Self-assembled gold nanoclusters on amine-functionalized glass substrate towards the development of biosensor based on light absorption

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Abstract
A technique for self-assembly of gold nanoclusters on amine-functionalized glass substrate is presented. The amount of self-assembled nanoclusters can be controlled by varying the time of immersion in the colloidal solution. The electrostatic interaction between the ionized amine group and the citrate anions is utilized for the self-assembly of gold nanoclusters on the amine glass substrate. The self-assembly process is studied by microscopy and spectroscopy techniques. The use of self-assembled gold nanoclusters as biosensor based on light absorption is demonstrated.
Key words: Gold Nanoclusters, Self-Assembled, Biosensor, Optical Absorption.

1. Introduction

Metallic nanoclusters research became a thrust area of interest among the scientific community due to the potential applications of this "neglected dimension" of matter in almost all the fields of science and technology [1, 2]. While the synthesis of these nanoclusters is a key approach important aspect of the research, the self-assembly (SA) of nanoclusters (NC) is important for various purposes such as environmental [3], biomedical [4] or (electro) chemical analytical applications [5]. The self-assembly of metallic nanoclusters is a process by which metallic particles spontaneously organize due to direct specific interactions or indirectly (for example, electrostatic or ion-dipole), through their environment [6].

In particular, self-assembly of gold nanoclusters on substrate [7] is crucial to the development of biosensors based on the light absorption that can be easily measured using absorption spectroscopy. It is well known that not only is the absorbance originating from localized surface plasmon resonance (LSPR) of the gold nanoclusters influenced by the dielectric properties of molecules attached to the nanoclusters but also the interband absorption of the nanoclusters changes [8]. Therefore, the adsorption of chemical or biological molecules on the surface will change the intensities and frequency of absorption bands that can be monitored by measuring the absorption spectra of the metallic nanoclusters.

In this paper it is reported the self-assembly of gold nanoclusters on amine-fucionalized glass substrate evaluating their application in the development of biosensors based on light absorption in the ultraviolet-visible (UV-Vis) spectral region (Figure 1).

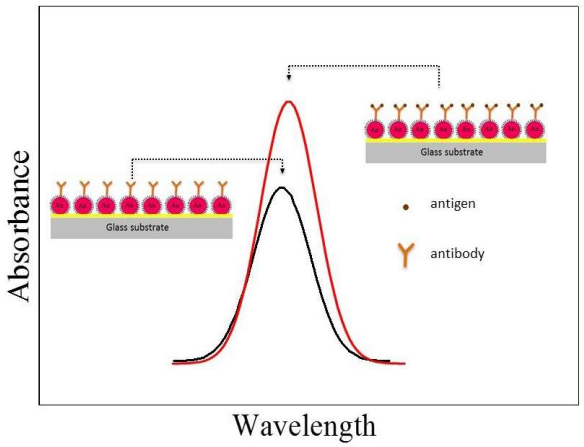

Figure 1. Schematic illustration of a biosensor based on self-assembly of gold nanoclusters on amine-functionalized glass substrate, and resulting absorbance spectra increase upon binding of analytes to the metallic nanoclusters.

2. Experimental Section

The synthesis of gold nanoclusters and self-assembly were studied using of UV-Vis absorption spectroscopy, IR and AFM techniques. Aminopropyltrimethoxysilane (APTES) was used to obtain amine-functionalized glass substrate (AFGS). The gold nanoclusters density self-assembled on AFGS was controlled by varying the immersion time. The biosensor activity was evaluated to detect the antibody (anti-human serum albumin, Anti-HSA) – antigen (human serum albumin, HAS) recognition reaction and monitored via change of light absorption when this binding event occurs.

Materials chemical

Hydrogen tetrachloroaurate ($HAuCl4 \cdot 3H2O$ and trisodium citrate dihydrate ($Na3C6H5O7 \cdot 2H2O$), Aminopropyltrimethoxysilane were purchased from Sigma-Aldrich, USA. Acetone, absolute ethanol, sulfuric acid ($H2SO4$, 64 %) and hydrogen peroxide ($H2O2$) were purchased from Merck, Germany. Deionized (DI) water (18.2 $M\Omega \cdot cm$) was used for preparation of solutions, synthesis and cleaning glass substrate. All the chemicals were used without further purification.

Preparation of colloidal gold nanoclusters

The colloidal gold nanoclusters were synthesized by the chemical reduction of corresponding metal ions [9]. In brief, an aqueous solution of $HAuCl4 \cdot 3H2O$ (0.033 mmol in 25 mL water) was refluxed for 30 min in a 100 mL tree-necked round-bottom flask. A condenser was utilized to prevent the evaporation of the solvent. Next, aqueous $Na3C6H5O7 \cdot 2H2O$ (0.033 mmol in 1 mL) was injected into the solution and then refluxed for 60 min to produce the stable red-aqueous solution of gold nanocluster. The obtained colloidal solution showed a pH=6.3 and the resulting gold nanoclusters are coated with negatively charged citrate ions [10].

Substrate amine-functionalized glass substrate

The glass substrates (2.5 x 7.5 cm) were immersed in chromic acid boiled for 4 h. Then they were then left to cool at room temperature in the acidic solution overnight. They were thoroughly rinsed with distilled water and left to dry for 1 hr at 100 °C. The substrates were then immersed in 1 % (v/v) solution of APTES for 24 h at room temperature. After soaking in APTES solutions, the substrates were rinsed with a copious amount of ethanol and DI water to remove any physisorbed molecules. The rinsed substrates were then treated ultrasonically in ethanol and left to dry for 1 h at 100 °C for finally obtain amine-functionalized glass substrates.

Self-Assembly of colloidal gold nanoclusters

As-prepared glass substrates were immersed in the colloidal dispersion of gold nanoclusters for a time in the range 5 to 25 hrs at room temperature, formation of a layer of gold nanoclusters on glass substrates was finally obtained. The glass substrates were then removed and rinsed with DI water and left to dry for 10 min in an oven at 80 oC.

3. Results and Discussion

A typical micrograph of the gold nanoclusters colloidal and its optical absorption spectrum are show in Figure 2. The size distribution of the metallic colloids is presented as the inset in Figure 2a. The absorption spectrum of the colloidal dispersion of metallic particles revealed a band with a peak near 525.1 nm (Figure 2b), which is attributed to the LSPR [11] band of monodispersed gold nanoclusters with size of 15.2 nm (Figure 2a).

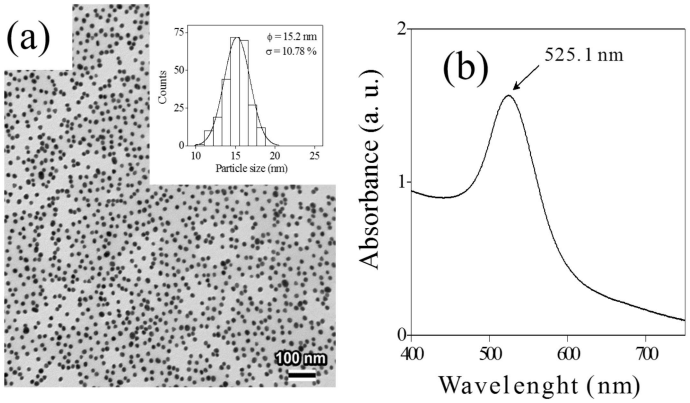

Figure 2. (a) A typical TEM micrograph of gold nanoclusters with size distribution, and (b) optical absorption spectrum of colloidal gold nanoclusters.

Figure 3, show the UV-Vis absorption spectra of the AFGS after immersion in the colloidal dispersion. Absorption spectral changes as a function of time of metallic colloidal were not observed, which indicated that the colloidal dispersion is stable over self-assembly time. However, after 25 hrs of immersion of the AFGS, the self-assembled on the glass substrates display a broadening of the band absorption and a shift about 65 nm towards the high wavelengths of the LSPR. The changes, with respect to the absorption spectra of the colloidal gold, it is result of the electromagnetic interaction between the gold nanoclusters on AFGS [8]. The presence of a single peak LSPR in the figure, suggest that gold nanoparticles tend to self-assembled on surface directly without forming aggregates, see Figure 4a. As the time immersion is incremented (25 hrs) and due to strong ionization of the amine (-NH2) groups, it is more coverage of gold nanocluster on AFGS. A red-ruby color is observed in the nanoclusters – coated substrates (Figure 4b).

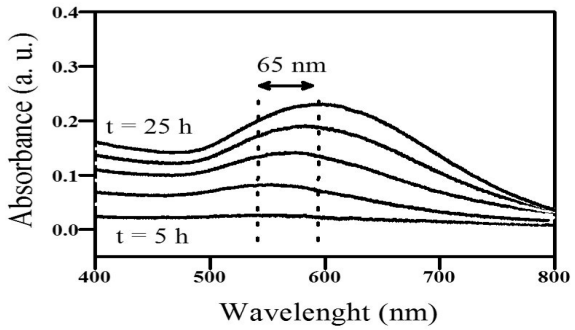

Figure 3. Optical absorption spectra of amine-functionalized glass substrate after 25 hrs of immersion in colloidal solution.

The process of self-assembly of the gold nanoclusters is characterized using the IR absorption spectra of the gold nanoclusters self-assembled on AFGS. In Figure 5, the IR absorption spectra of AFGS and after of

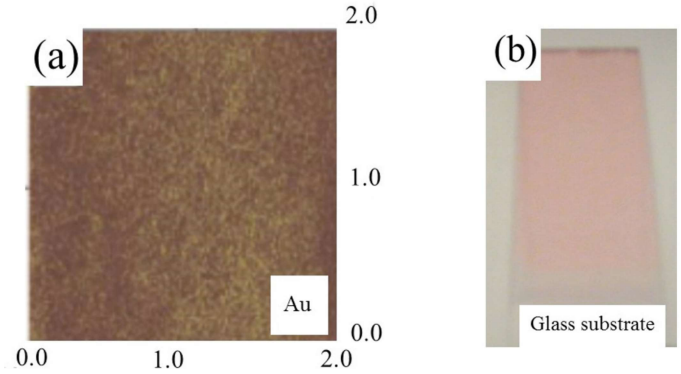

Figure 4. (a) AFM images of amine-functionalized glass substrates after self-assembly of gold nanoclusters for 25 hrs of immersion and (b) color of the amine-functionalized glass substrate after of immersed for 25 hrs in colloidal gold nanoclusters.

immersed for 25 hrs in the colloidal gold nanoclusters are shown. In the 3500-3200 cm-1 spectral range, showed an absorption band at about 3332 cm-1 for the AFGS, which corresponds to the antisymetric vibration frequency on the –NH2 as observed by other researchers [12]. This band disappears after immersion process for 25 hrs. The disappearance of the band indicates that all the –NH2 groups on the AFGS are completely ionized to interact electrostatically with the citrate anions that exist on the gold nanoclusters.

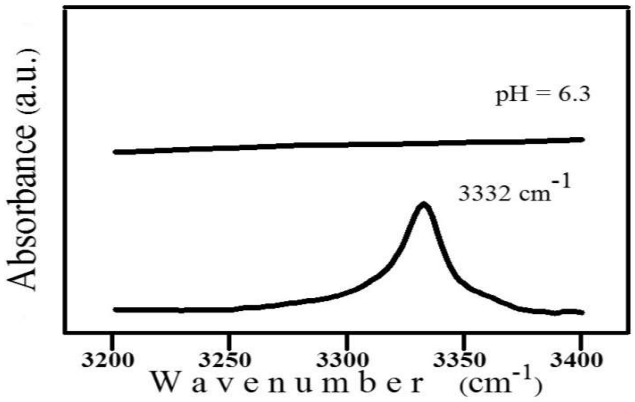

Figure 5. FTIR spectra of amine-functionalized glass substrate and after of immersed for 25 hrs in the colloidal gold nanoclusters.

The process of self-assembly of the gold nanoclusters on the amine-functionalized glass substrate is shown in Figure 6. As pH=6.3 all the -NH2 groups are expected to be fully ionized and that the gold nanoclusters are coated with negatively charged citrate ions, then it is expected that there is a strong electrostatic interaction between the $-NH3^{+}_{3}$ groups and citrate anions. So, the self-assembly of gold nanoclusters on the amine surface is performed, causing the formation of films of gold nanocluster on the AFGS.

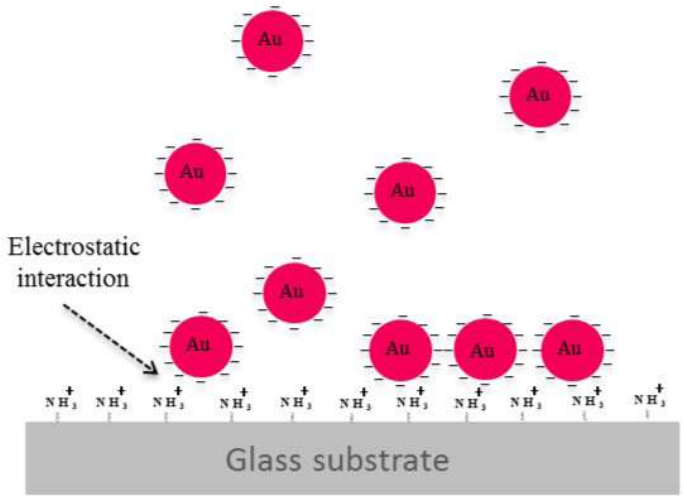

Figure 6. Schematic illustration of proposed self-assembly mechanism of gold nanoclusters on amine-functionalized glass substrate.

To demonstrate the biosensing ability of the self-assembled gold nanoclusters glass substrate, anti-HAS was directly adsorbed onto self-assembled gold nanoclusters on glass substrate. The experimental conditions of the antibody-antigen recognition reaction were performed according to the procedure described in the Ref. [13]. The antibody-antigen recognition reaction is corroborated as a change in the interband absorption at 280 nm (an increase in absorbance of 86 %) and as a shift to higher wavelength (from 580.1 to 584.0 nm) in the LSPR frequency. An increase is also noted of 5 % in the LSPR absorption to 584. Details of these results will be published in future reports.

5. Conclusions

By using electrostatic interaction between the ionized amine group of amine-functionalized glass substrate and the citrate anions, we could self-assemble gold nanoclusters on amine-functionalized glass substrate. By adjusting the immersion time, the amount of self-assembled in the amine-functionalized glass substrate could be controlled. The self-assembled gold nanoclusters were biofunctionalized and utilized as biosensor based on light absorption.

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