

Article

Biocompatible Osmium Telluride-Polypyrrole Nanocomposite Material: Application in Prostate Specific Antigen Immunosensing

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Abstract: Prostate cancer is a dominant global threat to society. It affects nearly 4000 men in South Africa annually, making it the second most threatening cancerous disease after lung cancer. A potential serological biomarker to monitor early diagnosis of prostate cancer is prostate specific antigen (PSA). We used the PSA biomarker in our work to develop an extremely sensitive electrochemical immunosensor to achieve low detection limits. The fabrication steps followed with the combination of thioglycolic acid capped osmium telluride quantum dots (TGA-OsTe₂QD)-polypyrrole (PPy) nanocomposite and prostate specific antigen modified on a glassy carbon electrode. The UV-Vis signatures of TGA-OsTe₂QD-PPy showed an absorption band at 262 nm which is attributed to the PPy and TGA-OsTe₂QD composite. This band corresponds to the energy band gap of 4.4 and 5.4 eV. The CV responses of BSA | Ab | TGA-OsTe₂QD | PPy | GCE modified electrode to prostate specific antigen (PSA) was studied within a range of 0–16 ng/mL PSA that was linear, herein referred to as linear range (LR), which produced a limit of detection (LOD) value of 0.36 ng/mL PSA. The values of the immunosensor's calibration parameters (LR and LOD) make them suitable for real sample application, due to their coverage of the PSA concentration range (0–14 ng/mL) that is of clinical importance.

Keywords: antibody; cyclic voltammetry; electrochemical immunosensor; osmium telluride quantum dots; polypyrrole; prostate specific antigen



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1. Introduction

Prostate cancer is a global threat to society due to its highly prevalent rate in males. The WHO determines prostate cancer to be the second leading cause of morbidity amongst the male population, counting more than 4000 cases in South Africa annually. The extent and morbidity ratio due to noncutaneous malignancy positions the cancer in the top five list of death-causing cancers worldwide [1,2]. The incidence of prostate cancer correlates to the factors of age and time of diagnosis. An early inspection of the malignancy seldom needs very little or no treatment at all. However, the asymptomatic behavior of cancerous cells at an early stage is a bottleneck for early cancer diagnosis, defining it as the most critical step. The typical symptoms do not manifest until the later stages of cancer, leaving out immediate care or are left treated due to hesitance on patient's part to seek medical attention. Many diagnostic centers determine the cancer on basis of elevated prostate specific antigen levels. However, high PSA levels and other cancer appearing symptoms such as infection, inflammation or enlargement of prostate may not directly relate to this disease. These limitations in clinical assessment could be misleading and has a considerable ambiguity in a proper diagnosis [3]. It is due to these reasons that early diagnosis faces hurdles, and the patient is unaware of the disease until serious symptoms

appear. ELISA test for quantitative monitoring of PSA in human serum, a physical examination of the prostate called Digital Rectum Examination (DRE), PSA blood test and MRI scan, are all clinical tests for this disease that may fail at some point of time due to very low sensitivity and detection limit. For example, ELISA has a detection range of 0–100 ng/mL which is quite low [4–6]. Therefore, objective strategies that could enable early diagnosis are overwhelmingly needed, not only due to the malignant disease but also to present cost-effective, sensitive, specific and non-invasive prognostic assessments in the market.

Numerous studies for cancer detection have been reported; however, the improved cancer survival rates are rather low. The limitation is due to the last stage detection and delayed diagnosis of cancerous cells, making tumors less curable. To achieve early detection of any cancerous disease, the discovery of specific, sensitive and reliable biomarkers is required [7]. Prostate specific antigen (PSA) is a potential biomarker considered as the most reliable tool for diagnostics of prostate cancers [8]. Studies illustrate more prominent detection of malignancies by PSA than DRE or any other isolated parameter. This theory led to the development of rapid and sensitive point of care testing (POCT) detection devices for PSA [9]. Electrochemical immunosensors in cancer clinical testing has outreached in this area and has potential advantages over other clinical analysis methods [10]. The portable detection systems are promising tools that hold the potential to convert complex assays in easy-to-use systems and distribute them amongst the community for their wide use [11]. Immunosensors can be designed to detect developing cancer biomarkers and to allow improved monitoring of cancer growth and patient therapy [12].

The introduction of nanomaterials in biosensing applications is due to their unique characteristic features desirable in this area such as small size, high surface area, excellent electronic properties, specific physicochemical characteristics and good biocompatibility [13,14]. The sensitivity of biosensors significantly increases by incorporation of nanomaterials at the interface, where most of the chemical reactions takes place [15]. At present, nanostructured platforms such as metals, metal oxides, conducting polymers and organic-inorganic hybrid nanocomposites are being used significantly as the immobilizing matrix to improve the signal and sensitivity of the biosensor [16]. Biocompatible thioglycolic acid (TGA) capped osmium telluride (TGA-OsTe₂) quantum dot (QD) material was used as a mediator in this study. Conducting polymers, PPy used in this work, are commonly chosen in electrochemical experiments due to their biocompatible and inert nature. They prevent fouling of electrodes and cause minimal interference to electroactive materials [16]. PPy is used mostly in biosensors and immunosensors due to biocompatibility and the ease of immobilization of biological compounds [17]. It is important to note that nanomaterials are therefore decorated with conductive polymers in order to enhance biocompatibility and to achieve precise targeting [18]. Hence, in this study PPy was electrosynthesised on the glassy carbon electrode surface using 30 cycles. This was followed by the incorporation of the biocompatible TGA-OsTe₂QD material onto the PPy | GCE modified electrode surface. Lastly, the electrochemical immunosensor for prostate specific antigen (PSA) was prepared by immobilizing anti-PSA antibody onto the TGA-OsTe₂QD | PPy | GCE modified electrode for 6 h.

2. Experimental Details

2.1. Chemicals and Sample Preparation

Analytical grade sodium hydroxide (NaOH, 98%), granular sodium borohydride (NaBH₄, 98%), osmium tetrachloride powder (OsCl₄, 99%), tellurium powder (Te, 99.99%), thioglycolic acid (TGA, 99%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 98%), N-hydroxysuccinimide (NHS, 98%), 0.1 M lithium perchlorate (LiClO₄, 98%), pyrrole (non-distilled C₄N₅H, 98%), absolute ethanol, disodium hydrogen phosphate (Na₂HPO₄, ≥98%), sodium dihydrogen phosphate (NaH₂PO₄, 99%), 1% bovine serum albumin (BSA, ≥97%), the anti-prostate specific antigen antibody (ab 33609) and native human PSA protein (ab 96163) were all purchased from Merck, Johannesburg, South Africa. Micropolish II alumina powder 0.05 µm, 0.3 µm and 1.0 µm was purchased from Buehler,

Lake Bluff, IL, USA. An amount of 0.1 M PBS (pH 7) was used as the working solution for all the electrochemical measurements. All the antibody and antigen stock solutions (100 µg/mL) and their working solutions (20 µg/mL) used in the experiment were prepared with 0.1 M PBS (pH 7, 4 °C) and kept in the fridge when not in use. All solutions were prepared using double distilled water and deoxygenated with nitrogen (N₂) prior to use.

2.2. Instrumentation

All electrochemical experiments were performed using a conventional three electrode set up. Herein, a glassy carbon electrode (GCE), platinum wire and Ag/AgCl (3 M NaCl) act as the working, counter, and reference electrode, respectively. Voltammetric experiments were carried with a CHI 760E Electrochemical Workstation (CH Instruments, Inc., Shanghai, China). Ultraviolet-visible (UV-Vis) absorption measurements of the prepared TGA-OsTe₂QD, PPy and TGA-OsTe₂QD-PPy were obtained using 1 cm quartz cuvette on a Nicolet Evolution 100 UV-Visible Spectrophotometer (Thermo Electron, Cheshire, UK) over a wavelength range of 200 to 800 nm. The morphology, size and shape of TGA-OsTe₂QD, PPy and TGA-OsTe₂QD-PPy were characterized by high-resolution transmission electron microscopy (HR-TEM), using a Tecnai G2 F20X-Twin MAT 200KV HRTEM Spectrometer equipped with an Energy-Dispersive X-ray Spectroscopy (EDS) detector—product of Field Electron and Ion Company (FEI) Europe, Eindhoven, The Netherlands. Copper grid (Cu) was used as a sample holder to immobilize 2 µL sample solution to obtain micrographs, at room temperature. Small Angle X-Ray Scattering (SAXS) spectroscopic measurements were obtained with Anton Paar SAXSpace Spectrometer (Anton Paar, GmbH, North Ryde, Australia). Samples were placed in a 1 mm diameter quartz capillary positioned at a distance of 317 mm from the SDD camera and temperature controlled at 20 °C.

2.3. Synthesis of TGA Capped OsTe₂QD

The synthesis of TGA-OsTe₂QD was achieved via bottom-up approach by following the method illustrated by Chang et al. [19], with slight modifications. Osmium tetrachloride and tellurium powder were used as Os and Te sources, respectively. As a first step, osmium tetrachloride solution was prepared by addition of 0.056 g OsCl₄ and 96 µL TGA capping in 25 mL distilled water under continuous stirring. Meanwhile, the solution was adjusted to a pH of ±12.06 using 0.1 M NaOH and was bubbled under nitrogen gas for 45 min at 25 °C. The second step involves preparation of NaHTe solution using NaBH₄ as a reducing agent. Chemical reduction of tellurium ions occurred by mixing 0.0638 g of tellurium powder and 0.03783 g of NaBH₄ in 25 mL distilled water under nitrogen gas for 30 min at 100 °C. Then, NaHTe precursor solution was introduced to OsCl₄-TGA solution under constant stirring until a brownish-red color solution was obtained which indicates the formation of TGA capped OsTe₂ quantum dots (TGA-OsTe₂). Aliquots at different reflux time of TGA-OsTe₂QD were collected at different time intervals (i.e., 10, 20, 40 and 60 min). This was done to study the effect of time on the formation of quantum dots. At 60 min final TGA-OsTe₂QD material was obtained followed by immediate quenching.

2.4. Immunosensor Fabrication

A stepwise schematic representing the fabrication of electrochemical immunosensor is illustrated in Figure 1. The fabrication starts with polishing of glassy carbon electrode (GCE) on micro cloth pad to a mirror-like finish with 1 µm, 0.3 µm, and 0.05 µm alumina slurries. The step removes any adsorbed organic matter of the electrode surface which is followed by a thorough rinse with de-ionized water after each polishing step. The electrode is successively ultrasonicated for 10 min with absolute ethanol and distilled water to remove any possible absorbed alumina crystals on the GCE surface. The pretreated electrode was then electropolymerized using pyrrole monomer to make the electrode conductive. Pyrrole was distilled prior to use and stored in refrigerator in an air tight vial.

Electropolymerization of pyrrole monomer was carried out in aqueous solutions using cyclic voltammetry in a three-electrode setup containing 100 μL monomer solution along with 0.1 M lithium perchlorate as the supporting electrolyte in 30 segments in the potential range of -0.8 to 0.8 V. The PPy|GCE modified electrode was functionalized with 8 μL of a solution consisting of TGA-OsTe₂QD in the presence of 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide/N-Hydroxysuccinimide (EDC/NHS, ratio 1:1) for 12 h at room temperature. The mixture served to activate the amine groups of the polypyrrole film for conjugation with the carboxyl groups of the TGA capped OsTe₂ quantum dots. A drop of 5 μL anti-PSA solution (10 $\mu\text{g}/\text{mL}$ of antibody in 0.1 M PBS, pH 7.0) was then drop casted onto TGA-OsTe₂QD|PPy|GCE modified electrode surface and was allowed to dry for 6 h at 4 $^{\circ}\text{C}$. The interaction of antibody with the modified electrode surface could leave free active sites and it is necessary to block them. For this, the modified electrode was immersed in 1% BSA solution (5 μL) for 30 min to block the active sites and to remove non-specific absorption on the surface. In each step electrochemical measurement were performed in a potential sweep between -1 to $+1$ V at 20 mV/s scan rate. The immunoreaction was performed by dipping Ab|TGA-OsTe₂QD|PPy|GCE modified bioelectrode in 0.1 M PBS, pH 7.0, containing different PSA concentrations for 10 min. The variation in current response of immunoreactions upon PSA interaction with anti-PSA modified electrode was detected.

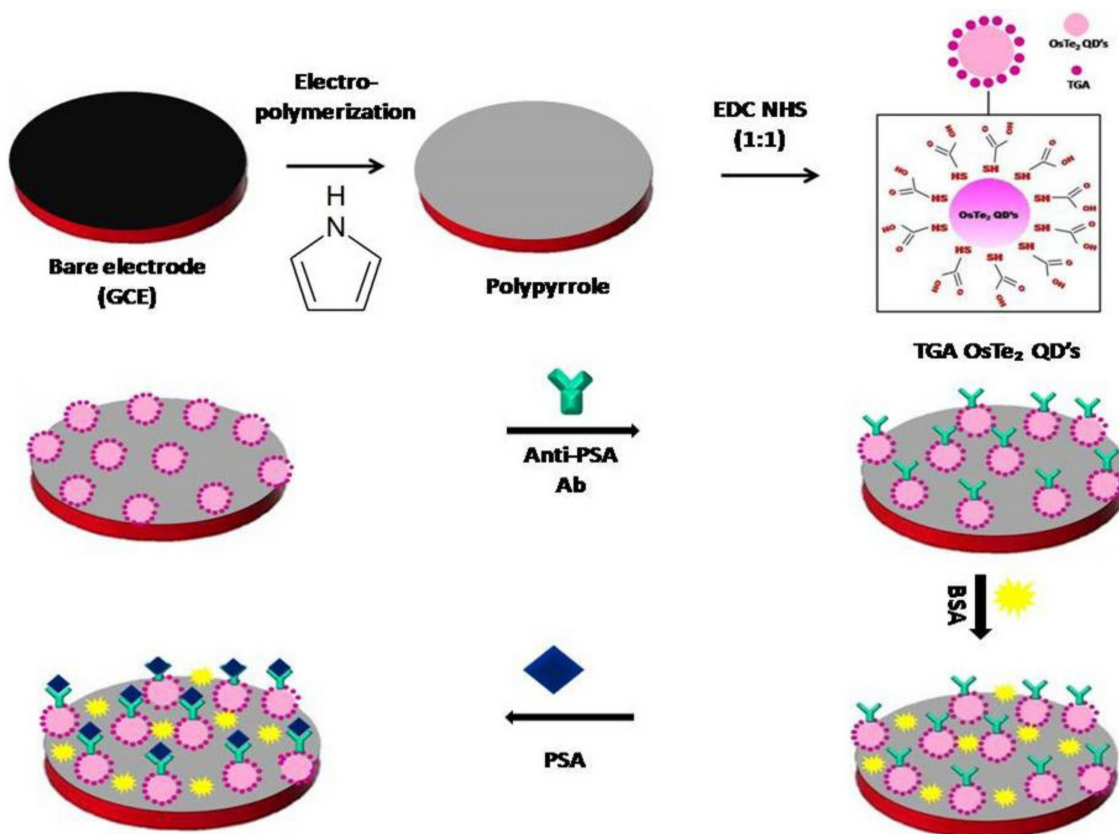


Figure 1. Schematic representation of electrochemical immunosensor.

3. Results and Discussion

3.1. Optical Properties of TGA-OsTe₂QD and PPy

UV-Vis spectrophotometer of PPy, TGA-OsTe₂QD, and TGA-OsTe₂QD-PPy materials are illustrated in Figure 2. As inspected carefully, Figure 2A line (a) shows two small absorbance bands at 262 nm and 295 nm which occurred due to π - π^* interaction of polypyrrole aromatic ring [20]. It is important to note that TGA-OsTe₂QD (Figure 2A, line(b)) exhibited three absorbance bands at 230 nm, 320 nm and 400 nm. The absorbance band at 230 nm is ascribed to the thioglycolic acid (TGA) capping agent which is in good agreement with previous findings on analogous mercaptocarboxylic acid molecules [21]. On the other

hand, the absorbance band at 320 nm is due to d valence band of Os^{8+} [22]. Lastly, the formation of TGA- OsTe_2QD was determined by the absorbance band appearing at 400 nm [23]. The incorporation of polypyrrole with TGA- OsTe_2QD (Figure 2A, line (c)) produced a more pronounced absorbance band at 262 nm. This is due to the interaction between the functionalized quantum dots and polypyrrole functionalities [24]. Figure 2B shows extrapolation values for Tauc plots. As can be seen in Figure 2(B1), polypyrrole exhibited an energy band gap value of 5.4 eV. The results obtained are in agreement with already reported studies for PPy [25]. Table 1 summarizes the energy band gap values of metal telluride chalcogenides and metal-polymer composites. As can be seen, the HgTe and WTe_2 obtained the lowest energy band gap values as compared to MoTe_2 and CdTe . Thus, the obtained energy band gap value for TGA- OsTe_2QD was found to be 4.5 eV (Figure 2(B2)). This value falls within the PbEuTe and PbSrTe band gap values of 4.5–4.7 eV [26]. On the other hand, the TGA- OsTe_2QD -PPy mixture exhibited two energy band gaps at 5.4 eV and 4.4 eV. These two values are attributed to the individual transition of PPy and TGA- OsTe_2QD as illustrated in Figure 2(B3,B4). Interestingly, these results are in accordance with the reported literature for ZnS -PPy (4.26–4.7 eV) and ZrO_2 -PPy (5.07 eV) [27,28].

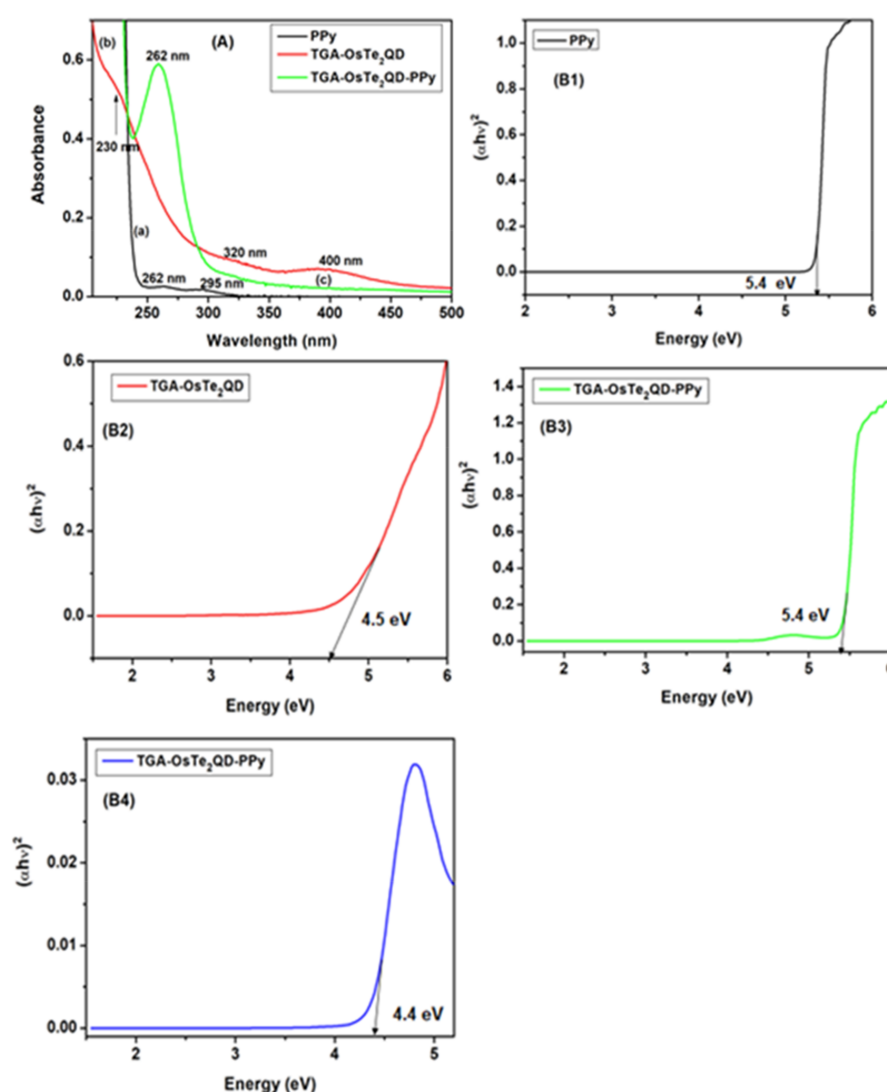


Figure 2. UV-Vis spectra and Tauc plot (A,B1–B4) of (a) PPy, (b) TGA- OsTe_2QD and (c) TGA- OsTe_2QD -PPy in a wavelength range of 200–500 nm.

Table 1. Energy band gap values of metal telluride chalcogenides and metal–polymer composites.

Metal Telluride Chalcogenides	Band Gap Energy (eV)	Reference	Metal–Polymer Composites	Band Gap Energy (eV)	Reference
PbEuTe	4.5	[26]	ZnS-PPy	4.26–4.7	[27]
PbSrTe	4.7				
Ag ₂ Te	3.64	[29]	ZrO ₂ -PPy	5.07	[28]
WTe ₂	0.2	[30]	NPs/rGO-PPy	3.58–3.85	[31]
CdTe	2.15–2.28	[32]	CdTe-PPy	1.45	[33]
MoTe ₂	1.15	[34]	CHI-PPy	1.30–2.32	[35]
OsTe ₂	4.5	This work	OsTe ₂ -PPy	4.4 and 5.4	This work

3.2. Internal Structure of TGA-OsTe₂QD and PPy

Small Angle X-ray Scattering provides suitable information regarding the overall shape and sizes of the as-prepared nanomaterials. The technique can quantify key features of the particles to identify the shape and peak symmetry into spherical (or globular), prolate (or cylindrical) and oblate (or lamellar) [36]. Figure 3A shows the PDDF of (a) pyrrole which exhibits almost bell-shaped symmetrical structure. According to the literature, the presence of spherical-shaped particles form a bell-shaped peak, and hence formation of globular particles could be concluded [37]. The results are in agreement with previous literature, where the structure of pyrrole as a bulky circular ring is reported. The small humps could be due to the aggregation of particles as it is having a bulky appearance. This behavior is also observed in HR-TEM where pyrrole exhibits a cloudy structure at the micro level and circular rings of pyrrole could be seen when zoomed to nanometer range [38]. As shown in Figure 3A line (b), TGA-OsTe₂QD exhibits a perfect bell-shaped structure indicating the presence of globular-shaped particles with maximum radius of 68 nm [39]. A small hump observed at 150 nm could be attributed to impurities and agglomeration. Interestingly, shown in Figure 3A line (c), TGA-OsTe₂QD-PPy produced two peaks at 44 nm and 114 nm. The two subunits of aggregates or dumbbell make a PDDF which is rather defined by the appearance of a second peak, the same as the case of TGA-OsTe₂QD-PPy. The two peaks represent the presence or interaction between TGA-OsTe₂QD and PPy.

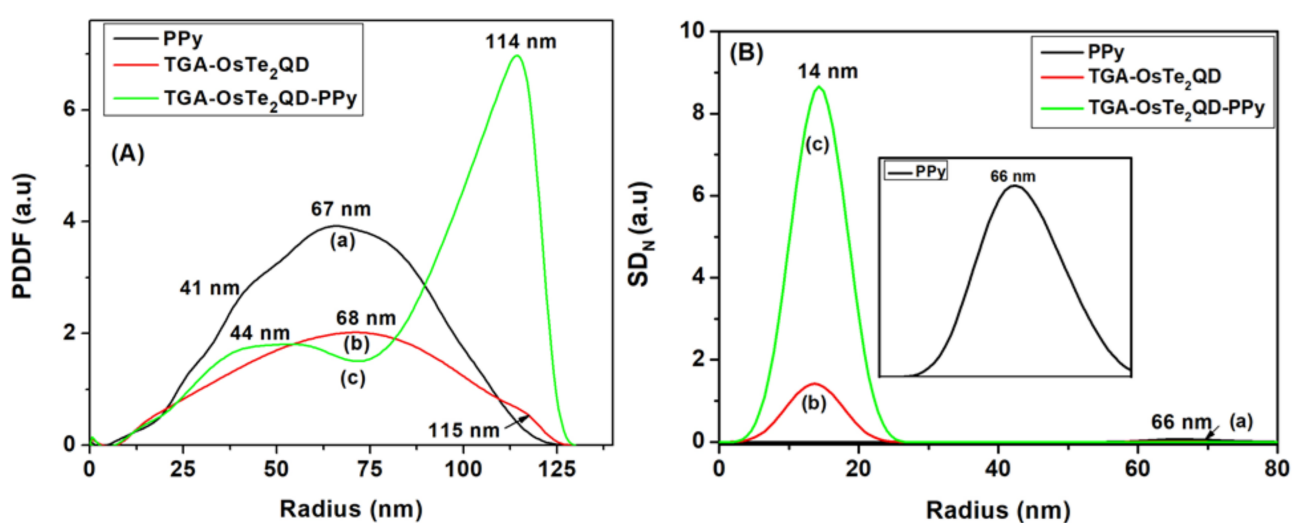


Figure 3. (A,B) SAXS free model PDDF and size distribution weighted by number for (a) PPy, (b) TGA-OsTe₂QD and (c) TGA-OsTe₂QD-PPy.

Figure 3B shows size distribution function weighted by number for PPy, TGA-OsTe₂QD and TGA-OsTe₂QD-PPy. It was observed that (a) PPy exhibited size distribution at 66 nm (insert a). However, (b) TGA OsTe₂QD and (c) TGA-OsTe₂QD-PPy show a narrow size distribution with a maximum radius of 14 nm. As inspected carefully, (c) TGA-OsTe₂QD-PPy exhibited an enhanced peak (95%) higher than the TGA-OsTe₂QD. This indicates that the combination of the materials enhances the signal.

3.3. Morphological Properties of TGA-OsTe₂QD and PPy

Further analysis of PPy, TGA-OsTe₂QD, and TGA-OsTe₂QD-PPy was performed by HR-TEM and corresponding statistical size distribution histograms (Figure 4), to validate the information about the shape, size and structure of the composite material. HR-TEM micrograph of PPy was taken at the scale view of 200 nm. The image shows a cloudy structure of aggregated polymeric particles, forming a bulky cloud with doughnut-shaped circular dark rings [40]. The corresponding size distribution ranges from 60–160 nm, with maximum size distribution of 107 nm (Figure 4B). TGA-OsTe₂QD (Figure 4C) exhibits globular-shaped particles with a homogeneous size distribution of 5 nm. The results are comparable and in good agreement with the QDs UV-Vis absorption spectra which showed a significant absorption band and could be attributed to refluxing process that allows the growth of globular sized QD material [41]. The affirmation of crystallinity was done by lattice fringes, estimating an average diameter of the QDs to be 1–2 nm. On the other hand, Figure 4C TGA-OsTe₂QD-PPy at a scale view of 20 nm shows aggregates of cloudy structure along with the presence of globular shaped particles [37]. The pyrrole monomer and the QD combined together to form aggregates, comparable to SAXS histogram. It can be seen that the particle sizes of TGA-OsTe₂QD-PPy were slightly bigger than in TGA-OsTe₂QD material. The increase in particle sizes are attributed to the interaction between TGA-OsTe₂QD and polypyrrole during the growth into larger particles.

3.4. Electroanalysis of Immunosensor Platform

3.4.1. Analysis of Biosensor Platform

Herein, antibody (Ab) exhibited two absorbance bands at 262 nm and 295 nm at a relatively low absorbance value due to quenching effect as shown in Figure 5A line (a). The presented spectral absorbance bands results from amino acid residues, particularly tryptophan (Trp, 295 nm), tyrosine (Tyr, 295 nm) and phenylalanine (Phe, 262 nm) [42]. The absorbance band at 262 nm is ascribed to π - π^* and n - π^* electronic transitions of aromatic heterocycle in Phe [43]. On the other hand, the disulfide bond of Trp residue (cystine) is responsible for the absorbance band at 295 nm [44]. It was noticed that BSA (Figure 5A, line (b)) showed a special broad absorbance band at 267 nm with high absorbance intensity value of 0.15. This band is ascribed to π - π^* transitions of some aromatic amino acids of BSA [45]. Noteworthy, a solution containing a mixture of BSA and antibody resulted in a slight increase of antibody absorbance intensity as illustrated in Figure 5A line (c). Lastly, Figure 5A line (d) shows the presence of PSA in a solution mixture of BSA and antibody which caused an increase in the absorbance band at 262 nm. The increase is caused by interactions among charged amino groups (NH₂) of PSA and amino acids arising from BSA-Ab [46].

The antibody has a quenching effect on the material which reduces the absorbance intensity when attached to the nanocomposite, as is evident from the UV-Vis spectra of antibody alone which has the lowest absorbance behavior (Figure 5A, line (a)). A similar response was observed when antibodies (Ab) were added to the solution containing a mixture of TGA-QsTe₂QD-PPy, which resulted in a decrease of absorbance band at 262 nm (Figure 5B, line (b)). This decrease is attributed to the covalent bonding interaction between the carboxylic group (COOH-) of TGA-OsTe₂QD and amino group (NH₂) of the antibody [47]. Figure 5B shows sequential addition of (c) BSA and (d) PSA in a solution mixture containing Ab-TGA-OsTe₂QD-PPy which leads to a gradual decrease in the absorbance band at 262 nm due to further quenching of biomolecules. The addition of

PSA in the solution mixture of BSA-Ab-TGA-OsTe₂QD-PPy reveals two small and broad peaks at 262 nm and 295 nm. The results are comparable to the absorbance spectra of PPy solution where the monomer shows an exact behavior due π - π^* interaction of polypyrrole aromatic ring (Figure 2A, line (a)).

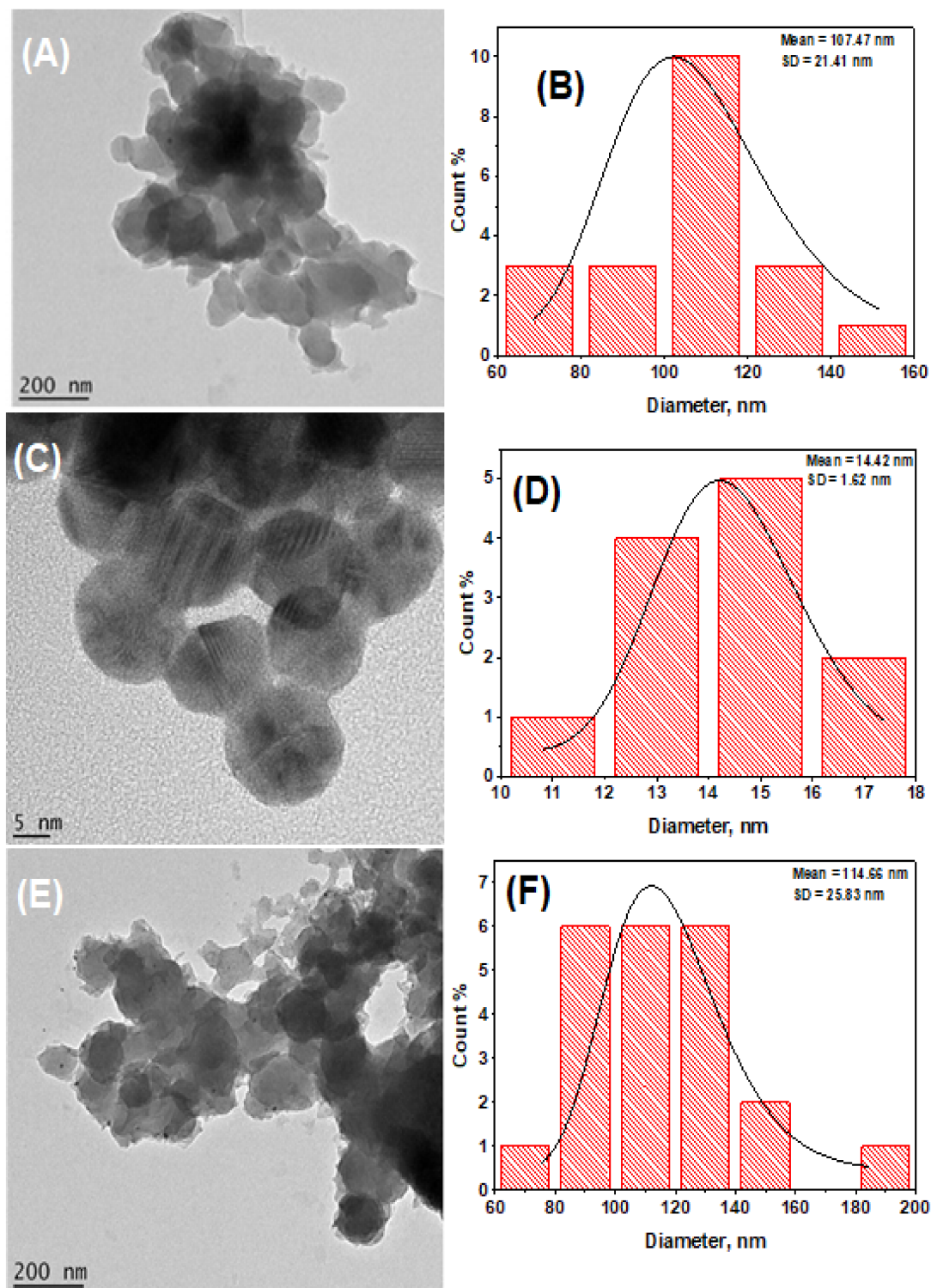


Figure 4. HR-TEM micrographs of (A) PPy, (C) TGA-OsTe₂QD and (E) TGA-OsTe₂QD-PPy at 200 nm and 5 nm scale view. (B,D,F) show statistical size distribution histograms of PPy, TGA-OsTe₂QD, and TGA-OsTe₂QD-PPy, respectively.

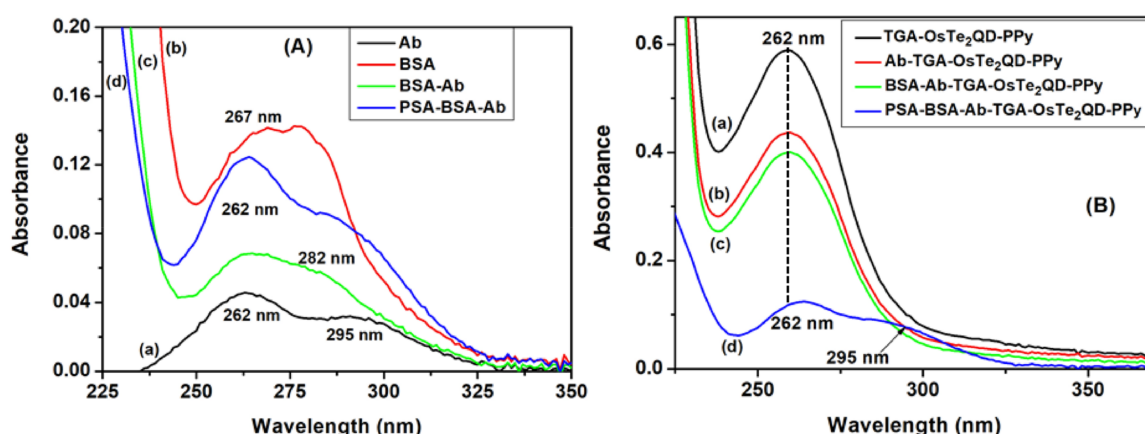


Figure 5. Optical signatures of (A): (a) Ab, (b) BSA, (c) BSA-Ab and (d) PSA-BSA-Ab. (B) UV-Vis spectrophotometer of (a) TGA-OsTe₂QD-PPy, (b) Ab-TGA-OsTe₂QD-PPy, (c) BSA-Ab-TGA-OsTe₂QD-PPy and (d) PSA-BSA-Ab-TGA-OsTe₂QD-PPy in 0.1 M PBS, pH 7.

3.4.2. Electrochemistry of the Immunosensor Platform

Electrochemical deposition is a convenient method for electropolymerization of PPY thin films on the electrode surface [48]. This method provides a better way of synthesizing PPY films and is able to monitor the properties including thickness and surface topography that can be easily controlled by the electrochemical polymerization conditions (e.g., electrode potential, current density, and electrolyte solutions). The crucial parameters of a thin film are its film thickness and topography, and electrochemical deposition holds a good control over it. These could be easily modified by altering the polymerization conditions, such as the number of cyclic scans, electrode potential and concentration of electrolyte solutions [49]. Figure 6A shows the electrosynthesis process of 0.1 M pyrrole (PPy) monomer in 0.1 M lithium perchlorate (LiClO₄) using cyclic voltammetry within a potential range of -1 and 1 V at a scan rate of 20 mV/s for 30 cycles. The growth of polypyrrole films on the electrode surface was measured by monitoring the increase in current waves. As can be seen, the oxidation peak observed at 0.21 V was due to the neutral species of PPy that is oxidized to PPy⁺ [50]. During the reduction process, one reduction peak was observed at 0.01 V due to the cationic species of PPy⁺ being reduced back to its original form (PPy).

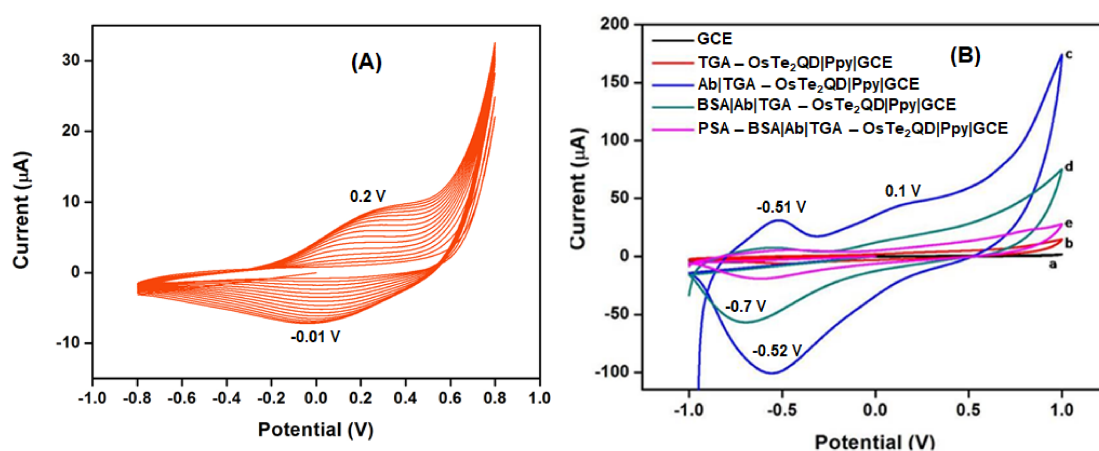


Figure 6. (A) Electrosynthesis of pyrrole monomer in 0.1 M LiOCl₄ solution at a potential window of -1 to 1 V at a scan rate of 20 mV/s for 30 cycles. (B) CV measurements of immunosensor platform where curve a represents GCE, curve b TGA-OsTe₂QD|PPy|GCE, curve c Ab|TGA-OsTe₂QD|PPy, curve d BSA|Ab|TGA-OsTe₂QD|PPy|GCE and curve e PSA-BSA|Ab|TGA-OsTe₂QD|PPy|GCE (for 10 ng/mL PSA) in 0.1 M PBS at 20 mV/s in the potential window of -1 to 1 V.

Figure 6B shows CV measurements comparing the characteristic features of (a) GCE, (b) TGA-OsTe₂QD|PPy|GCE, (c) Ab|TGA-OsTe₂QD|PPy|GCE, (d) BSA|Ab|TGA-OsTe₂QD|PPy|GCE and (e) PSA-BSA|Ab|TGA-OsTe₂QD|PPy|GCE at a potential window of -1 to 1 V in 0.1 M PBS, pH 7. As can be seen, the presence of the TGA-OsTe₂QD (curve b) on the PPy|GCE modified electrode resulted in an increase in current response. Two prominent oxidation peaks at -0.51 V and 0.1 V as well as one reduction peak at -0.52 V are observed for (c) Ab|TGA-OsTe₂QD|PPy|GCE (curve c). The peaks are assigned to the interactions between carboxylic and amino groups from TGA capped QDs and antibody. [51]. Such an enhancement is associated with an enhanced electron transfer from the electrode to buffer solution. On the other hand, electroactive charged TGA-OsTe₂QD|PPy|GCE surface serves as a good environment for antibody immobilization. This indeed improves PSA sensing limitations [52]. Furthermore, the electrochemical current response for the BSA|Ab|TGA-OsTe₂QD|PPy|GCE (curve d) decreased. At this point, a hinderance in charge transfer is expected due to the insulating characteristics of BSA. This confirms that BSA is effectively immobilized on electrode surface blocking most non-binding sites. The blockage results in less available electroactive surface which evidently decreases the current magnitude [53]. The PSA-BSA|Ab|TGA-OsTe₂QD|PPy|GCE for 10 ng/mL PSA (curve e) showed a decrease in current due to hindrance in electron transfer as a result of the capture of the PSA by its antibody. It is important to note that the capture promoted a slight shift of the reduction peak due to the immunosensor surface modification [54].

3.4.3. Electrochemical Responses for PSA

For an efficient electrochemical detection of PSA, important experimental parameters were optimized such as the incubation time and appropriate concentration of antibody incubation on the surface of TGA-OsTe₂QD|PPy|GCE. Firstly, the incubation time was varied to optimize the time period essential for the best response. Four sets of electrodes were fabricated with TGA-OsTe₂QD|PPy|GCE and subsequently incubated with 5 μ L of 20 μ g/mL Ab for 3, 6, 9, 15 and 24 h. As can be seen in Figure 7A, the current increases with increasing incubation time from 2 to 9 h, and thereafter starts decreasing at 15 and 24 h. The highest current response was obtained at 6 h, and hence was selected as an optimum time for antibody (Ab) incubation. The influence of increasing the concentration of the antibody was studied from 1 ng/mL Ab to 100 ng/mL Ab. It is evident from Figure 7B that 10 ng/mL Ab exhibited the maximum current response, and this concentration was employed for further analysis.

The interaction of analyte with antibodies causes immunoreactions at the electrode surface. These reactions cause alternation in the current response [53]. Figure 7C shows the CV responses of the immunosensor electrode (BSA|Ab|TGA-OsTe₂QD|PPy|GCE) in the presence of PSA concentrations. The presence of PSA resulted in a decrease in the cyclic voltammetric cathodic peak currents, as shown in Figure 7C for 10 – 16 ng/mL PSA. This decrease is ascribed to the interaction between the antigen and antibody which confirms the successful immuno-complex formation. The immuno-complex results in electron transfer hindrance to the electrode due to the insulating behavior of PSA. Figure 7D is the BSA|Ab|TGA-OsTe₂QD|PPy|GCE immunosensor's calibration plot, which gave a linear dependence of the decrease in the cathodic peak current (taken at -0.52 V of the CVs in Figure 7C) with increase in PSA concentration. A limit of detection (LOD) value of 0.36 ng/mL and a linear range (LR) value of 0 – 15 ng/mL, respectively, were calculated for the PSA concentrations studied. The linear regression analysis of the calibration plot in Figure 7D gave a R^2 value of 99.9% and a sensor sensitivity value of 2.56 ± 0.11 μ A/(ng/mL).

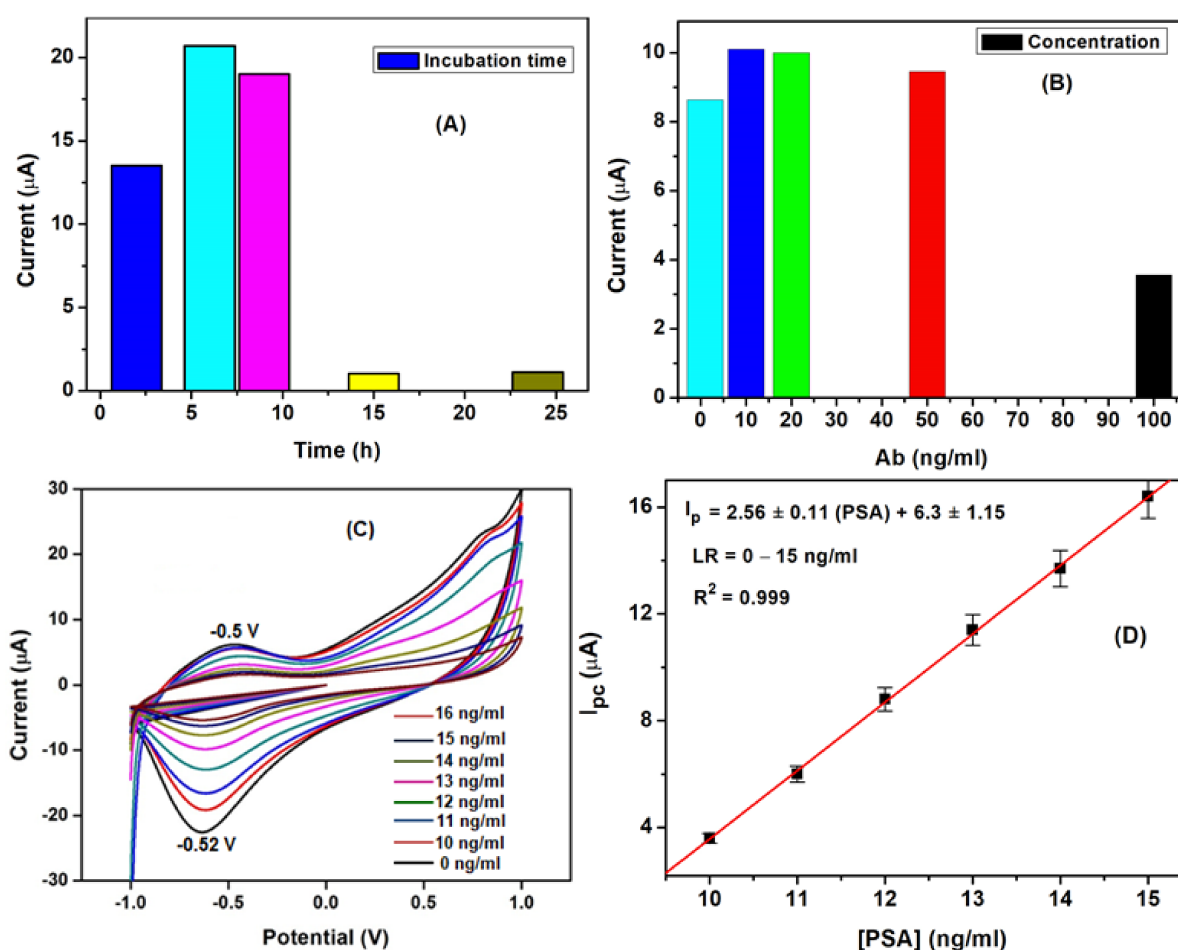


Figure 7. (A) Incubation time responses (i.e., CV cathodic peak currents at 20 mV/s) for the formation of Ab|TGA-OsTe₂QD|PPy|GCE immunosensor electrode with 5 μ L of 20 μ g/mL Ab in 0.1 M PBS pH 7. (B) Ab concentration dependence of the Ab|TGA-OsTe₂QD|PPy|GCE immunosensor electrode responses (i.e., CV cathodic peak currents at 20 mV/s) for 1–100 ng/mL Ab and 6 h incubation time in 0.1 M PBS pH 7. (C) CV responses of the PSA immunosensor at 20 mV/s, in 0.1 M PBS pH 7. (D) Linear calibration of the PSA immunosensor (i.e., BSA|Ab|TGA-OsTe₂QD|PPy|GCE) plotted with peak current values obtained from (C) at -0.52 V (standard errors of triplicate experiments are represented by error bars).

Jonoush et al., 2019 [55], reported an LOD value of 1.4 ng/mL for PSA immunosensor based on GO/AuNPs composite as a platform. Thus, the immunosensor's LOD value was four orders of magnitudes higher than what was obtained for the PSA immunosensor developed in this current work. Table 2 represents other PSA based immunosensors, which implies that the immunosensor reported in this study is very sensitive.

Table 2. Comparison of the proposed method with already reported immunosensor for PSA.

Electrode Materials	Detection Method	Linear Range (ng/mL)	Limit of Detection (ng/mL)	Ref.
Pd@Cu ₂ O NPs	EIS	10^{-5} – 1×10^2	2×10^{-6}	[56]
Au/Ag-rGO/Animated-GQD/Carboxyl-GQDs	ECL	1×10^{-3} –10	2.9×10^{-4}	[57]
AuNP-PANI/Au	DPV	1×10^{-3} – 1×10^2	6×10^{-4}	[58]
Au-NS/POEGMA-co-GMA brush	EIS	5×10^{-3} – 1×10^3	2.3×10^{-3}	[59]
GO/AuNPs	CV	-	1.4	[55]
TGA-OsTe ₂ QD-PPy	CV	0–15	0.36	This work

4. Conclusions

We designed a novel nanocomposite film composed of TGA-OsTe₂QD-PPy on a glassy carbon electrode. The film is designed to construct an electrochemical immunosensor for a rapid and sensitive detection of PSA. The proposed nanocomposite exhibited high current response. Its stable response and an advantage of being biocompatible makes the film well competent for electrochemical immunosensors. The proposed immunosensor integrates the advantages of sensitive electrochemical detection and specific immunoreaction with nanoparticle amplification technique, which could provide a promising tool for PSA detection. The BSA | Ab | TGA-OsTe₂QD-PPy immunosensor exhibits improved sensing characteristics such as a linear range of 0–15 ng/mL (i.e., the concentration range studied was linear) and the limit of detection value of 0.36 ng/mL. The linear range and the limit of detection values of the immunosensor cover the physiologically relevant PSA levels (0–14 ng/mL) [60] for ascertaining prostate cancer related health conditions. The immunosensor was constructed with a highly selective anti-PSA antibody, which was shown in previous work [61] not to exhibit false positive signal in the presence of other proteins (i.e., interferences), such as alpha fetal protein (AFP), carcinoembryonic antigen (CEA), human chorionic gonadotropin (HCG), immunoglobulin G (IgG) and thrombin.

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