

Gold nanoparticles assisted characterization of amine functionalized polystyrene multiwell plate and glass slide surfaces

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Abstract We demonstrated citrate-capped gold nanoparticles assisted characterization of amine functionalized polystyrene plate and glass slide surfaces through AuNPs staining method. The effect of AuNPs concentration on the characterization of amine modified surfaces was also studied with different concentration of AuNPs (ratios 1.0–0.0). 3-Aminopropyl triethoxy silane has been used as amine group source for the surface modification. The interactions of AuNPs on modified and unmodified surfaces were investigated using atomic force microscopy and the dispersibility, and the aggregation of AuNPs was analyzed using UV–visible spectrophotometer. Water contact angle measurement and X-ray photoelectron spectroscopy (XPS) were used to further confirmation of amine modified surfaces. The aggregation of AuNPs in modified multiwell plate leads to the color change from red to purple and they are found to be adsorbed on the modified surfaces. Aggregation and adsorption of AuNPs on the modified surfaces through the electrostatic interactions and the hydrogen bonds were revealed by XPS analysis. Remarkable results were found even in the very low concentration of AuNPs (ratio 0.2). This AuNPs staining method is simple, cost-effective, less time consuming, and required very low concentration of AuNPs. These results can be read out through the naked eye without the help of sophisticated equipments.

Keywords Polystyrene multiwell plate · Glass slides · Amine functionalization · Gold nanoparticles interaction

Introduction

Immobilization of biomolecules on the solid surface was the key step in the diagnosis of infectious and genetic diseases, analysis of gene expression, forensic science, toxicology, drug discovery, biosensing, and environmental monitoring (Kannoujia et al. 2010; North et al. 2010). In general, polystyrene (PS), polypropylene, polycarbonate, and glass slide (GS) have been used as a solid materials for the immobilization of biomolecules (DNA and protein) because of their versatile chemical, physical, and surface adherence properties. Among these, PS and GS are most commonly used solid materials because they readily adsorb to DNA and proteins (antibodies or antigens) via non-covalent interactions (Chu et al. 2005), excellent optical as well as mechanical properties, and cost-effective (North et al. 2010). However, the non-covalent interactions of DNA and proteins on their surfaces also have limitations like desorption during the time of washing, poor recognizing properties, protein denaturation, loss of bioactivity, and low sensitivity (Rebeski et al. 1999; Butler 2000). To overcome these problems, covalent immobilization protocols have been developed and it ensured with the strong attachment and displaying a reorganization site of interest with respect to its target (Goddard and Hotchkiss 2007). Covalent immobilization of biomolecules on PS and GS surfaces can be achieved using a variety of techniques including wet chemical treatments, silane monolayers, plasmas, flame, and UV irradiation (Goddard and Hotchkiss 2007). Among these, silane monolayer using

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3-aminopropyl triethoxy silane (APTES), 3-aminopropyl trimethoxy silane (APTMS), N-[3-(trimethoxysilyl) propyl]ethylenediamine (TMSPED), and 3-glycidoxy propyl trimethoxy silane (GOPS) for the functionalization of the amine and the epoxy groups on the PS plate and the GS surfaces (Huang et al. 2012; Moirangthem et al. 2012; Wang and Vaughn 2008; Yang et al. 2005; Lin et al. 2006; Kobayashi et al. 2007; Dixit et al. 2011). APTES functionalized PS plates and GS are used in the immobilization of antibodies for the detection of antigens. Similarly, oligonucleotide probes/DNA were used to detect target DNA sequences and single nucleotide polymorphisms (SNP) typing and assaying in the protein kinases activity (Dixit et al. 2010; Qin et al. 2007; Pack et al. 2007; Hirayama et al. 1996; Nikiforov et al. 1994; Kim et al. 2008).

The presence of functional groups and chemical status of elements, rough and smooth surface, hydrophobic and hydrophilic properties of materials was characterized by using X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and water contact angle (WCA) measurements (North et al. 2010; Qin et al. 2007; Idage and Badrinarayanan 1998). However, these characterization methods are cost-effective, time consuming and they are not affordable for routine modification process in the view of commercialization. Therefore, an essential need to develop a simple and effective characterization method was considered as an important one. The use of AuNPs to characterize the amine modified GS surfaces through the staining method was recently reported (Carré and Lacarrière 2007). Herein, we have demonstrated citrate-capped AuNPs assisted characterization of amine modified PS plate and GS surfaces based on the AuNPs aggregation, color changes and adsorption. This characterization was completed within 1 h and the results could be read out by the naked eye without the help of any sophisticated equipments.

Materials and methods

Materials

Sodium citrate was obtained from Sisco Research Laboratories Pvt. Ltd (Mumbai, India). PS 96-multiwell plate, nitric acid (HNO₃), sulphuric acid (H₂SO₄), and hydrogen peroxide (H₂O₂) were purchased from Thermo Fisher Scientific India Pvt. Ltd (Mumbai, India). Hydrogen tetrachloroaurate trihydrate (HAuCl₄•4H₂O) was purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). The glass slides were purchased from HIMedia Laboratories Pvt. Ltd (Mumbai, India), GOPS and APTES were obtained from Sigma Aldrich Co. (St. Louis, USA).

Synthesis of citrate-capped AuNPs

Citrate-capped AuNPs were synthesized based on the early described boiling method (Grabar et al. 1995). In brief, 100 mL of 1 mM HAuCl₄ was heated and added 10 mL of 38.8 mM sodium citrate at the boiling point and this solution was stirred continuously for 15 min. The solution was cooled at room temperature and characterized by UV–visible spectrophotometer (Shimadzu UV-1600, Japan) and HR-TEM [Model Fei Technai G² F30 S-TWIN with 250 kV high-resolution (UHR) pole piece].

Amine modification on PS plate and GS surfaces

Functionalization of amine group on the surface of PS plate and GS was carried out with earlier described methods (Kaur and Raman Suri 2007; Ji et al. 2011). The plates were treated with freshly prepared HNO₃ and H₂SO₄ mixtures (47:53 ratio, 250 μL/well) at room temperature for 30 min in a fume hood with constant shaking. The plates were washed three times with ddH₂O followed by the addition of 5 % of APTES (250 μL/well, pH 6.9) and incubated at room temperature for 2 h. The excess APTES was washed three times with ddH₂O and incubated at 60 °C for 2 h to enhance the APTES binding over the PS surface. Then the modified plates were kept at 4 °C for overnight before to use.

GS was first treated with piranha solution (H₂O₂/H₂SO₄, 30:70 ratio) for 12 h, washed thoroughly with ddH₂O and dried under a stream of N₂ gas. For the silanization, the treated GSs were immersed in a solution containing 1 % of AEPTS for 30 min at room temperature. After the incubation, the slides were rinsed with ddH₂O, dried under a stream of N₂ gas and the slides were kept for 30 min at 120 °C.

AuNPs staining for the characterization of amine modified PS plate and GS surfaces

Citrate-capped AuNPs solution (250 μL) was added to each well in amine modified PS plate and kept at room temperature for 1 h. The color changes were observed and the optical absorbance was measured using with UV–visible spectrophotometer. The modified GS was immersed in AuNPs solution for 1 h and rinsed with ddH₂O then dried at room temperature. The stained slides were scanned using a cannon flatbed scanner LiDE 110. Modified GS treated with AuNPs solution was also analyzed by UV–visible spectrophotometer along with AuNPs with unmodified GS as a control. Different ratios of AuNPs (ratios: 1.0, 0.8, 0.6, 0.4, 0.2 and 0.0) were prepared with ddH₂O to investigate the effect of AuNPs concentrations on this characterization method. In detail, 250 μL of different ratios of AuNPs

solutions was added to each well in amine modified and unmodified PS plate then incubated for 1 h at room temperature. The color changes were observed and corresponding optical absorbance of AuNPs solutions was analyzed by UV–visible spectrophotometer. Similarly, 1 cm × 2.5 cm pieces of amine modified GS were incubated with 3 mL of different ratios of AuNPs solutions (ratio 1.0–0.0) for 1 h at room temperature. After the incubation, GS pieces were removed from the AuNPs solutions then rinsed with ddH₂O and dried at room temperature. Air dried GS pieces were scanned using cannon flatbed scanner. Simultaneously GS pieces treated with AuNPs solutions were analyzed by UV–visible spectrophotometer along with the unmodified GS piece treated with different ratios of AuNPs solutions as control.

WCA measurement

WCA measurement was carried out using the static sessile drop technique. Contact angle measurements were performed at room temperature using a goniometer (GBX Digidrop, France) equipped with a microsyringe helps to control the volume of liquid drop (2 μL). Four water drops were placed at different locations on modified and the unmodified surfaces of PS plates and GS. Average contact angles were measured and images were recorded.

XPS and AFM analyses

Chemical composition of modified and AuNPs stained surfaces of PS plates and GS was revealed by XPS analysis. The samples were prepared in 1 × 0.4 cm size of the GS and the bottom of the well in PS plates. Prepared samples were placed on the sample holder and transferred

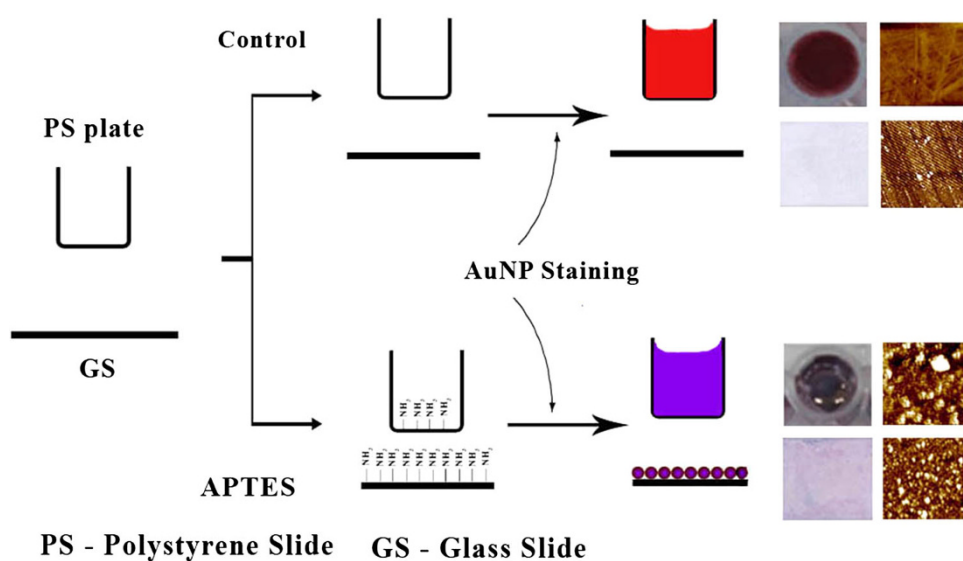
to analysis chamber under ultra high vacuum (UHV) of 3×10^{-10} mbar. Elements of modified surfaces were analyzed by the OMICRON™ XPS system (Omicron Nano Technology GmbH, Germany) using monochromatic Al K α X-ray source operated at 300 W (anode at 15 kV and emission at 20 mA). Survey the XPS data were recorded with using pass energy of 50 eV and sleep size 0.5 eV with 0.2 s dwell time. High-resolution scans were recorded with pass energy of 20 eV, sleep size 0.03 eV, and dwell time of 0.2 s with three sweeps. The recorded spectrum data analyses and multiple curve fitting were performed using Casa XPS software. Topology of modified and AuNPs stained PS plate and GS surfaces was analyzed using AFM (Park AFM System XE 70, South Korea) operated in a set frequency of 273.73 Hz with NC-AFM head mode.

Results and discussion

Synthesis and characterization of citrate-capped AuNPs

The amine functionalized PS plate and GS surfaces were characterized by using citrate-capped AuNPs based on their aggregation, color changes and adsorption were illustrated as schematic representation in Fig. 1. AuNPs were synthesized by earlier described boiling method (Grabar et al. 1995). The yellow color reaction solution was gradually changed to red color. It indicates that the formation of AuNPs and the corresponding SPR band of NPs was found at 520 nm in UV–visible spectra as shown in Fig. 2. The inset of the Fig. 2 (TEM image) shows the spherical shape of the NPs with an average diameter of 13 nm. The reduction of Au³⁺ ion to Au⁰ metal and control

Fig. 1 Schematic representation of the characterization of amine modified PS plate and GS surfaces using citrate-capped AuNPs



the further nucleation growth was achieved using sodium citrate because it acts as reducing and capping agent. The synthesized citrate-capped AuNPs were used for further studies.

WCA measurement of amine modified PS plate and GS surfaces

Amine group was functionalized on PS plate and GS surfaces through the silane monolayer method using APTES as a source of amine group. Nitro group was introduced on the benzene of PS after treated with a nitrating mixture (HNO_3 and H_2SO_4) and GS surfaces were treated with piranha solution. Then the treated surfaces were functionalized with amine functional group through the condensation reaction with organosilane (APTES) (Kaur and Raman Suri 2007; Ji et al. 2011). The hydrophobic and hydrophilic properties of modified and unmodified PS plate and GS

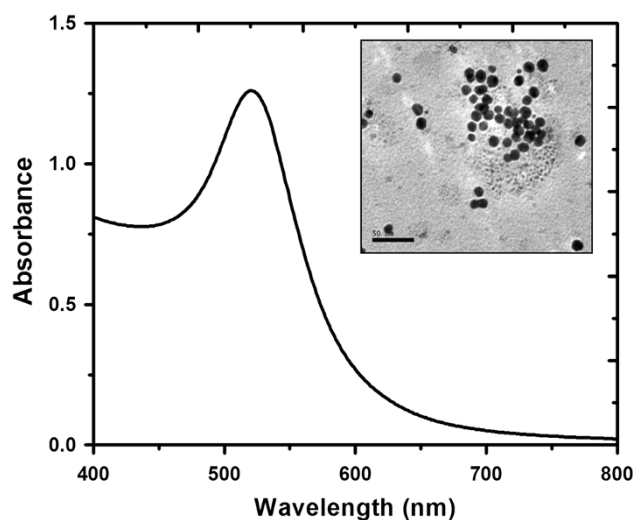
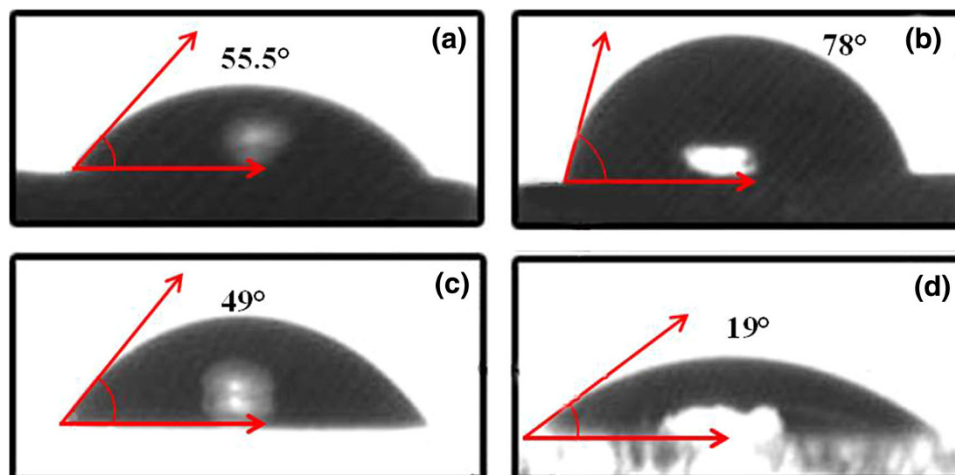


Fig. 2 UV–visible absorption spectra and the corresponding TEM image of synthesized citrate-capped AuNPs in aqueous medium

Fig. 3 Photographic images of water contact angle measurements of modified and unmodified PS plate and GS surfaces. **a** Amine modified PS plate surface, **b** unmodified PS plate surface, **c** amine modified GS surface, and **d** unmodified GS surface



surfaces were characterized using with WCA measurement method.

Figure 3a, b shows that the average WCA of the modified and unmodified PS plate surfaces was found to be 55.5° and 78° , respectively. The contact angle of water on the modified surface was lower than the unmodified surface. This could be due to the decrease of the hydrophobicity of PS plate surfaces by the amine modification, which facilitates the interaction of water molecules with amine group of the modified surfaces through hydrogen bonds. It proves that the PS plate surfaces were modified with the amine group (North et al. 2010). Similarly, Fig. 3c, d shows that the average contact angle of water on the amine modified and unmodified GS surfaces was found to be 49° and 19° , respectively. WCA of modified surface was higher than the unmodified one. This could be due to the increase of the hydrophobicity as well as decrease of the wettability of the surface by the presence of aliphatic carbon chains (ethyl and propyl stretches) as a result of silanation (Qin et al. 2007).

AuNPs staining of amine modified PS plate and GS surfaces

Amine modified PS plate and GS surfaces were investigated through AuNPs staining method. Figure 4a shows SPR band of AuNPs present in the unmodified multiwell PS plate which was found at 525 nm (black line) and SPR band was shifted towards the longer wavelength with the broadening of peak which was found in amine modified plate (red line). This could be due to the absence of AuNPs aggregation in the unmodified multiwell plate as evidenced from the un-changes in the color of the solution. The shift of the SPR band confirms that the AuNPs were getting aggregated in the amine functionalized plates and the color of the solution changes from red to blue as shown in the inset image of Fig. 4a. The mode of AuNPs aggregation in

Fig. 4 **a** UV–visible spectra of AuNPs in the unmodified PS plate (*black line*) and amine modified PS plate (*red line*) and **b** scanned image of control and AuNPs stained amine modified PS plate surface, respectively

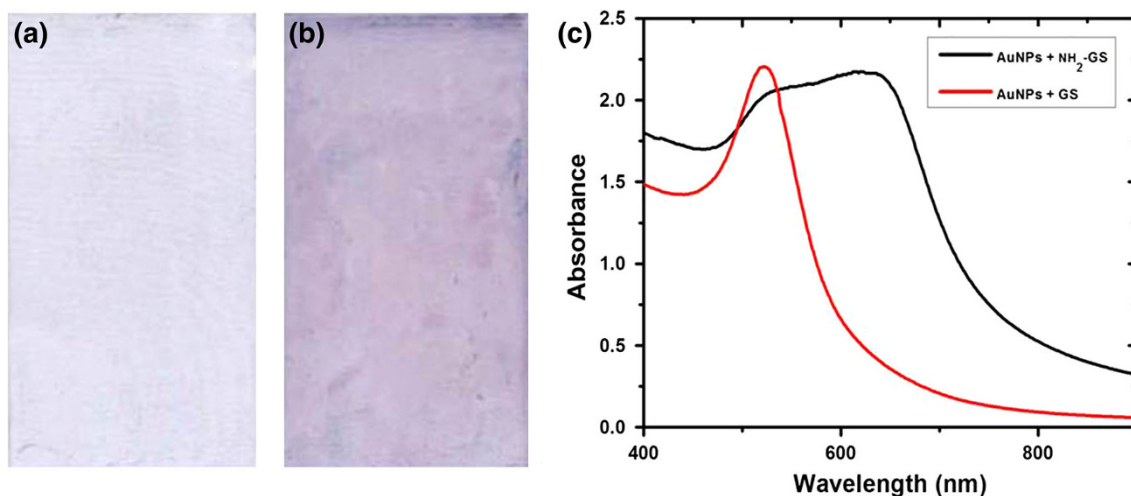
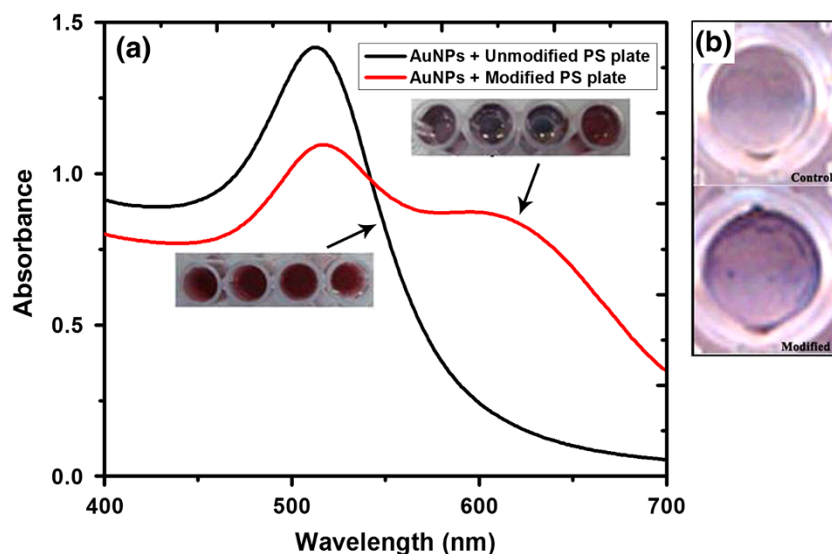


Fig. 5 Scanned images of AuNPs stained unmodified GS surface (**a**) and amine modified GS surface (**b**). (**c**) UV–visible absorbance spectra of AuNPs with modified GS (*black curve*) and unmodified GS (*red curve*)

modified plate might be due to the screening of electro-negative repulsion among the NPs by protonated amine groups present on the modified plate surface. Also, AuNPs were weakly adsorbed on the modified surface through the electrostatic interactions and the hydrogen bonds between the positively charged amine groups and the negatively charged AuNPs as shown in Fig. 4b. In the case of unmodified plate, AuNPs were not adsorbed on the surface (Moirangthem et al. 2012; Carré and Lacarrière 2007; Fujiwara et al. 2006). Figure 5a, b shows that the AuNPs stained modified and unmodified GS surface, respectively. Purple staining was observed only on the amine modified GS surface but not on the unmodified one, and corresponding UV–visible absorbance spectra of AuNPs treated with modified and unmodified GS were also taken (Fig. 5c). AuNPs with unmodified GS show a sharp SPR

band at 522 nm without any shift towards the longer wavelength region (Fig. 5c curve–red). But, the absorbance spectra of AuNPs with amine modified GS show a broadening peak towards the longer wavelength (above 600 nm) was shown in Fig. 5c (curve – black). These absorbance spectra prove that AuNPs dispersed with unmodified GS and aggregation with modified GS. Aggregation of AuNPs mainly caused by the protonated amine group present on the modified GS surface (Carré and Lacarrière 2007; Fujiwara et al. 2006; Huang et al. 2012).

The effect of AuNPs concentration on the characterization of amine modified surfaces was also revealed. The different concentrations of AuNPs were prepared by the dilution with water (1.0, 0.8, 0.6, 0.4, 0.2, and 0.0) and were treated with amine modified/unmodified PS plate and GS. The color of AuNPs solution was changed from red to

purple and blue in amine modified PS multiwell plate while decreasing the concentration of AuNPs from the ratio of 1.0 to 0.2 was illustrated in Fig. 6a. From red to purple color change was observed in the ratio from 1.0 to 0.6 and red to light blue color shift was found in the 0.2 ratio of AuNPs. The intermediate color between purple and blue was found in the ratio of 0.4 and no more color was found in the ratio 0.0 of AuNPs. But the case of AuNPs solutions in the unmodified PS multiwell plate shows that the gradual decrease of intensity of red color without any color changes, while decreasing the ratio of AuNPs from 1.0 to 0.0 (Fig. 6a). These color changes of AuNPs in the amine modified PS plate might be due to the aggregation of AuNPs caused by protonated amine group present on the surface. But in the unmodified PS plate, the AuNPs solution was remained in red color without any color changes and this could be due to the absence of the amine group on the unmodified plate surface as a result AuNPs not aggregated and present as such individual particles. In addition, UV–visible absorbance spectra of AuNPs with unmodified and modified PS plate were also taken for further confirmation. Figure 6b shows the maximum absorbance and a sharp SPR band of different ratios of AuNPs (from 1.0 to 0.0) with the unmodified PS plate were found to be at 522 nm. The intensity of the peak was decreased (OD: 0.76, 0.69, 0.49, 0.25, and 0.12) with decreasing the ratio of AuNPs from 1.0 to 0.2, respectively,

and no peak was found in 0.0 ratio. But an optical absorbance spectra of AuNPs from the amine modified PS plate show broadening peak towards the longer wavelengths (more than 650 nm), which corresponds to the purple and blue colors of aggregated AuNPs. In Fig. 6c, UV–visible absorbance spectra show the low intensity peak that was found at 522 nm and high intensity peak at more than 650 nm. These results confirm that AuNPs aggregation occurred only in amine modified PS plate but not in unmodified plate and also proved that even the very low concentration of AuNPs (ratio 0.2) enough to characterize the modified PS plate surface. The color changes can be observed through naked eye and to be revealed by UV–visible spectrophotometer.

Similarly, the amine modified and unmodified GS surfaces were also characterized by gold staining with different ratios of AuNPs (1.0–0.0). An intensity of AuNPs staining on the amine modified surface was gradually decreased while decreasing the ratio of AuNPs from 1.0 to 0.2 shown in Fig. 7a. Strong pinkish purple color stain was observed on the modified surface treated with 1.0 ratio of AuNPs which was shifted to purple color when decreasing the AuNPs ratio to 0.8 and finally the shift was reached to light blue color in 0.2 ratio of AuNPs. The color of staining was not observed in 0.0 ratio of AuNPs. These could be due to the aggregation and adsorption of AuNPs on the amine modified surfaces by electrostatic interaction

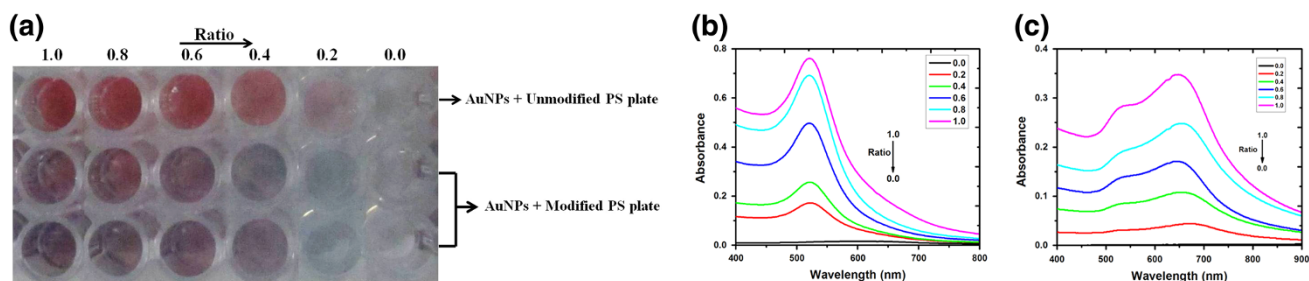


Fig. 6 (a) Characterization of amine modified and unmodified PS plate with different ratios of AuNPs (1.0, 0.8, 0.6, 0.4, 0.2, and 0.0). UV–visible absorbance spectra of different ratios of AuNPs with unmodified PS plate (b), and amine modified PS plate (c)

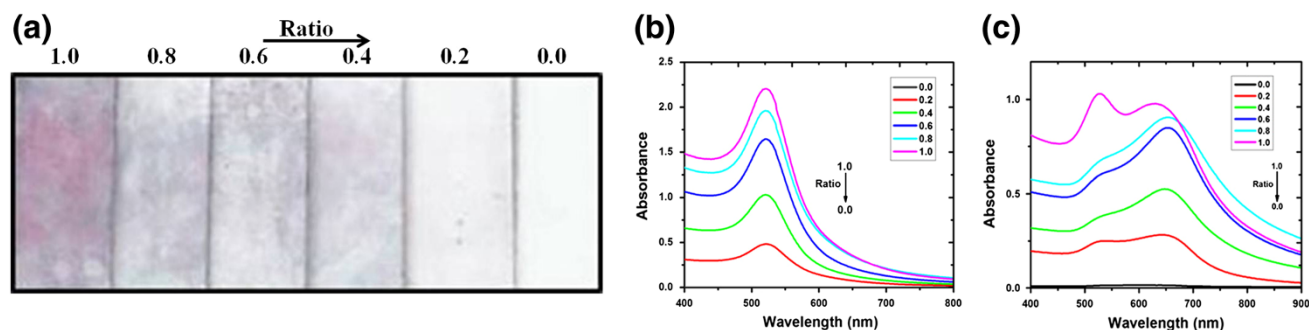


Fig. 7 a Characterization of amine modified GS surface using different ratios of AuNPs (1.0, 0.8, 0.6, 0.4, 0.2, and 0.0) through gold staining. UV–visible spectra of different ratios of AuNPs treated with unmodified GS b, c amine modified GS

between the protonated amine and the negative charged AuNPs. Where the AuNPs ratio is too low, the NPs easily get aggregated and adsorbed on the modified surface due to the presence of protonated amine group. The number of individual particles adsorbed on the modified surface was decreased by decreasing the ratio of AuNPs from 1.0 to 0.2 as a result less number of particles were adsorbed on the modified surface in the lower ratio of AuNPs (ratio 0.2), finally all particles get aggregated. Due to the huge population of NPs at higher concentration (ratio 1.0), complete aggregation of AuNPs could not be possible by defined modified surface of GS as a result more number of individual NPs adsorbed on the surface which make the AuNPs stained GS appeared in pinkish purple color. However, noticeable blue color stain was clearly found up to 0.4 ratios of AuNPs but not in the ratio 0.2 and below.

The optical absorbance of AuNPs solutions with unmodified and modified GS were measured by UV–visible spectrophotometer. In Fig. 7b, UV–visible spectra of different ratios of AuNPs with unmodified GS show the sharp SRP band at 521 nm without any shift. At the same time the peak intensity was decreased with decreasing the ratio of AuNPs from 1.0 to 0.2, and 0.0, respectively. In the case of AuNPs with amine modified GS indicates the shift of the SPR band towards the longer wavelengths (more than 600 nm) with broadening peaks were shown in Fig. 7c. These could be due to the dispersion and aggregation of AuNPs by the absence and presence of the amine group on the unmodified and the modified GS surfaces, respectively. The remarkable spectral shift was found up to 0.2 ratios of AuNPs with modified GS. These results were

similar to the findings of different ratios of AuNPs with modified and unmodified PS plate.

XPS characterization of amine modified and AuNPs stained PS plate and GS surfaces

The elements present on amine modified and AuNPs stained PS plates and GS surfaces were analyzed by using XPS and are summarized in Table 1. Figure 8a depicts the XPS survey spectra of amine modified PS plate surface and it indicates the presence of N, Si, C, and O. High-resolution spectra of C 1s show peaks at 285.2, 286.5, and 288.9 eV which were characteristic features of C–C/C–H, C–N, and C–O, respectively (Fig. 8b). Peak at 400.3 eV in N 1s spectra indicates the presence of protonated amine (NH_3^+) and O 1s spectra show that binding energy peak at 532.4 eV was specific for O–Si chemical bond (Fig. 8c, d). Appearance of binding energy peak at 103.6 eV was characteristic of Si–O/Si–C chemical composition found in Si 2p spectra (Fig. 8e). These characteristic features of XPS spectra prove the presence of the amine group on the PS plate surface. This could be the reason for AuNPs aggregation and adsorption as well as decreases the hydrophobicity of PS plates (Flores-Perez and Ivanisevic 2007; Durdureanu-Angheluta et al. 2012).

XPS survey spectra of AuNPs stained aminated PS plates (Fig. 9a) illustrate the presence of N, C, Si, Au, and O elements and binding energy 1 eV was subtracted for the carbon charge correction from the obtained spectra for curve fitting. The binding energy peaks at 285.1, 287.1, and 288.7 eV were found in C 1s spectra and they were

Table 1 XPS binding energy peaks of amine modified and AuNPs stained PS plate and GS surfaces with their possible assignments

Amine functionalized PS plate surface							
Elements	C 1s			N 1s	O 1s	Si 2p	
Binding energy (eV)	285.2	286.5	288.9	400.3	532.4	103.6	
Types of assignments	C–C/C–H	C–N	C–O	NH_3^+	O–Si	Si–O/Si–C	
Relative atomic concentration (%)	73.05			6.60	16.53	3.83	
AuNPs stained amine functionalized PS plate surface							
Elements	C 1s			N 1s	Si 2p	Au 4f	
Binding energy (eV)	285.1	287.1	288.7	400.5	102.8	83.8	87.4
Types of assignments	C–C/C–H	C–N	C–O	$\text{NH}_2/\text{NH}_3^+\text{–C}$	Si–O	Au 4f7/2	Au 4f5/2
Amine functionalized GS plate surface							
Elements	C 1s			N 1s	O 1s	Si 2p	
Binding energy (eV)	284.4	285.4	287.0	400.5	532.4	103.3	
Types of assignments	C–C	C–N	HN–CO	NH_3^+	O–Si	Si–O	
Relative atomic concentration (%)	53.67			1.25	24.04	21.04	
AuNPs stained amine functionalized PS plate surface							
Elements	C 1s			N 1s	Si 2p	Au 4f	
Binding energy (eV)	283.9	285.6	288.3	400.0	102.6	84.3	88.0
Types of assignments	C–C	C–N	HN–CO	$\text{NH}_2/\text{NH}_3^+$	Si–O	Au 4f7/2	Au 4f5/2

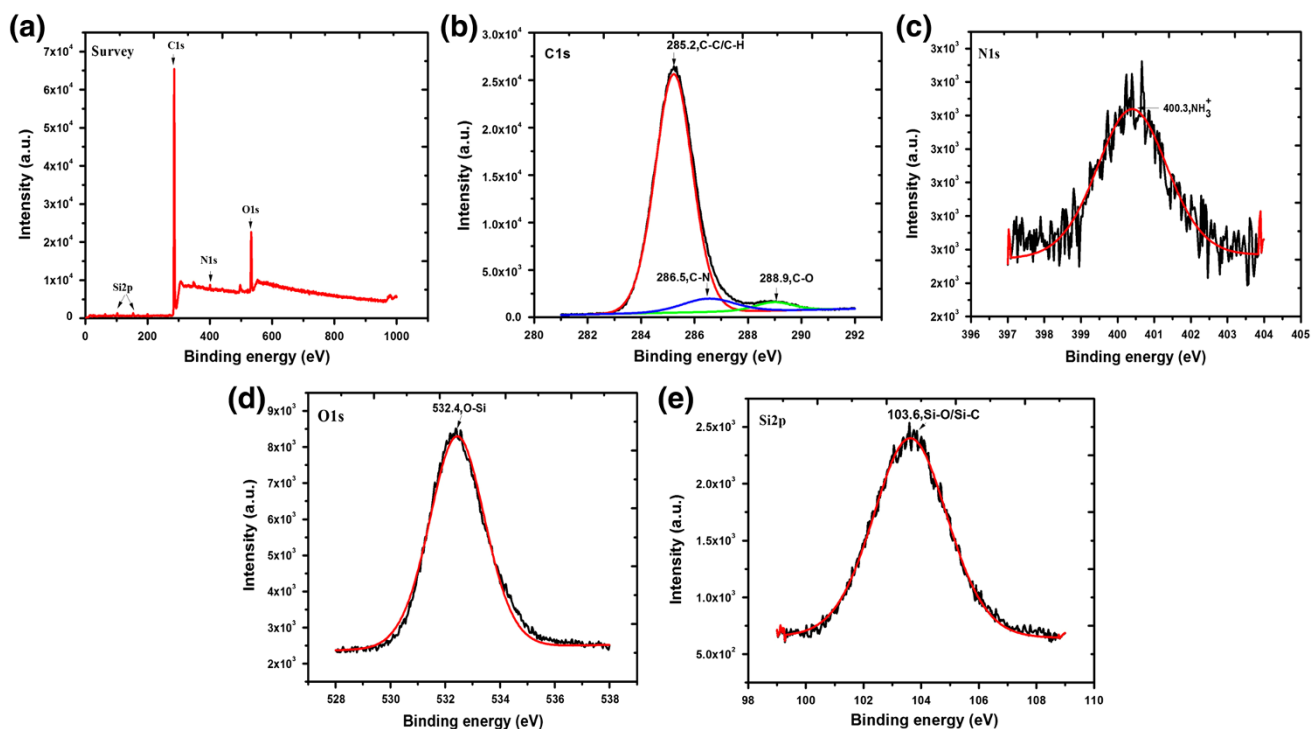


Fig. 8 XPS spectra of amine modified PS plate. **a** Survey scan, high-resolution scan in the **b** C 1s region, **c** N 1s region, **d** O 1s region, and **e** Si 2p region. N 1s, Si 2p and O 1s spectra indicate that the presence

of organosilane with the amine functional group on the APTES treated PS plate surface

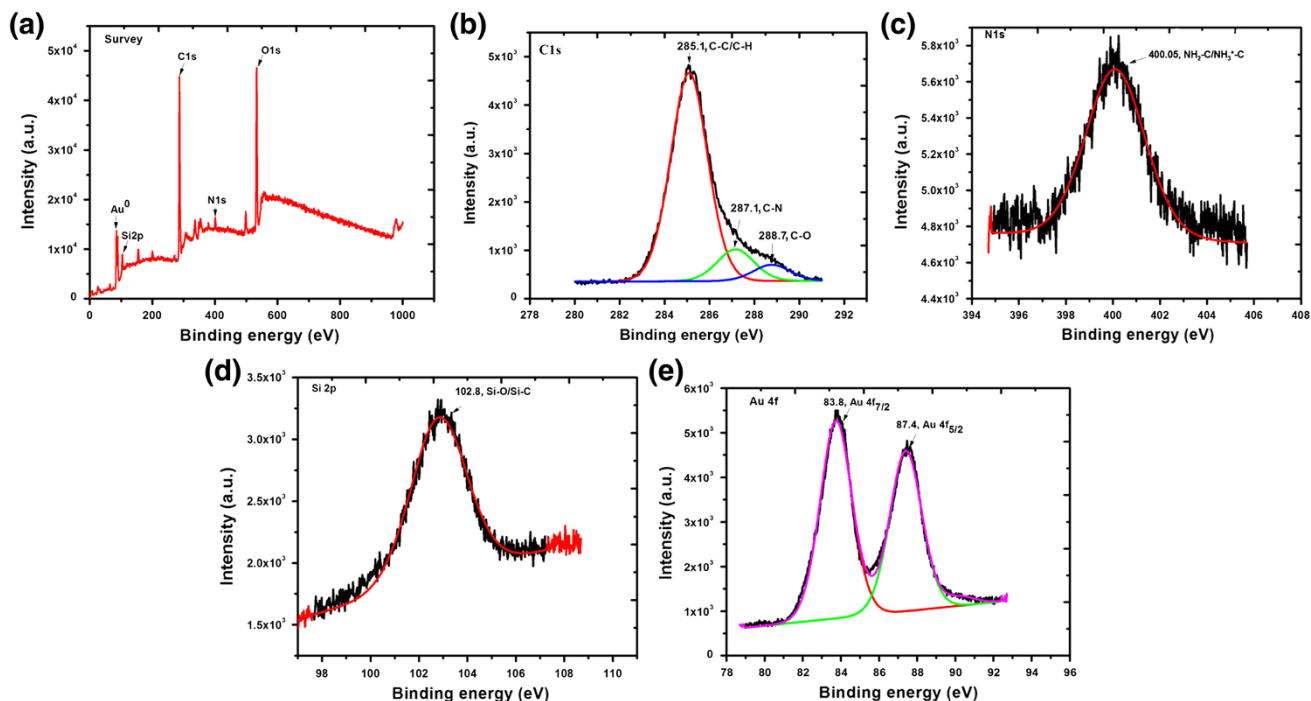


Fig. 9 XPS spectra of AuNPs stained amine modified PS plate. **a** Survey scan, high-resolution scan in the **b** C 1s region, **c** N 1s region, **d** Si 2p region, and **e** Au 4f region. N 1s spectra indicate that

the presence of the amine functional group and Au 4f region proves that the presence of metallic gold on the amine modified PS plate surface

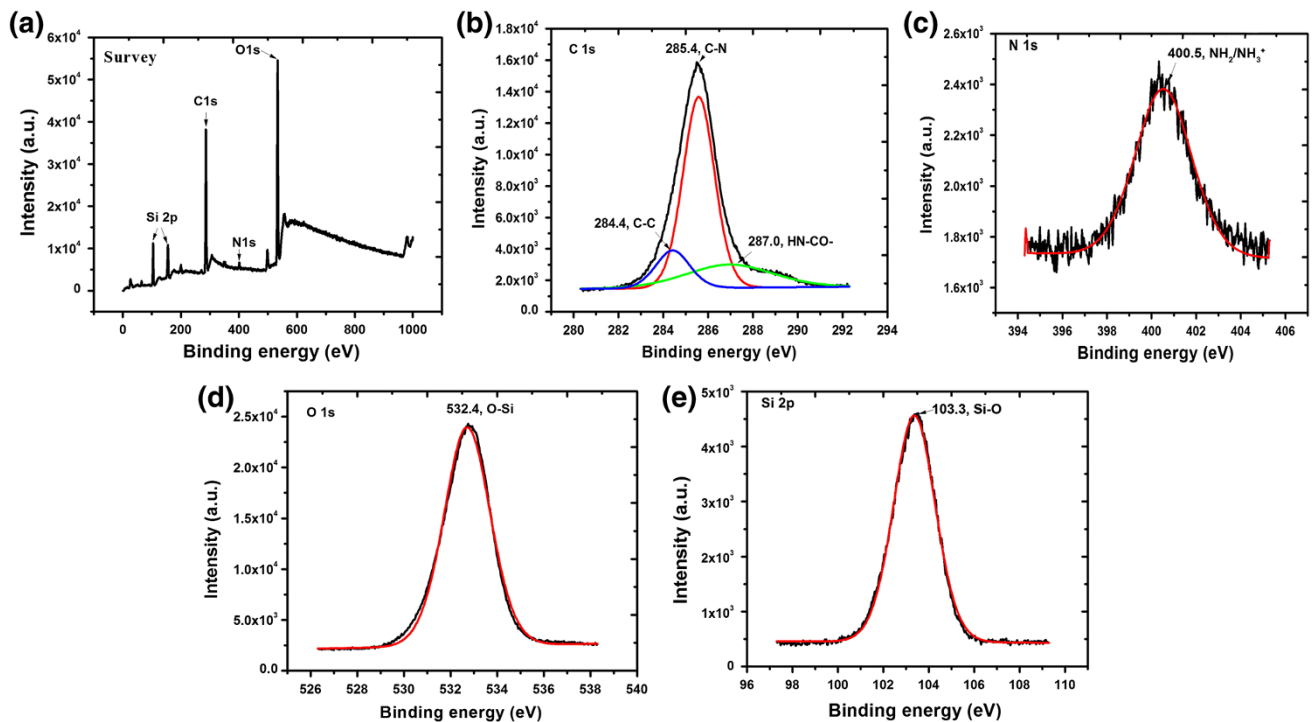


Fig. 10 XPS spectra of amine modified GS surface. **a** Survey scan, **b** high-resolution scan of C 1s region, **c** N 1s region, **d** O 1s region, and **e** Si 2p region. N 1s, Si 2p, and O 1s indicate that the presence of the amine functional group on the APTES treated GS surface

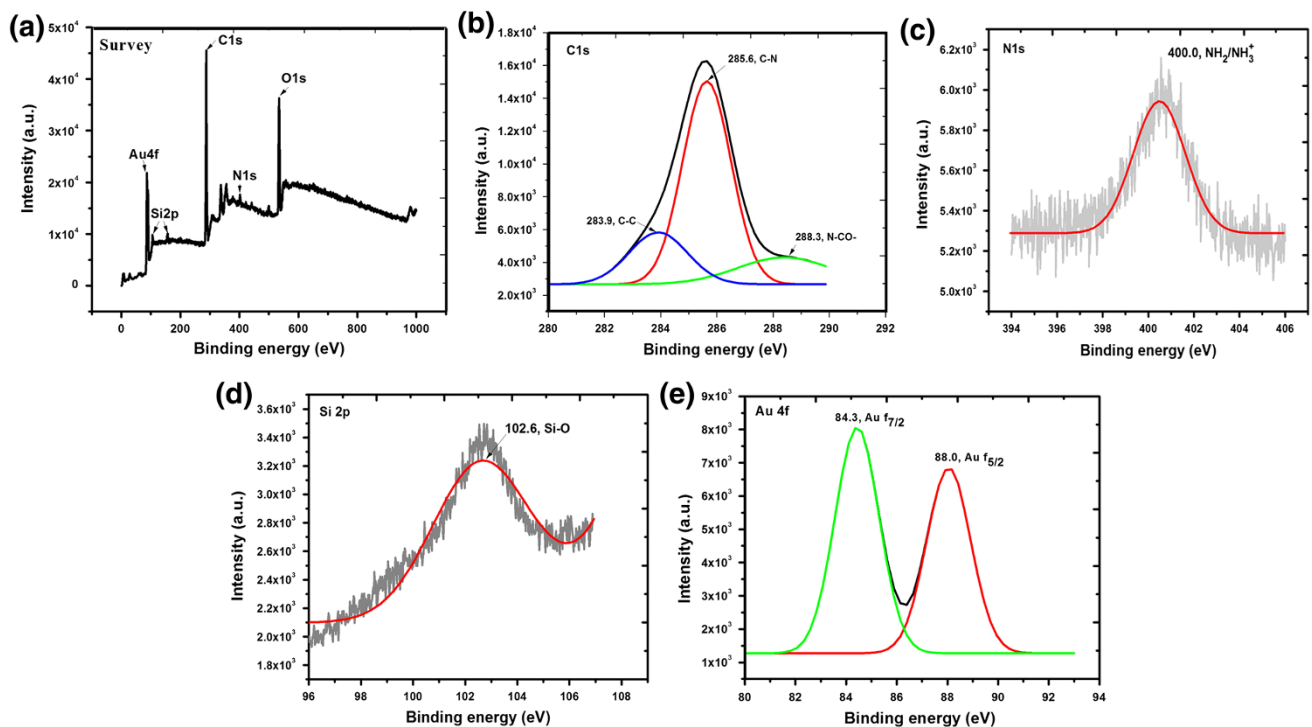


Fig. 11 XPS spectra of AuNPs stained amine modified GS surface. **a** Survey scan, high-resolution scan in the **b** C 1s region, **c** N 1s region, **d** Si 2p region, and **e** Au 4f region. N 1s region indicate that

the presence of the primary amine functional group and Au 4f region prove that the presence of a metallic gold layer on the amine modified GS surface

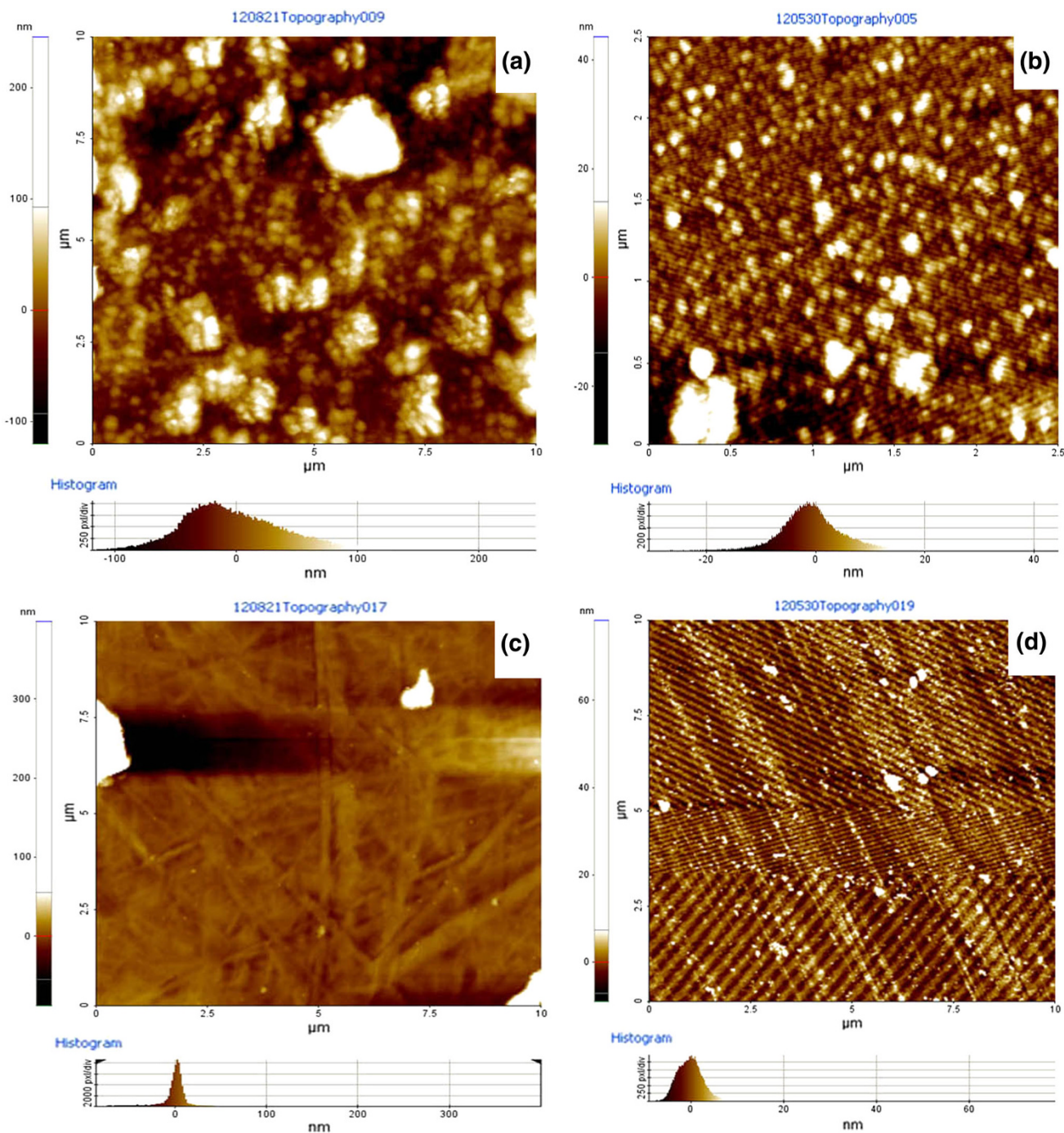


Fig. 12 AFM topographic images with histogram of AuNPs thin metallic film on the amine modified PS plate (a) and GS surfaces (b), respectively. c, d show AFM images with histogram of unmodified PS plate and GS surfaces, respectively

characteristics of C–C/C–H, C–N, and C–O bonding of APTES, respectively (Fig. 9b). Peak corresponding to the binding energy of primary/protonated amine (NH_2 or NH_3^+) was found at 400.5 eV (Fig. 9c). Peak for Si 2p was observed at 102.8 eV which was the characteristic feature of Si–O bonding nature (Fig. 9d). Figure 9e shows the binding energy peaks at 83.8 and 87.4 eV specific for Au

$4f_{7/2}$ and Au $4f_{5/2}$, respectively. The characteristic features of these peaks indicate the presence of AuNPs in the metallic state on the modified PS plate surface (Young et al. 2011). Also, it indicates that the physical adsorption of AuNPs on the aminated PS plate surface through the electrostatic interactions and the hydrogen bonds (Carré and Lacarrière 2007). This study confirms that the AuNPs

interact with modified PS plate surface through the weak interactions that lead to aggregation and the color changes of AuNPs from red to blue.

Figure 10a (XPS survey spectra) shows the presence of the C, N, Si, and O on the amine modified glass slide surface. The C 1s spectra show peaks at 284.4, 285.4, and 287.0 eV which correspond to C–C, C–N, and HN–CO, respectively (Fig. 10b). The N 1s and O 1s spectra show peaks at 400.5 and 532.4 eV, respectively, the characteristic features of protonated amine and O–Si (Fig. 10c, d). Also, Si–O corresponding binding energy peak was found at 103.3 eV in Si 2p spectra (Fig. 10e). AuNPs stained amine modified GS surface XPS survey spectra were shown in Fig. 11a and 1 eV of binding energy was subtracted for curve fitting with respect to carbon charge correction. Peaks at 283.9 eV (C–C), 285.6 eV (C–N), 288.3 eV (HN–CO), 400.0 eV ($\text{NH}_2/\text{NH}_3^+$), 102.6 eV (Si–O), 84.3 eV ($\text{Au } f_{7/2}$), and 88.0 eV ($\text{Au } f_{5/2}$) were found in C 1s, N 1s, Si 2p, and Au 4f of high-resolution spectra, respectively (Fig. 11b–e, respectively). These spectral data show that the AuNPs present in the metallic state even after the adsorption on the modified surface (Flores-Perez and Ivanisevic 2007; Durdureanu-Angheluta et al. 2012; Young et al. 2011).

AFM analysis of AuNPs stained PS plate and GS surfaces

Adsorption of AuNPs on the aminated PS plates and GS surfaces was reconfirmed by AFM topographical analysis. Figure 12 represents AFM images of AuNPs on the amine modified and unmodified PS plate and GS surfaces, respectively. In the modified PS plate and GS surfaces, the size of AuNPs was found to be 10–100 nm (Fig. 12a, b, respectively) and they are not found on the unmodified PS plate and GS surfaces (Fig. 12c, d, respectively) which could be observed with the corresponding histograms. The size of AuNPs up to 100 nm also indicates the aggregation of AuNPs is due to the presence of amine groups on the PS plate and GS surfaces. This result also proves that AuNPs adsorption and aggregation take place only on amine modified surfaces (Carré and Lacarrière 2007; Fujiwara et al. 2006; Huang et al. 2012).

Conclusion

Citrate-capped AuNPs used to characterize the amine functionalized PS multiwell plate and GS surfaces without help of sophisticated instruments were demonstrated. Here, the surface modification could be observed by naked eyes based on the color changes of AuNPs from red to purple or blue. This will clearly discriminate the modified and

unmodified surfaces by simply without the addition of salts. The effect of AuNPs concentration on the characterization of amine modified surfaces with AuNPs was also investigated and we found satisfactory results even in the very low concentration of AuNPs (0.2 ratio). This characterization explored that the interactions and aggregation of AuNPs on the modified PS and GS surfaces by colorimetric and simple scanning methods. These AuNPs assisted characterization method for amine modified surfaces is very simple, cost-effective, less time consuming, and required very low concentration of AuNPs (0.2 ratio) and we hope this study may help to characterize the amine modified solid surfaces rapidly in the routine process in commercial and research point of view.

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References

- Butler JE (2000) Solid supports in enzyme-linked immunosorbent assay and other solid-phase immunoassays. *Methods* 22:4–23
- Carré Birch W, Lacarrière V (2007) Glass substrates modified with organosilanes for DNA immobilization. *Silanes and Other Coupling Agents* 4:1–14
- Chu X, Xiang ZF, Fu X, Wang SP, Shen GL, Yu RQ (2005) Silver-enhanced colloidal gold metalloimmunoassay for *Schistosoma japonicum* antibody detection. *J Immunol Methods* 301:77–88
- Dixit CK, Vashist SK, O’Neill FT, O’Reilly B, MacCraith BD, O’Kennedy R (2010) Development of a high sensitivity rapid sandwich elisa procedure and its comparison with the conventional approach. *Anal Chem* 82:7049–7052
- Dixit CK, Vashist SK, MacCraith BD, O’Kennedy R (2011) Multisubstrate-compatible ELISA procedures for rapid and high-sensitivity immunoassays. *Nat Protoc* 6:439–445
- Durdureanu-Angheluta A, Dascalu A, Fifere A, Coroaba A, Pricop L, Chiriac H, Tura V, Pinteala M, Simionescu BC (2012) Progress in the synthesis and characterization of magnetite nanoparticles with amino groups on the surface. *J Magn Magn Mater* 324:1679–1689
- Flores-Perez R, Ivanisevic A (2007) Molecular recognition of chromophore molecules to amine terminated surfaces. *Appl Surf Sci* 253:4176–4181
- Fujiwara K, Watarai H, Itoh H, Nakahama E, Ogawa N (2006) Measurement of antibody binding to protein immobilized on

- gold nanoparticles by localized surface plasmon spectroscopy. *Anal Bioanal Chem* 386:639–644
- Goddard JM, Hotchkiss JH (2007) Polymer surface modification for the attachment of bioactive compounds. *Prog Polym Sci* 32:698–725
- Grabar KC, Freeman RG, Hommer MB, Natan MJ (1995) Preparation and characterization of Au colloid monolayers. *Anal Chem* 67:735–743
- Hirayama H, Tamaoka J, Horikoshi K (1996) Improved immobilization of DNA to microwell plates for DNA–DNA hybridization. *Nucleic Acids Res* 24:4098–4099
- Huang KW, Hsieh CW, Kan HC, Hsieh ML, Hsieh S, Chau LK, Cheng TE, Lin WT (2012) Improved performance of aminopropylsilatrane over amino propyltriethoxysilane as a linker for nanoparticle-based plasmon resonance sensors. *Sensor Actuat B-Chem* 163:207–215
- Idage SB, Badrinarayanan S (1998) Surface modification of polystyrene using nitrogen plasma: an X-ray photoelectron spectroscopy study. *Langmuir* 14:2780–2785
- Ji H, Dong H, Yan F, Lei J, Ding L, Gao W, Ju H (2011) Visual scanometric detection of DNA through silver enhancement regulated by gold-nanoparticle aggregation with a molecular beacon as the trigger. *Chem Eur J* 17:11344–11349
- Kannoujia DK, Ali S, Nahar P (2010) Single-step covalent immobilization of oligonucleotides onto solid surface. *Anal Methods* 2:212–216
- Kaur J, Raman Suri C (2007) Direct hapten coated ELISA for immunosensing of low molecular weight analytes. *Nat Protoc Exch*. doi:10.1038/nprot.2007.508
- Kim YP, Oh YH, Kim HS (2008) Protein kinase assay on peptide-conjugated gold nanoparticles. *Biosens Bioelectron* 23:980–986
- Kobayashi Y, Tadaki Y, Nagao D, Konno M (2007) Deposition of gold nanoparticles on polystyrene spheres by electroless metal plating technique. *J Phys: Conf Ser* 61:582–586
- Lin HY, Chen CT, Chen YC (2006) Detection of phosphopeptides by localized surface plasma resonance of titania-coated gold nanoparticles immobilized on glass substrates. *Anal Chem* 78:6873–6878
- Moirangthem RS, Yaseen MT, Wei PK, Cheng JY, Chang YC (2012) Enhanced localized plasmonic detections using partially-embedded gold nanoparticles and ellipsometric measurements. *Biomed Opt Express* 3:899–910
- Nikiforov TT, Rendle RB, Goelet P, Rogers YH, Kotewicz ML, Anderson S, Trainor GL, Knapp MR (1994) Genetic bit analysis: a solid phase method for typing single nucleotide polymorphisms. *Nucleic Acids Res* 22:4167–4175
- North SH, Lock EH, Cooper CJ, Franek JB, Taitt CR, Walton SG (2010) Plasma-based surface modification of polystyrene microtiter plates for covalent immobilization of biomolecules. *ACS Appl Mater Interfaces* 2:2884–2891
- Pack SP, Kamisetty NK, Nonogawa M, Devarayapalli KC, Ohtani K, Yamada K, Yoshida Y, Kodaki T, Makino K (2007) Direct immobilization of DNA oligomers onto the amine-functionalized glass surface for DNA microarray fabrication through the activation-free reaction of oxanine. *Nucleic Acids Res* 35:1–10
- Qin M, Hou S, Wang L, Feng X, Wang R, Yang Y, Wang C, Yu L, Shao B, Qiao M (2007) Two methods for glass surface modification and their application in protein immobilization. *Colloids Surf B* 60:243–249
- Rebeski DE, Winger EM, Shin YK, Leleta M, Robinson MM, Varecka R, Crowther JR (1999) Identification of unacceptable background caused by non-specific protein adsorption to the plastic surface of 96-well immunoassay plates using a standardized enzyme-linked immunosorbent assay procedure. *J Immunol Methods* 226:85–92
- Wang W, Vaughn MW (2008) Morphology and amine accessibility of (3-aminopropyl) triethoxysilane films on glass surfaces. *Scanning* 30:65–77
- Yang Y, Hori M, Hayakawa T, Nogami M (2005) Self-assembled 3-dimensional arrays of Au@SiO₂ core-shell nanoparticles for enhanced optical nonlinearities. *Surf Sci* 579:215–224
- Young JK, Lewinski NA, Langsner RJ, Kennedy LC, Satyanarayan A, Nammalvar V, Lin AY, Drezek RA (2011) Size-controlled synthesis of monodispersed gold nanoparticles via carbon monoxide gas reduction. *Nanoscale Res Lett* 6:4281–42811