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Electrochemical detection of HIV-1 by nanomaterials

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ABSTRACT

AIDS is one of the global pandemic diseases that results from infection by HIV and was estimated 34.2 million people infected in 2011 by this virus. The investigators had previously shown that by early antiretroviral treatment, the risk of AIDS/HIV-related illness and transmission reduced significantly. Nanomaterials could be applied to improving the ability and sensitivity of sensors to detect serum biomarkers with low-sample volume. Moreover, results can be obtained faster. In this paper, we present a review of several experimental studies for HIV electrochemical detection based on nanomaterials. Furthermore, we explained each assay and detection limited.

Abbreviation: NP(s): Nanoparticle(s); AuNP(s): Gold Nanoparticle(s); AuNR(s): Gold Nanorod(s); AgNP(s): Ag Nanoparticle(s); AgNR(s): Ag Nanorod(s); QD(s): Quantum Dot(s); ELISA: Enzyme-Linked Immunosorbent Assay; aM: Attomolar, $10-18$ mol/dm3; MB: Methylene Blue; AIDS: Acquired immunodeficiency syndrome; HIV: human immunodeficiency virus; HIV-1 PR: HIV-1 protease; CV: Cyclic Voltammetry; SWV: Square Wave Voltammetry; EIS: Electrochemical Impedance Spectroscopy; DPV: Difierential Pulse Voltammetry; SWCNT: Single-Walled Carbon Nanotube; MWCNT: Multi-Walled Carbon Nanotubes; HIV-1 RT: HIV-1 Reverse Transcriptase; Ab: Antibody; SPCE: Screen-Printed Carbon Electrode; HQ: O-Hydroxyl Phenol; ITO: Indium Tin Oxide; VLPs: Virus like particles; AC impedance: Alternating Current impedance; PPy: polypyrrole; AP1: auxiliary probe 1; AP2: auxiliary probe 2; CP: capture probe; TD: target DNA; SPE: screen printed electrode; AuNPs-SPCE: AuNPs-Functionalized Screen-Printed Carbon Electrode; AEP: Acetone-Extracted Propolis; HRP: Horseradish Peroxidase; MNPs: Magnetic Nanoparticles; MNPs-p24 Ab1: Magnetic NPs (MNPs) labeled with the primary p24 antibody; AuNPs/EV-p24 Ab2: EV incubated with the secondary antibody of p24 that immobilized on AuNPs. The EnVision reagent (EV) is a kind of enzyme–polymer complex which contains about 100 molecules of HRP and 15 molecules of anti-IgG antibody connected in a poly-dextrin amine skeleton.; GO: Graphene Oxide; TH: Thionine; Si-HRP: silica matrix with horseradish peroxidase (HRP) entrapped

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Introduction

Nanomaterials are materials by nanoscale dimension (at least in one dimension) that preparation and synthesis by various methods (Farkhani and Valizadeh 2014, Valizadeh and Farkhani 2014, Valizadeh et al. 2016), and used in many different ways (Ajayan and Zhou 2001, Badrzadeh et al. 2016, Farkhani et al. 2014, Sohrabi et al. 2016, Valizadeh and Khosroushahi 2015, Valizadeh et al. 2012, Valizadeh 2015). Nanomaterials applied for detecting and diagnosis of different targets include (Valizadeh 2015):

1. Macromolecules such as:
   a. Protein (Boeneman et al. 2010, Courty et al. 2006)
   b. RNA and DNA (Cao et al. 2002)
2. Virus such as HIV (Kim et al. 2009), hepatitis B (Wang et al. 2010), influenza A (Cui et al. 2011) and etc.
3. Cells such as:
   a. Bacteria such as *E. coli* (Hirschey et al. 2006), *Salmonella Typhimurium* (Yang and Li 2006), *Staphylococcus aureus* (Xue et al. 2009) and etc.
   b. Eukaryotic cells such as human mesenchymal stem cells (Seleverstov et al. 2006), cancer cells (El-Sayed et al. 2005) and etc.

One of the global pandemic diseases is Acquired immunodeficiency syndrome (AIDS) that result from infection by the human immunodeficiency virus (HIV). It was estimated 34.2 million people infected by this virus in 2011 (Mohammed Fayaz et al. 2012, Valizadeh 2015). The HIV virus is one of the most variable viruses with a diameter of about 120 nm that can be seen as a biological nanostructure (Valizadeh 2015). HIV genetic material is RNA form and containing 9 genes that

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encoding 19 proteins (Valizadeh 2015). The investigators had previously shown that by early antiretroviral treatment, the risk of AIDS/HIV-related illness and transmission reduced to 96% (Cohen et al. 2011, Valizadeh 2015). Nanomaterials could be applied to achieve this goal by improving the ability to detect serum biomarkers of the blood-borne infectious diseases with low sample volume, more sensitivity, and rapidity in compare with currently methods (such as ELISA, Western Blotting assay, and particle agglutination assay and etc.) (Klostranec et al. 2007, Valizadeh 2015). The major viral markers of HIV-1 that have been used for detecting HIV-1 infection, monitoring disease progression, screening blood donors, and evaluating HIV-1 therapy are RNA, capsid protein (Tat, JCV, and p24 antigen), and anti-HIV antibodies (Fiebig et al. 2003, Petersen et al. 1994, Tang et al. 2007). Also, there are many studies about Nanomaterials that applied for HIV/AIDS treatment (Kumar et al. 2015). Here, we review several electrochemical detection methods that applying nanomaterials for improving sensitivity in 5 categories including: (1) Cyclic Voltammetry (CV), (2) Square Wave Voltammetry (SWV), (3) Electrochemical Impedance Spectroscopy (EIS), (4) Difierential Pulse Voltammetry (DPV), (5) Hybrid method.

Electrochemical detection methods

Various electrochemical biosensors design and fabricated for detection of targets (Anik et al. 2016, Dalkıran et al. 2016, Erdem et al. 2014). There are several electrochemical detection methods such as:

Cyclic voltammetry

The CV is a type of potentiodynamic electrochemical measurement that the working electrode potential is ramped linearly versus time. In a cyclic voltammogram, the current at the working electrode is plotted versus the applied voltage to give the cyclic voltammogram trace. The CV is commonly applied for detection and characterization of the electrochemical properties of an analyte in solution (Bard and Faulkner 1980, Nicholson and Shain 1964).

Square wave voltammetry

The Square Wave Voltammetry (SWV) is a form of linear potential sweep voltammetry. In this method, the current at a working electrode is measured, while the potential between a reference electrode and the working electrode is swept linearly in time (Mirceski et al. 2007, Osteryoung and Osteryoung 1985).

Electrochemical impedance spectroscopy

Electrochemical impedance Spectroscopy (EIS) or the Alternating Current (AC) impedance is a sensitive technique, which in the studied system monitors the electrical response after application of a periodic small amplitude AC signal. In this method, the system response provides information concerning the interface state (presence of adsorbed analyte or species) and can detect the occurrence of interfacial reactions (Hassen et al. 2008). Because of using electrochemically active labels, the electrochemical-based detection methods can be considered as one of the most sensitive tools for the detection of enzymes, antibody and nucleic acids (Laczka et al. 2009). An electrochemical biosensor array has many advantages such as low-detection limit, inexpensive instrument, and simplicity due to ease of obtaining electrical signal (Zhang et al. 2010). EIS as Nyquist plots were reported as an effective method to monitor the surface characteristics and thus allow an understanding of the chemical transformation and processes associated with the conductive surface of electrode at different modification steps (Rezaei et al. 2011).

Differential pulse voltammetry

Differential Pulse Voltammetry (DPV) is sensitive and extremely selective for measuring trace levels and the quantitative determination of analytes (Wang 2006). In this method, the potential is scanned with a series of pulses, and it can superior elimination of the capacitive/background current (Scholz 2010).

Hybrid method

In hybrid method, scientific used 2 or more electrochemical methods for analyte detection.

Nanomaterials for electrochemical detection

Magnetic nanoparticles

Hassen et al. (2008) developed a biosensor based on streptavidin functionalized magnetic NPs for HIV DNA detection. In this study, the magnetic layer is composed of the streptavidin functionalized magnetic NPs immobilized on surface of a gold electrode via a 300 mT magnet. Then, the biotinylated HIV DNA probes were linked through biotin–streptavidin interaction to magnetic layer, and DNA hybridization detection was conducted by impedimetric measurements. The CV measurements were performed in a 5 mM solution of redox couple $[Fe(CN)_6]^{4-/3-}$ prepared in Phosphate-buffered saline (abbreviated PBS) buffer. Scanning potential was conducted between −600 mV and 600 mV, potential value of −300 mV and the frequency range between 100 mHz and 100 kHz. By this method, the lowest detection limit for HIV DNA target is obtained 160 pM. This approach can be considered as suitable method for PCR fragment detection whose length is often superior to 1000 bp (Hassen et al. 2008).

In Dai Tran and coworkers study, the detection of HIV-1 was demonstrated on chitosan/Fe_3O_4/GEM system with methylene blue (MB) Redox indicator, and using SWV and EIS techniques (Figure 1) (Dai Tran et al. 2011). GEM is abbreviation of 25-mer gene expression of modulator 91. The results suggest that the presence of Fe_3O_4 superparamagnetic NPs facilitates electron transfer. Thus, the current response and the sensitivity of the overall system showed enhancement. The scan rate 50 mV/s and potential range −0.5 V to +0.2 V vs. SCE (saturated calomel electrode) were the CV parameters.

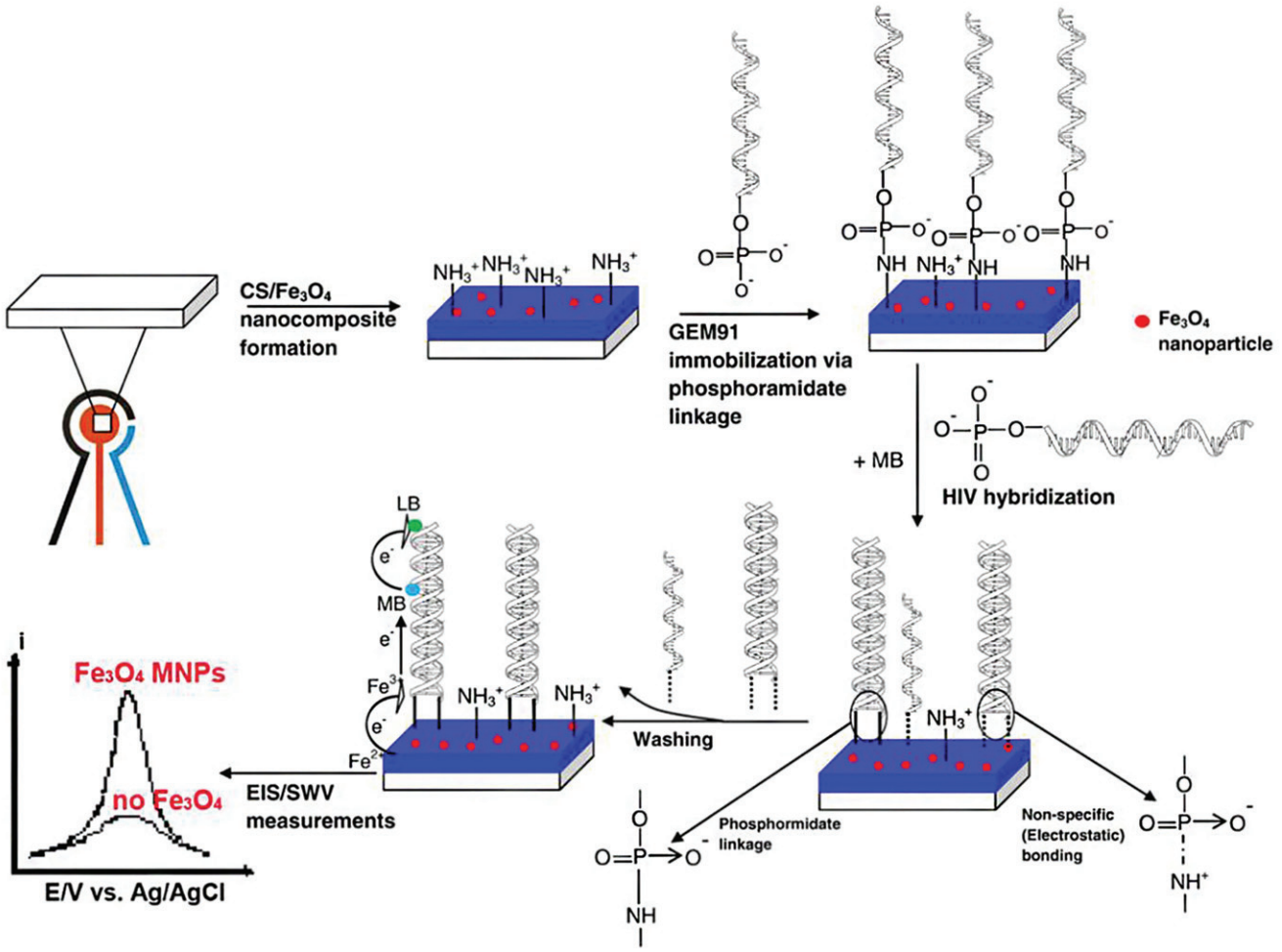

Figure 1. Schematic representation of HIV electrochemical detection on chitosan/Fe_3O_4 SPE, using MB as an intercalator (Reuse with permission from Elsevier) (Dai Tran et al. 2011).

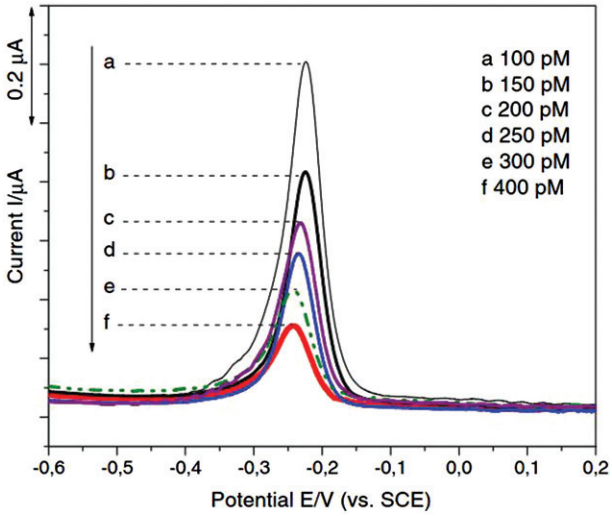

Figure 2. Quantitative detection of different target DNA concentrations by SWV is shown (Reuse with permission from Elsevier) (Dai Tran et al. 2011).

The frequency 12.5 Hz, the potential range from −600 to +400 mV, step 8 mV, amplitude 25 mV were optimized as SWV parameters. The frequency range from 200 kHz to 10 mHz using 5 mV alternating voltage superimposed on DC potential were optimized as EIS parameters. In this study, a detection limit is 50 pM (Dai Tran et al. 2011). The SWV response to different concentrations of the target DNA was displayed in Figure 2. In this figure, the decrease in the magnitude of the voltammetric signal was due to the extent of the hybrid formation. EIS was also used to study the effect of hybridization on the chitosan/Fe_3O_4 interfacial platform of screen printed electrode (SPE) surface.

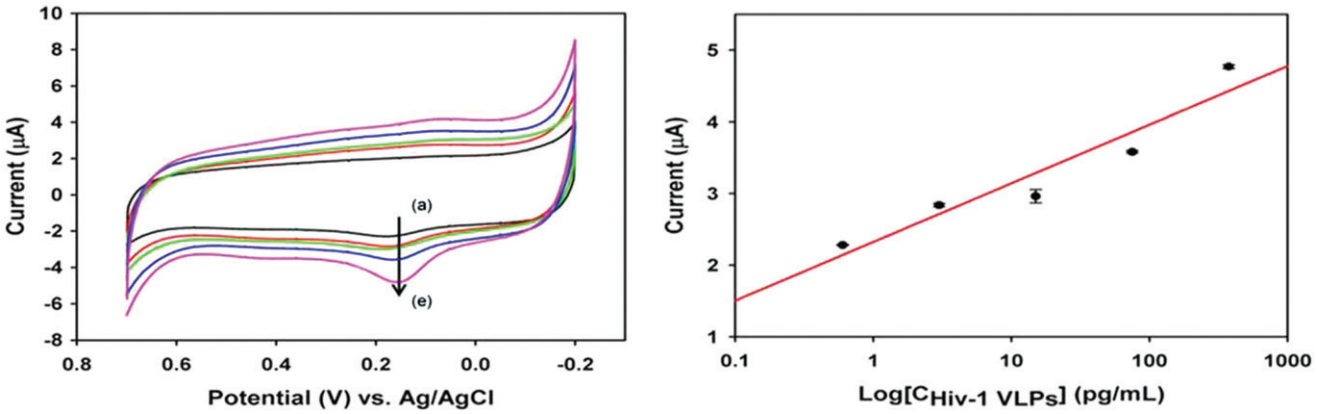

Figure 3. (Left) Cyclic voltammogram of different concentration of HIV-1 VLPs after immobilization of fragmented antibody (a) 600 fg/mL, (b) 3 pg/mL, (c) 15 pg/mL, (d) 75 pg/mL, and (e) 375 pg/m, respectively and (Right) linear plot of anodic current peak as a function of HIV-1 VLPs range from 600 fg/mL to 375 pg/mL ($R = 0.958$) (Reuse with permission from Elsevier) (Lee et al. 2013).

Gold-based nanomaterials

Lee et al. (2013) proposed a method based on CV without the use of any mediators to detect direct electron transfer signal from HIV-1 virus. For improving the surface area, they fabricated AuNPs-modified Indium Tin Oxide (ITO) electrode by electrochemical deposition (Lee et al. 2013). Subsequently, antibody fragment was immobilized by self-assembly method and different concentrations of HIV-1 virus-like particles (VLPs) on ITO electrode surface. In this systems, HIV-1 VLPs were measured from 600 fg/mL to 375 pg/mL that shown in Figure 3 (Left) (Lee et al. 2013). In Figure 3 (Right), the calibration plot, acquired from oxidation peak of gp120 at ca. 0.153V shows a linear relation in a range from 600 fg/mL to 375 pg/mL (correlation coefficient was 0.958). This technique provided better electron-transfer kinetics and higher background charging current than current methods (Lee et al. 2013).

Recently, Narang and coworkers applied AuNRs for amplification of electrochemical sensing of anti-HIV replication drug, deferiprone, by the EIS method (Narang et al. 2015). In this study, electrochemical cell composed of: 1- Horse radish peroxidase/AuNRs/chitosan/pencil graphite electrode as working electrode, 2- Ag/AgCl as reference electrode, 3- and Pt wire as auxiliary electrode. It was recorded in PBS (50 mM, pH 6.5, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and applied frequency range 0.01 Hz to10 KHz (Narang et al. 2015). Different concentrations of deferiprone drug were used into serum and urine samples, and after each addition, the potential between 0.0 and +1.75 V at a scan rate of 100 mV s^{-1} was recorded by cycling. The AuNRs sensor exhibits improved biosensing characteristics like fast response time of 15 s, linearity as 5 nM to 1000 μM, specific and anti-interferants. The response of AuNRs sensor with serum and urine samples showed its implications towards the development of biosensor for commercial drug monitoring device (Narang et al. 2015). In this study, a low detection limited 5 nM. Figure 4 showed Impedimetric response of AuNRs sensor for deferiprone.

Chen et al. (2012) fabricated a ultrasensitive electrochemical biosensor of DNA. In this method, long-range self-assembled DNA nanostructures were used as carriers for signal amplification. This method can achieve an impressive

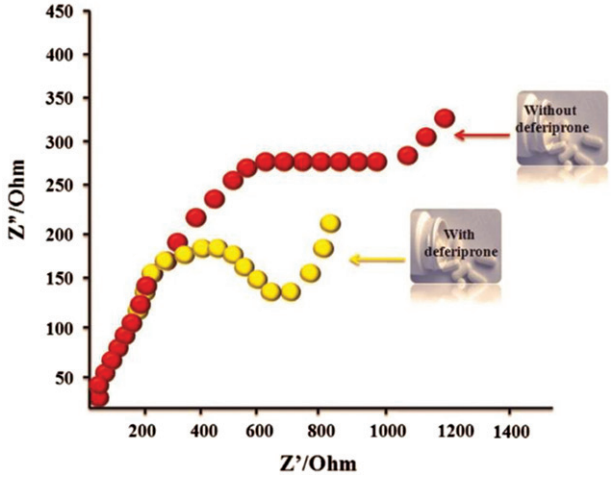

Figure 4. Impedimetric responses of AuNRs sensor with deferiprone and without deferiprone were shown (Reuse with permission from Elsevier) (Narang et al. 2015).

detection limit of 5 aM HIV DNA (38-base) even in complex biological samples such as cell lysates or human serum (Chen et al. 2012). In this work used a three electrode cell (capacity 10 mL; diameter 25 mm) consisting of Ag/AgCl reference electrode, working electrode (the assembled gold electrode, $d = 2$ mm), and platinum counter electrode was used for all electrochemical measurements. DPV was performed using a potential window of 0.2 to −0.6 V (versus Ag/AgCl) in 3 mL of RuHex solution ($[Ru(NH_3)_6]^{3+}$, Hexaammineruthenium(III) chloride), which was degassed with nitrogen for 15 min (Chen et al. 2012). In this protocol, designed two auxiliary DNA probes by name auxiliary probe 1 (AP1) and auxiliary probe 2 (AP2). The capture probes (CP) were immobilized on the gold electrode by Au-S chemistry at the beginning of fabricating the electrochemical DNA biosensor, and after being hybridized CP with target DNA (TD), some more RuHex cations were binding to CP and TD. Then, the solution containing 1 μM of each AP1 and AP2 was dripped on the surface of the electrode, the DNA nanostructures self-assembled by numerous AP1 and AP2. So, AP1 and AP2 were firmly immobilized on the gold electrode via CP and TD. At result, the large number of RuHex can be binding to DNA nanostructures that

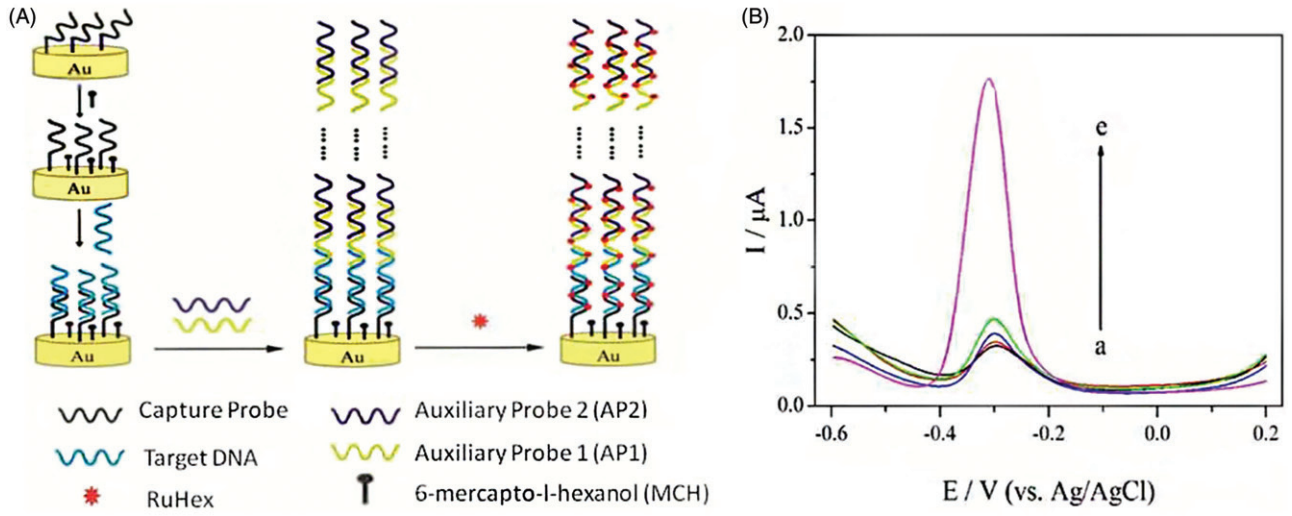

Figure 5. (A) Schematic representation of the enzyme-free and label-free ultrasensitive electrochemical DNA biosensor based on long-range self-assembled DNA nanostructures. (B) DPV responses of the gold electrode modified with various oligonucleotides: (a) CP, (b) CP + TD, (c) CP + AP1 + AP2, (d) CP + TD + AP1, and (e) CP + TD + AP1 + AP2. The concentration of TD is 10 pM. The concentrations of AP1 and AP2 are both 1 μM (Reprinted with permission from American Chemical Society) (Chen et al. 2012).

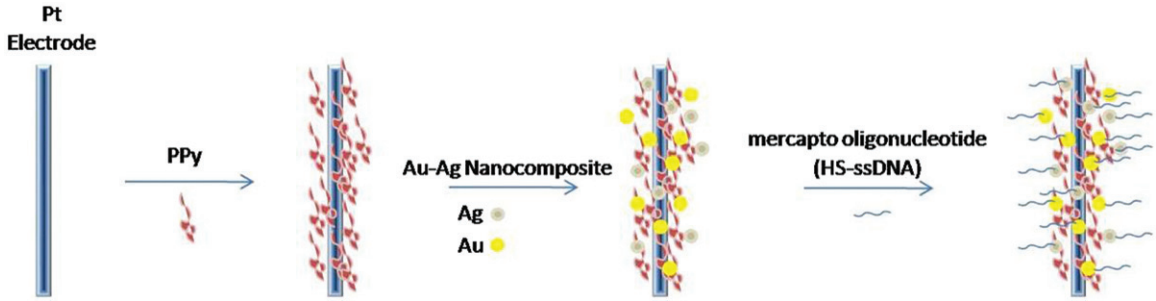

Figure 6. A layer of PPy film was electrodeposited on the platinum electrode surface, and then the Au–Ag nanocomposite was bonded directly onto the surface of PPy, which is a layer of polycation. Finally, mercapto oligonucleotide was self-assembled onto the Au–Ag nanocomposite surface.

produced a remarkable amplified electrochemical signal (Figure 5) (Chen et al. 2012).

The immobilization of probe DNA molecules onto electrode surface is a common method in studies of the electrochemical DNA diagnostics. Fu et al. (2006) immobilized a single strand DNA (ssDNA) on prefunctionalized platinum electrodes by combining Au–Ag nanoparticle composite and polymer polypyrrole (PPy) as shown in Figure 6. The electrochemical characteristics of the modified electrode carried out by using CV and EIS. The CV was measured in 2.5 mM $[Fe(CN)_6]^{4-/3-}$ (1:1) mixture as a redox probe in 0.1 M PBS, pH 7.0. EIS was measured in 0.3 M PBS, pH 7.0 at the frequency range from 10 to 10^5 Hz in a given open circuit voltage and amplitude was 5 mV. The detection limit is 0.5 pM (Fu et al. 2006).

Labib et al. (2011) used an organometallic peptide conjugate that is chemically linked to a nanostructured gold surface to the detection of the reverse transcriptase (RT) of HIV-1 in serum exploiting. The assay format is based on a thin film formation of a ferrocene-labeled lipoic acid onto an AuNPs-functionalized screen-printed carbon electrode (AuNPs-SPCE). Then, it modified with short peptide to bind to HIV-1 RT. This modified electrode is used to detect HIV-1 RT in human serum by 3 electrochemical forms, including CV (scan rate of 100 mV s^{-1} in the potential range from −100 to 350 mV), SWV (the potential range from −100 to 300 mV with a step potential of 5 mV, amplitude of 25 mV, and frequency of 10 Hz), and EIS (the frequency range of 100 kHz to 0.1 Hz, at a formal potential of 250 mV and AC amplitude of 5 mV) were performed. In this study, the SWV technique is rapid, sensitive, and capable of discriminating background capacitive currents. Also, it provides a two-dimensional robust measurement of RT. The AuNPs-SPCE with high surface-to-volume ratio can be considered as a nanodisc array that can enhance the electrode conductivity, and improve the detection limit of the target analyte (a detection limit of 0.8 pg/ml (0.7 fM) with a short response time) (Labib et al. 2011).

Recently, an electrochemistry biosensor based on graphene stabilized gold nanoclusters (GR/AuNCs) platform with exonuclease III-assisted DNA recycling amplification based on Cytosine-rich capture probe (Wang et al. 2015). The GR/AuNCs was prepared according to the one step ultrasonic method. Then, after fabrication and pretreatment of the bare GCE, GR/AuNCs dropped on its surface to construct GR/AuNCs-based biosensor. After dried of GR/AuNCs, capture probe was dropped onto GR/AuNCs modified electrode. Finally, the electrode was immersed in different concentration of target solution which contains exonuclease III (Wang et al. 2015). CV and EIS were performed in order to characterize

the modification process of the biosensor. A detection limit of 30 aM (30×10^{-18}) was recorded by this method ($S/N = 3$).

Carbon- based nanomaterials

Adam and colleagues used of paramagnetic microparticles with diameter of 2.8 μm that covered by streptavidin. Then this particle modified by an oligonucleotide probe with a specific viral sequence labeled by biotin to detect HIV (Adam et al. 2010). In this work, carbon nanotubes-based screen-printed electrodes were applied as the working electrode and SWV measurements were carried out (parameters: the potential step and the frequency were at 5 mV and 280 Hz, respectively). Target nucleic acids' sequences gave an oxidation signal of adenine at 1.15 ± 0.05 V using carbon nanotubes-based screen-printed working electrodes, and the detection limits were estimated down to 0.1 pg/μL that were 15 times higher as compared to hanging mercury drop electrode (Adam et al. 2010).

Recently, Fang and coworkers developed sandwich-type electrochemical immunoassay for the detection of HIV-p24 based on signal amplification strategy of multiple nanocomposites (Figure 7) (Fang et al. 2015). Nanocomposite made of enzyme encapsulated in carbon nanotubes-silica as a matrix and graphene oxide (GO) as a nanocarrier (Fang et al. 2015). The different concentrations of HIV-p24 or serum samples were dropped in the immunosensor array, and 10 μL of HRP-Ab2/TH/GO solution was deposited onto the electrode surface (HRP: horseradish peroxidase, TH: thionine). The electrode was recorded by CV from −200 to 600 mV, DPV from −100 to 600 mV with and without 3 mM H_2O_2 solution. EIS measurement were performed in 5.0 mM $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$ containing 0.1 M KCl. This proposed method shows increase of response current to the HIV-p24 concentration in the range of 0.5 pg/mL to 8.5 ng/mL with the detection limit of 0.15 pg/mL, which was remarkably lower than other current techniques (Fang et al. 2015).

The DNA biosensor was fabricated by drop-coating graphene oxide (GO) on a glassy carbon (GC) electrode. Then, the designed single-stranded DNA probe covalently immobilizing onto GO using carbodiimide chemistry (Gong et al. 2015). The GO was later electrochemically reduced and applied to genosensing. In presence of target DNA, DNA double strand was formed at the electrode surface, and the negative charge in the electrode/electrolyte interface couple was changed. Also, the electron transfer resistance of the

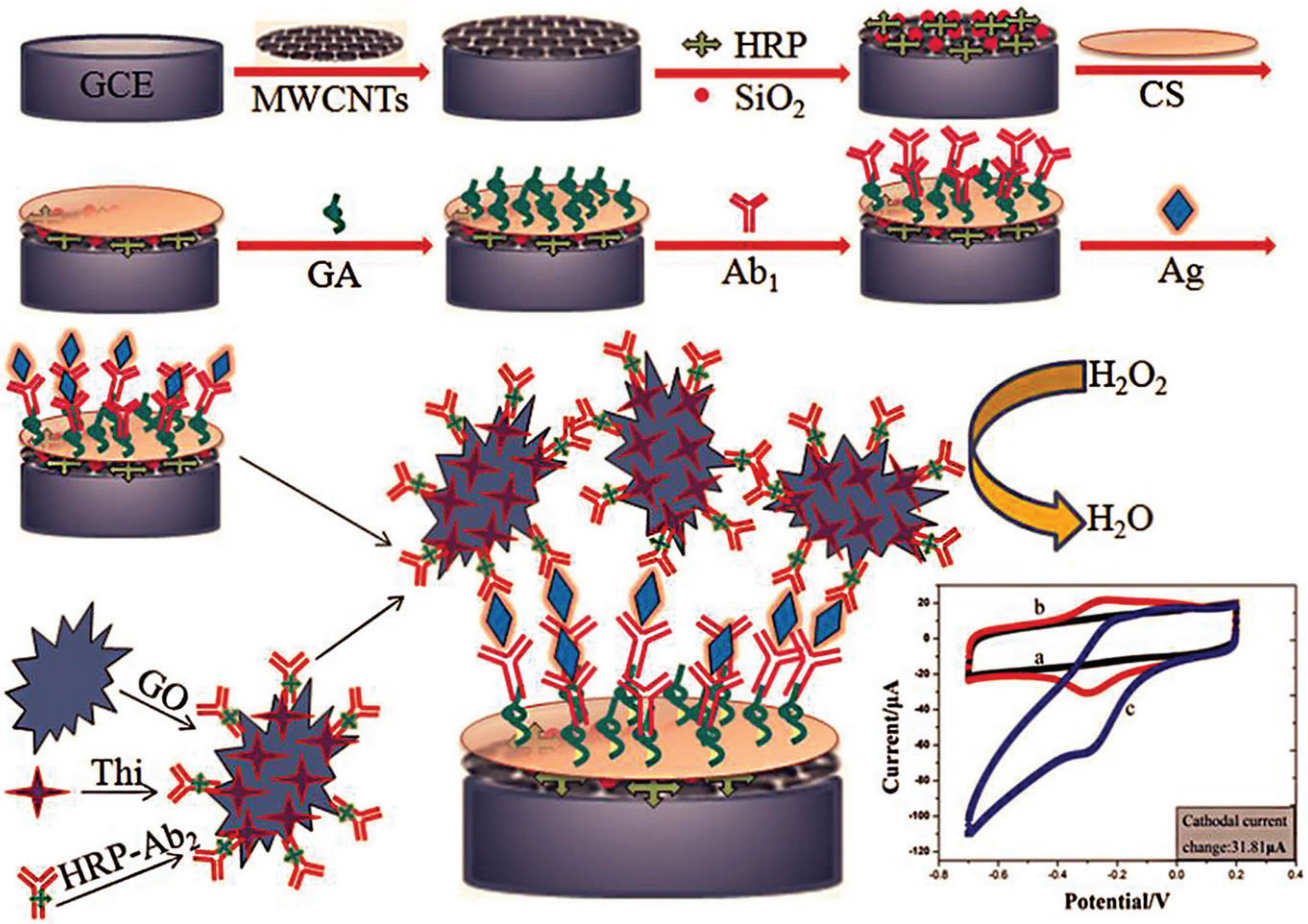

Figure 7. Schematic representation of preparation of immunosensor array and detection strategy by sandwich-type immunoassay (electrochemical voltammetry analysis) is shown. firstly, the immunosensors were fabricated by layer-by-layer coating MWCNTs, Si-HRP, Chitosan, glutaraldehyde composite on the working electrode; after that, the immunosensors were incubated with primary antibody 1 (Ab1); secondly, the primary Ab1 on the immunosensors were biorecognition with the corresponding antigens Ag; and thirdly, the immunocomplex-coated Ag were reacted with second antibodies labeled with TH/GO (Reprinted with permission from American Chemical Society) (Fang et al. 2015).

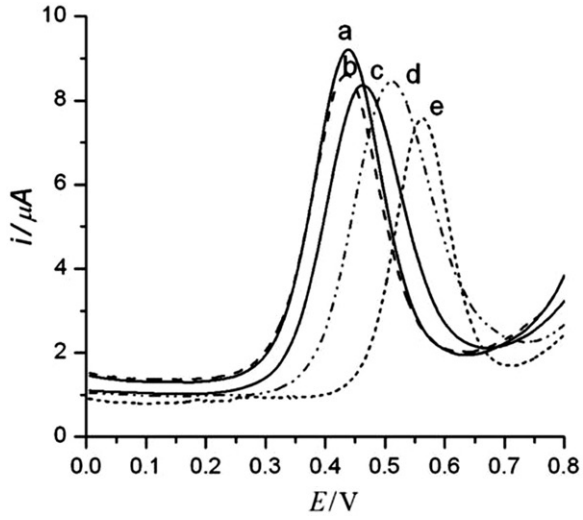

Figure 8. DPV of SWCNT/AuNP modified gold electrodes modified with Fc-pepstatin conjugate in the presence of different concentrations of HIV-1 PR at: (a) 0 pM, (b) 5 pM, (c) 10 pM, (d) 100 pM, and (e) 1000 pM. Ag/AgCl was used as the reference electrode at 100 mV/s. The assay buffer consisted of 0.1 M sodium acetate, 2 M NaClO4, 1 mM EDTA, 1 mM DTT, 10% DMSO, pH 7.4. The E of the Fc/Fc^{+} couple under the experimental conditions is 448 (5) mV (Reprinted with permission from Taylor & Francis) (Mahmoud and Luong 2010).

electrodes toward the $[Fe(CN)6]^{3-/4-}$ redox couple were changed. The detection limit of 3.0×10^{-13} M ($S/N = 3$) was recorded (Gong et al. 2015).

Hybrid nanomaterials

Mahmoud and Luong (2010) developed an ultrasensitive electrochemical method for the detection of HIV-1 PR by modifying a disposable screen printed gold electrode (SPGE) with AuNPs-SWCNT. The monolayer of cystamine containing the Fc-pepstatin conjugate was formed on surface of the SWCNT/AuNP modified electrode, and then S-terminated Fc-pepstatin is self-assembled on such surfaces as the sensing probe. By SEM, AFM, and DPV, The interaction and binding between the Fc-pepstatin-modified substrates and HIV-1 PR is studied. CV, DPV, and amperometric measurements were performed using an electrochemical analyzer coupled with a picoamp booster and a Faraday cage. A platinum wire as counter electrode and Ag/AgCl as reference electrode were used. The electrodes were conditioned by CV between 0 and +1.4 V, and Ag/AgCl in 0.5 M H_2SO_4 at 100 mVs^{-1} until a stable CV profile was obtained. The process electrode preparation and surface recognition of HIV-1 PR was first confirmed by DPV as shown in Figure 8. A strong potential shift of 98 mV was recorded in the presence of small viral amount (680 copies/mL). The peak shift (164 mV) was obtained at the highest viral replications of 1.14×10^{6} copies/mL (Mahmoud and Luong 2010).

Kheiri et al. immobilized a polyclonal antibody to HIV-1 p24 based on MWCNT, AuNPs and acetone-extracted propolis (AEP) to yield a hybrid film deposited on an Au electrode (Kheiri et al. 2011). AuNPs-MWCNTs and AuNPs-AEP film can enhance the conductivity of the propolis film. The cyclic voltammograms and electrochemical impedance spectra of different modified electrodes was carried out in the potential range from −0.2 to 0.4 V in the presence of 5 mM $Fe(CN)_6^{-3}$/$Fe(CN)_6^{-4}$ as a redox probe (0.1 M PBS, pH 7.0, 0.1 M KCl) and frequency range from 1.0×10^{-2} to 1.0×10^{5} Hz at 25 °C with signal amplitude of 5 mV. The optimum ratio of signal-to-noise was obtained at −0.32 V (versus reference electrode), which was selected as the applied potential for the amperometric measurements, and each amperometric immunosensor exhibits a fast response time for the whole concentration range of p24 Ag ((between 8 and 18 s for 0.01–60.00 ng/mL) (Kheiri et al. 2011)).

The HIV-1 virus encodes an aspartic protease (HIV-1 PR) that it is an essential enzyme for virion assembly and maturation. Methods for assaying HIV protease have been developed both *in vitro* and *in vivo*, and despite their advantages, such screening systems can only detect the inhibitor in the micromolar range, whereas only a nanomolar or lower range of such drugs can be used in HIV-1 therapy (Hu et al. 2005, Mahmoud and Luong 2008). So, there is a critical need for high throughput and sensitive protocols for detecting protease activity and/or screening novel protease inhibitors. Mahmoud and Luong demonstrated the use of nanomaterials in impedance spectroscopy for detecting HIV-1 protease and subsequent evaluation of the enzyme inhibitors at 10 pM levels. Thiolated single-walled carbon nanotubes beside AuNPs and a ferrocene-conjugate applied as sensitive enhancer tools in this assay format (Mahmoud and Luong 2008). The gold electrode surface is modified with thiolated single-walled carbon nanotube (SWCNT)/gold nanoparticle (AuNP) and thiol-terminated Fc-pepstatin is self-assembled on such surfaces as the sensing probe. The interaction and binding between HIV-1 PR and the Fc- pepstatin-modified substrates are studied by electrochemical impedance spectroscopy (EIS). Also, Differential Pulse Voltammetry (DPV) was performed to support the results obtained by EIS (Mahmoud and Luong 2008). Figure 9 is shown their protocol.

Gan et al. (2013), has been developing an ultrasensitive portable electrochemical immunosensor that its detection sensitivity for HIV p24 was 1000 times higher than the ELISA method. In their study, a novel horseradish peroxidase (HRP) enzyme–antibody copolymer was synthesized and incubated with the secondary antibody of p24 (EV-p24 Ab2), then immobilized on AuNPs to fabricate a novel signal tag (AuNPs/EV-p24 Ab2). In the next step, a sandwich-type immunoreaction would take place between the capture probe (silicon dioxide-coated magnetic Fe_3O_4 NPs (MNPs) labeled with the primary p24 antibody (MNPs-p24 Ab1)), p24 and the signal tag (AuNPs/EV-p24 Ab2) to form the immunocomplex. In the final step, the immunocomplex was absorbed on the surface of SPCE by a magnet and immersed in the o-hydroxyl phenol (HQ) and H_2O_2, which induce an amplified reductive current when a large amounts of HRP on the signal tag catalyze the oxidation of HQ by H_2O_2 [56]. These process steps were shown in Figure 10.

The vertical silicon nanowire electrode array (VSNEA) was fabricated and coated with thin layer of Au (Lee et al. 2016). Then, the peptide was immobilized on VSNEA surface by covalent interaction using cysteine (Figure 11) (Lee et al. 2016). Finally, CV and DPV measurements were performed for detection of HIV-1 Rev response element RNA. Potential

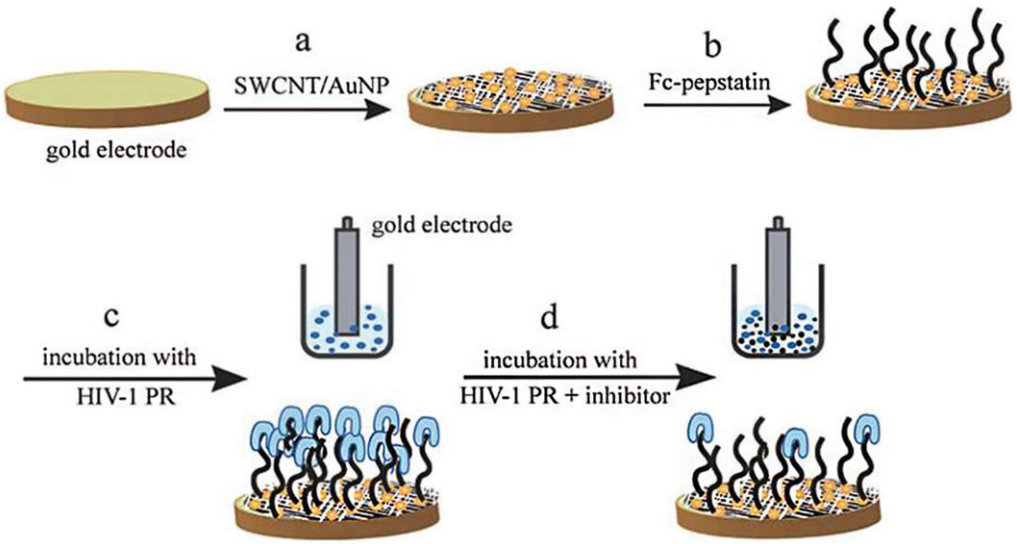

Figure 9. Schematic illustration protocol of the preparation of ferrocene- pepstatin conjugate/thiolated SWCNT and AuNP modified electrodes and their use for detecting HIV-1 protease and the subsequent assay of HIV-1 protease inhibitors. (Reproduced by permission of The American Chemical Society) (Mahmoud and Luong 2008).

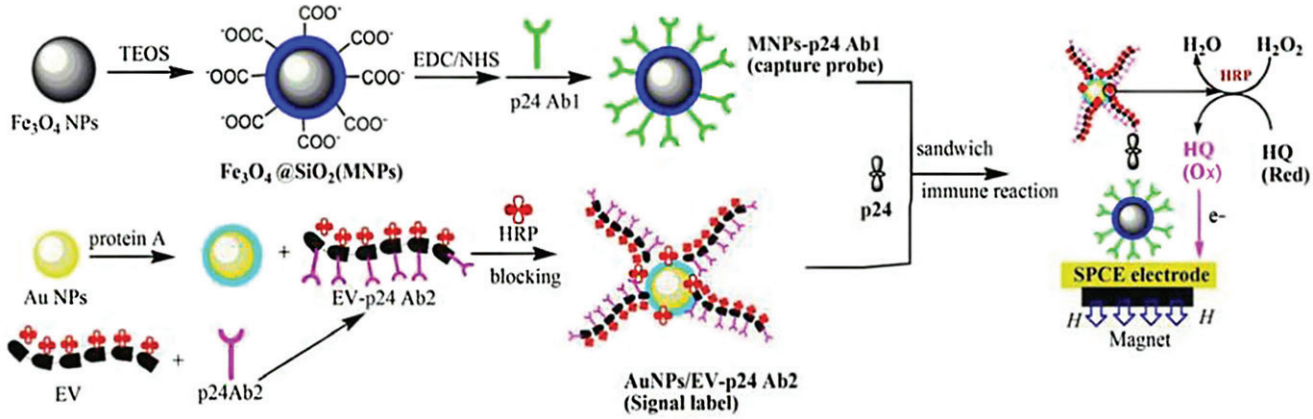

Figure 10. Demonstrated of the fabrication and detection procedure of the immunosensor (by permission from the authors (Gan et al. 2013)).

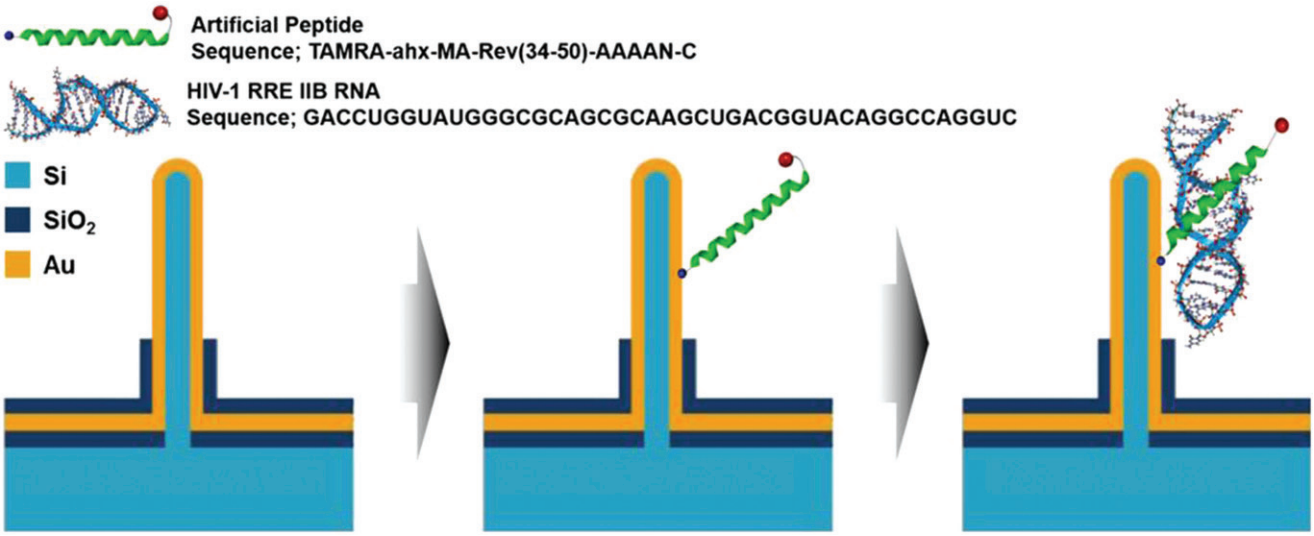

Figure 11. Schematic illustration of principle of immunosensor using VSNEA. (Left) The VSNEA fabricated and coated by Au. (Center) Artificial peptide sequences attached on Au-coated VSNEA by covalent interaction. (Right) RNAs attached to peptides and current path is blocked reducing Fe(CN)63-/4- redox reaction (Lee et al. 2016).

Table 1. Some of the studies listed based on electrochemical detection methods.

Electrochemical Detection Method	Nanomaterials	Target	Parameters	Limited Detection	Ref
Cyclic Voltammetry (CV)	Magnetic NPs	HIV DNA Sequence	Scanning Potential: −600 mV to 600 mV Frequency Range: 100 mHz to 100 kHz Potential Value: −300 mV	160 pM	Hassen et al., (2008)
	AuNPs	HIV gp120	Scan Rate: 50 mV/s Scanning Potential: −200 to 800 mV	600 fg/mL	Lee et al. (2013)
Square Wave Voltammetry (SWV)	carbon nanotubes-based screen-printed electrodes	HIV DNA Sequence	Potential Step: 5 mV Frequency: 280 Hz	0.1 Pg/μL	Adam et al. (2010)
Electrochemical Impedance Spectroscopy (EIS)	single-walled carbon nanotube (SWCNT)/ gold nanoparticle (AuNP)	HIV-1 protease	Frequency: 0.1 Hz to 100 kHz Bias Potential: 0.45 V (versus Ag/AgCl) Alternate Voltage: 5 mV	–	Mahmoud and Luong (2008)
	AuNRs	Deferiprone (anti-HIV replication drug)	Frequency: 0.01 Hz to 10 kHz Potential Rang: 0.0 to +1.75 V Scan Rate: 100 mV/s	5 nM	Narang et al. (2015)
Differential Pulse Voltammetry (DPV)	self-assembled DNA nanostructures	HIV DNA Sequence	Potential: 200 to −600 mV (versus Ag/AgCl) Scan Rate: 100 mV/s	5 aM	Chen et al. (2012)
Hybrid method EIS-CV	Au-Ag nanoparticle composite	HIV DNA Sequence	Frequency: 10 to 10^5 Hz Amplitude: 5 mV Swept Potential: -200 to +600 mV sweeping rate: 20 mV/s	0.5 pM	Fu et al. (2006)
CV, DPV, and amperometric measurements	AuNPs-SWCNT	HIV-1 Protease	Potential: 0 to +1.4 V Scan Rate: 100 mV/s E/V: 200 to 800 mV	680 copies/mL or 0.8 pM	Mahmoud and Luong (2010)
CV, SWV, EIS	AuNPs-functionalized screen-printed carbon electrode	Reverse Transcriptase (RT) of HIV-1	Scan Rate (CV): 100 mV/s Potential Range (CV): −100 to 350 mV Potential Range (SWV): −100 to 300 mV Step Potential (SWV): 5 mV Aplitude (SWV): 25 mV Frequency (SWV): 10 Hz Frequency (EIS): 100 kHz to 0.1 Hz Formal Potential (EIS): 250 mV Amplitude (ESI): 5 mV	0.8 pg/ml (0.7 fM)	Labib et al. (2011)
CV, ESI	AuNPs-MWCNTs	HIV-1 p24	Frequency: 0.01 to 100 KHz Amplitude: 5 mV Potential Range: −0.2 to 0.4 V	0.01–60.00 ng/mL	Kheiri et al. (2011)
CV, SWV, and EIS	Fe_3O_4 superparamagnetic NPs	HIV DNA Sequence	Scan Rate (CV): 50 mV/s Potential Range (CV): −500 to +200 mV Frequency: 12.5 Hz Potential Range: −600 to +400 Step Potential (SWV): 8 mV Amplitude: 25 mV Frequency: 200 kHz to 10 mHz Alternate Voltage: 5 mV	50 pM	Dai Tran et al. (2011)

range and the scan rate of CV were 0.8 to −0.8 V and 20 mV/s, respectively. DPV was carried out with pulse amplitude of 50 mV, a potential range from −0.2 to 0.6 V, and pulse width of 10 ms. By this method, it is possible to detect RNA concentrations of up to 1.513 fM and distinguish between wild-type RNA and mutant RNA (Lee et al. 2016).

Some of the experimental studies based on electrochemical detection of HIV were listed in Table 1.

Conclusion

In this paper, we demonstrated various electrochemical diagnosis methods based on nanomaterials for HIV detection, including CV, SWV, EIS, DPV, and hybrid methods. In each method, several examples were listed and explained, and noted its detection limit. Compared to other methods, such as optical biosensor array, electrochemical biosensor array has many advantages such as inexpensive instrument, low-detection limit and simplicity due to ease of obtaining electrical signal. So, in this paper, we tried to cover most experiments in this field.

Future prospects

In the future, by the development of new devices and materials in nanotechnology will be offered that it will bring a new revolution in the detection incurable diseases such as HIV/AIDS. These developments based on nanotechnology are:

1. The availability of sensitive and specific detection methods to identify HIV-1 using nanobiosensors
2. Prevention of sexual transmission of HIV by early diagnosis of infection with promotion sensitivity of methods

Disclosure statement

The authors report no conflicts of interest in this work.

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