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*Review*

# A Review on the Development of Aptamer Immobilization Techniques in Aptamer-Based Electrochemical Biosensors for Viruses Detection

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**Abstract**- Electrochemical biosensors have been widely used to detect several biomolecules, such as viruses, because they have been shown to have several advantages, including portability, good sensitivity, high specificity, fast response, and easy to use. A biosensor that utilizes aptamer as a bioreceptor is known as an aptasensor. Compared with antibodies, aptamer has several advantages, such as smaller size, easy synthesis and chemical modification, thermal stability, lower toxicity, high affinity, and excellent sensitivity. Aptasensor exhibits very high sensitivity, specificity, and reproducibility to a wide variety of target analytes. This review explains, that the aptamer can be immobilized on the surface of the electrode by various immobilization techniques. This review describes the use of the aptamer-based biosensor for viruses detection including the development of the aptamer immobilization technique in an electrochemical aptasensor. Several applications of using the aptamer-based biosensor for viruses detection including the development of the aptamer immobilization technique in electrochemical aptasensor in the last past eight years are proposed in this review.

**Keywords-** Electrochemical biosensor; Aptamer; Immobilization technique, Viruses detection, Diagnosis

## 1. INTRODUCTION

Viruses are protein packets that surround genetic material, they cannot survive without a host. The basic structure of the viruses consists of a genome in the form of DNA or RNA, a capsid protein that functions to protect the viral genome (nucleocapsid), and a phospholipid membrane called an envelope that surrounds the nucleocapsid [1]. Despite their simple structure, viruses are one of the main causes of disease. Viruses lack a cytoplasmic membrane, cytosol, or functioning organelles, yet they can infect any cell type, including bacteria. Viruses are unable to carry out their metabolic functions, thus they invade and exploit other cells to create viral genomes and proteins, as well as assemble new viral particles [2].

For an efficient response to viral infections, a rapid, sensitive, and selective approach for early viruses detection are required. The capacity to detect individual viruses with great sensitivity has a big influence on health care since it allows doctors to diagnose at the very beginning of viral infections [2].

Currently, viruses detection methods can be classified into two categories, gene-based detection method, and antibody-based detection method. Gene-based detection methods are based on detecting viral genetic material by polymerase chain reaction (PCR), such as real-time PCR. This method is usually sensitive but has debility including a long process and expensive. Antibody-based detection methods simplify the detection process and increase the specificity of the assay by directly detecting viral proteins or host cell antibodies. However, detection methods that target antibodies are not suitable for early diagnosis, as most patients have an antibody response around 7 to 21 days after infection [3-5].

The electrochemical biosensor has been widely used to detect several biomolecules, such as viruses [6], because they have been shown to have several advantages, including portability, ease of use, fast response, good sensitivity, and high specificity [7,8]. Several bioreceptors such as antibodies, nucleic acids, and aptamer can be well immobilized on the electrode surface for detection purposes [6].

The aptamer is a single-stranded DNA or RNA molecule that is selected through an in vitro method, known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [9], which may bind a wide range of targets with great selectivity and specificity, including small molecules, metal ions, amino acids, peptides, protein, microorganisms, viruses, and nucleic acids [10–13]. Aptamers have several advantages over antibodies, including smaller size, ease of synthesis and chemical modification, thermal stability, low toxicity, high affinity, and excellent sensitivity [9]. The development of the aptamer immobilization method in an electrochemical aptasensor was discussed in this review of aptamer-based biosensors for viruses detection.

## 2. APTAMER BASED BIOSENSOR: APTASENSOR

A biosensor is a scientific device made up of immobilized bioreceptors like enzymes, antibodies, nucleic acids, hormones, organelles, or cells that can interact with the target analyte and produce measurable physical, chemical, or electrical signals [14]. As shown in Figure 1, biosensors are made up of three basic components: biological sensing elements, also known as bioreceptors, a transducer, and a signal processing system. The basic principle of a biosensor is to detect molecular recognition and convert it into a type of signal using transducers [14,15]. A biological recognition system, also known as a bioreceptor, is a sensor that can detect a specific analyte with great selectivity. Biosensors have been used in medical, food, security, environmental, and industrial testing [16].

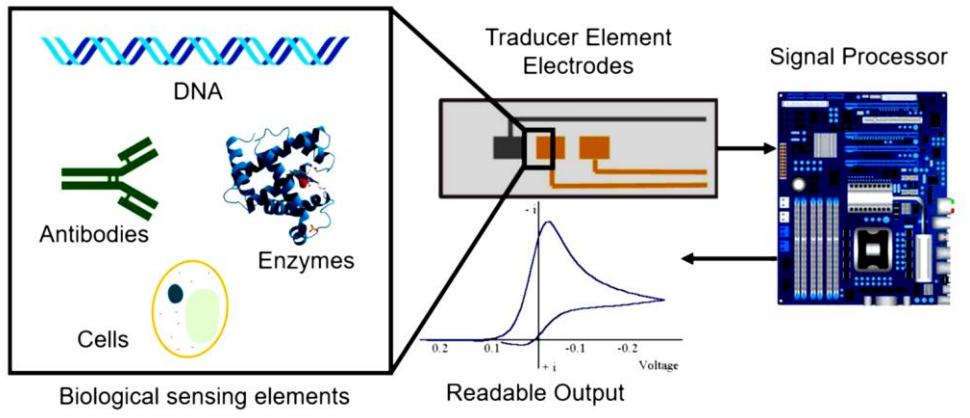


**Figure 1.** The basic principle of the biosensor; reprinted with the permission from [17]

A biosensor can be classified based on its bioreceptor or type of transducer [14]. Bioreceptors are a molecule that allows binding with the analyte [18]. Bioreceptors are molecules that recognize analytes through chemical reactions or interactions. They are in charge of attaching the analyte to the sensor surface so that it may be measured [15]. A transducer is a biosensor component that has an important role in the signal detection process and converts a signal into a detectable signal. A biosensor can be classified based on the transduction method, such as optical, electrochemical, and mass-based detection methods [15].

A biosensor that uses aptamer as a bioreceptor is known as aptasensor [19]. To a wide range of target analytes, aptasensor has extremely high sensitivity, specificity, and repeatability. Aptasensor was developed to detect small molecules and various contaminants, such as heavy metals, pesticides, antibiotics, and toxins [20,21].

Aptamer originated from the Latin word "aptus" which means fit and the Greek word "meros" which means part. The aptamer is a nucleic acid molecule either in the form of DNA or single-stranded RNA, consisting of 40 to 50 bases with a molecular mass between 10 to 30 kDa [22] that can bind various targets including small molecules, metal ions, amino acid,

peptides, protein, cell, microorganism, viruses, and other nucleic acids with high selectivity and specificity [10–13]. The aptamer is also often referred to as a "chemical antibody" because it is synthesized in vitro through a selection process based on Systematic Evolution of Ligands by Exponential Enrichment (SELEX) from random combinatorial libraries [23].

SELEX is a technique used to isolate aptamer with high affinity for target analyte from approximately 1012 to 1015 oligonucleotide combinations [24,25]. The selection procedure is separated into three stages, as illustrated in Figure 2, library formation, binding and separation, and amplification, which are repeated to get nucleotides with superior binding capabilities to the intended target analyte [26].

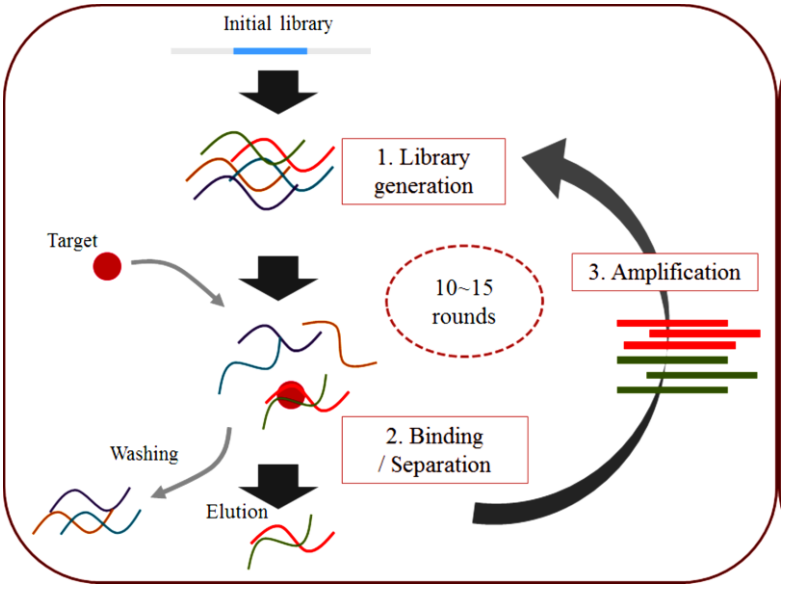


**Figure 2.** Systematic Evolution of Ligands by Exponential Enrichment (SELEX); reprinted with permission from [26]

Compared with antibodies, aptamer has several significant advantages such as easily modify and synthesized in vitro, cost-effectiveness, high affinity and excellent specificity, high stability, excellent flexibility and has been widely used as an excellent bioreceptor for aptamer-based sensors (aptasensor), such as electrochemical sensor [13,23,27]. The interaction between the aptamer and the target analyte can be compared to the interaction between antibodies and antigens since it has great selectivity and affinity for the target analyte [23].

Aptamer has a specific and complex three-dimensional structure as shown in Figure 3 so that it can make interactions with a variety of targets with the same affinity as antibodies [23], such as stems, loops, purine-rich bulges, hairpins, tetraloops, pseudoknots, kissing complexes, triplexes, and G-quadruplex [28,29]. The interaction between the aptamer and target compounds can be based on hydrogen bonds, van der Waals forces, electrostatic interactions, and π-π* stacking interactions [28,30].

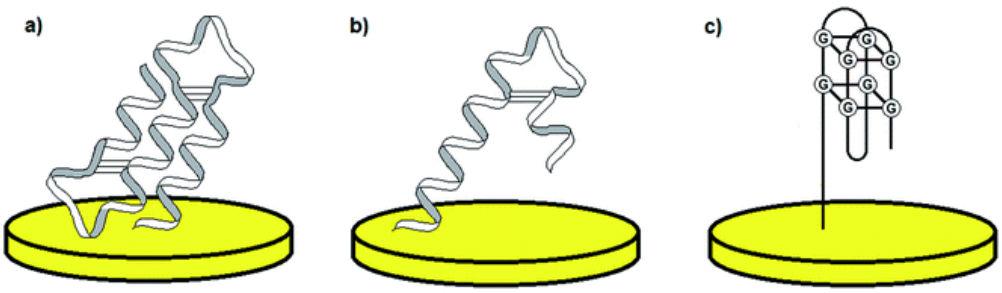


**Figure 3.** The most common examples of aptamer conformations, (a) pseudo-knot, (b) hairpin, and (c) G-quadruplex; reprinted with the permission from [31]

Chemical modification of aptamer at the 5' or 3' is frequently performed to facilitate aptamer immobilization [29]. Chemical modification of aptamer by thiol, biotin, or amino groups allows immobilization of aptamer on various surfaces, so that aptamer can function as a bioreceptor in the biosensor [30].

The dissociation constant (Kd) is one of the important characteristics of an aptamer. An aptamer's dissociation constant with the analyte is in the micromolar, nanomolar, and picomolar ranges; a lower Kd indicates a more selective interaction [23,30,32].

## 3. ELECTROCHEMICAL APTASENSORS

A chemical recognition system and a physical transducer are the two essential components of electrochemical sensors. Physical transducers are electrodes that convert a recognition system's signal into an electroanalytical signal that can be measured [33]. An electrochemical aptasensor is a biosensor with an electrochemical transducer. Electrodes are used as transduction elements [15]. On the electrode surface, electron transport, diffusion, and oxidation-reduction reactions all take place [34]. The electrochemical aptasensor works on the idea that the aptamer, which is immobilized on the electrode surface as a bioreceptor, interacts with the target analyte, changing the electrochemical signal [35].

The detection methods for electrochemical aptasensors can be classified into five types: amperometric, conductometry, potentiometric, voltammetry, and impedimetric [15], each of which depends on the parameters that can be measured as current, conductance, potential, current and potential, and also impedance [36].

### 3.1. Aptamer Immobilization Techniques

Aptamer immobilization technique is another important step in the development of electrochemical aptasensor because it plays a major role in the overall performance of an aptasensor [36]. Immobilization is a method used to attach or conjugate a bioreceptor to a transducer [37]. The technique used for immobilization on the electrode surface influences the stability, affinity, and specificity of the aptamer to the target analyte. The preference of

immobilization technique depends on several factors including the surface of the electrode, the properties of the bioreceptor, the physicochemical properties of the target analyte, and the fabrication condition of the biosensor. Thus, the technique immobilization chosen must assure that the affinity and selectivity of aptamer are maintained to obtain a good time immobilized right as a bioreceptor [36].

The most commonly used aptamer immobilization technique is physical adsorption and covalent bonding, such as self-assembled monolayers (SAM) with thiol-based interactions, streptavidin-biotin interactions, and surface activation with EDC/NHS as shown in Figure 4. The aptamer immobilization technique influences the surface coverage of the aptamer, which might affect the detection efficiency of the binding of the aptamer to the target analyte [31]. Several studies related to the development of the aptamer immobilization technique in electrochemical aptasensor for viruses detection in the last eight years are presented in Table 1.

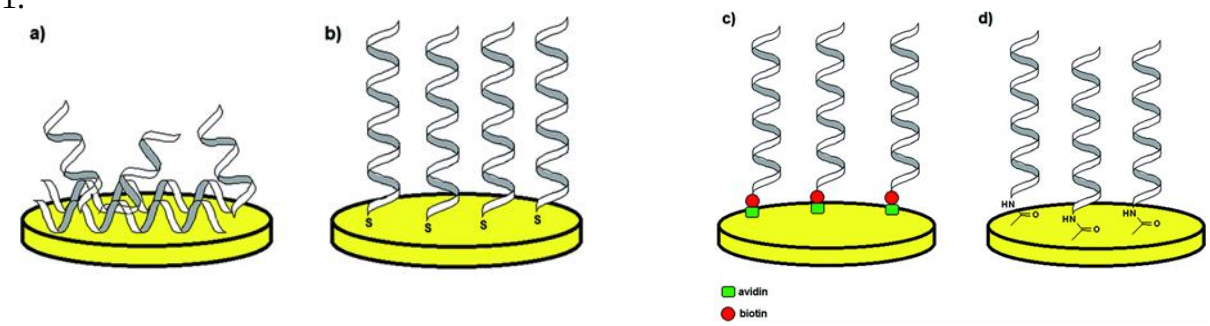


**Figure 4.** Examples of techniques immobilization of aptamer through (a) physical adsorption, (b) self-assembled monolayers (SAM) based on thiol, (c) avidin-biotin, and (d) surface activation with EDC/NHS; reprinted with the permission from [31]

In the immobilization technique with physical adsorption, there is no need for aptamer modification. The applicator which is immobilized on the surface of the electrode is carried out using an electrostatic force. This method is simple but not suitable for the development of aptasensor because of the low stability due to the desorption of aptamer on the electrode surface [36].

Gong et al. [38] was developed an electrochemical aptasensor based on the use of a graphene-Nafion composite to detect the HIV-1 gene using the electrochemical detection technique EIS (Electrochemical Impedance Spectroscopy). This electrochemical aptasensor is made by adsorbing single-stranded DNA (ssDNA) through π-π* stacking interactions on the GCE (Glassy Carbon Electrode) surface that modified graphene-Nafion. Due to steric hindrance, the electron transfer resistance of the electrode with the $K_3[Fe(CN)_6]$ redox system becomes difficult, so the value of the electron transfer resistance increases. On the measurement of the HIV-1 gene, the ssDNA probe interacts with the DNA of the target analyte to form double-stranded DNA (dsDNA), thus inducing dsDNA to leave the aptasensor surface

because the interaction between dsDNA and graphene is weaker than the interaction between ssDNA and complementary DNA [38].

In other immobilization techniques, an aptamer can be modified with thiol groups (-SH), biotin, and chemical functional groups such as amino (-$NH_2$). This modified applicator can then be immobilized onto the surface of the electrode both at the 3' and 5' ends. Self-assembled monolayers (SAM), such as a thiol based interaction is a technique that is simple and effective based on the strong affinity of thiol groups (-SH) in the surface of the gold electrode which allows the formation of covalent bonding between sulfur and gold as shown in Figure 5 [36,39].

In the study of Mohsin et al. [9], an electrochemical aptasensor was developed using the technique of electrochemical detection CV (Cyclic Voltammetry) for Hepatitis B virus detection. The surface of the GCE is modified with gold nanoparticles (AuNPs) that function with graphene oxide. The aptamer modified thiol group that recognized HBsAg (Hepatitis B surface antigen) covalently immobilized on the modified GCE surface with AuNPs through its strong gold and sulfur affinity. Methylene blue (MB) was used as a redox probe which was intercalated into the aptamer structure through electrostatic interactions between the guanine base and MB. The aptasensor function relies on the specific binding between the aptamer and the HBsAg target analyte. In the absence of the target analyte, a strong electrochemical signal is generated. Meanwhile, if there is a target analyte, the aptamer intercalated by the MB redox probe will be forced to release MB from the surface of the aptasensor after dissociation of the aptamer structure caused by the presence of the target analyte, so the resulting electrochemical signal is weak. This reduction in an electrochemical signal can be attributed to the formation of a layer of the aptamer complex and the target analyte on the electrode surface due to the specific binding between the target analyte and the aptamer, which acts as a barrier to the $K_3[Fe(CN)_6]$ redox system to the electrode surface [9].

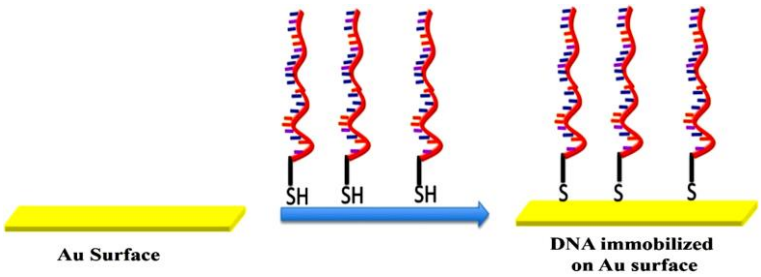


**Figure 5.** Self-assembled monolayers (SAM) immobilization technique, thiol based interaction; reprinted with the permission from [40]

The aptamer immobilization technique is based on the streptavidin-biotin interaction, based on the specific affinity between streptavidin and biotin. Streptavidin-biotin is used because it has a high affinity and specificity. Streptavidin is a protein consisting of four

subunits (tetrameric), each of which can interact with one biotin molecule as shown in Figure 6. The interactions that occur between streptavidin and biotin are Van der Waals forces and hydrogen bonds [41]. Streptavidin can be easily immobilized to the electrode surface through noncovalent bonding or physical adsorption [36,39].

Karash et al. [42], was developed an electrochemical aptasensor using a specific aptamer and a microelectrode to detect the H5N1 virus. Streptavidin is immobilized on the microelectrode surface by physical adsorption. Subsequently, the aptamer labeled biotin was tied to streptavidin. The surface of the microelectrode is blocked with polyethylene glycol. Furthermore, the H5N1 virus was incubated on the microelectrode surface, thus allowing the virus to bind to the aptamer and form the streptavidin/aptamer/H5N1 virus complex. The changes of impedance were measured using the electrochemical detection technique EIS. The EIS is based on Faradaic impedance measurements in the presence of $K_3[Fe(CN)_6]$ as a redox probe. The electron transfer of $K_3[Fe(CN)_6]$ can be inhibited by the formation of the streptavidin/aptamer/H5N1 virus complex on the electrode surface, which increases the electron transfer resistance of $K_3[Fe(CN)_6]$. To improve signal impedance, nanoparticle-based signal amplifiers are designed and implemented to form gold nanoparticles /aptamer/thiocyanuric acid [42].

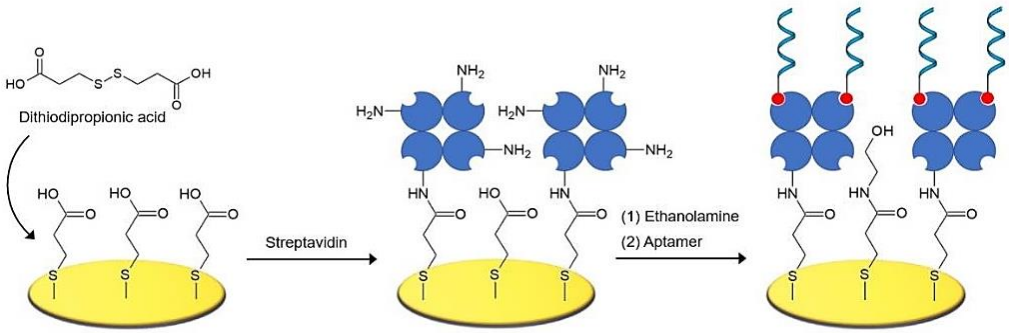


**Figure 6.** Schematic of electrochemical aptasensor with immobilization technique based on the streptavidin-biotin interaction; reprinted with the permission from [43]

In the covalent bond based immobilization technique with surface activation using EDC/NHS, the electrode surface is modified with chemical groups such as hydroxyl (-OH), the carboxylic acid (-COOH), an aldehyde (-CHO), and amino ($-NH_2$), which will interact with a modified aptamer with a suitable chemical functional group by forming a regular layer of aptamer on the electrode surface. Typically, aptamer modified with an amino group ($-NH_2$) is covalently immobilized onto the surface of the electrode with the carboxylate (-COOH) group activated before 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/ *N*-hydroxysuccinimide (NHS) to provide reactive succinimide ester. Then the succinimide ester will react spontaneously with primary amines or other nucleophiles as shown in Figure 7. However, the immobilization technique with surface activation to be applied depends on the

type of terminal functional group to which the aptamer is connected. This method allows increased sensor specificity and decreased nonspecific adsorption [36,39].

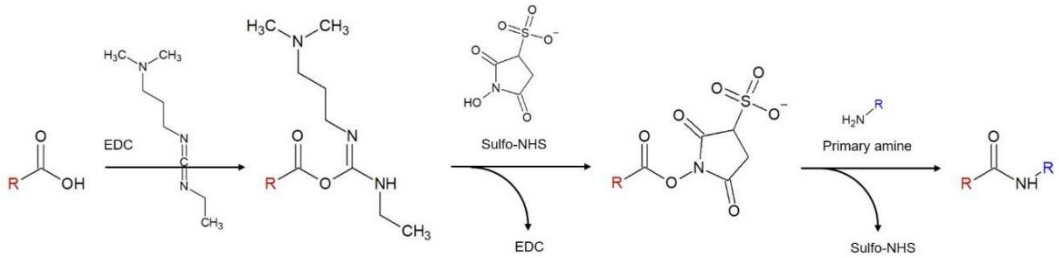


**Figure 7.** Reaction for EDC/NHS-assisted amide bond formation between carboxylic acids and primary amines [43]

Gong et al. [44] was developed an electrochemical aptasensor with a sensitive electrochemical detection technique EIS for HIV-1 gene detection using reduced graphene oxide (ERGO) as a biosensor platform. The ssDNA probe that was modified with an amino group was covalently immobilized onto the GCE surface modified with graphene oxide drop-coating (GO) followed by electrochemical reduction to the ERGO/GCE electrode. Hybridization of DNA probe with the DNA complementary, causing the resistance of the transfer of electrons to the redox system ferricyanide $K_3[Fe(CN)_6]$ negatively charged greatly increased due to electrostatic repulsion enhanced between duplex dsDNA and redox systems ferricyanide $K_3[Fe(CN)_6]$. The increased electron transfer resistance can be used as an aptasensor determination [44].

Pei et al. [45] proposed a new concept to improve the recognition performance of target analyte with 3D structured DNA. For example, DNA in the form of a tetrahedron with three thiol groups was designed and used for biosensor development. Tetrahedron DNA consists of four or more single-stranded DNA. This technique is expected to be immobilized to the gold surface via a thiol-Au bond and leave the probe free at the top end. Tetrahedron DNA can quickly and strongly attach to the gold surface due to the presence of three thiol groups so that increasing stability compared to monothiolate DNA, which is useful for designing stable and strong biosensors [45]. The immobilized tetrahedron DNA on the surface of the electrode can increase the efficiency of molecular recognition and avoid excessive electrode modification steps [46].

In the study of Dong et al. [47], an electrochemical aptasensor based on the tetrahedron DNA structure was developed for the avian influenza A (H7N9) virus detection by recognizing a fragment of the hemagglutinin (HA) gene sequence. The tetrahedron DNA was immobilized onto the surface of the gold electrode with three thiol modified nucleotide sequences and a nucleotide sequence containing complementary DNA to hybridize with the single-strand DNA of the target analyte. The target analyte hybridized with the biotinylated ssDNA oligonucleotide as a detection probe, and then avidin-horseradish peroxidase (HRP) was used

to generate the aptasensor signal through interaction with the 3,3′,5,5′-tetramethylbenzidine substrate. The biosensor fabrication process was characterized by CV, and EIS. The results obtained indicate that the use of tetrahedron DNA can improve the detection performance of electrochemical biosensors compared to ssDNA. The electrochemical aptasensor scheme based on the tetrahedron DNA structure is shown in Figure 8 [47].

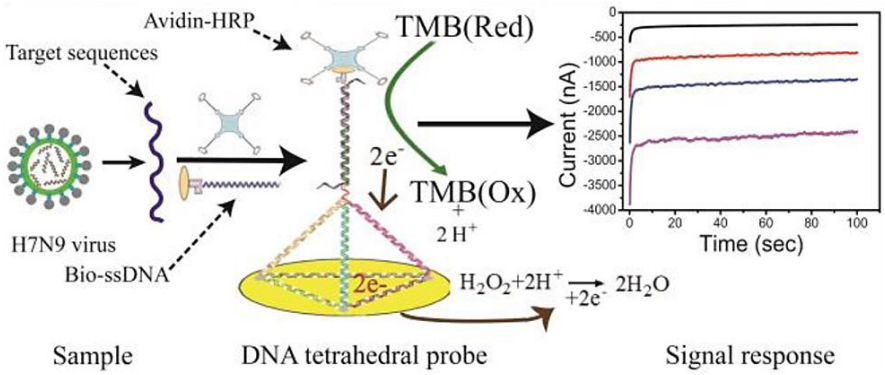


**Figure 8.** Electrochemical aptasensor scheme based on tetrahedron DNA structure to detect avian influenza A (H7N9) virus; reprinted with the permission from [47]

The aptamer immobilization technique with the triple-helix molecular switch (THMS) system has been widely applied as a strategy to detect different target compounds. THMS generally consists of two parts, the sequence of aptamer which is called the signal transduction probe (STP), and the aptamer which is specific to the compound of the target with two fragments of DNA sequences that form hairpin structures as shown in Figure 9. The STP is labeled with methylene blue (MB) and thiol group at the 5' or 3' end. The THMS structure is formed through the Watson-Crick and Hoogsteen base pairs under optimal conditions. The aptamer can hybridize with STP, so the aptamer to form the structure of the hairpin specific to the compound of the target system with triple-helix molecular switch (THMs). THMS is then modified on the surface of the electrode through the Au-S bond [48].

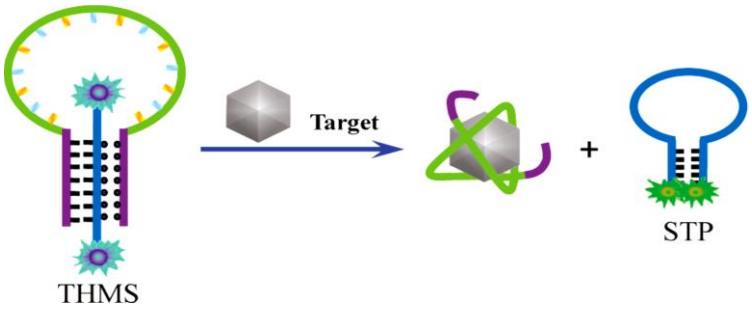


**Figure 9.** Triple-helix molecular switch (THMS) system through the Watson-Crick and Hoogsteen base pairs; reprinted with the permission from [49]

**Table 1.** The development of the aptamer immobilization technique in electrochemical aptasensor for viruses detection in 2013-2020

| Target Analyte | Aptamer Immobilization Technique | Aptamer | Nanomaterial | Detection Technique | LOD | Ref. |
|---|---|---|---|---|---|---|
| Hepatitis C Virus Core Antigen | Physical Adsorption | 5'-ACT ATA CAC AAA AAT AAC ACG ACC GAC GAA AAA ACA CAA C-3' | Graphene quantum dots (GQD) | CV EIS DPV | 3.3 pg/mL | [51] |
| HIV Gene | Physical Adsorption | Aptamer sequence is not available | Graphene-Nafion | EIS | $2.3\times10^{-14}$ M | [38] |
| Hepatitis C Virus Core Antigen | Physical Adsorption | 5'-CCA ACA CAA AAA AGC AGC CAG CAC AAT AAA AAC ACA TAT CA-3' | Multi-walled carbon nanotubes-chitosan nanocomposite (MWCNTs-Chit) | CV EIS DPV | 1.67 fg/mL | [52] |
| Human Noroviruses | Thiol-based Self-Assembled Monolayers (SAM) | 5'-SH-CCT AGG GGG CCC GAC GTC GGG TTA CAC CTT AAG CGG GAA GCA TGC CTT AAG CGA TCG-3' | Gold nanoparticle (AuNPs) | CV EIS SWV | - | [53] |
| Hepatitis B Virus | Thiol-based Self-Assembled Monolayers (SAM) | 5′ -SH-$(CH_2)_6$-GGG AAT TCG AGC TCG GTA CCG GCA CAA GCA TAT GGA CTC CTC TGA ACC TAC GAT GTA GTA CCT GCA GGC ATG CAA GCT TGG-3′ | Reduced graphene oxide–gold NPs (rGO/Au) | CV EIS DPV | 0.0014 fg/mL | [9] |
| Inactivated H1N1 Virus | Thiol-based Self-Assembled Monolayers (SAM) | 5'-SH-GCA ATG GTA CGG TAC TTC CGG ACC AGT TGT CTT TCG GTC TCT ACC CCA GCC CGT CAA AAG TGC ACG CTA CTT TGC TAA-3' | Gold electrode surface | EIS | 0.9 pg/$\mu$L | [3] |
| Avian Influenza Viruses H5N1 | Thiol-based Self-Assembled Monolayers (SAM) | (Probe 1) 5'-SH-CAA CAG GAC AAC TAT-3'<br><br>(Probe 2) 5'-SH-GCT ATC CAT GCA CAC-3'<br><br>5'-GTG TGC ATG GAT AGC ACG TAA CGG TGT AGT AGA TAC GTG CGG GTA GGA AGA AAG GGA AAT AGT TGT CCT GTT G-3' | Nanoporous gold film | CV | $2^{-9}$ HAU | [2] |
| Norovirus | Streptavidin-biotin interactions | 5'-Biotin-AGT ATA CCG TAT TAC CTG CAG CCA TGT TTT GTA GGT GTA ATA GGT CAT GTT AGG GTT TCT GCG ATA TCT CGG AGA TCT TGC-3' | Graphene-AuNPs | DPV | 100 pM | [6] |
| Avian Influenza Virus H5N1 | Streptavidin-biotin interactions | 5′-Biotin-GTG TGC ATG GAT AGC ACG TAA CGG TGT AGT AGA TAC GTG CGG GTA GGA AGA AAG GGA AAT AGT TGT CCT GTT G-3′ | Gold electrode surface | EIS | 0.25 HAU | [42] |
| Avian Influenza Virus Proteins | Surface Activation with EDC/NHS | 5'- TTG GGG TTA TTT TGG GAG GGC GGG GGT T-$NH_2$-3' | Gold nanoparticle deposition | CV DPV | 100 fM | [54] |

| | | | | | | |
|---|---|---|---|---|---|---|
| HIV Gene | Surface Activation with EDC/NHS | 5'-CAG TGT GGA AAA TCT CTA GC-$(CH_2)_6$-$NH_2$-3' | Graphene oxide | EIS | $3.0\times10^{-13}$ M | [44] |
| Virus Avian Influenza A (H7N9) | Tetrahedron DNA | 5'-CCC GCA GAT GAC TAA TTT TTT TTT TAC ATT CCT AAG TCT GAA ACA TTA CAG CTT GCT ACA CGA GAA GAG CCG CCA TAG TA-3'<br><br>5'-SH-$C_6$-TAT CAC CAG GCA GTT GAC AGT GTA GCA AGC TGT AAT AGA TGC GAG GGT CCA ATA C-3'<br><br>5'-SH-$C_6$-TCA ACT GCC TGG TGA TAA AAC GAC ACT ACG TGG GAA TCT ACT ATG GCG GCT CTT C-3'<br><br>5'-SH-$C_6$-TTC AGA CTT AGG AAT GTG CTT CCC ACG TAG TGT CGT TTG TAT TGG ACC CTC GCA T-3' | Gold electrode surface | CV<br>EIS | 100 fM | [47] |
| Human Immunodeficiency Virus tipe 1 (HIV-1) | THMS | Capture probe (CP)<br>5'-SH-$(CH_2)_6$-ATA TGG GAA GGG AGG GAT GGG T-3'<br><br>Molecular beacon 1 (MB 1)<br>5'-CTT CCC TAT GTG GAA AAT CTC TAG CAG TTC CCT TC-3'<br><br>Molecular beacon 2 (MB 2)<br>5'-CTT CCC TAT GTG GAA AAT CTC TAG CAG T-3'<br><br>Molecular beacon 3 (MB 3)<br>5'-CTT CCA TGT GGA AAA TCT CTA GCA GTC CTT C-3'<br><br>Molecular beacon 4 (MB 4)<br>5'-CTT CCC ATG TGG AAA ATC TCT AGC AGT CCC TTC-3'<br><br>Molecular beacon 5 (MB 5)<br>5'-CTT CCC TCA TGT GGA AAA TCT CTA GCA GTC TCC CTT C-3'<br><br>Molecular beacon 6 (MB 6)<br>5'-CTT CCC TCC ATG TGG AAA ATC TCT AGC AGT CCT CCC TTC-3' | Gold electrode surface | EIS<br>DPV | 0.054 nM | [50] |

Wang et al. [50], developed an electrochemical aptasensor using the THMS approach, based on the formation of a triple-helix structure between the STP which is immobilized to the surface of the gold electrode via Au-S bonds. The aptamer sequence specific to the target compound with two fragments of the DNA sequence that form the structure hairpin through intermolecular DNA hybridization induced by Watson-Crick and Hoogsteen base pairs to detect Human Immunodeficiency Virus type 1 (HIV-1) as the target analyte. In the absence of a target analyte, it forms a triple-helix structure with a capture probe based on Watson-Crick and Hoogsteen base pairs via hybridization of intermolecular DNA. Due to the formation of the triple-helix structure conformation, aptamer hemin cannot interact with hemin even in the presence of hemin and $K^+$, resulting in a weak electrochemical signal. However, after recognition with a target analyte, the triple-helix region is released to free aptamer hemin and a G-quadruplex hemin complex can be formed in the presence of $K^+$ and hemin on the electrode surface to provide an electrochemical response. The biosensor fabrication process was characterized by electrochemical detection techniques EIS and DPV (Differential Pulse Voltammetry) [50].

The binding of the aptamer to the target analyte usually relies on certain conformations, such as hairpin and G-quadruplex. Significantly, electrochemical aptasensor based hairpin structures have demonstrated the performance remarkable. This type of biosensor makes use of the conformational changes induced by hybridization that can be detected electrochemically against distance-dependent electron transfer [50].

## 4. APPLICATION OF ELECTROCHEMICAL APTASENSOR FOR VIRUSES DETECTION

Since Clark and Lyons developed a biosensor by immobilizing the enzyme glucose oxidase on the electrode surface to detect blood glucose in 1962, biosensor technology has developed very rapidly, one of which is a biosensor to detect viruses [55].

Recently aptasensor with electrochemical methods has attracted a lot of attention because it provides great advantages over optical and piezoelectric detection methods. Electrochemical detection has several advantages, including portability, ease of use, fast response, good sensitivity, and high specificity [7,8], and has been widely used to detect several biomolecules, such as viruses [6].

Human Norovirus (HuNoV), is a common cause of food poisoning worldwide. Norovirus is genetically categorized into GI to GV, with Norovirus GII mediated infection predominantly infecting humans. Chand & Neethirajan [6] have developed an aptasensor electrochemical to detect viruses. The selective detection of norovirus was carried out by using a modified graphene-gold nanoparticle (Grp-AuNP) composite on a carbon electrode with a specific norovirus aptamer. The norovirus-specific aptamer is modified with biotin and characterized by ferrocene molecules, which act as redox probes. The use of ferrocene as a redox probe

increases the portability of the biosensor. Grp-AuNP composites provide a stable substrate for electrochemical signal amplification and aptamer immobilization. The norovirus capsid protein was selectively captured by Bt-Apt-Fc immobilized on the Grp-AuNPs composite electrode. The interaction of aptamer and norovirus resulted in decreased electrochemical signals from ferrocene. Using the DPV electrochemical detection technique, a detection limit of 100 µm was obtained. This electrochemical aptasensor can also be applied to detect norovirus in blood samples [6].

There are three types of avian influenza viruses, namely types A, B, and C. Influenza virus type A is the most virulent human pathogens and the most varied with the threat of epidemics and even pandemics. Rapid detection of viruses is desirable for protecting public health and minimizing the spread of infectious diseases. Diba et al. [54] developed an electrochemical aptasensor using a sandwich system that involved the formation of an aptamer-protein-antibody complex developed for avian influenza virus type A H5N1 protein detection based on gold nanoparticle (AuNPs) modified electrodes. AuNPs modified SPCE electrodes functioned by covalent immobilization of the specific aptamer followed by adsorption of H5N1 protein. The alkaline phosphatase (ALP) conjugated monoclonal antibody is then adsorbed to form the AuNP-aptamer/H5N1/antiH5N1-ALP sandwich complex which binds to the surface of the SPCE, which is then reacted with the enzyme-substrate, 4-amino phenyl phosphate (APP). The current associated with the electrocatalytic reaction of ALP bound to APP increases with increasing H5N1 concentration. The lowest concentration that could be detected was 100 fM using the DPV electrochemical detection technique [54].

Electrochemical aptasensor to detect viruses have also been developed by Ghanbari et al. [51] for antigen detection of hepatitis C virus (HCV). Hepatitis C virus infects hepatocytes in the liver, which enter via the liver sinusoids which are linked to several lipoproteins. HCV is a single positive RNA virus that is a member of the genus Hepacivirus. The surface of the GCE was modified with graphene quantum dots (GQD). GQD is a new substrate and is suitable for aptamer immobilization through π-π* stacking interactions, which can increase the absorption of aptamer on the electrode surface. Immobilization of aptamer on the surface of GQD via chemisorption between GQD and the amine group of the aptamer. The carboxyl groups of GQD allow strong adsorption of the aptamer onto the GQD surface. Electrochemical detection techniques such as EIS, CV, and DPV are used at every step of the modification process. The electrochemical signal changes were performed using EIS as a measurement technique. In the presence of the target compound antigen, the electron transfer of the redox ferricyanide $K_3[Fe(CN)_6]$ system is significantly increased, which confirms the formation of the aptamer/antigen complex. EIS is used as an efficient alternative detection system for the measurement of hepatitis C virus antigen with a detection limit of 3.3 $pg/mL^{-1}$. This electrochemical aptasensor can accurately detect the hepatitis C virus antigen concentration in human serum samples [51].

## 5. CONCLUSION

Diseases caused by viruses are a public health problem. Currently, viruses diagnosis is based on the direct detection of viral components or indirect detection by measuring the antibodies produced in response to viral infection. Compared to antibody-based detection, electrochemical aptasensor has been widely used to detect several biomolecules, such as viruses, as they have been shown to have several advantages. Aptamer presents several advantages in its use as an excellent bioreceptor for aptamer-based sensors (aptasensor) which can be immobilized on different transducer surfaces, such as electrochemical sensors. The aptamer immobilization based on covalent bonding is the strongest immobilization method when compared to adsorption immobilization because the bond formation between the bioreceptor and the transducer is stronger.

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