The Stability Study of Electrochemical Aptasensor to Detect SARS-CoV-2 Spike Protein and Its Application for Clinical Samples of Nasopharyngeal Swab

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Abstract: *The stability characteristics associated with the shelf life of a biosensor are rarely investigated, however, they are important factors for real applications. Stability is the variation in the detection signal over a long period of storage. This study aims to determine the effect of storage time on the stability of SARS-CoV-2 receptor binding domain (RBD) spike protein aptamers related to shelf life and the performance of an electrochemical aptasensor on clinical samples. The research method includes a stability study conducted using the accelerated stability method based on the Arrhenius equation at three variations of temperature and storage time. The electrochemical aptasensor's performance was evaluated on clinical samples of 32 nasopharyngeal swabs at biosafety level 3 and its potential on clinical saliva samples. The results indicated that the developed electrochemical aptasensor was stable for ± 15 days with a shelf life of 18, 17 and 16 days, respectively, at 25, 40 and 50 °C. This electrochemical aptasensor has the potential to be a Point of Care (POC) device for the clinical detection of SARS-CoV-2 because it can be tested on clinical samples of nasopharyngeal swabs and the results show its potential application to detect in clinical saliva samples.*

Keywords: *stability; aptasensor; SARS-CoV-2 RBD S Protein*

■ INTRODUCTION

In late December 2019, a case caused by an unidentified pneumonia outbreak was first reported in Wuhan, Hubei Province, China. This disease outbreak originated in the Huanan seafood market, which has since grown rapidly and begun to spread throughout the world. The new coronavirus caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was later identified as the cause of the Coronavirus Disease 2019 (COVID-19) outbreak. SARS-CoV-2 is a positive single-strand RNA virus with a size of 29.9 kb. SARS-CoV-2 has four structural proteins, namely spike protein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N) [1-2]. The S protein consists of two functional subunits, namely the S1 subunit, responsible for the attachment of the virus to the receptor on the host cell surface via the receptor-binding domain (RBD), and S2 subunit, responsible for the fusion of the viral membrane with the host cell to facilitate the entry of the virus into the host cell [3-4]. Therefore, SARS-CoV-2 RBD S protein can be used as the main target for diagnosis, treatment, and vaccination [5].

Currently, there are several types of methods used to detect SARS-CoV-2, namely molecular tests based on the detection of viral RNA, antigen tests based on the detection of viral proteins, and antibody tests based on the detection of specific antibodies against viral proteins. Reverse Transcription Polymerase Chain Reaction (RT-PCR) is the gold standard method for the detection of SARS-CoV-2 virus RNA. The main limitations of this method are the expensive equipment requirements and

the need for highly qualified experts [6]. In the case of the molecular approach, a long sample processing time is required, which also requires sophisticated and expensive facilities. Lateral Flow Immunoassays (LFIAs) provide a faster response but it has lower sensitivity [6]. Meanwhile, detection methods targeting antibodies based on Enzyme-Linked Immunosorbent Assay (ELISA) are not suitable for early diagnosis because most patients have antibody responses around 7 to 21 days after infection [7-8].

Alternative diagnostic methods for the detection of SARS-CoV-2 can be done using a biosensor-based approach. Electrochemical biosensors have been widely used to detect several biomolecules, such as proteins as the biomarker of disease [9-11] or a marker of bacterial [12-13] or viral infection [14-15]. They have been shown to have several advantages, including portability, good sensitivity, high specificity, fast response, and ease of use [16-18]. Several bioreceptors such as antibodies, aptamers, and nucleic acids can be immobilized on the electrode surface for detection purposes [15]. Electrochemical biosensor methods have also been reported to be used for the detection of SARS-CoV-2 using various bioreceptors, such as antibodies [19-20], ssDNA [21-22], antigens [23-25], and aptamers [26-27].

In the preliminary research that has been carried out, an electrochemical aptasensor method was developed using a screen-printed carbon electrode/AuNP to detect the SARS-CoV-2 RBD S protein. In the creation of disposable electrodes for electrochemical biosensors, screen-printed carbon electrodes (SPCE), which incorporate three electrodes (WE, RE, and CE) into a single design, are frequently employed [28]. AuNPs are used to improve the performance of electrochemical biosensors by modifying the electrodes with nanomaterials, thereby increasing the electroactive surface area and increasing electron transfer between the electrodes and analytes [14]. Aptamers as bioreceptors for detecting SARS-CoV-2 have been reported by Song et al. [5] of the SELEX method that can bind to the SARS-CoV-2 RBD S protein. The RBD S protein of SARS-CoV-2 and the aptamer CoV2-RBD form hydrogen bonds with amino acids of the SARS-CoV-2 RBD S protein [5]. The aptamer was immobilized to the electrode surface by the streptavidin-biotin system via the MPA (3-mercaptopropionic acid) linker [29]. The aptamer will then bind to the SARS-CoV-2 RBD S protein, which can be detected electrochemically using the $K_3[Fe(CN)_6]$ solution redox system and the differential pulse voltammetry electrochemical detection technique. This electrochemical aptasensor with the streptavidin-biotin system has a low detection limit of 2.6308 ng/mL, a quantification limit of 7.9720 ng/mL, an accuracy of 99.89%, and a precision of 99.61%. Based on the results of several analytical parameters that have been obtained, this electrochemical aptasensor has the potential to be a method of detecting SARS-CoV-2 [14].

Biosensors generally consist of three basic components, namely bioreceptors, transducers, and signal processing systems [30]. Factors that affect stability are bioreceptor affinity (level of analyte binding to bioreceptors) and bioreceptor degradation over time [31]. Usually, bioreceptor limits the shelf life and stability of a biosensor [32]. Bioreceptors are quite susceptible to environmental changes, which can be characterized as a decrease in signaling over time. It is therefore very important to test the stability of a biosensor, especially for commercial purposes and because biosensors are currently being applied to more and more diverse applications. Stability characteristics related to shelf life are often under-investigated or underreported in the literature, even though they are important factors. The instability of a biosensor is referred to as a decrease in sensitivity over a certain period of time.

SARS-CoV-2 has been detected in various human body samples, including saliva, nasopharyngeal swabs, and oropharyngeal swabs [33]. A nasopharyngeal swab is a commonly used sample to detect respiratory viruses such as SARS-CoV-2. Currently, nasopharyngeal swabs are used in the RT-PCR method [34]. However, nasopharyngeal swab sampling is an invasive technique that can cause patients to cough or bleed, increasing the risk of transmitting the virus to healthcare workers. The use of saliva samples may offer a way to reduce this limitation, as sampling can be done individually, thereby

reducing the likelihood of exposure to the virus to healthcare workers. Thus, saliva samples can also be used as an alternative for the detection of SARS-CoV-2 [33-35].

Liv [36] studied the interference effect of several enzymes, compounds, and ions that can be found in saliva on electrochemical immunosensors to detect SARS-CoV-2 antibodies, namely α-amylase, lipase, Na^+, K^+, Ca^{2+}, Mg^{2+}, $H_2PO_4^-$, HPO_4^{2-}, urea, HCO_3^-, and NH_3. The presence of interference in saliva samples is the reason for the need for selectivity studies first before electrochemical aptasensors are applied to clinical saliva samples.

In this research, a stability study was conducted as proof of the quality of a sensor that will be developed and has the potential to become an applicable and commercial method. The stability of the biosensor was evaluated by comparing the response of peak currents measured on different days (1, 15, 30, and 60 days) in temperature variations of 25, 40 and 50 °C. The estimation of the shelf life of the biosensor can be done by the accelerated stability method using the Arrhenius equation. The performance of the electrochemical aptasensor was then tested on clinical samples of nasopharyngeal swabs and their potential against other clinical samples, namely saliva.

■ EXPERIMENTAL SECTION

Materials

The materials used in this study were 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) (Sigma Aldrich, Singapore), 3-mercaptopropionic acid (MPA) (Sigma Aldrich, Singapore), demineralized water (PT Ikapharmindo Putramas, Indonesia), biotinylated aptamer (biotin 5'- CAG CAC CGA CCT TGT GCT TTG GGA GTG CTG GTC CAA GGG CGT TAA TGG ACA-3') (Bioneer, Korea), chloroauric acid trihydrate ($HAuCl_4{\cdot}3H_2O$) (synthesized by the Chemical Analysis and Separation Laboratory, 2018, Indonesia), ethanolamine (Merck, Germany), potassium ferricyanide ($K_3[Fe(CN)_6]$) (Sigma Aldrich, Singapore), potassium chloride (KCl) (Merck, Germany), SARS-CoV-2 RBD S protein solution (GenScript, USA), trisodium citrate dihydrate ($Na_3C_6H_5O_7{\cdot}2H_2O$) (Merck, Germany), *N*-hydroxysuccinimide (NHS) (Sigma Aldrich, Singapore), phosphate-buffered saline (PBS) pH 7.4 (Merck, Germany), and streptavidin (Promega, USA).

Instrumentation

The SPCE (GSI Technologies, USA) and homemade SPCE were used carbon as a working and auxiliary electrode, and Ag/AgCl was used as a reference electrode for the electrochemical transducer. The electrochemistry measurements were conducted using a Zimmer & Peacock potentiostat connected to a computer using PSTRACE 5.8 software (Zeamer & Peacock, UK).

Procedure

SPCE modification with AuNP

The overall schematic of the electrochemical aptasensor method is shown in Fig. 1. The SPCE surface was rinsed with demineralized water and dried at room temperature. Then, 25 µL of colloidal AuNP solution was dropped onto the surface of the SPCE and incubated at room temperature for 24 h. The SPCE/AuNP was rinsed with demineralized water and dried at room temperature before being electrochemically characterized by differential pulse voltammetry over a potential range of −1.0 to 1.0 V at a scan rate of 0.008 V/s, step potential (E_{step}) 0.004 V with a pulse potential (E_{pulse}) of 0.025 V and the pulse time (t_{pulse}) of 0.05 s.

Fabrication of the electrochemical aptasensor

The SPCE/AuNP was incubated with 0.01 M MPA for 20 min at 25 °C. After that, the SPCE/AuNP/MPA were rinsed with ethanol. Then 0.1 M EDC solution and 0.1 M NHS solution (1:1 v/v) were incubated for 60 min at 25 °C, and rinsed with demineralized water. The streptavidin solution was incubated overnight at 4 °C on the surface of the SPCE/AuNP/MPA/EDC:NHS, and then rinsed with PBS solution 0.01 M. Ethanolamine was dropped onto the surface of the SPCE/AuNP/MPA/EDC:NHS/streptavidin for 20 min at 25 °C, and then rinsed with demineralized water. Furthermore, the 0.5 µg/mL biotinylated aptamer was immobilized on the SPCE/AuNP surface using a streptavidin-biotin system for 40 min at 25 °C, and then rinsed with PBS solution 0.01 M.

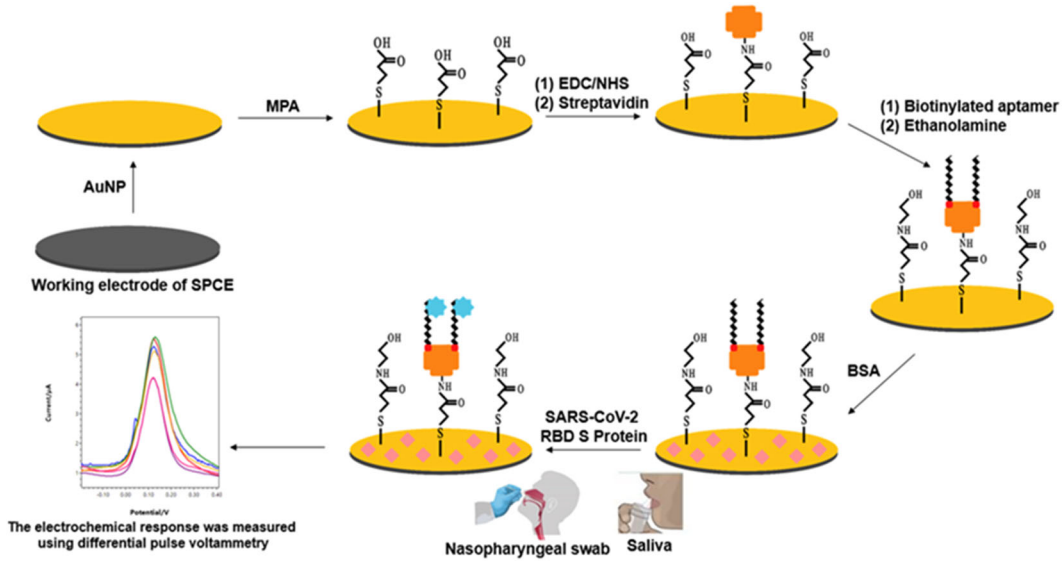

Fig 1. Schematic of an electrochemical aptasensor for SARS-CoV-2 RBD S protein detection

After the aptamer was successfully immobilized on the surface of the SPCE/AuNP electrode with the streptavidin-biotin system, the non-specific binding site on the electrode surface was incubated using a 1% BSA solution for 15 min at 25 °C, and then rinsed with PBS solution 0.01 M. After that, a solution of SARS-CoV-2 RBD S protein with a certain concentration was dropped on the electrode and incubated for 60 min at 25 °C. The differential pulse voltammetry was then performed using a redox system of 10 mM $K_3[Fe(CN)_6]$ solution in 0.1 M KCl over a potential range of −1.0 to 1.0 V at a scan rate of 0.008 V/s, E_{step} 0.004 V with an E_{pulse} of 0.025 V and a t_{pulse} of 0.05 s.

Stability

Electrochemical aptasensors were stored for 1, 15, 30, and 60 days at 25, 40 and 50 °C. After the aptasensor was stored, BSA (20 µL 1%) was added, and then SARS-CoV-2 RBD S protein was added with a concentration of 30 ng/mL. The electrochemical response was measured using differential pulse voltammetry on a redox system containing 10 mM $K_3[Fe(CN)_6]$ solution in 0.1 M KCl at a potential range of −1.0 to 1.0 V at a scan rate of 0.008 V/s, E_{step} 0.004 V with an E_{pulse} of 0.025 V and a t_{pulse} of 0.05 s. The stability was evaluated by looking at the peak current response at each different temperature and storage time.

Clinical samples analysis

After the aptamer was successfully immobilized on the surface of the SPCE/AuNP electrode with the streptavidin-biotin system, the non-specific binding site on the electrode surface was incubated using 1% BSA solution and incubated for 15 min. A total of 32 clinical samples were collected from nasopharyngeal swabs and tested using aptasensors at biosafety level 3. As required by current law, clinical samples of nasopharyngeal swabs were obtained from patients after receiving the explicit agreement of the person whose material was being obtained. Next, the sample solution was dropped on the surface of the electrode and incubated for 60 min. Differential pulse voltammetry was used to measure current response in a redox system of 10 mM $K_3[Fe(CN)_6]$ solution in 0.1 M KCl over a potential range of −1.0 to 1.0 V at a scan rate of 0.008 V/s, E_{step} 0.004 V with an E_{pulse} of 0.025 V and a t_{pulse} of 0.05 s.

Selectivity to interference in saliva samples

Selectivity was determined by measuring the current response of the aptamer as a negative control, SARS-CoV-2 RBD S protein as a positive control, and Na^+, K^+, Ca^{2+}, and Mg^{2+} ions to the electrochemical aptasensor. The electrochemical response was measured using differential pulse voltammetry with a redox system of 10 mM $K_3[Fe(CN)_6]$ solution in 0.1 M KCl at a

potential range of −1.0 to 1.0 V at a scan rate of 0.008 V/s, E_{step} 0.004 V with an E_{pulse} of 0.025 V and a t_{pulse} of 0.05 s, and the relative% response was calculated.

■ RESULTS AND DISCUSSION

Stability

Biosensor stability is an important aspect in terms of long-term application requirements. To study the stability of the aptasensor, the current response of the aptasensor to 30 ng/mL SARS-CoV-2 RBD S protein was recorded after 1, 15, 30, and 60 days of storage at 25 °C. Higher temperatures, such as 40 and 50 °C are also used for tests that allow accelerated stability prediction. Fig. 2 shows that the current response decreases with increasing storage time. Within 15 days, the current response decreased at 25, 40 and 50 °C, respectively, 6, 8 and 8% of the initial current response. Thirty days later, the sensor was re-measured and only maintained at 83, 82 and 82% of current response activity, at 25, 40 and 50 °C, respectively. Meanwhile, after 60 days, the sensor can only maintain current response activity of 60% at 25 °C, 56% at 40 °C, and 52% at 50 °C. These results indicate that the aptasensor is able to detect well up to ± 15 days after fabrication. The electrochemical aptasensor that was developed was able to produce > 90% current response activity on the 15th day compared to 100% of the current response activity on the first day, so that the aptasensor had good stability up to ± 15 days.

The determination of shelf-life stability is usually estimated by two different stability testing procedures, namely the real-time stability test and the accelerated stability test. In accelerated stability testing, the product is stored under conditions such as high temperatures. Temperature is the most commonly used acceleration factor for chemicals, pharmaceuticals, and biological products because of its relationship with the rate of degradation characterized by the Arrhenius equation [37]. The accelerated stability method is the determination of product shelf life by accelerating quality changes in critical parameters. This method uses environmental conditions that can accelerate the decline in product quality. With this method, product storage uses three temperatures that are able to predict shelf life at the desired storage temperature [38].

Based on the results of the current response in Table 1, it is made in graphic form with the x-axis being the storage time in days and the y-axis being the current response value for the linear equation and the coefficient of determination (R^2) of order 0. The graph of the current response of the order 0 electrochemical aptasensor

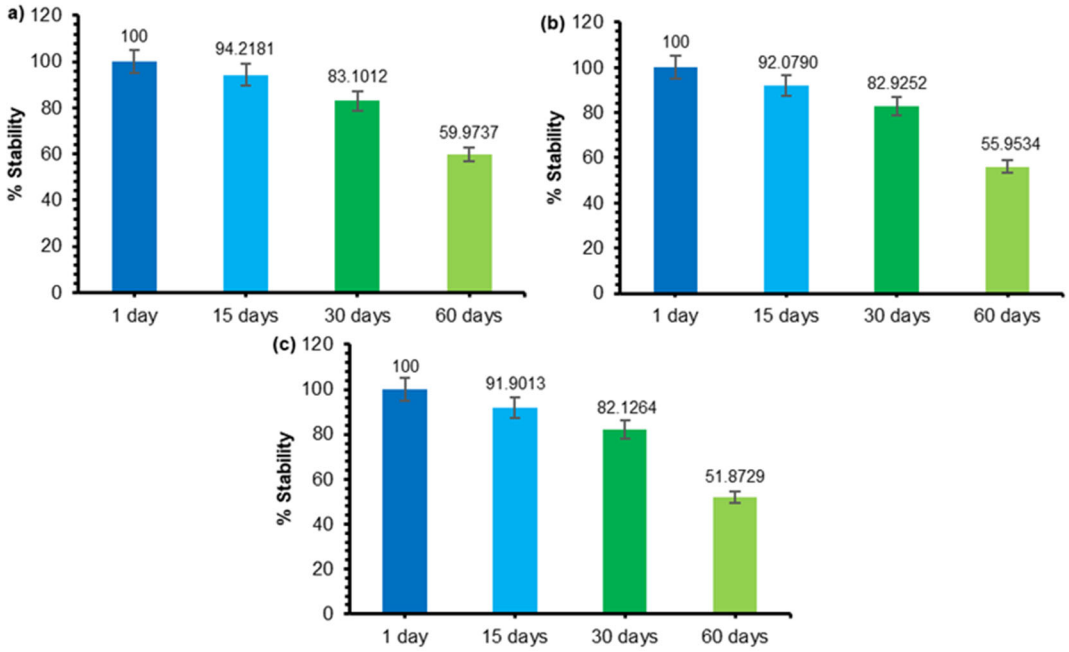

Fig 2. Stability of the aptasensor after 1, 15, 30, and 60 days of storage at (a) 25 °C, (b) 40 °C, and (c) 50 °C

Table 1. Current response of the electrochemical aptasensor to 30 ng/mL SARS-CoV-2 RBD S Protein recorded after 1, 15, 30, and 60 days of storage at 25, 40 and 50 °C

Storage time (day)	Average current response (μA)		
	25 °C	40 °C	50 °C
Aptamer	6.2820	4.4570	6.1540
1	3.8050	2.9605	3.9780
15	4.0250	3.1950	4.1845
30	4.4480	3.4660	4.5625
60	5.3280	4.2645	5.7340

can be seen in Fig. 3. In the first order graph, the current response value is converted to the form ln before being made in the form of a graph with the storage time as shown in Fig. 4. From Fig. 3 and 4, it can be seen that the largest value of R^2 is 0.9957, namely R^2 of the current response for order 1 at a temperature of 40 °C so the estimated shelf-life calculation uses a linear equation plot of order 1. The value of k is the slope value, which is obtained from the value of b in the equation y = bx + a. The value of k is then converted into the form ln k and then used to create an Arrhenius graph between ln k and 1/T. The Arrhenius graph can be seen in Fig. 5. Based on the linear regression equation obtained in Fig. 5, the Arrhenius equation can be determined as follows:

y = -328.79x – 4.0318

ln k = –328.79 (1/T) – 4.0318

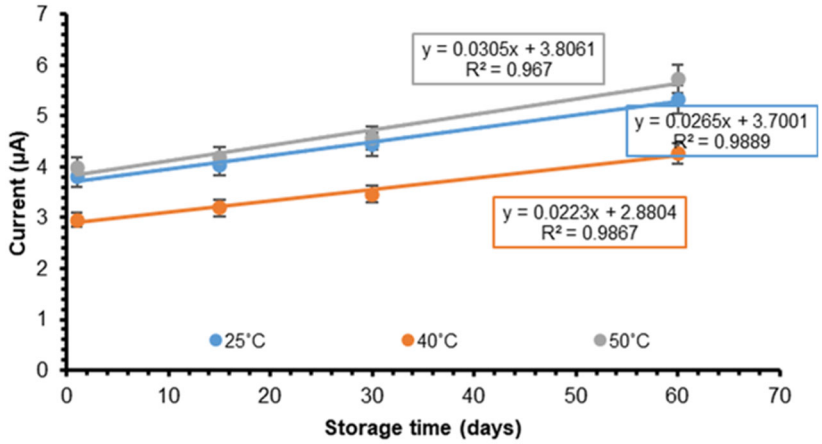

Fig 3. Electrochemical aptasensor current response to storage time of order 0

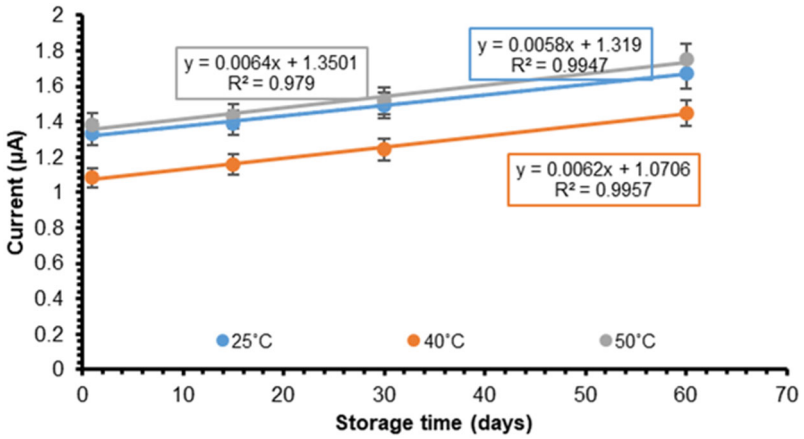

Fig 4. Electrochemical aptasensor current response to storage time of order 1

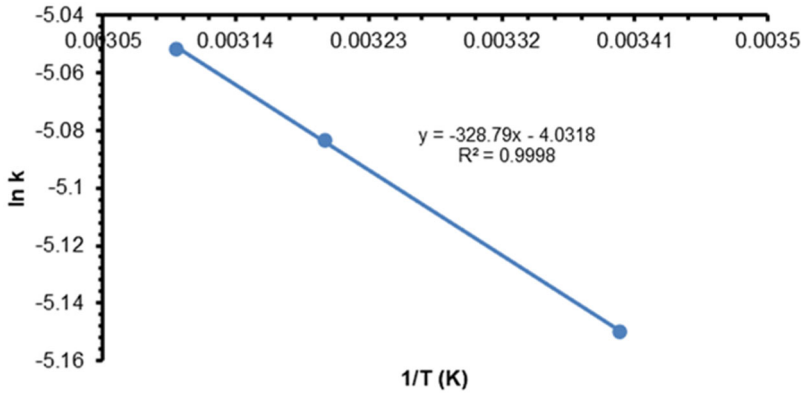

Fig 5. Correlation of ln k to temperature in the Arrhenius equation

If the slope value of the linear regression equation for each temperature at order 1 is changed to the ln value and plotted with 1/T (units of degrees Kelvin), the Arrhenius equation is obtained as shown in Fig. 5. The value of the degradation reaction rate constant (k) at each storage temperature can be estimated by the Arrhenius equation obtained as shown in Table 2. The results of determining the shelf life of electrochemical aptasensors can be seen in Table 2. The optimum storage of aptasensor is at 25 °C, because it has a longer shelf life than other storage temperatures.

The shelf life of the aptasensor at different temperatures can also be calculated using the Arrhenius equation. The Arrhenius equation obtained is ln k = −328.79x − 4.0318, so the shelf life of the product if stored at a temperature of 4 °C or 277 °K will produce a value of ln k = −5.2188 or k = 0.3051. This means that there will be a decrease in the current response of 0.0054 units per day. Thus, the estimated storage time (90% activity maintained) at 4 °C can be estimated at about 20 days.

Clinical Samples Analysis

To assess the feasibility of electrochemical aptasensors in detecting SARS-CoV-2 RBD S protein in clinical samples, the detection performance was tested using clinical samples and compared with the RT-PCR method. A total of 32 samples (labeled S1 to S32) were collected from nasopharyngeal swabs and tested using aptasensors. In the electrochemical aptasensor method, if the peak current response is smaller than the peak current of the aptamer as a negative control or not much different from the peak current response of RBD as a positive control, then the sample is positive for COVID-19. Meanwhile, if the peak current response is greater than or equal to the peak current response of the aptamer, then the sample is negative for COVID-19. The peak current of the aptamer as a negative control is 30.124 μA, while the peak current of RBD as a positive control is 19.857 μA. The RT-PCR method used to diagnose COVID-19 provides an overview of the Ct value or Cycle threshold, which is the size of the viral load in the sample. A low Ct value indicates a high viral load. The Ct result is inversely proportional to the amount of viral nucleic acid present in the sample. The low Ct value indicates that the amplification cycle required to reach the threshold is shorter because the nucleic acid contained in the sample is high. Of the 32 samples tested, 3 samples were confirmed positive for SARS-CoV-2 and 29 samples were confirmed negative, as shown in Table 3. Based on the results obtained, this developed electrochemical aptasensor can be applied to detect SARS-CoV-2 RBD S protein in clinical samples of nasopharyngeal swabs.

Table 2. Determination of shelf life of electrochemical aptasensors

Temperature (°C)	k	A_0	A_t	ln A_0	ln A_t	t (days)
25	0.0058	100	90	4.6052	4.4998	18
40	0.0062	100	90	4.6052	4.4998	17
50	0.0064	100	90	4.6052	4.4998	16

Table 3. Application of electrochemical aptasensors to detect SARS-CoV-2 on clinical samples compared with RT-PCR. S# is the number of clinical samples tested

Sample of patient	Ct value			RT-PCR	Aptasensor
	ORF1ab	N gene	E gene		
S1	**27.50**	**22.52**	**25.55**	**(+)**	**(+) 20.885 µA**
S2	**17.13**	**14.32**	**16.64**	**(+)**	**(+) 20.550 µA**
S3	-	-	-	(-)	(-)
S4	-	-	-	(-)	(-)
S5	-	-	-	(-)	(-)
S6	-	-	-	(-)	(-)
S7	-	-	-	(-)	(-)
S8	-	-	-	(-)	(-)
S9	-	-	-	(-)	(-)
S10	-	-	-	(-)	(-)
S11	-	-	-	(-)	(-)
S12	-	-	-	(-)	(-)
S13	-	-	-	(-)	(-)
S14	-	-	-	(-)	(-)
S15	-	-	-	(-)	(-)
S16	-	-	-	(-)	(-)
S17	-	-	-	(-)	(-)
S18	-	-	-	(-)	(-)
S19	-	-	-	(-)	(-)
S20	-	-	-	(-)	(-)
S21	-	-	-	(-)	(-)
S22	**20.31**	**18.68**	**20.46**	**(+)**	**(+) 20,578 µA**
S23	-	-	-	(-)	(-)
S24	-	-	-	(-)	(-)
S25	-	-	-	(-)	(-)
S26	-	-	-	(-)	(-)
S27	-	-	-	(-)	(-)
S28	-	-	-	(-)	(-)
S29	-	-	-	(-)	(-)
S30	-	-	-	(-)	(-)
S31	-	-	-	(-)	(-)
S32	-	-	-	(-)	(-)

Selectivity to Interference in Saliva Samples

Selectivity is a very important characteristic, especially in clinical applications where the sample matrix, contains many molecules that are very similar to the target analyte and compete for binding to bioreceptors [39]. Salivary components can be divided into organic and inorganic components. However, this level is still low compared to the main component of saliva, which is water, which is around 99.5% [40-41].

Selectivity was determined by the interference ions present in saliva, namely Na^+, K^+, Ca^{2+}, and Mg^{2+}. The measured current response was compared with the RBD peak response of SARS-CoV-2 S protein as a positive (+) control and the aptamer as a negative control (-) as shown in Fig. 6. The results obtained indicate that the measured ion current response is not much different from the aptamer. This indicates that there is no binding between the aptamer and the interference ions.

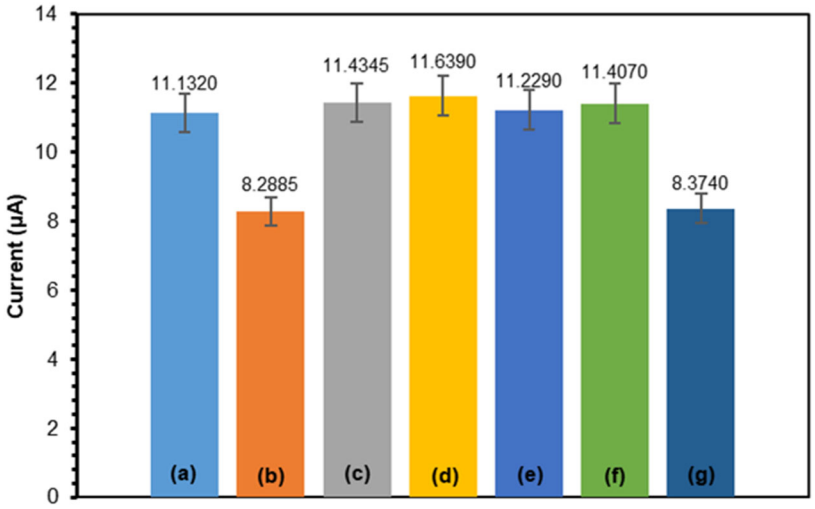

Fig 6. Selectivity of electrochemical aptasensor to interference in the form of ions. Current response (a) aptamer, (b) SARS-CoV-2 RBD S protein, (c) Na^+ ions, (d) K^+ ions, (e) Ca^{2+} ions, (f) Mg^{2+} ions, and (g) RBD + Na^+ + K^+ + Ca^{2+} + Mg^{2+}

As for other components in saliva, namely enzymes, such as the results of research by Liv [36]. Interferences in saliva samples are α-amylase and lipase enzymes, but due to the unavailability of enzyme reagents, the alternative is to use individual saliva, because the saliva component itself contains the required enzymes. From the results of the salivary flow response obtained, when compared with the aptamer current response as a negative control (-) and SARS-CoV-2 RBD S protein as a positive control (+), the salivary flow response was not much different from the aptamer current response as shown in Fig. 7. This means that there is no interaction between enzyme interference in saliva and the aptamer. Meanwhile, when saliva was added to SARS-CoV-2 RBD S protein and then tested on the aptasensor, the current response obtained was not much different from the current response to SARS-CoV-2 RBD S protein alone. This shows that the presence of interference or interference does not affect the activity of the aptamer against SARS-CoV-2 RBD S protein.

Aptasensor selectivity testing was also carried out on the saliva of other individuals. The results also showed

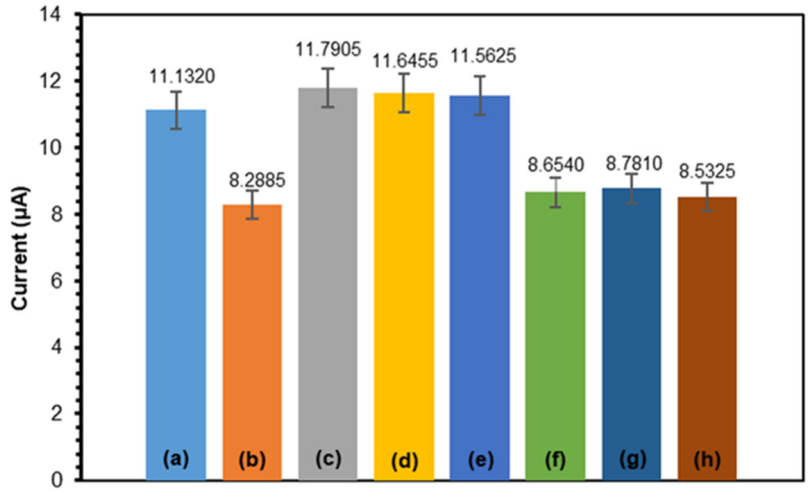

Fig 7. Selectivity of electrochemical aptasensors in saliva samples. Current response (a) aptamer, (b) SARS-CoV-2 RBD S protein, (c) Saliva 1, (d) Saliva 2, (e) Saliva 3, (f) Saliva 1 + RBD, (g) Saliva 1 + RBD, and (h) Saliva 3 + RBD

Table 4. Electrochemical aptasensor studies to detect SARS-CoV-2

Method	% Recovery	Limit of detection, ng/mL	Ref.
Two-dimensional (2D) metal–organic framework (MOF)-based photoelectrochemical (PEC) aptasensor for SARS-CoV-2 spike glycoprotein (S protein) detection	104–106%	72	[42]
Electrochemical dual-aptamer biosensor based on the metal-organic frameworks MIL-53(Al) decorated with Au@Pt nanoparticles and enzymes to determine SARS-CoV-2 nucleocapsid protein (2019-nCoV-NP) via co-catalysis of the nanomaterials, horseradish peroxidase (HRP) and G-quadruplex DNAzyme	92–110%	0.00833	[43]
CRISPR-Cas12a-mediated label-free electrochemical aptamer-based sensor for SARS-CoV-2 antigen detection	98–105%	0.077	[44]
Electrochemical aptasensor to detect SARS-CoV-2 S protein RBD as a biomarker of COVID-19 disease using a screen-printed carbon electrode/AuNP	95–99%	2.63	This research
CRISPR/Cas12a-derived electrochemical aptasensor for ultrasensitive detection of COVID-19 nucleocapsid protein	99–101%	0.0165	[45]

that interference in saliva samples did not interfere with the binding activity of the aptamer with SARS-CoV-2 RBD S protein, which means that the developed aptasensor can detect SARS-CoV-2 RBD S protein in saliva samples non-invasively. Aptamer selectively only recognized its target, namely SARS-CoV-2 RBD S protein and obtained the percentage selectivity value, which was 97% for ions, 87% for saliva 1, 82% for saliva 2, and 91% for saliva 3.

The developed aptasensor method was also used to determine the amount of SARS-CoV-2 RBD S protein in spiked-saliva samples. Saliva samples were taken from negative individuals which were then dissolved in PBS pH 7.4 0.01 M. The saliva samples were then tested on the aptasensor with 100 ng/mL of SARS-CoV-2 RBD S protein. The resulting peak current response is entered into the equation $y = 0.0634x - 0.4449$. The % recovery values obtained were 99.85% and 95.22%, respectively. Thus, the results show that this method also provides good accuracy. The quantitative analysis of SARS-CoV-2 RBD S protein in spiked-saliva samples for the early diagnosis of COVID-19 showed reliability and accuracy, as shown in Table 4, and the results were comparable to those of the electrochemical aptasensor studies to detect SARS-CoV-2. The results obtained with this external standard calibration curve also illustrate that the developed method is not affected by the sample matrix.

■ CONCLUSION

The accelerated stability method can be used to determine the shelf-life stability of electrochemical aptasensors, namely 18 days at 25 °C, 17 days at 40 °C, and 16 days at 50 °C. Electrochemical aptasensors can be applied to detect SARS-CoV-2 RBD S protein in clinical samples of nasopharyngeal swabs. Of the 32 samples tested, 3 samples were confirmed positive and 29 samples were confirmed negative for COVID-19. This electrochemical aptasensor also shows the potential to detect SARS-CoV-2 RBD S protein in clinical saliva samples.

■ ETHICAL APPROVAL

The ethical approval has been given by Research Ethics Committee Universitas Padjadjaran Bandung, Molecular Biotechnology and Bioinformatics Research Center No. 860/UN6.KEP/EC/2022.

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■ AUTHOR CONTRIBUTIONS

Yeni Wahyuni Hartati and Shabarni Gaffar designed the experiment, evaluated, and corrected the manuscript. Irkham guided the experiment. Muhammad Yusuf prepared the ethical approval and clinical samples for aptasensor. Arum Kurnia Sari and Ghina Nur Fadhilah conducted the experiment, wrote, and revised the manuscript. Yeni Wahyuni Hartati was the funding leader of the Academic Leadership Grant (ALG). All authors agreed to the final version of this manuscript.

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