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Star shaped zinc sulphide quantum dots self-assembled monolayers: Preparation and applications in food toxin detection

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ABSTRACT

We report the results of studies relating to the fabrication of an electrochemical immunosensor based on self-assembled of star shaped thioglycolic acid capped zinc sulphide quantum dots (ZnS QDs) synthesized by hot injection method. Structural and morphological investigations of ZnS QDs have been accomplished via transmission electron microscopy (TEM), X-ray diffraction spectroscopy (XRD), UV–visible spectroscopy (UV–vis) and Photoluminescence spectroscopic (PL) techniques. Further, monoclonal aflatoxin-B_1 antibodies (anti-AFB_1) have been covalently immobilized onto the ZnS/APTES/ITO electrode surface for the detection of aflatoxin-B_1, a potential food toxin using electrochemical impedance spectroscopy. The impedemetric response of the proposed immunosensor shows high sensitivity (176.58 Ω/(ng/mL)/cm^2), improved detection limit (0.02 ng/mL) and wide detection range (0.25–2.0 ng/mL). The ZnS QDs based platform provides an efficient platform for the fabrication of immunosensor for the detection of aflatoxin- B_1 and these results have implications in the design of highly efficient biosensors for other potential toxins.

1. Introduction

Quantum dots (QDs) have now been attracting tremendous attention due to potential application in various fields such as lasers, third generation solar cells, single photon emitters, QD memories, biosensing and clinical diagnostics [1,2]. Uniform QD films and monolayers having precisely controlled size and shape may serve as a template for the next generation of nanoelectronic and optoelectronic devices. Several different synthetic routes have been proposed for the preparation of QDs. Among various QDs, ZnS QDs have attained remarkable interest especially for the application in biological field due to the apparent reasons such as less toxicity among other II–VI semiconductor nanoparticles [3]. However, highly ordered ZnS nanocrystals having uniform particle size, shape and good optical properties have rarely been reported in the literature for biomedical applications.

Aflatoxins are secondary metabolites that are produced by Aspergillus *flavus* and Aspergillus *parasiticus*. Aflatoxin is highly toxic chemical compound that can cause both acute and chronic toxicity in human and animals [4]. There are four major members of aflatoxins as B_1, B_2, G_1 and G_2 among which aflatoxin-B_1 is known to be most toxic chemical substance and it is responsible for human hepatocellular carcinoma. Aflatoxin generally enters through the food chain mainly by injection via dietary route. The presence of very small amount of aflatoxin can cause a major problem leading to liver failure or liver cancer. There are conventional techniques which are used for the detection of aflatoxin-B_1 such as high pressure liquid chromatography (HPLC), thin layer chromatography (TLC), gas liquid chromatography (GLC) [5]. However, these techniques are very expensive and time consuming. Due to the complex implications of food toxins, efforts are being made to develop rapid and sensitive method for the detection of minute amount in the food samples [6,7]. In this regards, development of electrochemical biosensor may offer a suitable platform for sensitive, rapid and reliable detection of food toxins.

Application of nanoparticles in electrochemical biosensor plays a significant role as transducer for the immobilization of biomolecules. Nanoparticles offer high surface to volume ratio enabling high loading of biomolecules on the transducer surface. In addition to this, they offer enhanced electron transport properties, improving biosensing characteristics like sensitivity and detection limit. In this context, QDs have been considered to have great potential in biosensing due to small size, quantum confinement, size tunability, band gap tunability, high surface reactivity, wide absorption, narrow emission band spectra with excellent photostability and quantum yield [8,9]. The performance of biosensor

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mainly depends on the immobilization of biomolecules onto suitable matrices. Development of hybrid materials for tailoring the biosensor to obtain improved sensitivity and stability that may lead to evolution of advanced biosensor. It has been suggested that QDs can be used as functional materials for electrochemical sensing, owing to their high reactivity and ability to participate in charge transfer [10–12]. The electrochemical performance of the immunosensor mainly depends upon the selection of the materials, the properties of materials, availability of the conducive functional groups for the covalent immobilization of antibodies and activity of the antibodies on electrode surface [38,39]. Besides this, QDs play an important role towards enhancing the electron transfer between the immobilized biomolecules and electrode surface, which may be attributed to higher charge detaching efficiency arising from their quantum size effect which also enhance electron transfer between the immobilized biomolecules resulting improved performance of electrochemical biosensor [13–15]. The anisotropic shape of QDs have very significant role in enhancing the conductivity of the nanostructured matrices [40].

Sharma et al. have reported the results of nanostructured composited of chitosan-cadmium telluride (CdTe) QDs and TGA capped CdTe QDs for chronic myelogenous leukemia detection which shows a detection limit as 5.9×10^{-8} M [16]. Further, Zhang et al. have reported core-shell structure of glucose oxidase capped CdSe/ZnS QDs for glucose detection having wide detection range from 4.4–6.6 mM [17]. Although, they exhibit improved biosensing having heavy-metal toxicity limits their application in biomedical field.

The fabrications of ordered and oriented architectures of QDs have lots of scope in futuristic nanotechnology and biomedical applications. In this context, self-assembly of ordered structures of nanomaterials has become major area of research owing to their potential applications. Shen et al. have synthesized ZnS nanowires based on self-assembly and observed that formation of three fold tetrapod like and six fold heptapod like structures [18]. ZnS structures having each pod diameter of 50–70 nm by maintaining the temperature at 1300 °C. Similarly, wurtzite ZnS 3-Dimensional architecture has been synthesized such as nanorods network by the self-assembly from nanorods and ZnS nanostructures has been transformed from 3-D architecture to 1-D nanowires by providing the temperature variation of 160 °C to 200–240 °C [19].

The specific shape and size of the ZnS QDs are probably the most significant aspect in achieving superior immunosensing. Formation of well ordered structures may introduce anisotropic mobility though the different edges of the QDs and facilitating the electron transfer and improving the electrochemical immunosensing response characteristics [4,5]. Therefore, in the present manuscript, star shaped ZnS QDs self-assembled monolayers have been fabricated by optimizing various physical parameters and detailed structural and morphological investigations have been carried out. We report a facile, reproducible and optimized approach for the synthesis and self-assembly of thioglycolic acid capped star shaped ZnS QDs for the applications in toxic and carcinogenic food toxin, aflatoxin detection for the first time.

2. Materials and methods

2.1. Chemicals

Zinc acetate, sodium sulphide, thioglycolic acid (TGA), (3-aminopropyl) triethoxysilane (APTES), toluene, Aflatoxin-B_1 (AFB_1), anti-Aflatoxin mouse monoclonal antibodies (anti-AFB_1) and bovine serum albumin (BSA) have been purchased from Sigma-Aldrich, India. Deionized water (Milli-Q, Millipore, 18.2 M Ωcm) has been used to prepare buffers and aqueous solutions. All the reagents used for this study are of analytical grade and have been used without further purification.

2.2. Instruments

Spectroscopic characterization has been carried out for structural analysis using UV–vis spectrophotometer (Lambda 950, PerkinElmer), Photoluminescence spectrometer (Jobin Yvon-Horiba, model 3–11), Fourier transform infrared (FT-IR) spectroscopy (Spectrum BX, PerkinElmer) and X-Ray Diffractometer (Cu Kα radiation, Rigaku). Morphological investigations of zinc sulphide QDs have been studied using high resolution transmission electron microscope (HR-TEM, Tecnaii-G2F30 STWIN) and scanning electron microscope (SEM, LEO 440). Electrochemical characterization has been conducted using Autolab Potentiostat/Galvanostat (Eco Chemie, The Netherlands) in phosphate buffer saline (PBS, 50 mM, 0.9% NaCl, pH 7.4) containing 5 mM $[Fe(CN)\ 6]^{3-/4-}$ (redox species) using three electrode cell having Ag/AgCl as reference electrode and Platinum (Pt) as the counter electrode.

2.3. Synthesis of zinc sulphide quantum dots

TGA capped ZnS QDs have been synthesized using previously reported hot injection method with slight modification [20]. In a three necked round bottom flask, 100 mL of aqueous solution of TGA (10.3 mmol) is taken and kept on stirring under inert atmosphere. After 1 h, 100 mL 10.26 mmol of zinc acetate is added at pH-11.5 for 12 hrs by continuous stirring under inert atmosphere. Sodium sulphide is slowly and successively added to this mixture till white dense precipitate appear [21]. Stirring is further continued for 2 hrs at a temperature of 50–70 °C. Thus synthesized QDs are thoroughly washed several times with distilled water followed by acetone to remove impurities from the solution. The QDs are collected after repeated washing and centrifuging cycles.

2.4. Deposition of zinc sulphide quantum dots by self-assembly

Firstly, indium tin oxide (ITO) coated glass substrates ($1 \times 2\ cm^2$) are hydrolyzed with $H_2O_2/NH_4OH/H_2O$ (1:1:5, v/v) solution for 30 min at 80 °C. For self-assembled monolayers (SAM) formation, the hydrolyzed ITO coated glass substrates are immersed in aqueous solution of APTES-toluene (0.2%, v/v) for about 2 hrs followed by washing with distilled water to remove any unbound APTES molecules. Subsequently, the silanized ITO substrates are dipped overnight in ZnS solution containing *N*-Ethyl-*N*'-(3-dimethylaminopropyl) carbodiimide (EDC;0.2 M) and *N*-hydroxy succinide (NHS;0.05 M). The presence of EDC:NHS leads to the activation of carboxylic groups of TGA capped ZnS QDs, restoring into the self-assembly on APTES/ITO electrode via amide bond formation [22]. The silanized ITO coated electrode substrates are subsequently rinsed with distilled water to remove any unbound ZnS QDs and dried in air (Fig. 1).

2.5. Immobilization of antibody of aflatoxin (anti-AFB_1) onto fabricated electrode

The monoclonal aflatoxin (anti-AFB_1) antibodies have been covalently immobilized onto self-assembled ZnS/APTES/ITO electrodes surface. The concentration of antibody (anti-AFB_1) to be immobilized on the ZnS/APTES/ITO matrices has been optimized by varying the concentration of antibodies from (2–15 μg/mL) as shown in (Fig. S5). The optimum concentration of 10 μg/mL anti-AFB_1 in phosphate buffer saline (PBS, pH 7.4) has been uniformly spread onto ZnS/APTES/ITO electrodes surface which has