe should be designed so as not to be denatured on the substrate. Hence, the functional molecules being reactive with a terminal of the biological probe should be designed because the reaction efficiency and the denaturation of the probe depend on the density of the reactive group at the surface.

Biomolecules bound to carboxyl-terminated self-assembled monolayer (SAM) could be applied for biological probes. An alkanethiol SAM has recently become very attractive for well ordered thin-film fabrication, by which thiol or disulfide derivatives can spontaneously form a closely packed monolayer on a gold surface. Thiol molecules

attached to a gold surface make the strongest bond and less oxidation, compared to other metals. Bain *et al* have reported that gold substrates for SAM-based alkanethiols have been more widely used than others because thiol molecules are well bound to gold surface with high affinity [1–3]. Moreover, functional molecules like primary amine (NH₂), carboxyl (COOH), or hydroxyl (O–H) at the free end at the opposite side of S–H molecules of the SAM could also be bound to biomolecules such as proteins and bacteria [4, 5].

To further study biochip fabrication, we need to determine a number of biomolecules serving as probe molecules. Hence, quantifying probe molecules, which would be bound to the functional molecules, must be necessary. As we suppose that one functional molecule like COOH or NH₂ could be bound to one probe biomolecule, we can determine the functional molecule instead of quantifying the number of biomolecules. Cuong and Basarir [5] have determined the number of functional molecules such as NH₂ which

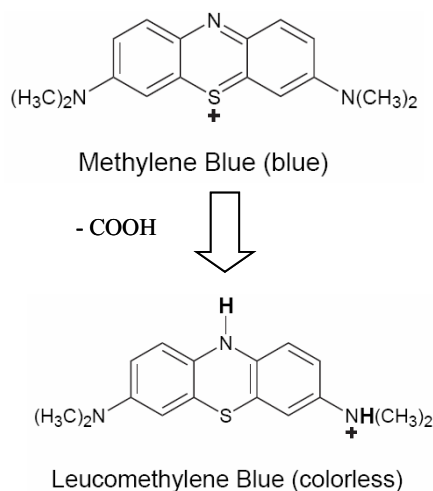
immunoglobulin G (IgG) molecules were immobilized onto. Continuing this work, they attempt to quantify a number of carboxyl molecules on a gold surface for binding biological molecules.

This paper focuses on quantitative analysis of carboxyl (COOH) molecules immobilized on a gold nanoparticle (AuNP) surface. We first study the distribution of COOH-terminated alkanethiol molecules through determining the presence of the gold–sulfur (Au–S) interaction and typical bonds such as C=O, C–H, C–H₂ and O–H. Then, quantifying COOH-functional molecules on the AuNP surface, we estimate carboxyl density per unit area of the SAM by the detection of methylene blue (MB) bleaching using an UV-Vis spectrometer. The roughly estimated COOH molecules would be useful and valuable for preparation of probe biomolecules for further biochip studies.

2. Experimental

2.1. Theoretical approach to quantifying COOH molecules based on their reaction with methylene blue

The chemical reaction between the methylene blue (MB) molecule and a COOH one in solution leads to the bleaching of MB diluted solution. The bleaching mechanism in acidic media is attributed to a pair of electrons belonging to nitrogen (N) atoms which would receive H⁺ (proton). This leads to a resonance with the benzene rings inside the MB molecule that is then itself reduced, forming a leucomethylene blue molecule (MB colorless solution).



The bleaching of given MB concentration due to the formation of leucomethylene blue in MB solution is determined by UV-Vis absorption detection and the empirically plotted calibration curves.

According to the Beer–Lambert law, the absorbance for the liquids, denoted as A , is linearly proportional to the molar absorptivity (extinction coefficient) of the absorber (ϵ), the molar concentration (C) of absorbing species in the material and the width (L) of the cuvette:

$$A = -\lg \frac{I}{I_0} = \epsilon CL \lg e, \quad (1)$$

where I_0 and I denote intensities of the incident light and the transmitted one, respectively. This formula would be

correct only when the concentration of the solution is rather small (limit of linear response is a solution range in which the Beer law is correct). If the solution's concentration increases, the distance between molecules decreases (a shorter range). This causes the interaction between molecules to be considerably enhanced. Hence, measured values of the solution concentration would have a much larger margin of error.

Based on the linear proportion of the MB absorbance to its solution concentration, we empirically measured the MB absorbance of given different concentrations. Changes in the linear relationship between MB absorbance versus its concentration, caused by the interaction of MB molecules and COOH ones, were determined using a UV-Vis spectrometer at a specific absorption peak at 662 nm. The difference of the MB concentration in a given solution before and after the chemical reaction is converted to the number of MB molecules which already reacted with the COOH molecules. Empirically plotting a calibration curve, we determine values of the molar absorptivity and intensity. As the chemical reaction only completely occurs between one MB molecule and one carboxyl molecule, we can estimate the number of carboxyl molecules per unit area of the SAM through the MB molecules instead of carboxyl ones.

2.2. Experimental quantitative analysis

Thioglycolic acid 99% (TGA, $-\text{HS}-\text{CH}_2-\text{COOH}$), as received, bought from Merck Co. (Germany) was diluted in absolute ethanol (EtOH) to prepare a solution at appropriate concentration of 10 mM. Methylene blue solution was diluted in distilled water at different concentrations. The sputtered gold-surface on a silicon wafer had less than 100 nm in thickness. All gold-coated substrates, ultrasonic cleaned in acetone solvent for 15 min, were dried in a vacuum oven at 60 °C prior to their immersion in the TGA 10 mM solution. Thiol molecules of the TGA solution reacting with AuNPs could create Au–S bonds when an Au-surfaced sample had been immersed in the solution. Details of the chemical reaction during the time of self-assembled alkanethiols had been clearly described in the previous paper [6].

To prove the interaction of COOH-terminated alkanethiol molecules with AuNP surface, we employed a Fourier transform infrared (FTIR) spectrophotometer (GX-Perkin Elmer, USA) using the reflection mode at a resolution of 4 cm^{-1} over the 4000–600 cm^{-1} spectral region to reveal the Au–S bond and functional groups such as carbonyl (C=O) and hydroxyl (OH) groups belonging to COOH-terminated alkanethiol SAMs. The presence and disappearance of the thiol (S–H) bond, before and after dipping AuNP surface samples in TGA 10 mM solution, were characterized to confirm the formation of Au–S bond.

We quantified molecular density of COOH molecules per unit area of the alkanethiol SAM on the AuNP surface by empirically plotting a calibration curve of the MB at the typical absorption peak of 662 nm. To fulfill this work, we prepared MB solution at concentration ranging from 2×10^{-4} wt% to 14×10^{-4} wt%, and measured the absorption of the MB standard solution at given concentration. A calibration curve showing the linear relationship between the given

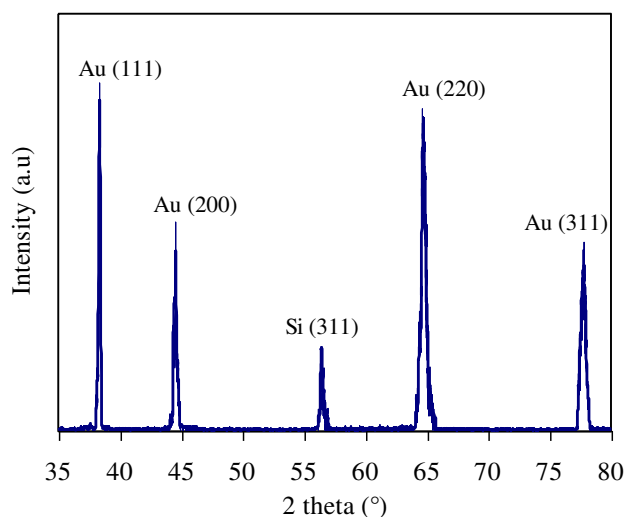


Figure 1. XRD spectrum of gold sputtered on silicon wafer showing (111), (200), (220) and (311)-oriented Au structure.

MB concentration and its absorbance was plotted. Then, we immersed SAM samples into the MB standard solution at undetermined concentration. The chemical reaction between the MB molecules and carboxyl molecules on SAM for a few minutes led to an MB colorless solution. After that we took the SAM samples out of the MB colorless solution, measured its absorbance, and then determined MB solution concentration using the calibration curve and the absorbance intensity measured in the previous step. We calculated the number of MB molecules in the MB solution after its reaction with COOH-terminated SAMs, and estimated MB molecules instead of determining carboxyl molecules because one MB molecule could only react with one COOH molecule, so both MB and COOH had an equal number of molecules in their chemical reaction. Finally, we determined the number of MB molecules that are equal to those of the COOH molecules we need to quantify.

3. Results and discussion

3.1. Structure analysis and AuNP size

X-ray diffraction (XRD) spectra were measured at 2θ ranging from 30° to 80° on a D8 advance x-ray diffractometer (Bruker, Germany) for the Au-deposited surface. It can be seen that XRD peaks were located at a diffractometry angle of $2\theta = 38.20^\circ, 44.45^\circ, 64.50^\circ$ and 77.50° which could be assigned to Au (111), (200), (220) and (311) planes, respectively (figure 1). Moreover, (111) and (220)-oriented Au structure possesses the higher density of sputtered nanoparticles compared to the other ones. Also, one peak with low intensity located at an angle of $2\theta = 56.20^\circ$ is ascribed to Si (311) plane. This revealed that silicon substrate was covered by gold thin film.

Using the Scherrer formula, we calculated the gold nanocrystal size as follows:

$$\tau = \frac{0.9\lambda}{\beta \cos \theta_\beta}, \quad (2)$$

where β is the full-width at half-maximum (FWHM) of the x-ray peak and the x-ray source of 1.54056 \AA for Cu-K α was

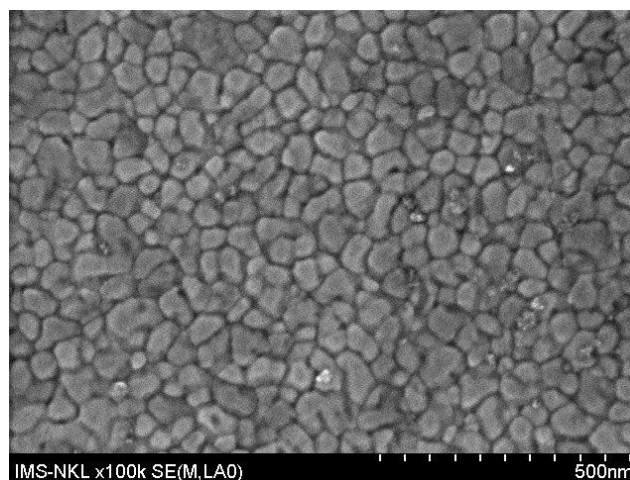


Figure 2. SEM micrograph showing gold nanocrystals deposited on the silicon wafer sample.

used. The FWHM value of the peak oriented to (111) was estimated to be 0.0028 rad . Therefore, the average size of Au-nanocrystals was about 52 nm .

Moreover, SEM micrographs also show the gold surface with Au-nanocrystals that were well uniformed and distributed on silicon wafer and their approximate size of 50 nm was estimated (figure 2).

3.2. Au-S identification

Figure 3 shows FTIR spectra of TGA diluted solution of 10 mM (upper spectrum) and SAMs COOH-terminated alkanethiol SAMs (bottom spectrum) to identify the interaction between thiol (S-H) molecules and gold nanoparticles on a Si-substrate. Thiol molecules bound to the AuNP surface through the Au-S bond were confirmed by revealing the presence of S-H groups located at the band of 2565 cm^{-1} for TGA diluted solution (upper spectrum) and their disappearance at the same band on the Au surface (bottom spectrum), respectively.

The most important distinction between two FTIR spectra could be clearly determined by the band at 2565 cm^{-1} assigned to S-H bonding on the upper spectrum (figure 3). However, this band disappeared in the bottom one. It might be attributed to the cleavage of S-H bond that led to the formation of a new bond, i.e. S-Au bond. This phenomenon proved the fact that thiol-terminated SAMs could be bound onto the gold surface. These facts further proved that the TGA molecules were bonded to the Au surface through the S-Au bond at one end while the functional carboxyl groups (-COOH) freely moved to the other end. These remarks are in a good agreement with those reported by Aryal *et al* who have discovered the formation of new Au-S bonding related to the absence of thiol (S-H) molecules of cystein covering Au grains when using FTIR spectrometer [7].

3.3. Carboxyl molecule identification

The COOH molecules could be directly identified through C=O and O-H bonds by FTIR detection. Figure 3 shows that the bands at 2925 and 2976 cm^{-1} assigned to vibration of C-H clearly exist in the bottom spectrum. These peaks seem to

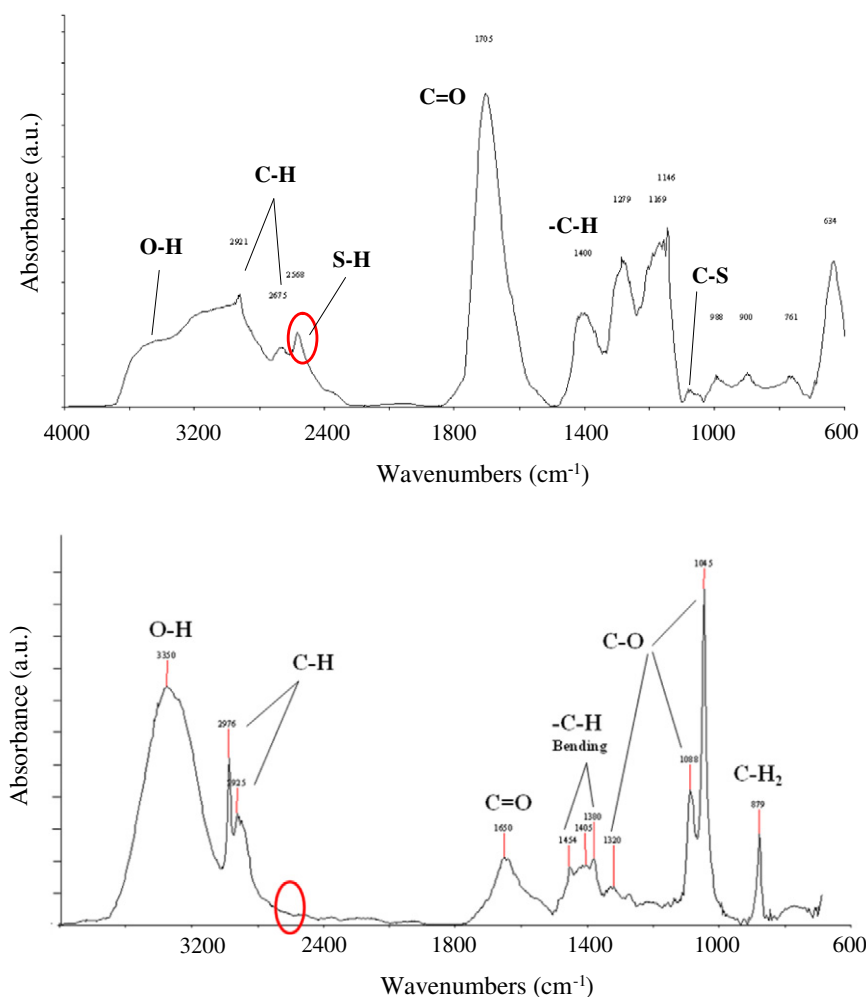


Figure 3. FTIR spectra of TGA-based SAM on the Au surface (bottom) and of TGA solution at concentration 10 mM (upper) showing the COOH existence and disappearance of S–H bonding, also the presence of hydroxyl (O–H), carbonyl C=O and C–H groups.

be split from one at 2921 cm^{-1} , also assigned to C–H (see the upper spectrum). The splitting of the peak at 2921 cm^{-1} might be attributed to SAM's structure defects during the SAM creation when immersing the AuNP surface sample into TGA diluted solution. Besides, C–H stretching vibration of the alkyl chain, which is very sensitive to incident light intensity, is the other reason causing the peak splitting.

Two split peaks at 2925 and 2976 cm^{-1} as well as blue-shifting of all peaks of the SAM are attributed to changing in the SAM structure which considerably affected symmetric and asymmetric stretching vibration, shifting and adsorption intensity of C–H bonding. We also found the peaks at 1676 , 1045 and 3350 cm^{-1} assigned to the C=O, C–C and O–H bonds, respectively. The existence of these peaks is clear evidence showing the presence of carboxyl (O=C–OH) groups on the one side of the SAMs.

This confirmed the presence of COOH-terminated alkanethiol SAMs bound to the Au surface through Au–S bonding and was also in agreement with the results reported by Krolkowska *et al* [8]. In addition, the bands of the C–H, C–O and C–C bonding of the SAMs were blue-shifted while their C=O bonding was red-shifted compared to those of the TGA diluted solution. This may be ascribed to the interaction affinity of S–H groups to Au surface that changed the vibration of carbon molecules with respect to oxygen and

hydrogen ones as well as the zigzagged pattern of the SAM's molecular chains bound to the AuNP surface.

3.4. Quantitative analysis of COOH molecules

Carboxyl molecules on the free end of the thiol-ended SAM (as a linker molecule) could be actively bound to probe biomolecules. Therefore, if we know a number of COOH molecules, we could also roughly calculate the number of probe biomolecules which could be bound to COOH ones.

UV-Vis absorption spectrum of MB solution shows two typical peaks near 612 and 662 nm (figure 4). We selected the peaks at the wavelength of 662 nm to plot a calibration curve for the MB solution.

Figure 5 shows absorbent intensities of the MB solution at the wavelength of 662 nm in response to the given concentration. It can be seen that absorbent peak intensities increased with increasing the MB solution concentration ranging from 0.2×10^{-3} to $1.4 \times 10^{-3}\text{ wt\%}$. We built an equation of a calibration curve coupled with the relative coefficient of $R^2 = 0.994$:

$$y = 2.407x + 0.264. \quad (3)$$

Concentration of the MB solution (denoted as C) was calculated following the determination of intensity of

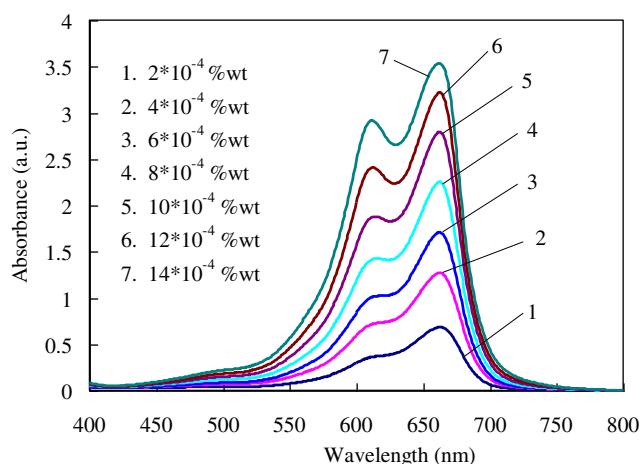


Figure 4. Visible absorption peaks of the MB solution at different concentrations.

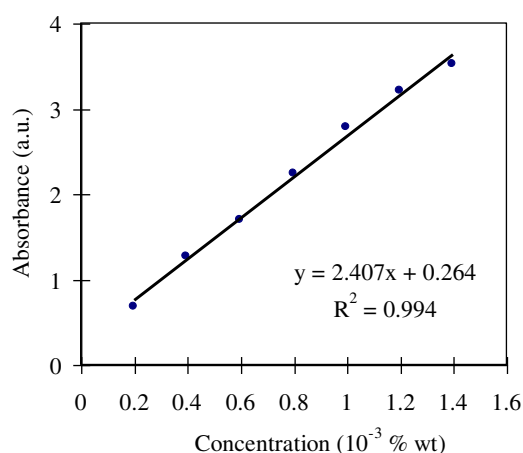


Figure 5. The calibration curve showing the relation between absorbance and concentration was plotted based on empirical measurement.

absorbance peak at 662 nm (denoted as I_{662}). We get:

$$C = \frac{I_{662} - 0.3}{2.6 \times 10^5}. \quad (4)$$

The number of COOH molecules per unit of square area was quantified by the following equation:

$$n = m \frac{(C_1 - C_2)}{M_{MB}}, \quad (5)$$

where C_1 , C_2 are MB concentrations before and after the reaction of MB molecules with $-\text{COOH}$ molecules, m is the mass of the MB solution used for the immersion of a SAM layer, M_{MB} is a molar mass of the MB equal to $319.85 \text{ g mol}^{-1}$. As the number of COOH molecules was equal to that of the MB in the same reaction, we calculated molecular density of COOH-terminated alkanethiol SAM layer by using formula

$$D = \frac{(C_1 - C_2) m N_A}{S M_{MB}}, \quad (6)$$

where N_A is Avogadro constant, $N_A = 6.02214 \times 10^{23} \text{ mol}^{-1}$, S is the area of a gold surface on which a SAM layer was deposited. S was estimated as 2.25 cm^2 .

Molecular density of COOH-terminated alkanethiol SAMs on a unit area of the gold layer ranging from 3.7×10^{14} to 4.2×10^{14} was calculated from formula (6). Their average value was about 3.9×10^{14} molecules per cm^2 compared to that of 4.5×10^{14} molecules per cm^2 calculated by theory [9]. It was found that the molecular density determined empirically is less than that calculated theoretically due to defects of the gold sputtered layer as well as the chemical reaction between MB molecules and COOH molecules not completely occurring during the time of the reaction.

4. Conclusion

Thioglycolic acid (TGA, $\text{HS}-\text{CH}_2-\text{COOH}$) molecules have been bound to AuNP surface by self-assembled monolayer at the most appropriate concentration (10 mM TGA solution). COOH-terminated alkanethiol SAMs were well uniformed on the AuNP surface through the gold-sulfur (Au-S) bond which has been revealed by FTIR detection. The COOH functional molecules at the free end of Au-S bonded SAMs were also identified by FTIR spectra. The density of the COOH molecules was quantified by the MB color reduction coupled with UV-Vis detection. The quantitative analysis revealed that the COOH molecular density averaged about 3.9×10^{14} molecules per cm^2 . These results clearly showed the number of COOH molecules that were immobilized on gold nanoparticle surface. As the number of COOH molecules was well determined, we would pre-prepare appropriate probe biomolecules that could be bound to COOH molecules for further research in biochip fabrication.

Acknowledgments

The work for this paper could not have been well-performed without the financial support of the project (QC.09.19) granted by Vietnam National University (VNU) in Hanoi. The author would like to thank Mr Bui Dinh Tu and Mr Do Tuan, postgraduate students of Engineering Physics and Nanotechnology Faculty, School of Engineering and Technology, VNU, for the preparation of gold-film sputtered on a silicon wafer and SAM sample, respectively.

References

- [1] Bryant M A and Pemberton J E 1991 *J. Am. Chem. Soc.* **113** 8284
- [2] Bain C D, Troughton E B, Tao Y T, Evall J, Whitesides G M and Nuzzo R G 1989 *J. Am. Chem. Soc.* **111** 321
- [3] Cousty J and Marchenko A 2002 *Surf. Sci.* **520** 128
- [4] Basarir F, Cuong N K, Song W K and Yoon T H 2007 *Macromol. Symp. (Wiley Inter. Sci.)* **249** 61
- [5] Cuong N K and Basarir F 2008 *Adv. Nat. Sci.* **9** 95
- [6] Cuong N K 2011 *J. Chem. (VAST)* **49** 585 (in Vietnamese)
- [7] Aryal S, Remant B K C, Dharmaraj N, Bhattarai N, Kim C H and Kim H Y 2006 *Spectrochim. Acta, A* **63** 160
- [8] Królikowska A, Kudelski A, Michota A and Bukowska J 2003 *Surf. Sci.* **532** 227
- [9] Bain C D, Evall J and Whitesides G M 1989 *J. Am. Chem. Soc.* **111** 7155