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A photo-electrochemical aptasensor for the determination of severe acute respiratory syndrome coronavirus 2 receptor-binding domain by using graphitic carbon nitride-cadmium sulfide quantum dots nanocomposite

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1. Introduction

The coronavirus disease 2019 (COVID-19) that is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a global healthcare challenge these days. Hence, there is an urgent need to fabricate a highly selective, sensitive, and cost-effective device for the diagnosis of SARS-CoV-2 RBD. Since SARS-CoV-2 RBD is a dangerous virus and those who work on it can get infected on COVID-19, the researchers prefer to measure the related biomaterials to the SARS-CoV-2 virus such as its RNA [1], nucleocapsid protein (Np) [2,3], spike protein [4], and receptor-binding domain (SARS-CoV-2 RBD) [5] for its detection. By now, various methods have been reported for the diagnosing of biomaterials related to SARS-CoV-2 such as immunosensor [4], clustered regularly interspaced short palindromic repeats (CRISPR) [6], reverse transcription-polymerase chain reaction (RT-PCR) [1], and fluorescent aptasensor [5]. However, these methods have several disadvantages such as unportable, high expensive, time-consuming, and require operators with a high level of experience.

The electrochemical aptasensors are good candidates to solve these disadvantages. The electrochemical aptasensors are the type of biosensor where an aptamer probe is used as the bio-recognizer element on the surface of a working electrode [7]. The aptamer is an artificial antibody that binds to its target with high affinity and specificity [8]. As the target binds to the immobilized aptamer on the surface of the working electrode, the working electrode then converts the aptamer-target interaction into a diagnostic electrical signal.

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ABSTRACT

Herein, a photoelectrochemical aptasensor for the quantitative measurement of the severe acute respiratory syndrome coronavirus-2 receptor-binding domain (SARS-CoV-2 RBD) has been reported for the first time. For this purpose, first, graphitic carbon nitride and (gC₃N₄) and cadmium sulfide (CdS) quantum dots were fabricated and characterized. After that, gC₃N₄ and CdS were mixed well. The fabricated nanomaterials were characterized by scanning transmission electron microscopy. Then, the CdS QDs-gC₃N₄ nanocomposite was added to the solution containing chitosan as an amine-rich polymer to generate a Chitosan/CdS-gC₃N₄ nanocomposite. Subsequently, the surface of the ITO electrode was modified with Chitosan/CdS-gC₃N₄. After that, the amine-terminal aptamer probes were immobilized on the surface of the Chitosan/CdS QDs-gC₃N₄/ITO electrode by using glutaraldehyde as an amine-amine crosslinker. The electrochemical performances of the electrodes were studied using cyclic voltammetry (CV), electrochemical Impedance Spectroscopy (EIS), and photo-electrochemistry (PEC). The surface coverage of the immobilized aptamer probe was founded to be 26.2 pmol.cm⁻². The obtained results demonstrated that the proposed photo-electrochemical aptasensor can be used for the measurement of Sars-Cov-2 RBD within 0.5–32.0 nM. The limit of detection (LOD) was obtained to be 0.12 nM (at 3σ slope). The affinity of the Aptamer/Chitosan/CdS QDs-gC₃N₄/ITO was also founded to be 3.4 nM by using Langmuir-typical adsorption systems. The proposed photo-electrochemical aptasensor was applied for the measurement of the spiked Sars-Cov-2 RBD in human saliva samples at two concentrations. The effect of the interfering biomaterials such as human immunoglobulin G human immunoglobulin A, human immunoglobulin M, and human serum albumin was also studied.
Compared to antibodies, aptamers are about 10 times smaller [10]. Therefore, more aptamers can be immobilized on the surface of the transducer. Also, aptamers are cheaper and can be synthesized with several functional groups [7]. Up to now, several electrochemical aptasensors have been designed for the diagnosis of viruses such as hepatitis B, [11,12], hepatitis C [13], virion [14], murine norovirus [15], human immunodeficiency virus (HIV) [16] influenza H1N1 [17, 18], and influenza H5N1 [19,20].

Photo-electrochemical (PEC) based-aptasensor is one of the highly sensitive electrochemical biosensors that can be used for the determination of biomarkers. In addition to the previously enumerated advantages of aptasensors, the PEC-based aptasensors have several additional advantages such as low background noise, low cost, simple operation, fast response, wide dynamic response range, and good stability, making them good candidates to fabricate the point of care biosensors [21, 22].

To the best of our knowledge, a photo-electrochemical aptasensor for the determination of the receptor-binding domain of Sars-Cov-2 RBD has not yet been reported.

To fabricate a photo-electrochemical biosensor, photoactive materials such as quantum dots (QD) [23–26], titanium dioxide (TiO2) [25, 27], and graphitic carbon nitride (gC3N4) [27] have been used to convert photons to electrons and then generate the photo-current. Cadmium sulfide quantum dots (CdS QDs) are one of the well-liked semiconductor materials that has been used for the fabrication of the photo-electrochemical-based biosensor because of its visible light activity and narrow band gap (2.4 eV) between the valence band and conduction band. However, the high recombination rate of photo-generated electrons and holes is its biggest disadvantage. To solve that, CdS QDs must be mixed with a second photo-active material that its band gap can overlap with the band gap of CdS QDs very well, separating the photo-generated electrons and holes each other. gC3N4/gC3N4 is a strong peak about at 27° and 53° are seen [31]. These results confirmed that CdS QDs and gC3N4 have several advantages such as non-hazardous, cheap, metal-free, two-dimensional π-conjugated nano-sheet, good chemical and thermal stability, and good electrical and optical properties, high-performance photo-activity and photo-stability [29,30].

In this study, CdS QDs and gC3N4 as photo-active materials, ascorbic acid as an electron donor molecule, chitosan as a binder, and an amine-terminal aptamer as a bio-recogizer have been used for the fabrication of the photo-electrochemical aptasensor. The results clearly display that the proposed aptasensor (Aptamer/Chitosan/CdS QDs-gC3N4/ITO electrode) can detect SARS-CoV-2 RBD in human saliva samples. The Aptamer/Chitosan/CdS QDs-gC3N4/ITO electrode revealed outstanding analytical performance in terms of dynamic response range, low detection limit, selectivity, sensitivity, and stability. The affinity of the proposed aptasensor to Sars-Cov-2 RBD was much better (about 4 times) than the previous reported method [5]. The proposed aptasensor is not only cheaper than the immunosensors that have been reported for diagnosing COVID-19 but also it has high sensitivity, low cost, and a portable device compared to the fluorescence apta-assay that has been reported before. Therefore, it has a great potential to be a point-of-care device to recognize COVID-19.

2. Experimental

2.1. Reagents and chemicals

The aptamer with the following sequences was obtained from Newtech: 5′-NH2(CH2)6-CAG CAC CGA CCT TGT TTG GGA GTG CTG GTCA AAG CGT TAA TGA ACA-3′ [5]. Potassium hexacyanoferrate (III) (K3[Fe(CN)6]), potassium hexacyanoferrate (II) (K4[Fe(CN)6]), potassium chloride (KCl), ascorbic acid (AA), phosphoric acid (H3PO4), potassium hydroxide (KOH), melamine, cadmium chloride (CdCl2), sodium sulfide (Na2S), thioglycolic acid (TGA), and isopropanol alcohol were purchased from Merck. Severe acute respiratory syndrome coronavirus 2 receptor-binding domain (Sars-CoV-2 RBD), glutaraldehyde, chitosan, 6-amino-1-hexanol, and indium tin oxide (ITO) on the polyethylene terephthalate substrate were obtained from Sigma-Aldrich. Deionized (DI) water (18.6 MΩ) was used throughout.

2.2. Fabrication of nanomaterials

CdS QDs [26] and gC3N4 [27] have been fabricated according to our previous research work.

Briefly, 2.0 g of melamine powder was transferred into an oven and the temperature of the oven was then increased to 520 °C for 4.0 h under argon conditions with a ramp rate of about 3 °C/min. Finally, gC3N4 was grounded in ammort with a pestle for 10 min. The yellow fine powder of gC3N4 was then dispersed in 1 mL of isopropanol alcohol in an ultrasonic agitation for 2 h to achieve a well-dispersed suspension. gC3N4 powder was then centrifuged, washed with isopropanol alcohol, re-dispersed in an ultrasonic agitation, and centrifuged again. This process was repeated 10 times to reach a thin layer nano-sheet of gC3N4. Finally, the obtained fine powder was dried at 60 °C.

To fabricate CdS QDs, 1.3 mL of 0.15 M thioglycolic acid solution and 80.0 mL of 0.001 M CdCl2 solution were mixed in a round bottom flask. After that, 3.0 mL of 0.1 M NaOH was added gradually into the above solution to reach the pH value of the solution to 8.0. Subsequently, nitrogen gas was then purged through the above solution for 30 min. 20.0 mL of 0.002 M of Na2S was added into the flask and the mixture was allowed to react for 30 min at room temperature under a nitrogen gas atmosphere. Finally, CdS QDs powder was centrifuged, washed with ethanol, re-dispersed in ethanol and centrifuged several times, and dried at 60 °C.

To fabricate CdS QDs-gC3N4 nanocomposite, 5 mg of gC3N4 and 1 mg of CdS QDs were added in 10 mL DI water and mixed together by using ultrasound for 2 h. The obtained nanocomposite was characterized by Energy-dispersive X-ray spectroscopy (EDX), and X-ray powder diffraction (XRD) (Fig. S2). The EDX result (Fig.S2A) specified that the composite contains nitrogen, carbon, cadmium, and sulfide elements. XRD analysis of CdS QDs-gC3N4 was also obtained (Fig.S2B). As shown, a strong peak appears at 27° related to gC3N4 and two small peaks related to CdS QDs at about 45° and 53° are seen [31]. These results confirmed that CdS QDs and gC3N4 were mixed together.

2.3. Fabrication of the aptasensor

1 mL of chitosan solution (0.5 wt %) was added to the solution containing the CdS QDs-gC3N4 nanocomposites and ultrasonicated for 2 h. After that, 5.0 μL of Chitosan/CdS QDs-gC3N4 solution was dropped onto the surface of the ITO electrode (2 mm) and allowed to dry at room temperature. Then, 6 μL of 2.5 % glutaraldehyde solution was cast on the surface of the Chitosan/CdS QDs-gC3N4/ITO electrode to attach to the amine groups of chitosan for 45 min. Glutaraldehyde acted as a cross-linker to attach amino-terminal aptamer probes to amine groups of chitosan via shift base interaction between the aldehyde groups of glutaraldehyde and amine groups [32]. The surface coverage of the aptamer (Γaptamer) was founded to be 26.2 pmol.cm−2 or 1.58 × 1013 molecules. cm−2. The details of the calculation of the Γaptamer are mention in the supplementary data (Fig. S1).

Then, the electrode was rinsed with 0.1 M phosphate buffer (PB, pH 7.4) solution. Subsequently, the electrode was deep in 0.5 mg/mL of aptamer solution (0.1 M PB, pH 7.4) for 4 h at 4 °C to attach the amine terminal aptamer to the surface of the Chitosan/CdS QDs-gC3N4/ITO electrode. The electrode was then washed with 0.1 M PB (pH 7.4) solution to remove the unattached aptamer probes. After that, the fabricated aptasensor was immersed in 0.1 M PB (pH 7.4) solution containing 0.1 M of 6-amino-1-hexanol for 1.0 h at room temperature to block non-specific sites. Finally, the fabricated aptasensor was washed with 0.1 M PB (pH 7.4) solution and kept in a refrigerator (4 °C) when not in use.
Fig. 1 shows the schematic illustration of the proposed aptasensor (Aptamer/Chitosan/CdS QDs-gC₃N₄/ITO electrode) fabrication.

2.4. Measurement procedure and the mechanism of PEC based-biosensor

During the measurement procedure, different concentration of Sars-Cov-2 RBD was dropped on the surface of the Aptamer/Chitosan/CdS QDs-gC₃N₄/ITO electrode to interact with the aptamer probe for 40 min. After that, the aptasensor was rinsed with 0.1 M PB solution (pH 7.4) to wash away any loosely interacted Sars-Cov-2 RBD from the surface of aptasensor. Finally, the aptasensor was dipped in the measurement cell 0.1 M PB containing 0.1 M AA as an electron donor molecule to photo-excitable nanocomposite (CdS QDs-gC₃N₄).

As CdS QDs-gC₃N₄ on the surface of the aptasensor was irradiated with a xenon lamp, CdS QDs that have a narrow band gap were photo-excited, generating hole and electron. The generated electron can back to the valance band and then recombine with the hole or transfer to the conductance band of gC₃N₄. If it recombines with a hole, the photocurrent won’t be generated. To avoid that, the photo-generated electron should be transferred anyhow. For this purpose, the photo-excitable nanomaterial (CdS QDs) must be mixed with another photo-excitable one whose band gap (the gap between the conduction band and valence band) is larger than the first one but close to it to overlap with each other. The band gap of gC₃N₄ is 2.7 ev that is close to the band gap of CdS QDs (2.4 ev). Therefore, the generated photo-electron will transfer from the conduction band of CdS QD to the conduction band of gC₃N₄, avoiding the recombination of an electron with a hole.

Besides that, gC₃N₄ can be photo-excited by a xenon lamp and generate a hole and electrode. The transferred electron from the conduction band of CdS QDs to the conduction band of gC₃N₄ as well as the photo-generated electron from gC₃N₄ transferred to the ITO electrode, generating photo-current. To have a stable photocurrent, the holes on the valance bands should be scavenged. For this purpose, an electron-donor molecule like AA is used. During the scavenging holes, the holes from the valance band of g-C₃N₄ transferred to the valance band of CdS QDs. After that, the holes oxidized AA. Any limitation in the diffusion of AA to the surface of electrode modified with photo-excitable nanomaterials will increase the possibility of the recombination of holes and electrons and subsequently decrease the intensity of the photocurrent. In the absence of Sars-Cov-2 RBD (Fig. 2A), AA can reach the surface of the electrode via diffusion without any limitation. As Sars-Cov-2 RBD that has a high molecular weight (35 kDa) incubated with the immobilized aptamer probes on the surface of the electrode (Fig. 2B), the mass transfer limitation for AA increased. Because Sars-Cov-2 RBD hindered AA to scavenge the holes and subsequently, the possibility of the recombination of holes and electrons increased. Therefore, the photo-current of the aptasensor in the presence of Sars-Cov-2 RBD decreased.

Fig. 2 demonstrates the PEC mechanism resonse of the proposed aptasensor before (A) and after (B) the incubation of Sars-Cov-2 RBD with aptamer probes.

3. Results and discussion

3.1. The morphology characterization of nanomaterials

Fig. 3 shows the scanning transmission electron microscopy (STEM)
images in high angle annular dark field (left) and bright field (right) for CdS QDs (A,B), gC₃N₄ (C,D), CdS QDs-gC₃N₄ (E,F).

As can be seen in Fig. 3.A and B, CdS QDs have a spherical shape with an average diameter of 2 ± 0.5 nm. Also, STEM images of gC₃N₄ (Fig. 3 C and D) show that it has a wrinkled nanosheet structure. After mixing gC₃N₄ with CdS QDs, the gC₃N₄ was decorated with CdS QD uniformly (Fig. 3 E and F). The obtained STEM images confirmed that the nano-composites were prepared successfully.

3.2. Surface characterization of the Aptamer/Chitosan/CdS QDs-gC₃N₄/ITO electrode

The Aptamer/Chitosan/CdS QDs-gC₃N₄ /ITO electrode was characterized. Fig. 4.A and B show the high-resolution scanning electron microscopy images (HR-SEM) obtained with a secondary electron detector and a backscattered electron detector (Fig. 4.A, B). As shown in Fig. 4.A, the surface of the electrode was decorated with the CdS QDs-gC₃N₄ nano-sheet composite, uniformly. Although due to the excess of a thin layer of chitosan and the immobilized aptamer probe, CdS QDs cannot be observed, but the image obtained with a backscattered electron detector (Fig. 4B) showed that the surface of the electrode was coated with CdS QDs-gC₃N₄. The atomic force microscopy image and the surface profiles of the aptasensor were shown in Fig. 4C and D, respectively. As it can be seen, the surface of the electrode was modified with a thin layer of CdS QDs-gC₃N₄ nano-composite. The thickness of the Aptamer/Chitosan/CdS QDs-gC₃N₄ was about 13.6 nm.

The Aptamer/Chitosan/CdS QDs-gC₃N₄/gC₃N₄ ITO electrode was also characterized with an infrared attenuated total reflectance (IR-ATR) spectroscopy (Fig.S3). As shown, a broad absorption band around 3300 cm⁻¹ attributed to the –OH group stretching of chitosan, two small absorption bands at 2930 cm⁻¹ and 2850 cm⁻¹, attributed to the symmetric and asymmetric stretching of the –CH₂, and –CH₃ groups, an absorption band at 1640 cm⁻¹ attributed to the vibration of the –C = O group of guanine or thymine, an absorption band at 1570 cm⁻¹

Fig. 2. Schematic illustration of separation mechanism of photogenerated electron – hole pairs between g-C₃N₄ and CdS QD on the proposed aptasensor before (A) and after (B) the incubation of Sars-Cov-2 RBD with aptamer probes.
attributed to the –NH group deforming vibrations of the imidazole ring (nucleotide bases), an absorption band at 1230 cm\(^{-1}\) attributed to the –PO\(_2\)- group of aptamer, and, an absorption band at 1050 cm\(^{-1}\) attributed to the –C-O-P- sugar-phosphate chin of aptamer are clearly seen. It demonstrated that the aptamer probe was immobilized on the surface of the electrode, successfully.

3.3. Electrochemical properties of the electrodes

Cyclic voltammetry (CV) (A), electrochemical impedance spectroscopy (EIS) (B), and PEC (C) methods were used to prove the stepwise changes in the electrochemical properties of the electrode surface (Fig. 5). CV and EIS signals were recorded in 0.1 M PB (pH 7.4) solution containing 0.5 mM Fe(CN)\(_{6}^{3-/4-}\) as an electrochemical probe. Also, PEC signals were recorded in 0.1 M PB (pH 7.4) solution containing 0.1 mM AA as an electron donor.

Fig. 5A shows the cyclic voltammogram of electrodes. As shown in Fig. 5A (a), the ITO electrode showed a well-known one-electron redox couple for Fe(CN)\(_{6}^{3-/4-}\). After the modification of the ITO electrode with Chitosan/CdS-gC\(_{3}N_{4}\) (b), the intensity of the redox couple increased, indicating that the Chitosan/CdS QDs-gC\(_{3}N_{4}\) facilitated the electron transfer rate of Fe(CN)\(_{6}^{3-/4-}\) probe on the electrode interface. After the immobilization of the aptamer on the surface of the Chitosan/CdS QDs-gC\(_{3}N_{4}\) /ITO electrode (C), the intensity of the redox couple not only

Fig. 3. Scanning transmission electron microscopy (STEM) images in high angle annular dark (left) and bright fields (right) for CdS QD (A,B), gC\(_{3}N_{4}\) (C,D), CdS QDs-gC\(_{3}N_{4}\) (E,F).
decreased but also the peak potential separation ($\Delta E_p$) of Fe(CN)$_{6}^{3-/4-}$ recorded by the Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode (309 mV) increased compared to the Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode (139 mV). It indicates that the immobilized aptamer probes decreased the electron-transfer kinetics of Fe(CN)$_{6}^{3-/4-}$. The reasonable explanation is that the negatively charged Fe(CN)$_{6}^{3-/4-}$ molecules were repulsed from the surface of the electrode by the negatively charged aptamer probes via the electrostatic interaction. After the incubation of Sars-Cov-2 RBD with aptamer on Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode (d), the intensity of the signal decreased more and the $\Delta E_p$ increased (360 mV). This is due to the mass-transfer limitation of Fe(CN)$_{6}^{3-/4-}$ to the electrode surface caused by the Sars-Cov-2 RBD that has a big size (MW~35 kDa).

Fig. 5 B shows the EIS of ITO electrode (a), Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode (b), Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode (c), and SARS-Cov-2 RBD /Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode (d). As can be seen, the diameter of the semicircles in a Nyquist plot of the EIS that shows the electron transfer resistance ($R_{et}$) decreased from 6105 $\Omega$ to 3765 $\Omega$ as the ITO electrode (a) was modified with Chitosan/CdS QDs-gC$_3$N$_4$ (b). It indicates that the Chitosan/CdS QDs-gC$_3$N$_4$ nano-composite facilitated the rate of electron transfer.

However, the $R_{et}$ of the electrode increased to 11,200 $\Omega$ (c) as aptamer probes were immobilized on CdS QDs-gC$_3$N$_4$/ITO electrode. The $R_{et}$ of the electrode increased to 17,628 $\Omega$ (d) as Sars-Cov-2 RBD (3 nM) interacted with the immobilized aptamer on the surface of the electrode. All these results demonstrated that the surface property of the electrode has been changed after each modification step.

Fig. 5C shows the PEC responses of the ITO electrode (a), Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode (b), Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode (c), and SARS-Cov-2 RBD /Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode (d) in 0.1 M PB (pH 7.5) solution containing 0.1 mM AA. As shown, the ITO electrode did not show any PEC signal. After the modification of the ITO electrode with Chitosan/CdS QDs-gC$_3$N$_4$, the recorded PEC signal dramatically increased, verifying that the Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode is a suitable electrode for obtaining the PEC signal. After the immobilization of the aptamer probes, the PEC signal decreased due to the diffusion limitation of the electron donor molecule (AA) to the ITO electrode surface coated with a photoactive nanocomposite (CdS QDs-gC$_3$N$_4$) and the electrostatic repulsion interaction between negatively charged AA and negatively charged aptamer probes. As SARS-Cov-2 RBD (3 nM) interacted with the immobilized aptamer on the surface of the electrode, the PEC signal decreased even more than before. The reasonable explanation is that the attachment of a high-molecular-weight protein like SARS-Cov-2 RBD [33] to the aptamer hindered the diffusion of electron donor (AA) to the electrode surface.

The effect of the incubation time on the response of the proposed aptasensor was also studied. As it can be seen in Fig. 6A, the response of the proposed aptasensor to 3 nM of SARS-Cov-2 RBD decreased as the incubation time increased up to 40 min and after that, the signal did not
change anymore. The reasonable explanation is that the interaction of the immobilized aptamers on the surface of the electrode and SARS-CoV-2 RBD reached the saturation level.

The concentration of the amino-terminal aptamer was also optimized (Fig. 6B). As it can be seen, the sensitivity of the Aptamer/Chitosan/CdS QDs-gC₃N₄/ITO electrode to 3 nM Sars-Cov-2 RBD during 40 min increased from 0.1 mg/mL to 0.5 mg/mL and then decreased as it reached to 0.75 mg/mL. The reasonable explanation is that the negatively charged aptamers repelled each other in the high concentration of aptamer [34]. Therefore, 0.5 mg/mL of amino-terminal aptamer and 40 min incubation time between the aptamer and Sars-Cov-2 RBD were selected as optimum conditions.

3.4. Detection of Sars-Cov-2 RBD and study the stability, selectivity, and reproducibility of the proposed aptasensor

Fig. 7A and B show the PEC response of the Aptamer/Chitosan/CdS QDs-gC₃N₄/ITO electrode to various concentrations of Sars-Cov-2 RBD (A) and the associated calibration curve (B), respectively. As shown in Fig. 7A, the intensity of the photocurrent kept decreasing as the concentration in the measurement solution of Sars-Cov-2 RBD increased. Fig. 7B shows that the Aptamer/Chitosan/CdS QDs-gC₃N₄/ITO electrode has a good linear relationship with the logarithm of the concentration of Sars-Cov-2 RBD in the range of 0.5 nM to 32 nM. The linear regression equation of the calibration curve is expressed as 

\[ \Delta I (\mu A) = -0.54 \log [\text{Sars-Cov-2 RBD} (\text{nM})] + 1.04 \] with a correlation coefficient of \( R^2 = 0.995 \) (n = 7). The limit of detection (LOD) was calculated to be 0.12 nM (3\( \sigma \)/S), where \( \sigma \) is the standard deviation of the blank measurements and S is the slope. The denoted error bars in Fig. 7B were calculated from the standard deviation for five measurements.

The values of the dissociation constant (K_d) that shows the affinity of the proposed aptasensor to the target was also calculated using the Langmuir-typical adsorption systems (Fig. S4):

\[ C_{\text{Sars-Cov-2 RBD}} = \frac{1}{KL \times I_{\text{max}}} + \frac{C_{\text{Sars-Cov-2 RBD}}}{I_{\text{max}}} \]  

(1)
The value of $K_d$ ($1/K_L$) was found to be 3.4 nM that was lower than the previous apta-assay method [5], indicating the high affinity of the proposed aptasensor to Sars-Cov-2 RBD. In this equation, $I_{\text{max}}$ is the maximum number of binding sites, and $K_L$ is the Langmuir isotherm constant.

Fig. 7 C shows the interfering effect of HIgG (a), HSA (b), HIgA (c), and HIgM (d) antigens (5-fold quantities) on the PEC signal of the Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode to 1 nM Sars-Cov-2 RBD. As shown, no interference was observed, indicating the high selectivity of the proposed aptasensor. The stability of the Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode has been studied (Fig. 7 D). After 3 weeks, the PEC signal of the aptasensor retained 96 % of its original response, suggesting good stability of the proposed aptasensor.

Also, the obtained results showed that the signals of the Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode were stable after 10-times repetitions (Fig. S5).

The reproducibility was also studied for determinations of 0.1 nM of Sars-Cov-2 RBD with six different aptasensors. The relative standard deviation (RSD) was calculated to be 5.8 %.

3.5. Analytical application of the Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode

The applicability of the Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode was also studied. To consider that, the proposed aptasensor was used for the measurement of 0.1 nM and 16 nM Sars-Cov-2 RBD in two different normal human saliva samples (Fig.S6). 1 mL of the first normal human saliva sample was diluted with 0.2 M PB solution containing 2 nM Sars-Cov-2 RBD, and 0.2 mM AA, and 1 mL of the second normal human saliva sample was diluted with 0.2 M PB solution containing 32 nM Sars-Cov-2 RBD, and 0.2 mM AA. The Sars-Cov-2 RBD concentration in the normal human saliva samples were estimated by using Fig. 7 B. The recovery of the analysis was obtained to be about 95.7 % for first sample 96.4 % for the second sample. The values were determined by the standard method that was used for florescence apta-assay of SARS-Cov-2 RBD [5]. It indicated that the Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$/ITO electrode has outstanding applicability for the diagnosis of COVID-19.

4. Conclusions

In summary, a photoelectrochemical aptasensor for the measurement of SARS-Cov-2 RBD has been developed by using CdS QDs-gC$_3$N$_4$ entrapped in chitosan as a photoactive nanocomposite. The response mechanism of the aptasensor is based on the change in the photoactive property of the surface of the ITO electrode modified with the Aptamer/Chitosan/CdS QDs-gC$_3$N$_4$. After the interaction of Sars-Cov-2 RBD with the immobilized aptamer probe on the surface of the electrode, the mass transfer limitation for AA as an electron donor molecule to the photoactive nanocomposite (CdS QDs-gC$_3$N$_4$) increased. Consequently, the intensity of the photo-current decreased. Therefore, the proposed aptasensor is considered as a signal-off-based aptasensor. The proposed aptasensor could be used for the determination of Sars-Cov-2 RBD in the dynamic range of 0.5–32 nM with a LOD of 0.12 nM. The aptasensor showed good selectivity in the presence of HIgG, HIgA, HIgM, and HSA.

CRediT authorship contribution statement

Mahmoud Amouzadeh Tabrizi: Supervision, Writing - review & editing, Resources, Project administration, Funding acquisition, Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. Leila Nazari: Formal analysis. Pablo Acedo: Formal analysis, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Appendix A. Supplementary data

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