Characteristics of pH sensors fabricated by using protein-mediated CdSe/ZnS quantum dots

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1. Introduction

Biosensors have drawn major attention as a diagnostic tool especially in the clinical laboratories and pharmaceutical companies [1,2]. Biosensors are being used in a wide range of applications from medical diagnostics [3], food quality assurance [4], environmental monitoring [5], industrial process control [6,7] to biological warfare agent detection [8]. Recently, there is a great need of biosensors for the early detection of infected biomolecules as the conventional detection methods that provide detailed information regarding the nature of infection involve, suffer from relatively poor sensitivity and slow response. In addition, the conventional biosensors require relatively large number of infected bio-molecules to be reliably detected. Therefore, researchers are focusing on nanostructured biosensors including quantum dots, nanowires, etc., as detecting probes for bio-molecules because these nano-biosensors are ultra-sensitive, fast, and can detect even few molecules. One of the obvious reasons of the improved sensing properties is that these nanoparticles have high surface to volume ratio which makes the binding sites for bio-molecules more pronounced as compared to their bulk counterpart parts. If quantum dots or other nanoparticles will be present on the SiO2 surface, it is easy to attach antibodies which will further detect the antigens [9,10]. To obtain the quantum dots, various methods have been investigated. Among them synthesis using biological templates such as DNA, virus, protein templates have many advantages such as room temperature process and natural availability. The detailed study of the nano-biosensors is one of the most challenging but of immense importance research topic in the field of bioelectronics. The tremendous research activities are carried out involving semiconductor based biosensors, such as ion sensitive field effect transistor (ISFET), capacitive electrolyte–insulator–semiconductor (EIS), and light-addressable potentiometric sensor (LAPS) have been reported by many groups [11–13]. Among all these kinds of structures, EIS sensors [14,15] have demonstrated because of their potential in terms of simplicity in layout, level free detection and above all their easy and low cost fabrication. To get a good EIS sensor, the chaperonin protein (GroEL) can be used as the templates to deposit CdSe/ZnS quantum dot membrane on SiO2 surface. The chaperonins are high-molecular weight protein complexes present in all living cells. The GroEL is a porous thick-walled cylinder that contains a substantial central cavity or channel which has a diameter of 4.5 nm and a wall thickness of 4.6 nm [16]. Although, the pH sensors using CdSe/ZnS quantum dots [17,18] are studied by optical method [19], the electronic-based pH sensor using CdSe/ZnS quantum dots is not reported yet.

In this study, a novel CdSe/ZnS quantum dot based pH sensor has been investigated. The CdSe/ZnS core–shell structured quantum dots over coated with hydrophobic triocylphosphin oxide (TOPO) ligands are trapped by hydrophobic cylindrical cavity of chaperonin protein templates and was used as sensing membrane. A bare SiO2 membrane is also used for comparison of pH sensor.
2. Experimental

Standard Radio Corporation of America (RCA) cleaned 4° p-type (<10 0 0>, resistivity: 5–10 Ω·cm) Si wafer was used to fabricate EIS-based sensors. After RCA cleaning, a 40 nm-thick SiO₂ layer was grown as an insulating layer by dry oxidation process at a temperature of 1000 °C. The wafer was then cut into pieces (5 cm × 1.5 cm), sonicated in absolute ethanol (Sigma–Aldrich) for 5 min and dried under nitrogen gun. Next, samples were soaked in freshly prepared piranha solution (conc. H₂SO₄: 30% H₂O₂, 7:3) for 30 min at 90 °C. Samples were then taken out, cooled for 5 min, rinsed in running deionized (DI) water and sonicated in spectroscopic grade methanol (Sigma–Aldrich) for 5 min followed by drying in an oven for 30 min at 100 °C. Silanization was carried out next in order to pre-treat the surface. The samples were soaked in 5% (v/v) phenyltriethoxysilane (PTS) solution (Sigma–Aldrich) in dry toluene for 60 min under nitrogen atmosphere. Then, the samples were taken out, cooled for 5 min, rinsed three times with dry toluene (Sigma–Aldrich) to remove un-reacted silane and then three times in spectroscopic grade methanol followed by drying in an oven for 2 h at 100 °C to remove the adsorbed water molecules.

Then, the samples were floated on chaperonin protein solution (0.1 mg/ml) for 15 min with the oxide side down, rinsed thoroughly in DI water and blown dry in N₂ flow. Then, the samples were then immersed in CdSe/ZnS quantum dot solution (0.1 mg/ml) in toluene for 30 min and blown dry in N₂ flow. Chaperonin protein (GroEL) was obtained from Takara Bio Inc., Japan and was used as-received. Trioctylphosphin oxide (TOPO) capped CdSe/ZnS core–shell type quantum dots with a nominal core diameter of 2.5 nm were obtained from Plasmachem Gmbh (Germany) in powder form. The process flow of the sensor fabrication is shown in Fig. 1.

The detail of the EIS chip fabrication is as follows. First, a 300 nm-thick aluminum (Al) film was deposited on the back side of the samples after removing the back side oxide using buffer oxide etching solution (BOE). The sensing membrane area was then defined by standard photolithography process using a negative photoresist-SU8-2005 (Micro Chem Inc.). Then, EIS devices were attached on a printed circuit board having copper lines. Silver gel was used to make the electrical contact between aluminum (back side of EIS sensors) and copper lines. Then, an epoxy package was employed to encapsulate the EIS structure and the copper line. The schematic cross-section of the fabricated EIS sensor is shown in Fig. 2. A sensor without CdSe/ZnS quantum dots (i.e., a bare SiO₂ membrane) was also fabricated for comparison. The pH sensitivities of the CdSe/ZnS modified sensing membranes were determined from capacitance–voltage (C–V) curves using various values of pH buffer solutions (Merck Inc.). The C–V measurements were performed with substrate bias through an Ag/AgCl reference electrode. The operating frequency of the ac signal was 100 Hz. All the measurements were carried out in a black box to avoid interference from light.

3. Results and discussion

The atomic force microscopy (AFM) images of the sensor surface with CdSe/ZnS quantum dots are shown in Fig. 3a and b. Both images show quantum dots explicitly. The average size of the CdSe/ZnS quantum dots is showing 4–10 nm. The diameter of the CdSe/ZnS quantum dot is 3.983 nm, which is estimated from the line profile (Fig. 3c). A large size (<10 nm) of the CdSe/ZnS quantum dots with respect to the nominal diameter of 2.5 nm is due to the large AFM tip diameter. The mechanism of the entrapment of
quantum dots is the hydrophobic–hydrophobic interaction between chaperonin protein cavity and CdSe/ZnS quantum dots. When the substrate (Si/SiO2) was dipped in the piranha solution, a number of hydroxyl groups (−OH) was attached on the surface and surface became hydrophilic. On immersion of this substrate in phenyltrithoxysilane solution, the silane group chemically reacts and covalently bonded to SiO2 surface. This process results the formation of a self-assembled monolayer containing water-repellent organic phenyl group (−C6H5) at the end and hence surface became hydrophobic. The removed −H and ethoxy groups react to form ethanol as a by product. This process is called silanization. When this sample was floated in chaperonin protein solution, one side of the hydrophobic chaperonin cavity was attached to surface and results a monolayer of cavities because of hydrophobic–hydrophobic interaction. After immersing of the sample having chaperonin monolayer on the surface in the hydrophobic CdSe/ZnS quantum dot solution, the quantum dots will be trapped in the chaperonin protein cavity due to hydrophobic–hydrophobic interaction. This interaction is not the same on the surface. As a result, the randomly distributed quantum dots are observed on the SiO2 surface. Furthermore, the concentration of chaperonin protein solution as well as CdSe/ZnS quantum dot solution plays also an important role to get the quantum dot arrays.

Fig. 4 shows capacitance–voltage (C–V) characteristics of bare SiO2 and CdSe/ZnS sensors. The sensitivity of the EIS sensors depends on the properties of the membrane surface. A comparison of the sensitivity and linearity of the EIS sensor with CdSe/ZnS quantum dot sensing membrane and bare SiO2 sensing membrane are shown in Fig. 5. The flat–band voltages (VFB) of SiO2 EIS sensor are found to be +1.47 V for pH 4 and +1.05 V for pH 12, while those values are found to be +1.92 V and +1.61 V for CdSe/ZnS quantum dot sensors. On the other hand, the VFB value of SiO2 EIS sensor in pH 2 buffer solution is 1.5 V (Fig. 5). The pH sensitivity in pH 2 buffer solution is not as good as the sensitivity in pH 4–pH 12 buffer solutions owing to more acidic nature of pH 2. Due to this reason, the pH 2 sensing is not included to calculate the sensitivity. The voltage shift in each pH solution was extracted from the flat-band voltage (VFB) position of the C–V curves. Assuming the minimum normalized capacitance (C/Cox) of 0.6, the normalized flat-band capacitance could be 0.7. The values of VFB are decreased with increasing the pH value for both sensors, which can be explained as follows. According to the site binding model [20] of the electrolyte–oxide interface, the oxide surface is assumed to contain silanol groups. When the SiO2 surface comes in contact with the electrolyte solution, a pH dependent electrochemical equilibrium is established. Fig. 6a and b show the energy band diagrams of SiO2/p-Si structure without and with electrolyte, respectively. Without electrolyte, no charges on SiO2 surface are assumed. So
ties (the energy band is flat [Fig. 5].

Sensitivity and linearity for the bare SiO2 and CdSe/ZnS quantum dot based sensors in different pH buffer solutions.

Normalized capacitance (C/Cox) of SiO2 and Si are 0.95 eV and 4.05 eV, respectively, where

\[
D = \left\{ \frac{E_{\text{FV}}}{E_{\text{FV}}} \right\}
\]

Normalized capacitance (C/Cox) (VFB) pH4 = 1.47 V, owing to the initial negative surface charges [21,22]. It is considered to be an n-type semiconductor. The energy gap (Eg) of CdSe quantum dot with a small diameter of 2.5 nm is 3.1 eV [21] and electron affinity is 3.8–4.6 eV [23,24]. The value of X is considered to be 3.8 eV in this study, as shown in Fig. 6d. Although the electron density on the QDs is unknown but the energy band is assumed to be flat [(E_{\text{FV}})QD = (E_{\text{FV}})] initially. The quantum dot will attract more H+ ions than that of bare SiO2 surface, due to negative surface charges and the quantum dot surface will be more positive. Due to this reason, the VFB values of quantum dot based sensors are higher than that of bare SiO2 sensors. This results in a positive directional shift of VFB for the quantum dot based sensors relative to the bare SiO2 sensors. It is interesting to note that the quantum dot based sensor shows higher VFB shift at pH 12 [(V_{\text{FB}})pH 12 of quantum dot sensor – (V_{\text{FB}})pH 12 of bare SiO2 sensor = 1.61 V – 1.05 V = 0.56 V] than that of the VFB shift at pH 4 [1.92 V – 1.47 V = 0.45 V], owing to the initial negative surface charges of the quantum dot membrane [21,22]. At higher pH value (i.e., pH 12) for quantum dot sensor, the H+ ions can be trapped more on the quantum dot surface as compared to that of bare SiO2 surface owing to higher repulsion of OH– ions. A similar phenomenon is also observed for Au quantum dot based EIS pH sensor [25]. It indicates that the QD based sensor has higher flat-band voltage and different response as compared to that of bare SiO2 sensor, which can be useful in future capture probe of different bio-markers. The sensitivity of the biosensors can be calculated using the following equation,

\[
\text{Sensitivity} = \frac{\Delta V}{\Delta \text{pH}}
\]
where $\Delta V$ is the change in flat-band voltage with changing in pH. The calculated sensitivity is $\approx 39$ mV/pH for the CdSe/ZnS sensors and it is $\approx 53$ mV/pH for the bare SiO$_2$ sensors. The linearity of the CdSe/ZnS and bare SiO$_2$ sensors are found to be 99.48% and 99.95%, respectively. The sensitivity and linearity of the CdSe/ZnS quantum dot sensors are lower (slightly), due to the initial negative charges on the CdSe/ZnS quantum dot membrane.

4. Conclusions

The pH sensors using CdSe/ZnS quantum dots have been studied. The CdSe/ZnS quantum dot with a small diameter of 3.98 nm is confirmed by AFM. The sensitivity of CdSe/ZnS QD based sensors is 39 mV/pH, while that value is 53 mV/pH for bare SiO$_2$ sensors. A lower (slightly) sensitivity of CdSe/ZnS sensor as compared to bare SiO$_2$ based sensor is due to the initial negative charges on quantum dot, which is described by energy band diagrams. Due to a moderate response of CdSe/ZnS quantum dot based sensor, the quantum dot membrane can be used for future capture probes of biomarkers.

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References


Fig. 6. Schematic view of energy band diagrams for bare SiO$_2$ EIS sensors: (a) without buffer solution, (b) pH 4 buffer solution, and (c) pH 12 buffer solution and CdSe/ZnS QD sensors: (d) without buffer solution, (e) pH 4 buffer solution and (f) pH 12 buffer solution.


