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Synthesis of Silica Nanoparticle and Cd-Based Carboxyl Quantum Dots (SiNP@QDs) Conjugates as Biosensing Platform: A Pleriminary Study

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Abstract. Silica nanoparticles (SiNP) are one of the most promising materials for biosensors. The introduction of functional groups make silica nanoparticles highly versatile. In this work, the silica nanoparticle was synthesized using sol-gel method from natural based precursor, which is geothermal precipitate. Based on the characterization result of SiNP, the specific surface area is 138 m^2/g and nanoparticle size is 43.56 nm. The XRD result shows SiNP in amorphous phase. The surface of SiNP was modified using silanization method with 3-Aminopropyl triethoxysilane (APTES) as an amino silane to immobilize the quantum dot and *E coli* antibody. The FTIR analysis shows that amine group in SiNP@QDs surface reacted with carboxyl group of antibodies indicated by the presence of a peak at the wavelength of 1620 cm^{-1}. The detection of *E. coli* bacteria was carried out using fluorescence spectroscopy through the reduced of maximum intensity of SiNP@QDs-Ab before and after *E coli* addition at wavelength of 595 nm. The fluorescence intensity of SiNP@QDs-Ab is 633.10 a.u and the intensity is reduced to 281.86 a.u. in the presence of *E. coli* (1 x 10^7 CFU/mL).

Keywords: *Biosensors; E. coli, quantum dots; silica nanoparticles; silanization*

1 Introduction

Biosensors have been applied for clinical, environmental, forensic and food monitoring purposes and have shown some promise in detecting foodborne bacteria[1]. Biosensors technology has many advantage such as rapid, selective and sensitive[2]. Silica nanoparticles are one of the most promising materials for biosensors due to their remarkable properties comprising relative inertness in many conditions, stability over a wide range of pH (excluding alkaline), and transparency in the UV-vis spectrum [3].

Surface modification by various functional groups make silica nanoparticles highly versatile[4]. 3-Aminopropyl triethoxysilane (APTES), as source of amino silane group is a coupling agents that is frequently used for the silanization in silica nanoparticle surface[5]. The silane layer obtained from APTES produce amine functionality on the surface[6] making APTES is mainly attractive for biotechnology application as it acts like a glue layer[7]. Goftman et al (2016) used APTES to functionalize silica to create silica coated CdSe QDs, the silica nanoparticle encapsulating the quantum dot through a water in oil reverse microemulsion process[8].

Silica is commonly used to stabilize quantum dot in harsh condition. The advantage of silica is its ability to conjugate with various functional groups with excellent biocompatibility, stability and low toxicity. Lv et. al. conducted a research on silica encapsulated CdSe/ZnS quantum dots and biomolecule immobilization to create stable fluorescence immunoassay[9].

In this paper study on the effect of silica addition to quantum dot for *E coli* bacteria fluorescence sensing, was carried out. Natural based silica precursor was used to obtain silica nanoparticles (SiNP) through the sol-gel method. The SiNP surface was then modified using silanization method to support immobilization of quantum dot and *E. coli* antibody.

2 Materials and Method

2.1 Materials

The materials used in this study were geothermal precipitate, as the raw material, supplied from the Geodipa geothermal power plants at Dieng, Central Java, Indonesia. Sodium hydroxide (NaOH), Hydrochloric acid (HCl) 37%, Cetyltrimethylammonium Bromide (CTAB), Toluene, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N- Hydroxysuccinimide (NHS), 3-Aminopropyl Triethoxysilane (APTES) Escherichia coli (*E.coli*) antibody, and Phospate Buffer Silane (PBS) were purchased from Sigma-Aldrich, Cd-based

Carboxyl Quantum Dots were purchased from Ocean Nanotech, and *E. coli* ATCC 25922 were obtained from National Food and Drug Agency Republic Indonesia.

2.2 Synthesis of Silica Nanoparticles (SiNP)

A total of 20 g of washed silica geothermal powder was mixed with 800 mL of 1.5 N NaOH in a beaker glass to form sodium silicate. The mixture was then stirred using a magnetic stirrer for 60 minutes and heated at 90 °C. The mixture was filtered through filter paper to separate the solution with solids. The sodium silicate solution, then titrated with 2 N HCl to form the gel until pH 5. The formed gel was added with 2% of CTAB (w/w). The formed gel was allowed to stand for 18 hours and washed with DI water until pH 7 and dried in an oven at 100 ºC, overnight. The dried samples were labelled as Silica Nanoparticles (SiNP)

2.3 Surface modification of silica nanoparticles through silanization method

1 g of SiNP was added to 5% (v/v) APTES in toluene solution and shaked at room temperature for 1 hour. The precipitate was rinsed with toluene 2 times, then dried at room temperature, overnight resulted SiNP-APT.

2.4 Preparation of silica nanoparticle encapsulated quantum dot (SiNP@QDs)

30 mg of SiNP-APT was added to the mixture of 200 µL of EDC (5 mg/mL), 100 µL of NHS (5 mg/mL) and 100 µL of Cd-based Carboxyl Quantum Dots in 600 µL of PBS. The mixture was shaked for 24 hours at room temperature. The mixture was then centrifuged for 3 minutes at 10,000 rpm, the resulting supernatant was discarded and then washed with PBS 2 times. Samples were dried at room temperature and labelled as SiNP@QDs

2.5 Preparation of conjugation SiNP@QDs with *E. coli* antibody

The SiNP@QDs was added with 1 mL of PBS and sonicated for 10 minutes. The solution was added into the mixture containing 10 mL of EDC (10 mg/mL) and 100 µL NHS (5 mg/mL), then was shaked at room temperature for 30 minutes. The SiNP@QDs were precipitated and separated using centrifuge for 20 minutes at 10,000 rpm, the precipitate was added to 250 µL of PBS and 2 µl of *E. coli* aliquot antibody (1 µg/µL). The samples were labelled as antibody modified silica nanoparticles, SiNP@QDs-Ab. The SiNP@QDs-Ab sample was shaked for 1 hour, then added with 2.5 µL BSA 1%, then shaked again for 60 minutes. The antibody modified SiNP@QDs-Ab was stored in refrigerator 4 °C prior to the application.

2.6 *E. coli* sensing experiments using SiNP@QDs-Ab

A total of 4500 µL of *E. coli* bacteria with concentration of 1 x 10^7 CFU/mL was added to 500 µL SiNP@QDs-Ab (1 mg/mL) and mixed for 15 minutes. The mixture was measured using a fluorescence spectrometer at an excitation wavelength of 360 nm and emission wavelength at 400-800 nm.

2.7 Characterization of nanoparticles

Nitrogen absorption-desorption isotherm was conducted in 77 K on Microtrimetritics Tristar II 3020, USA to obtain the Brunauer-Emmertt-Teller (BET) surface area. The samples were degassed at 300 °C and 10^{-4-} Torr preasure prior to the measurement.

X-ray diffraction (XRD) analysis was carried out for crystallographic phases identification of the silica nanoparticles using a Philip PW 1710 diffractometer, using Cu Kα radition at 40 kV and 30 mA, and a secondary graphite monochromator.

Fourier Transform Infrared (FTIR) spectra was recorded on a FTIR Prestige-21 in transmittance mode, at 16 cm^{-1} resolution, over the range of 300-400 cm^{-1} with an accumulating average of 10 scans. The software used to generate the spectra was IR solution (Shimadzu).

Fluorescence intensity was recorded with a fluorescence spectrophotometer (Agilent, Singapore) at an excitation wavelength of 360 nm and the emission was recorded in the wavelength range 400-800 nm.

3 Result and Discussion

In this study, the precursor of the nanoparticle was natural-based silica from geothermal precipitate. The synthesis was conducted using sol-gel method which produces very homogeneous composites with very high purity (99.99% purity)[10][11]. Silica nanoparticles are formed by hydrolysis and condensation reactions. Hydrolysis was conducted in acid conditions, hence the addition of HCl causes protonation of the siloxy group ($Si-O^-$) to become silanol (Si-OH). The silanol groupsare then further attacked by siloxy groups ($Si-O^-$) with the help of an acid catalyst to form siloxane bonds (Si-O-Si) [12]. The optimum of gelling time was chosen based on our previous works which is 18 h with NaOH concentration NaOH of 1.5 N[13][14].

The reaction in the synthesis of SiNP by sol-gel method can be formulated as follow:

SiO_2 (geothermal precipitate)$_{(s)}$ + 2 NaOH $_{(aq)}$ → Na_2SiO_3 $_{(aq)}$ + H_2O $_{(aq)}$

Na_2SiO_3 $_{(aq)}$ + 2HCl $_{(aq)}$ → SiO_2 (nanoparticles)$_{(s)}$ + 2NaCl $_{(aq)}$ + H_2O $_{(g)}$

The addition of CTAB as the surfactant resulted in a uniform particle size and dispersion[15]. Surface area was measured using BET method with a degassing temperature of 300 °C for 3 hours.

Table 1 Result of BET analysis

No	Specification	Value
1	Surface Area	138 m^2/g
2	Pore Size	21.34 nm
3	Pore Volume	0.72 m^2/g
4	Nanoparticle Size	43.5 nm

Figure 1 shows the XRD diffraction pattern of SiNP. Based on the results, the sample shows a widening peak in the range 2θ of 20-30°, referring to JCPDS 47-0715 which indicates that the SiNP is in its amorph phase[16].

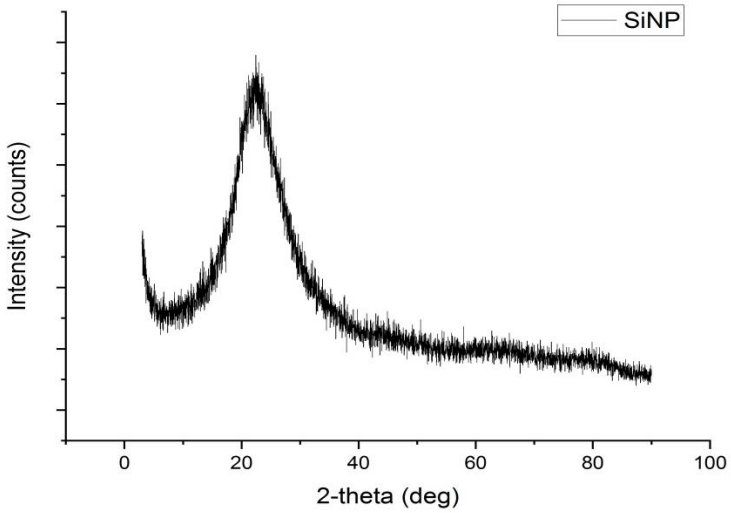

Figure 1. Crystallographic pattern of SiNP

The conjugation reaction between SiNP@QDs and biomolecule *E coli* antibody were observed using FTIR analysis. The FTIR result are shown in Figure 2. The functionalization of nanoparticles using APTES, replaced the –OH group of SiNP

by an amine group that is more reactive and has a charge on the surface[6]. The blue spectra (SiNP APT), shows bands in the region 1400–1700 cm^{-1} which corresponds to the absorption bands of amine group I at 1610-1690 cm^{-1} from the C=O stretching vibration, and amine II group around 1500-1600 from the N-H bending and C-N stretching [17]. Meanwhile the peaks at 2800–2980 cm^{-1} were assigned to N-CH_2 stretch indicating the presence of APTES on the surface [18] .

The amine in group in SiNP@QDs surface was reacted to the carboxyl group of antibody and created acylamide binding in the presence of EDC as the activator. The red spectra (SiNP@QDs-Ab) shows characteristic bands at 1620 cm^{-1} indicating carboxyl stretching vibration. The C=O stretching vibration was derived from carboxyl group on the *E coli* antibody[17].

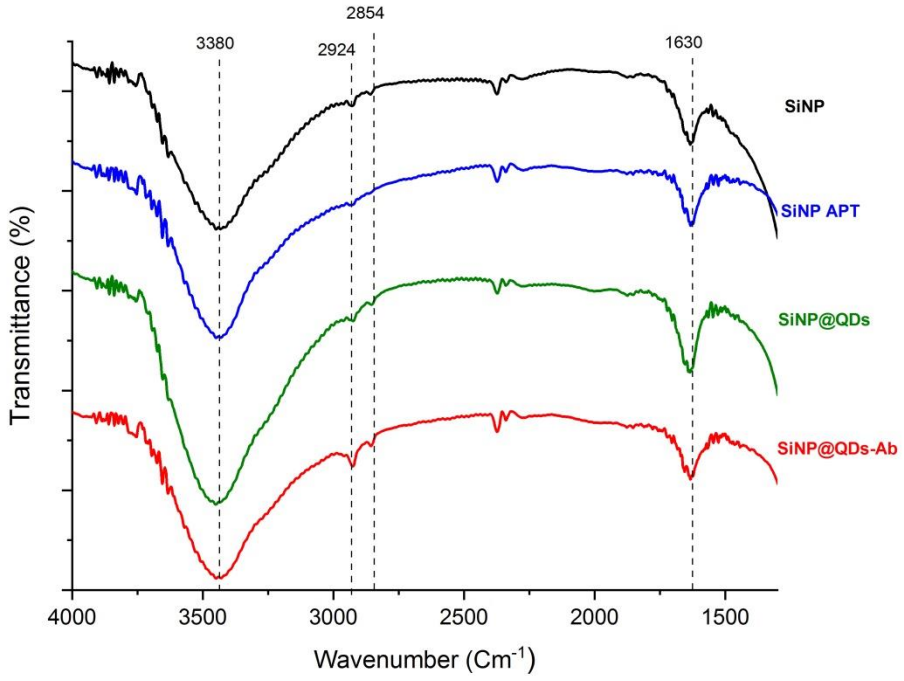

Figure 2. FTIR spectra of SiNP (black), SiNP-APTES (blue), SiNP@QDs (green), SiNP@QDs-Ab (red)

To measure the fluorescence intensity, 1 mg of SiNP@QDs-Ab were dispersed in 1 mL of PBS (Figure 3). The fluorescence intensity of SiNP@QDs-Ab were observed using fluorescence spectrophotometer and the maximum intensity was observed at wavelength of 595 nm is being 633.10 a.u.

The *E coli* detection was also observed using fluorescence spectrophotometer which resulted in maximum intensity of 281.86 a.u at the same wavelength of 595 nm. The decrease in fluorescence intensity indicated that the presence of *E. coli* (1 x 10^7 CFU/mL) caused fluorescence quenching of the SiNP@QDs-Ab. The maximum fluorescence intensity change was used as the indication of sensing due to its sensitivity[19].

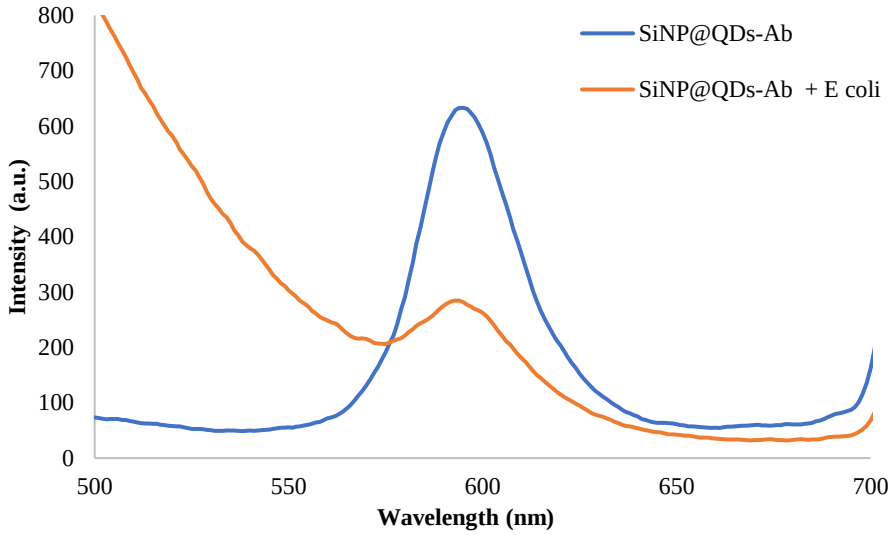

Figure 3. The fluorescence emission spectra of SiNP@QDs-Ab in PBS with concentration 1 mg/mL (blue) and SiNP@QDs-Ab in the presence of 1 x 10^7 CFU/mL of *E. coli* (red)

4 Conclusion

The synthesis of SiNP from natural based silica using sol-gel method was successfully carried out, with the specific surface area of 138 m^2/g and nanoparticle size of 43.5 nm. The XRD result shows the SiNP is in its amorphous phase. The surface of SiNP was modified using silanization method to immobilize the quantum dots and *E coli* antibody, the amide bonds between antibody and modified nanoparticles were confirmed by FTIR at wavenumber 1610-1690 cm^{-1} . The detection of *E coli* bacteria resulted in the decreaseingof the maximum intensity at 595 nm of SiNP@QDs-Ab before and after *E coli*, which are 633.10 a.u and 281.86 a.u, respectively. This preliminary study of the surface modification of SiNP using silanization method for SiNP@QDs as *E. coli*

detection opens up to the possibilities of employing such system in bacteria detection in the future.

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