Journal of Physical Science, Vol. 26(2), 1–10, 2015

Fabrication of a New Amine Functionalised Bi-layered Gold/Silver SPR Sensor Chip

Mohammad Ghorbanpour*

Chemical Engineering Department, University of Mohaghegh Ardabili, P.O. Box 56199-11367, Ardabil, Iran

*Corresponding author: Ghorbanpour@uma.ac.ir

Abstract: *This work describes a protocol for producing 3-Aminopropyltriethoxysilane (APTES)-treated bi-layered gold/silver films on glass substrates without an intermediate chromium or titanium adhesive layer but with a high adherence of the metal layer to the glass as well as suitable optical properties for use as an Surface Plasmon Resonance (SPR) chip. Different media (aqueous, organic and vapour phases) were applied. The final products were analysed using SPR, Atomic Force Microscopy (AFM) and contact angle analysis methods. Finally, the amine accessibility of the final prepared silanised chip was measured. There were considerable differences in the morphology and accessibility of the APTES films prepared by the four methods. Films prepared by Dilute Vapor Phase Deposition (DVPD) were uniform and had many available reactive amines. Films prepared by the Organic Phase Deposition (OPD) method appeared to be the thickest and bore the most accessible amine groups. Films prepared by Aqueous Phase Deposition (APD) were thin, very stable and smooth but did not possess a high density of available reactive amines. Films prepared by the concentrate vapour-phase deposition method appeared to be relatively thick and had a medium number of accessible amine groups.*

Keywords: Bi-layer, gold, silver, surface plasmon resonance, 3-aminopropyl triethoxysilane

1. INTRODUCTION

Gold and silver are typically used as Surface Plasmon Resonance (SPR) transducing metals. Gold is generally preferred over silver because it is more chemically inert. An intrinsic defect of Ag is its high chemical instability. This instability means that a silver layer deteriorates easily upon contact with the air or certain chemicals. Various advances have been suggested to improve the stability of silver films, such as bimetallic Au/Ag layers[1–5] and coating with slight strata of various dielectrics (SiO_x, ITO, or SnO_2)[6–7] to protect the underlying silver film. In a previous study[2], our work on optimising the Au/Ag thickness using SPR analysis and stability tests showed that a 20/30 gold/silver composite results in better resolution, higher precision and a more stable SPR sensing chip.

The SPR method for bio-sensing requires the immobilisation of biomolecules onto the sensor surface. Various techniques have been used to functionalise metal transducer surfaces for biosensing. Direct covalent attachment of biomolecules with suitable functional groups[8], formation of Langmuir-Blodgett films,[9,10] self-assembled monolayers (SAMs) of alkanethioles with various terminal groups[11,12] and functionalised plasma-polymerised films[13,14] have all been reported for the immobilisation of biomolecules on metal surfaces.

Silanes can serve as coupling agents for attaching organic molecules to various substrates.[15,16] Among the varieties of organosilane, a popular choice is 3-aminopropyltriethoxysilane (APTES), which allows the further attachment of molecules through its terminal amines and exhibits self-assembly. The density and orientation of organosilane molecules are the main parameters that affect the silanisation reaction. This mechanism of reaction has been discussed in existing accounts. The choice of solvent strongly affects the density and shape of the covalently attached APTES layer.[15,17]

Previously, our group worked on the silanisation of gold chips with APTES.[18] This work describes a protocol for the production of APTES-treated gold/silver bimetallic SPR chips with high adherence of the metal layer to the glass substrate, good SPR response and suitable amine accessibility of the final prepared films.

2. EXPERIMENTAL

2.1 Materials

Toluene, NaCl, H_2O_2 (30% w/w) and H_2SO_4 (94 wt. %) were purchased from MERCK. 3-Aminopropyltriethoxysilane (APTES), bovine serum albumin and glutaraldehyde were purchased from Sigma-Aldrich. Triply distilled water was used throughout the experiment.

2.2 Bimetallic Chip Preparation

The glass substrates (microscope slides, soda-lime glass) were cut into 10 × 10 × 1 mm^3 pieces. The substrate surfaces were first thoroughly treated in piranha solution. Exposing the glass surface to piranha solution (3:1 vol. ratio, H_2SO_4: H_2O_2) for 30 minutes at 80°C cleans the surface. After chilling, the samples were rinsed with triply distilled water and dried under a nitrogen gas stream.

Au and Ag layers with thicknesses of approximately 20 and 30 nm were sputter-deposited on the glass slides using a BAL-TEC SCD 005DC sputtering system. No intermediate chromium or titanium layer was used.

2.3 Bimetallic Chip Silanisation

The silane films on the sputtered bimetallic layer were prepared by Wang and Vaughn[15] silanisation methods with slight modifications, as described previously.[18]

2.4 Surface Analysis

The surface topology and SPR response of the original and treated bimetallic films were analysed using a Nanoeducator AFM apparatus in non-contact mode and an Iranian commercial SPR instrument (Nano SPR). The sputtered metal thickness was studied using a DEKTAK3 (Veeco) instrument. The contact angle of water along the treated films was evaluated according to the ASTM D 724-99 test method at three different spots on each sample.

Amine accessibility was assayed by the immobilisation of biomolecules onto the bimetallic SPR surface. First, 5% aqueous glutaraldehyde was injected into the system for 20 minutes at a flow rate of 5 ml/minute(s) such that the amino groups on the airfoil of the chip could react with the aldehyde group. Then, the chips were washed repeatedly using the washing command of the system. Later in this treatment, 1 mg/ml BSA was injected into the system for 30 minutes at the same flow rate, and 0.1 M NaCl was injected for one minute at the same flow rate to wash out unbound protein.

3. RESULT AND DISCUSSION

3.1 Topography of Chips

The topography of the silanised chips was characterised by atomic force microscopy, and one image for each deposition method is displayed in Figure 1. There are appreciable differences between the films produced by each method. The surface of the parent bimetallic chip is relatively flat. Silanisation via the OPD, Concentrated Vapor Phase Deposition (CVPD) and DVPD methods induces changes in the surface roughness (Table 1). The AFM images of the surface covered with silane film via the OPD and CVPD methods exhibit increased roughness (13.56 and 13.56 nm) compared with the native chip surfaces (Figure 1[a]), whereas the APD films show no obvious change in roughness (Figure 1[b]).

Table 1: Effect of silanisation method on the average roughness (R_a) of the chips.

Sample	R_a (nm)
Parent bimetallic chips	11.49
CVPD	14.80
DVPD	12.56
OPD	13.56
APD	11.57

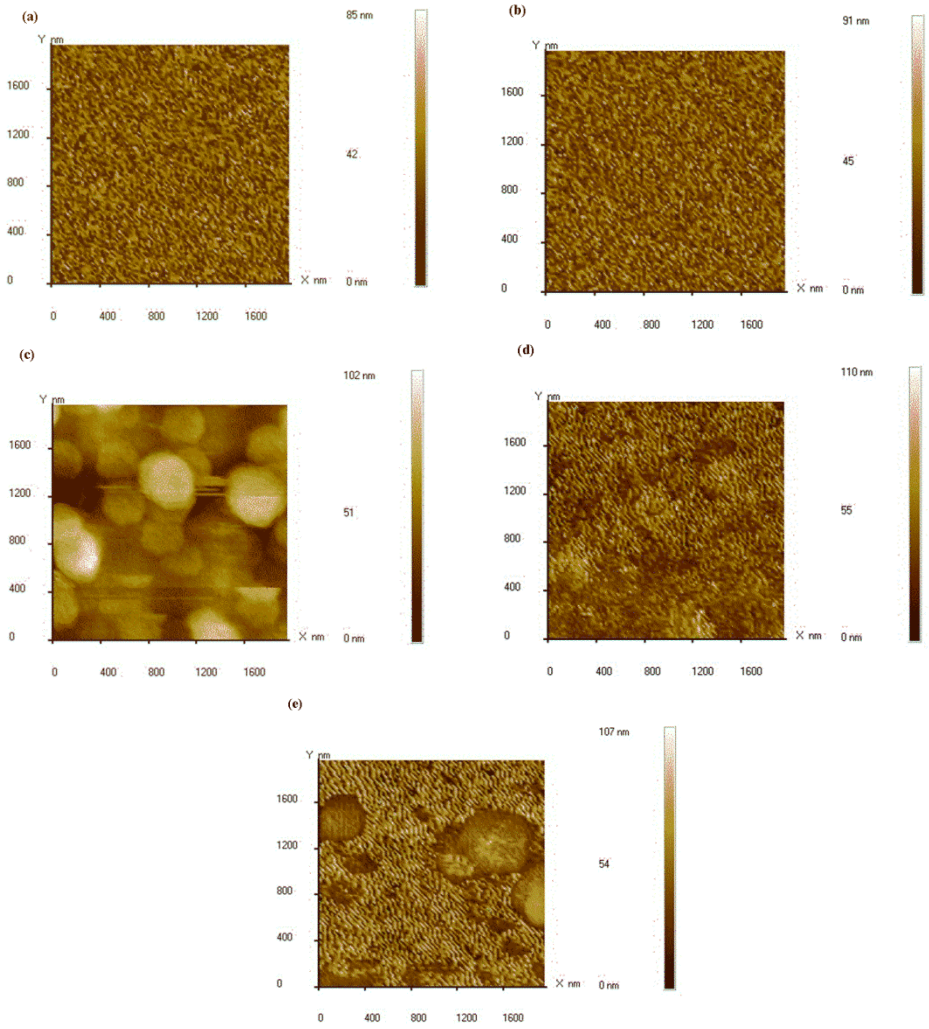

Figure 1: AFM images of bimetallic chips (a) silanised with APTES by (b) APD, (c) OPD, (d) CVPD and (e) DVPD methods.

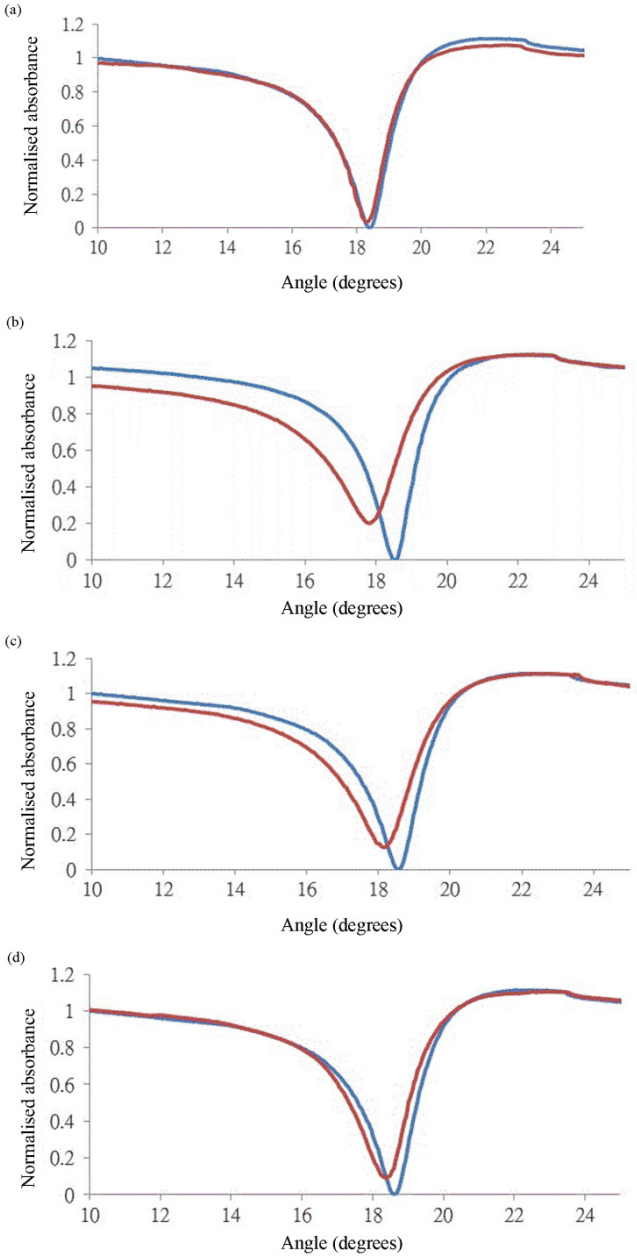

Figure 2: SPR response of sputtered thin Au/Ag films silanised with APTES by (a) APD, (b) OPD, (c) CVPD and (d) DVPD methods.

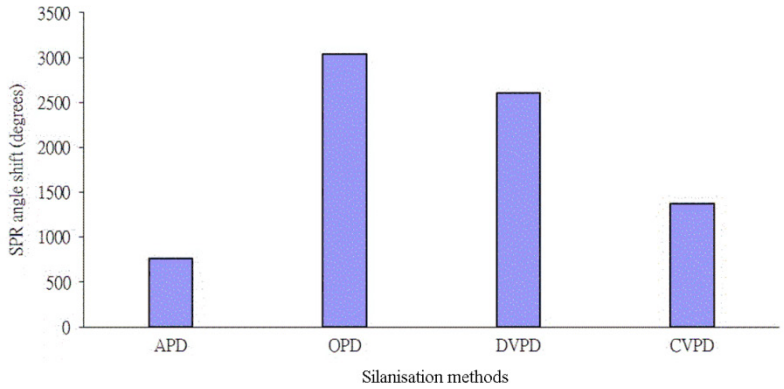

Figure 3: Amine accessibility for various silanisation methods.

3.2 SPR Analysis of Chips

Thin films of gold/silver bimetallic film were deposited on white glass slides without any Ti or Cr adhesion layer by sputtering at ambient temperature. The shape and lower limit of the SPR signal depended strongly on the thickness and overall roughness of the deposited bimetallic layer.[1,2] The effect of the silanisation method on the SPR response of the APTES-coated samples is shown in Figures 2(a) to 2(d). The alteration in the SPR angle and the percentage change in SPR depth are summarised in Table 2.

Table 2: Effect of silanisation method on the SPR response of Au/Ag coated glass slides.

Silanisation method	Resonance angle shift (°)	Minimum reflectivity change (%)
CVPD	0.46	15.76
DVPD	0.18	9.11
OPD	0.88	20.03
APD	0.14	3.90

The best SPR signal was observed for chips prepared via the APD method. This silanisation method, which caused small changes in the SPR response of the parent bimetallic chips (due to the silanisation reaction), is considered the optimal method. It is observed that the OPD method exhibits the largest decrease in SPR depth (20.03%) and angle (0.88°) with respect to the parent bimetallic substrate, which is most likely due to the increased surface roughness and the thickness of the silane film.

The SPR angle shift is related to the silane film thickness.[19] Accordingly, the APD method results in the minimal and OPD in the maximal layer thickness.

The CVPD and DVPD methods result in an intermediate layer thickness. Regarding the thickness, our results are consistent with the results reported by Wang and Vaughn[15] and Kim et al.[20] and with our own previous work.[18]

3.3 Contact Angle Measurement

The contact angles for various reaction conditions of APTES on the prepared chips are listed in Table 3. All cleaned glass slides were very hydrophilic, with a measured contact angle of 13°. The APTES layer increased the surface hydrophobicity, with contact angles ranging between 63.8° and 69.9°. The APD sample shows the minimal contact angle, i.e., 60.36°.

Table 3: Contact angles for various silanisation methods.

Silanisation method	Contact angle
CVPD	64.83 ± 2.73
DVPD	62.34 ± 1.49
OPD	63.23 ± 2.31
APD	60.36 ± 1.59

3.4 Amine Accessibility

Protein adsorption experiments were performed with bovine serum albumin as a representative example. Figure 3 shows the resonance angle shift induced by the immobilisation of BSA on silanised bimetallic chips that were produced via the APD, OPD and CVPD methods. It can be observed that the amine accessibility of the silane film produced via the OPD method is higher than that for the films created by the other methods. This figure also shows that the APD method produces the smallest resonance angle shift. The amine accessibility of the silane film produced via DVPD is very similar to the OPD film. These results show that the chips produced via all four methods are able to bind with biomolecules.

Because APTES forms an internal zwitterion in water, anhydrous solvent is applied to obtain a more uniform monolayer deposition. In this form, the amine group is aimed away from the bulk sample (hydrophobic gold) and thus points toward the liquid or gas phase, leaving free amine groups available for further functionalisation. Nevertheless, obtaining a uniform monolayer with the amine groups oriented away from the underlying substrate can be a complex matter because APTES is very sensitive to a number of reaction conditions. Horizontal polymerisation can occur when ethoxy groups are hydrolysed due to the water present in the organisation. The resulting silanol moieties can then react with

each other via a condensation reaction to produce silicon bonds. Vertical multi-layering can also occur when APTES molecules physisorb to each other on an already APTES-treated surface.[15]

Knowledge of the density of available reactive groups on a functionalised surface is important for many applications. Wang and Vaughn established a larger set of accessible amino groups on films prepared by vapour-phase deposition compared with films prepared by solution deposition.[15] According to their findings, the dilute vapour phase deposition technique produced relatively few domains. Wang and Vaughn also indicate that such differences arise from differences in film rearrangement during annealing. The differences between their results and ours seemed to arise from differences in the substrates used. Our substrate was hydrophobic gold, whereas theirs was hydrophilic glass.

3.5 Stability Tests

A simple qualitative test was used to examine the adhesion of Au/Ag films. A piece of adhesive tape was firmly placed over the flake and the surrounding glass substrate and was then withdrawn. The fraction of the film that was transferred to the tape then provided a relative measure of the film adhesion. Sputtered gold film onto glass substrates was completely removed from the chip. In fact, such a film was removed by simply rinsing the slide with water. No transfer to the tape was observed when any 20 nm silver under layer was applied. This result implies that the samples produced via all four methods passed stability tests.

4. CONCLUSIONS

There were considerable differences in the morphology and accessibility of APTES films prepared by the four methods. The films prepared by DVPD were uniform and relatively static and had many available reactive amines. The films prepared by the OPD method appeared to be the thickest and bore the most accessible amine groups. The films prepared by APD were thin, very stable and smooth but did not possess a high density of available reactive amines. The films prepared from the concentrate vapour-phase deposition method appeared to be relatively thick and had a medium number of accessible amine groups. For bioconjugation or macromolecule immobilisation applications, the dilute vapour-phase deposition method produced chips that were uniform and relatively stable and provided a high density of accessible amino groups.

5. REFERENCES

1. Ghorbanpour, M. & Falamaki, C. (2013). A novel method for the production of highly adherent Au layers on glass substrates used in surface plasmon resonance analysis: Substitution of Cr or Ti intermediate layers with Ag layer followed by an optimal annealing treatment. *Journal of Nanostructure in Chemistry*, 3(66), 1–7.
2. Ghorbanpour, M. (2013). Optimization of sensitivity and stability of Au/Ag bilayer thin films used in surface plasmon resonance chips. *Journal of NanoStructures*, 3, 309–313.
3. Ghorbanpour, M. & Falamaki, C. (2012). Micro energy dispersive x-ray fluorescence as a powerful complementary technique for the analysis of bimetallic Au/Ag/glass nanolayer composites used in surface plasmon resonance sensors. *Appl. Opt.*, 51(32), 7733–7738.
4. Yuan, X. C. et al. (2006). Sensitivity–stability-optimized surface plasmon resonance sensing with double metal layers. *J. Opt. A: Pure Appl. Opt.*, 8(11), 959–963.
5. Zynio, S. A. et al. (2002). Bimetallic layers increase sensitivity of affinity sensors based on surface plasmon resonance. *Sensors*, 2(2), 62–70.
6. Manesse, M. et al. (2008). Preparation and characterization of antimony-doped SnO_2 thin films on silver and silver substrates for electrochemical and surface plasmon resonance studies. *Electrochem. Commun.*, 10(7), 1041–1043.
7. Szunerits, S. & Boukherroub, R. (2006). Electrochemical investigation of a novel interface for electrochemical surface plasmon resonance studies (E-SPR). *Electrochem. Commun.*, 8, 439–444.
8. Homola, J. (2008). Surface plasmon resonance sensors for detection of chemical and biological species. *Chem. Rev.*, 108(2), 462−493.
9. Hou, Y. et al. (2006). Immobilization of rhodopsin on a self-assembled multilayer and its specific detection by electrochemical impedance spectroscopy. *Biosens. Bioelectron.*, 21(7), 1393–1402.
10. Davis, F. & Higson, S. P. J. (2005). Structured thin films as functional components within biosensors. *Biosens. Bioelectron.*, 21(1), 1–20.
11. Arya, S. S. K. et al. (2006). Application of octadecanethiol self-assembled monolayer to cholesterol biosensor based on surface plasmon resonance technique. *Talanta*, 69(4), 918–926.
12. Lee, J. W. et al. (2005). Characterization of a self-assembled monolayer of thiol on a gold surface and the fabrication of a biosensor chip based on surface plasmon resonance for detecting anti-GAD antibody. *Biosens. Bioelectron.*, 20(7), 1422–1427.
13. Szunerits, S. & Boukherroub, R. (2006). Preparation and characterization of thin films of SiO_x on gold substrates for surface plasmon resonance studies. *Langmuir*, 22(4), 1660–1663.

14. Szunerits, S. et al. (2006). Stability of the gold/silica thin film interface: Electrochemical and surface plasmon resonance studies. *Langmuir*, 22(25), 10716–10722.
15. Wang, W. & Vaughn, M. W. (2008). Morphology and amine accessibility of (3-aminopropyl) triethoxysilane films on glass surfaces. *Scanning*, 30(2), 65–77.
16. Weiping, Q. et al. (1999). Site-directed immobilization of immunoglobulin G on 3-aminopropyltriethoxylsilane modified silicon wafer surfaces. *Mater. Sci. Eng. C*, 8, 475–480.
17. Acres, R. G. et al. (2012). Molecular structure of 3-aminopropyltriethoxysilane layers formed on silanol-terminated silicon surfaces. *J. Phys. Chem. C*, 116(10), 6289−6297.
18. Ghorbanpour, M. & Falamaki, C. (2014). A novel method for the fabrication of ATPES silanized SPR sensor chips: Exclusion of Cr or Ti intermediate layers and optimization of optical/adherence properties. *Appl. Surf. Sci.*, 301, 544–550.
19. Wijaya, E. et al. (2011). Surface plasmon resonance-based biosensors: From the development of different SPR structures to novel surface functionalization strategies. *Curr. Opin. Solid State Mater. Sci.*, 15(5), 208–224.
20. Kim, J., Seidler, P., Wan Sze L. & Fill, C. (2009). Formation, structure, and reactivity of amino-terminated organic films on silicon substrates. *J. Colloid Interface Sci.*, 329(1), 114–119.