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## Review—Nanostructured Electrochemical Sensors for Determination of the First Generation of the NNRTIs for HIV-1

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# Review—Nanostructured Electrochemical Sensors for Determination of the First Generation of the NNRTIs for HIV-1

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Since the 1980s, the HIV/AIDS pandemic has been a global concern and remains despite the tremendous efforts and successes in the development of antiviral drugs. The drug development and formulation process are not without challenges. However, the effective use of excipients can allow formulators to circumvent or at least minimize the impact of these challenges. Some of the challenges include ensuring a high quality of actives, free from impurities, and ensuring administration in appropriate quantities. Incorrect dosing can have a detrimental impact on the body. Evaluation of the quality and physiological function of these drugs in pharmaceutical and biological samples is vital. Considering this, we explore the potential application of electrochemical methods for the detection of these drugs. Electrochemical methods are versatile and enable faster and cheaper detection in comparison to conventional techniques. This review paper provides an outline of the latest developments and new trends in electroanalysis of the first generation of nonnucleoside reverse transcriptase inhibitors class of drugs. Furthermore, we highlight several phenomena occurring on bare solid electrodes and modified electrodes, including underlying mechanisms. In addition, we investigate the physical and electrochemical properties together with their figures of merits. The review covers only the reactions occurring at the surface of bare and modified electrodes and the reader is referred to the cited literature for the corresponding voltammograms of these studies. Recent data shows that modified electrodes based on nanoparticles present substantial advantages over their non-modified counterparts enabling portability, higher selectivity, and multiple detection capabilities. These characteristics make nanoparticles promising candidates for the design of sensors.

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Early detection of diseases such as HIV, cancer, diabetes, corona, influenza, stroke, and cardiovascular diseases is required. Therefore, to improve the state of health and the quality of life of people, new reliable electrochemical sensors need to be developed.<sup>1</sup> In the fields of clinical research, medicines, food, and the environment, accurate detection of these diseases is critically important for ecological safety. The demand for sensors is an indispensable requirement in many areas i.e., healthcare, safety, environmental remediation, wearable gadgets, and agriculture with the aid of monitoring, control, and detection.<sup>2</sup>

HIV/AIDS remains a global health problem, posing a huge economic burden despite concerted efforts to date. Acquired immune deficiency syndrome (AIDS) is a disease that is caused by the human immunodeficiency virus (HIV).<sup>3</sup> HIV is a retrovirus, meaning it carries single-stranded DNA. The main HIV subtypes are referred to as HIV-1 and HIV-2, but studies on HIV mostly focus on HIV-1 which is more fatal and transmissible.<sup>4</sup> The vital target for the treatment of HIV virus is reverse transcriptase because of its major role in virus multiplication.<sup>5</sup> HIV viruses use the host cell's machinery to replicate. Their proteins and genetic information are encoded in and transmitted by RNA. The virus invades T-helper cells containing CD4 protein and employs the cell to replicate and proliferate within the host.<sup>6</sup> The infectious mechanism of the HIV virus to the cells is illustrated in Fig. 1.

While the prognosis of HIV/AIDS has significantly improved over the decades, from a death sentence to a controllable chronic condition, there is still no cure. Early diagnosis and appropriate treatment remain the best strategy for controlling the pandemic.<sup>7</sup> The Food and drug administration (FDA) has approved 25 drugs to combat HIV to date. The drugs target various stages of the viral life cycle as illustrated in Fig. 1. Indeed, the treatment of viral infection is highly challenging, therefore, the development of methods to identify and quantify antiviral drugs to ensure that they serve their purpose is a dire requirement.

An overview describing developments reported based on the highlighted systems for investigation of the electrochemical oxidation of these drugs is presented. This review summarizes the

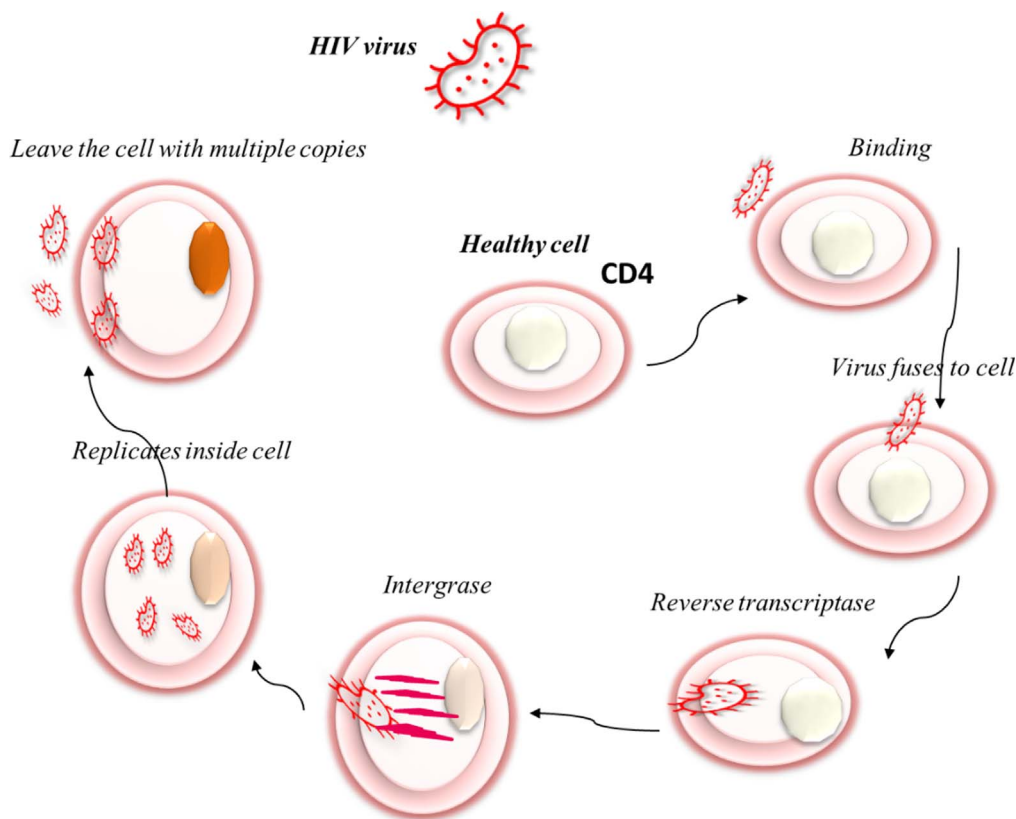
literature on the 1ST generation of NNRTIs detections by various electrochemical sensors in recent years. It focused “purely” on first-generation of NNRTIs, bare solid electrodes, and modified electrodes used in sensors with no participation of biological components (enzymes, antibodies, etc.); i.e., bio-electrochemical sensing is outside the scope of this review. The electrode modification methods, materials, and their applications in practical samples are discussed. A comprehensive review of the different types of electrode modifications in electrochemistry for determining these drugs during the last decade has not yet been reported. Therefore, this review will discuss voltammetry/amperometry-based electrochemical methods, ascribed to the first generation of anti-HIV drugs (NNRTIs) for detection of dosages and quantification in biological, medicine, food, and environmental samples. Sensors will be evaluated in terms of electrode type, application, advantages, disadvantages, and detection limits to compile a complete review of bare and modified electrodes for the detection of first-generation NNRTIs. The authors hope that this review can deliver imperative knowledge and inspires researchers to continue with this kind of research, especially in the fields this kind of study is lacking as mentioned in Table 1 and give a new direction for future development.

According to a Google scholar survey, only 18 articles focusing on the electrochemical sensors for the determination of the first generation of NNTIs have been published globally in the last 12 years.

Figure 2 displays data for several citations and publications for the electrochemical sensing of Nevirapine (NVP) and Efavirenz (EFV) anti-HIV drugs. In all the antiretrovirals (ARVs) analysed during the mentioned period, NVP is the most frequently studied. Despite the high number of citations of EFV in the year 2009, the data also shows a decrease in the number of publications. After 2009, only two articles were published with an overall of 13 citations. It is also important to mention that all the studies were in real samples. GCE and CPE are the dominating platforms commonly employed in these studies for electrochemical detection in clinical research and the medical field.

In Table 1, the types of electrodes, advantages, and disadvantages can be found as well as the fields that have not been studied. As can be seen on the table, there is a lack of research in the food and

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**Figure 1.** HIV life stages and mechanism for the target of HIV drugs.

environment industry that needs to be done, since only one publication has been documented recently in the environmental field. Most of the research increase greatly in medicine and clinical samples which indicates that attention is paid to these fields.

### Anti- HIV Drugs

HIV/AIDS drug development remains an area of intense research because of the several side effects associated with current ARVs. Nonnucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside analog reverse-transcriptase inhibitors (NARTIs), protease inhibitors (PIs), fusion Inhibitors, entry inhibitors, and integrase inhibitors, all fall under the class of HIV medication recommended by the FDA.<sup>8</sup> NARTIs and NNRTIs disrupt the polymerase activity of reverse-transcriptase. The NNRTIs are generally synthetic products and have turned into necessary components in treatments comprising of drug combinations. NNRTIs are only effective for the treatment of HIV-1, binding reversibly at a position specific to the deoxyribonucleic acid polymerase active site of the enzyme.<sup>9</sup> The members of this group are very potent and include dipyrindiazepinones such as nevirapine (NVP), which was first approved in 1996 followed by bi(heteroaryl)piperazine compounds such as delavirdine in 1997, benzoxamines such as efavirenz (EFV) in 1998, diarylpyrimidine such as etravirine (ETV) in 2008, and rilpivirine (RPV) in 2011.

The first line therapy of the first generation included one of the NVP, EFV, or RPV in combination with two NARTIs inhibitors. The chemical structures of the first generation of NNRTIs are shown in Fig. 3. At various processes of their development, transportation, and storage, these drugs may pick up impurities, which make them risky to be administered.<sup>10</sup> In addition, the potency may be affected, potentially leading to underdosing or overdosing, which can be dangerous and harmful to health. Detection and quantification of these drugs are very critical to avoid the effects. Various methods have been investigated for the detection and/or quantification of drug

molecules and their metabolites in real samples, such as spectroscopy and chromatography. Despite the success of these techniques, they require expensive equipment, that tends to involve time-consuming sample preparation, and may involve the use of harmful solvents for extraction of target molecules. Electrochemical methods overcome these challenges as they require minimal sample preparation, are highly sensitive and reliable, and usually require comparatively inexpensive equipment.

Electrochemical testing is becoming a method of choice across many analytical laboratories for testing anti-HIV drugs. Voltammetry has proven to be a powerful and adaptable analytical technique for the detection of drugs due to its simplicity and speedy measurements.<sup>11,12</sup> Portable point-of-care devices can enable convenient monitoring of the concentration of the drugs and their by-products in a patient's serum.

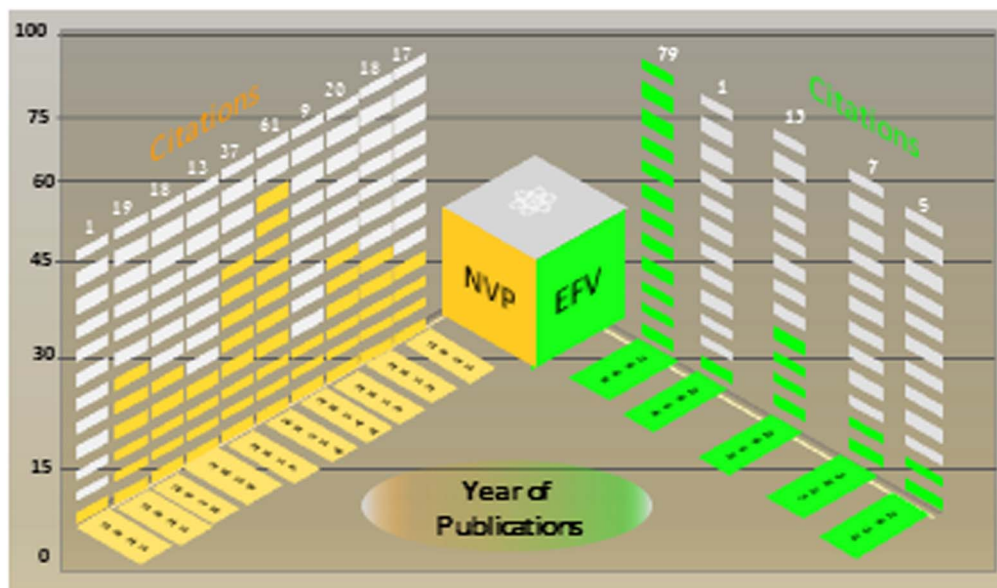
The focus of attention recently is the modification of electrodes with nanostructured particles since they convey huge significance with the peculiar physiochemical properties, presenting obvious advantages in the field of medical science, technology like biomedical, optical, electroanalysis, and electrocatalysis.<sup>13</sup>

### Electroanalytical Techniques for Detection of Pharmaceuticals

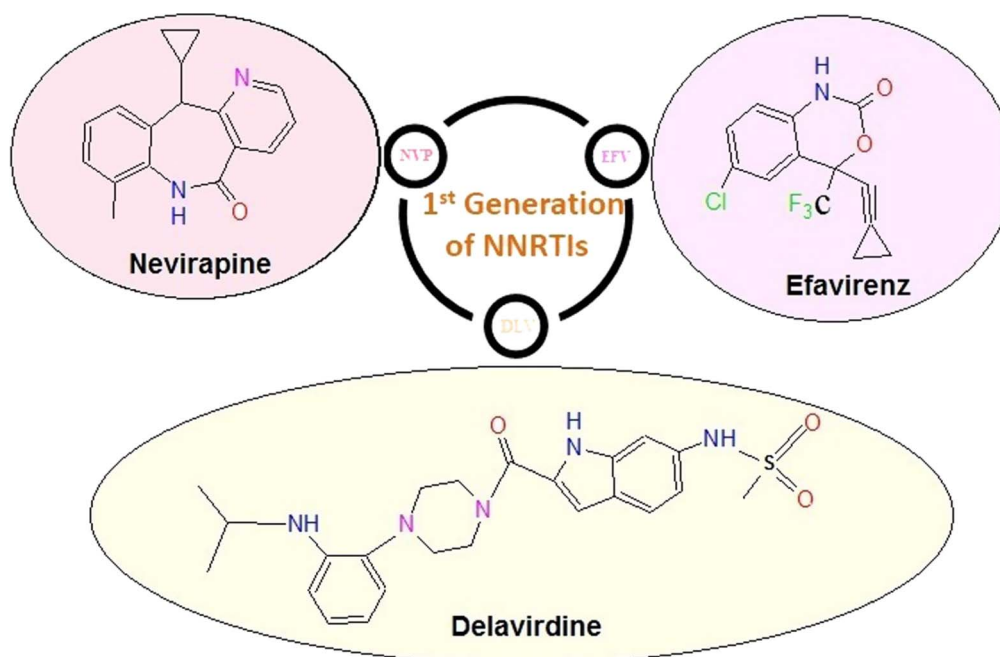
A significant number of researchers in many fields including, biochemistry, material chemistry, electrical engineering, and physical and analytical chemistry find applications in the field of electrochemical sensors. The versatility, potential for miniaturisation, simplicity, notable fast detection ability, low costs, and the ability to measure complex samples of these sensors, make electrochemical sensing attractive. Electrochemical methods can be categorized into three categories: potentiometry, amperometry, and voltammetry. Many analytical methods devoted to determining one or several pharmaceutical compounds in different matrices have been reported.<sup>10,12,14</sup> This review focuses on amperometric and voltammetric sensors. Sensors based on electrochemical methods are

**Table I. Comprehensive summary of electrodes used for the detection of the first generations of NNRTIs in a period between 2009 and 2022 with their advantages, limitations, and practical applications.**

| Bare Solid Electrodes    |                                                                                                                 |                                                                                                            |                                                      |                          |
|--------------------------|-----------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------|
| GCE                      | Non-toxic and environmentally friendly.                                                                         | Small surface area.                                                                                        | Human body Fluids                                    | Serum                    |
| CPE                      | The Preparation process is relatively easy.                                                                     | Poorer specificity                                                                                         |                                                      |                          |
| AuE                      | The surface can be used with a simple pre-treatment                                                             | The lack of special functional groups on the surface results in poor enrichment and catalytic performance. | Medicine                                             | Urine                    |
| PtE                      |                                                                                                                 |                                                                                                            | Food                                                 | Tablet                   |
| PGE                      |                                                                                                                 |                                                                                                            | Environment                                          |                          |
| Thin Mercury Film        | Provides a smooth, fresh surface for the reaction. It has a high hydrogen overvoltage.                          | <b>Phased out Modifiers</b><br>volatile and toxic heavy metal.<br>It has been withdrawn.                   |                                                      |                          |
| Carbon nanomaterials.    | Non-toxic and environmentally friendly. Good conductivity, large surface area, and high electron transfer rate. | <b>Current Modifiers</b>                                                                                   | Human body Fluids                                    | Serum                    |
| Metal nanomaterials.     | Chemical inertness, good thermal stability, and low toxicity.                                                   | not stable enough to anti-oxidize in aqueous solutions                                                     | Medicine                                             | Urine                    |
| Metal oxide nanomaterial | Large specific surface area and good biocompatibility, High aspect ratio, biocompatibility, catalytic.          | wide bandgap                                                                                               | Food<br>Environment                                  | Tablet<br>River<br>water |
| Quantum dots             |                                                                                                                 | <b>Organic Modifiers</b><br>insolubility and high                                                          |                                                      |                          |
| Polyaniline (PANI)       | Good antifouling ability.                                                                                       |                                                                                                            | Are added during electrode preparation and are often |                          |
| Nafion                   | Fast response speed, repeatability, and good stability.                                                         | brittleness                                                                                                | used in the preparation of MIP electrodes            |                          |
| Chitosan                 | Many amino and hydroxyl functional groups.                                                                      |                                                                                                            |                                                      |                          |



**Figure 2.** Number of publications and citations of the first generation of NNRTIs electrochemical sensing since 2009. Data gathered from Google Scholar.



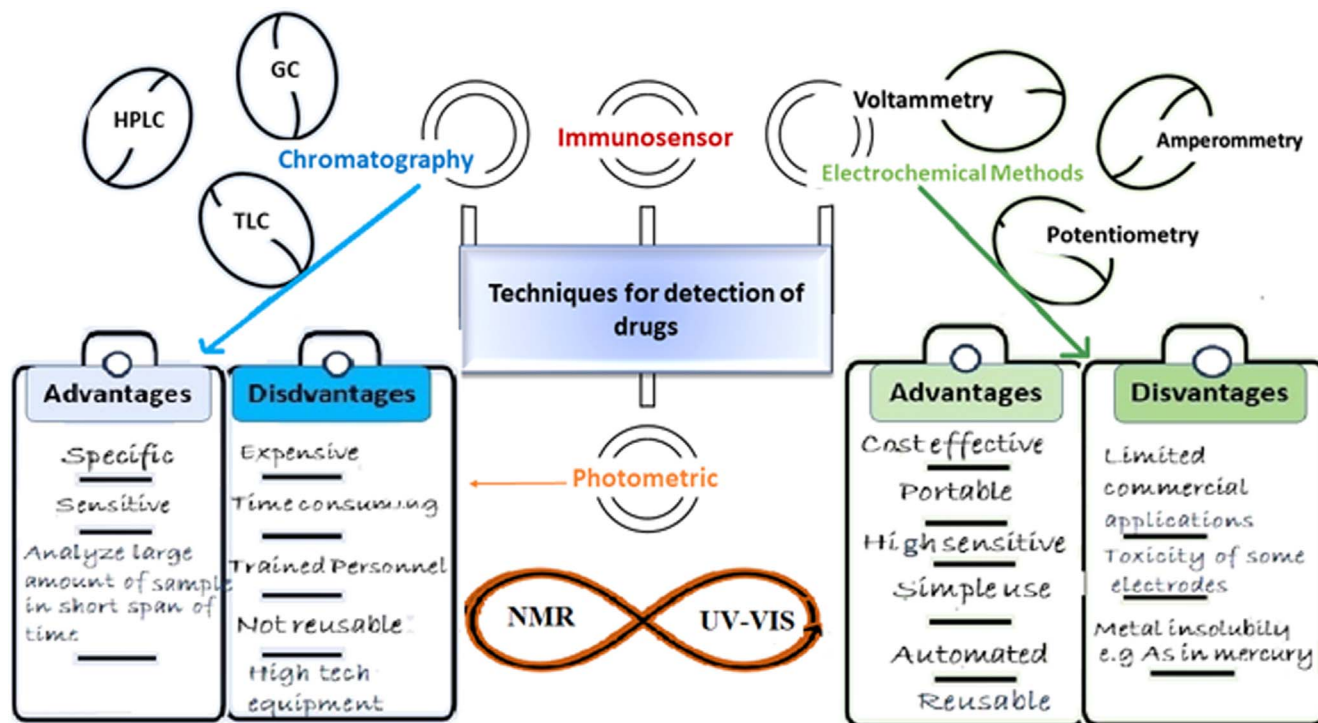
**Figure 3.** Structures of the first generation of the non-nucleoside reverse transcriptase inhibitors.

fascinating because of their remarkable sensitivity, simplicity, and low cost. Carbon electrodes, though used in conventional electrochemical sensors, they can be unstable and tend to have low sensitivity when not modified or enhanced, which is not ideal.<sup>15–17</sup> In recent years, a plethora of new electrode materials have been developed. Coating the electrode surface with an active material is has been demonstrated to enhance the sensitivity of the sensors.<sup>18,19</sup> Various voltammetry methods: cyclic, differential pulse, and square wave are the most widely used, many reports attest to their importance in detection of pharmaceutical compounds. Other sophisticated methods which are quantitative and qualitative for HIV drugs in biological samples such as chromatography<sup>20</sup> (HPLC, TLC, and GC) immunoassays, photometric (nuclear magnetic resonance and ultraviolet–visible)<sup>21</sup> have been reported in literature. Even though these methods are accurate and reliable, they are not portable and cannot be used for point-of-care device applications.

Presently, three-dimension printing technology (3D) is booming in the field of sensors. Recently, a new class of vapochromic sensing materials (VSMs) for chemical sensors has been introduced by Stevens and co-workers.<sup>22</sup> This inexpensive novel immobilization method uses  $\text{Zn}[\text{Au}(\text{CN})_2]_2$  together with polylactic acid (PLA) that can be used as a base materials for 3D printing, and additive manufacturing processes to create geometrically complex sensor surfaces. Figure 4 illustrates schematically some of the sophisticated methods and electrochemical methods applied for HIV drugs in biological samples with their advantages and disadvantages.

#### Nanoparticles Used for Modification of Sensors

Nanoparticle based electrodes can be assembled on the surface by creating nanoscale patterns on the substrate or the assembly of individual or bulk nanostructures on the electrode surface.<sup>23</sup>



**Figure 4.** Some of the methods used for detection of drugs and their advantages and disadvantages.

Nanoparticles can be prepared by different methods based on the nature and type of the material, either using a bottom-up and top-down approach. The nanoparticles are prepared from the elementary level in the case of bottom-up and can be produced by means of chemical and biological processes via self-assembly of atoms to fresh nuclei which develops into a particle with the size range lied in nanoscale.<sup>24</sup> In the top-down approach the material is reduced into nanoparticles from its bulk state.

Nanoparticles can either be inorganic or organic, and organic are classified as 0D (nanoparticles and quantum dots),<sup>25</sup> 1D (nanorods and nanowires), 2D (graphene and borophene),<sup>26,27</sup> and 3D (polycrystals). An efficient electrochemical sensor can be fabricated under these categories depending on the dimensionality of their features. Comparing these structures to conventional sensors, nanostructured sensors can detect at a scale of 1–100 nanometres. The main advantages of nanoparticle-based sensors include their high sensitivity, high carrier mobility, and their ability to detect minute sample volumes. Different types of surface modification methods have been illustrated in Fig. 5. The field of nanotechnology encompasses a wide range of applications: from biosensors to quantum computing.

Nanoparticles used in diagnostic and therapeutic applications include metallic, semiconductor, and polymeric nanomaterials. The surface of these nanomaterials can be tuned by covalent or non-covalent functionalisation to enhance solubility in aqueous solvents and impart added functionality. Due to their small size, nanoparticles have an exceptionally high surface area, which enhances their sensitivity, unlocking lower limits of detection (LOD). In drug delivery applications, the high surface area increases the loading capacity and may enable targeted delivery, thus reducing side effects.<sup>28</sup> Based on the significant ongoing research and applications, it is expected that surface modification of the surface plays an outstanding role not only in medicine but also in other important areas.

#### Electrochemical Detection of Nevirapine, (NVP)

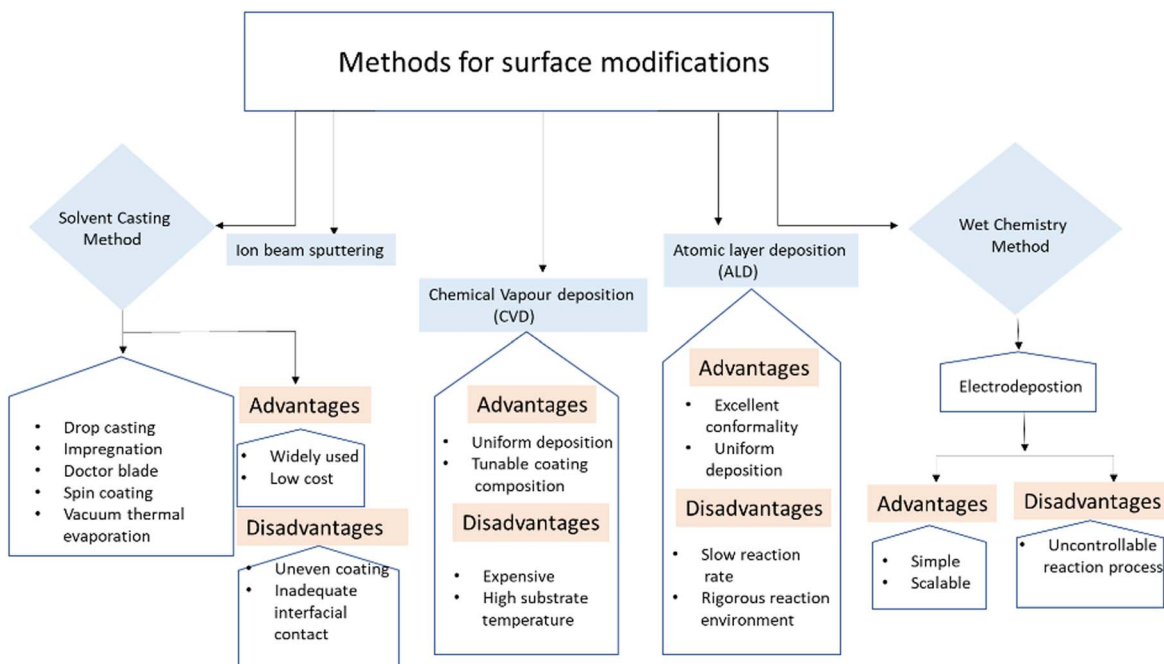
NVP with the brand name viramune (Fig. 1) is recommended for use in treating specifically type 1 (HIV-1) infections in adults jointly with the stavudine and lamivudine.<sup>29</sup> It directly binds reversibly to

the catalytic site of enzyme responsible for HIV.<sup>30</sup> Bare electrodes and modifiers of different materials have been used to detect this drug.

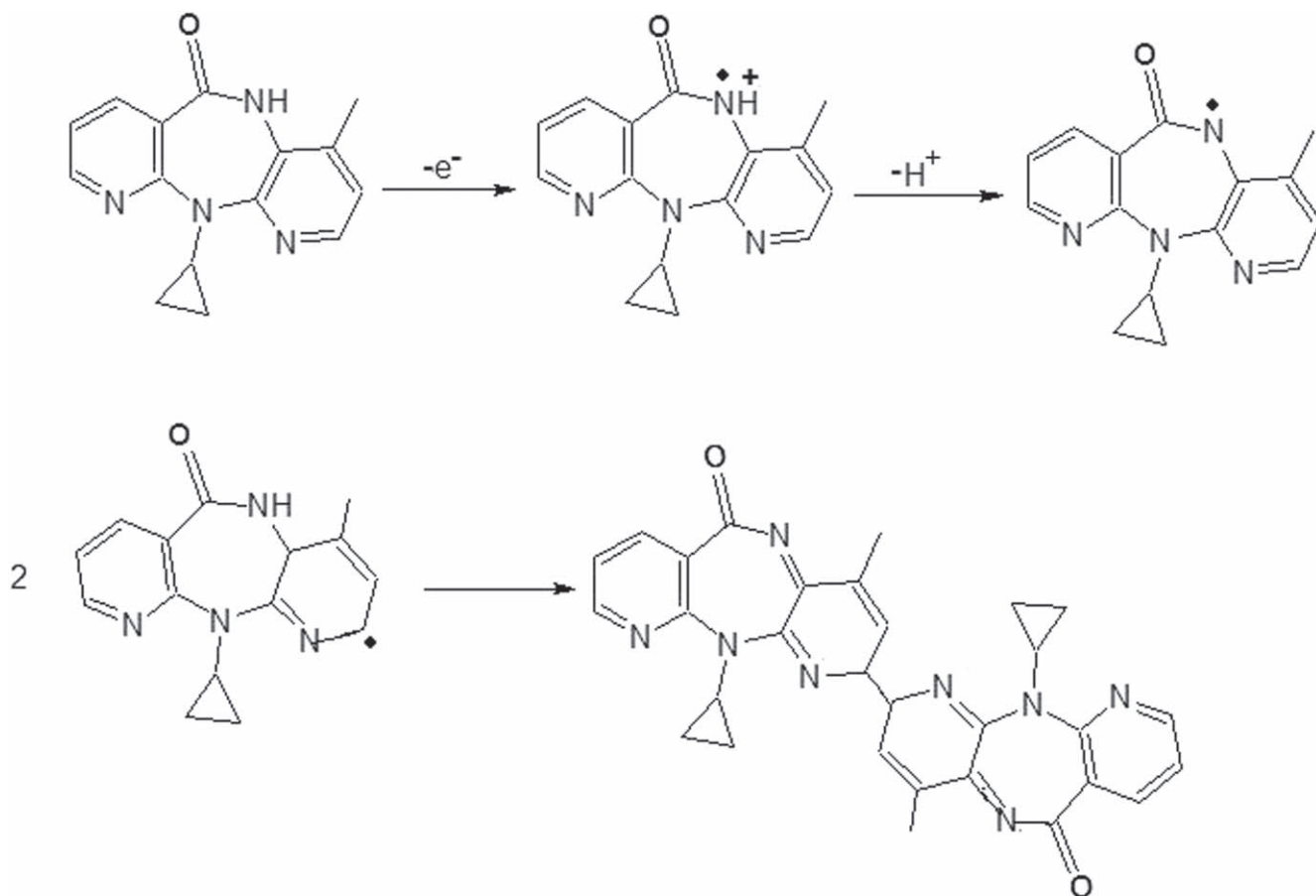
**Nevirapine detection at unmodified electrodes.**—Glassy carbon electrode (GCE).—Bare GCE is an interesting sensor for surface adsorption but faces the problem of peak decline of the analyte during electrochemical measurements. This is due to the surface passivation by the components of the reaction. The successful electrochemical detection of NVP using uncoated GCE was reported in the literature by Naggapa and co-workers.<sup>31</sup> The experiments showed reduction in intensity, indicating fouling of the GC surface due to non-specific adsorption. The suitability of bare GCE for this differential pulse voltammetric analysis showed an anodic peak potential at 0.749 V in PBS, pH 10. The linear range between 5.0–350  $\mu\text{M}$  with calculated detection and quantification limits values of 1.026 and 3.420  $\mu\text{M}$ , respectively were obtained. The authors confirmed that the kinetic reaction is irreversible with loss of  $1e^-$  that occurs at the secondary ring nitrogen and the formation of a radical cation which was further deprotonated to form a radical, followed by the combination of free radical with another radical to form a dimerized product as shown in Scheme 1.

**Nevirapine detection at modified electrodes.**—The modification of electrode surfaces has gained so much interest in electrochemical detection. Sensors based on electrochemical devices comprise of a transducer coated with an active material having conducted properties.<sup>32</sup> Modified electrodes are highly sensitive and can detect and quantify NVP, in very small quantities using with the help of different kinds of modifiers (Table II). The sensor permits improved properties and boosting of the signal related to the oxidation of NVP.

**GCE/MIP/ErGO.**—Recently, a molecular imprinting polymer (MIP) sensor was fabricated by Pour and co-workers to develop the electro-polymerization of pyrrole (Py) on electrochemically reduced graphene oxide (ErGO) immobilized on a glassy carbon electrode (GCE) and successfully used for NVP analysis.<sup>33</sup> The NVP measurements were tested using DPV in an alkaline medium under



**Figure 5.** Various methods for surface modification with advantages and disadvantages.



**Scheme 1.** Proposed reaction mechanism for the electrooxidation of nevirapine.<sup>31</sup>

optimized environment. The DPV signal of NVP increased linearly with concentration the range of  $0.005\ \mu\text{M}$  and  $400\ \mu\text{M}$  and was highly stable for an extended period. The modified electrode was then used to detect NVP in real samples. The limit of detection and

limit of quantification of the MIP/ErGO/GCE modified electrode was found to be  $2.00$  and  $6.66\ \text{nM}$ , respectively. The MIP/ErGO nanocomposite exhibited superior behaviour than other nanocomposite toward NVP detection as listed in Table II. The oxidation

**Table II.** Summary of the analytical performances of the recent literature on NVP analysis by electrochemical sensors.

| Sensor                                          | Method Analysis | LOD (nM) | Linear range ( $\mu\text{M}$ )     | Electrolyte solution | Real Sample          | References |
|-------------------------------------------------|-----------------|----------|------------------------------------|----------------------|----------------------|------------|
| GCE/MIP/ErGO                                    | DPASV           | 2.0      | 0.005–400                          | NaOH + KCl           | Serum, tablet        | 33         |
| GCE/ $\text{Eu}^{3+}/\text{Cu}_2\text{O}$ CLFNs | DPV             | 3.6      | 0.01–750                           | 0.1 M PBS, pH 11     | —                    | 34         |
| GCE/MWCNT/Ag-Pt                                 | DPV             | 21.0     | —                                  | 0.01 M NaOH          | Milk, urine, tablet  | 35         |
| CPE/MIP                                         | SWSV            | 10.0     | 0.05–300                           | PBS, pH 11           | Serum, tablet        | 36         |
| GCE/ $\text{TiO}_2$ /GNR                        | DPV             | 43.0     | 0.02–0.14                          | PBS, pH 11           | Tablet               | 37         |
| GCE/MUHC-AgNPs/c-MWCNTs                         | CA              | 14.0     | 0.05–21                            | 0.01 M NaOH M        | Serum, tablet        | 38         |
| GCE/Pd@rGO/MoS <sub>2</sub> QDs                 | DPV             | 50.0     | 0.1–80                             | PBS, pH 10           | Serum                | 39         |
| GE/AuNPs/p(MB)/f-MWCNTs                         | DPASV           | 53.0     | 0.1–50                             | PBS, pH 11           | Serum, tablet        | 40         |
| GCE/CuO/CNP                                     | LSV             | 66.0     | 0.1–100                            | PBS, pH 7            | Serum                | 41         |
| AuE                                             | DPV             | —        | 0.3–1.6                            | 0.04 M BR, pH 4      | Urine, tablet        | 42         |
| CPE/Ura                                         | DPV             | 50.0     | 0.1–70                             | 0.1 M NaOH           | Serum                | 43         |
| GCE                                             | DPV             | 102.6    | 5–350                              | PBS, pH 10           | Urine, serum, tablet | 31         |
| GCE/HgF                                         | LSV             | 3.0      | 0.04–0.53                          | 2 mM NaOH            | —                    | 44         |
| Pt/CoPc-cou-f-MWCNTs/Naf-5                      | LSV             | 0.2      | $6 \times 10^{-4}$ –30             | 0.1 M KCl            | River water          | 45         |
|                                                 | CA              | 0.21     | $4 \times 10^{-3}$ –0.13<br>2.5–30 |                      |                      |            |

mechanism proposed for NVP is shown in Scheme 2 which agrees with the mechanism proposed in literature.<sup>40,43</sup>

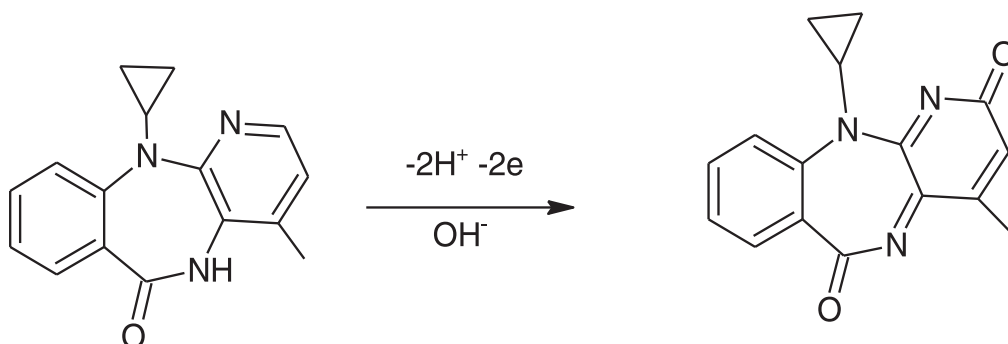
**GCE/ $\text{Eu}^{3+}/\text{Cu}_2\text{O}$  CLFNs.**—An interesting strategy to detect NVP has been proposed by Foroughi and co-workers using DPV on a three-dimensional cubic of europium ( $\text{Eu}^{3+}$ )/cuprous oxide ( $\text{Cu}_2\text{O}$ ) with clover-like face-centred nanostructures.<sup>34</sup> Other various approaches of electrochemical characterization like CV and EIS were also employed. In this approach, a GCE was modified with  $\text{Eu}^{3+}/\text{Cu}_2\text{O}$  CLFN and then applied to distinguish NVP. In this experiment, DPV showed a pronounced reduction peak around 0.52 V. This method resulted in a response of linear range between 0.01 and 750.0  $\mu\text{M}$  with an impressive LOD of 3.6 nM and a LOQ of 11.8 nM, whereas the approved sensor sensitivity was 0.1244 a  $\mu\text{M}^{-1}$ . The sensor was also tested milk and water samples and still successfully detected NVP.

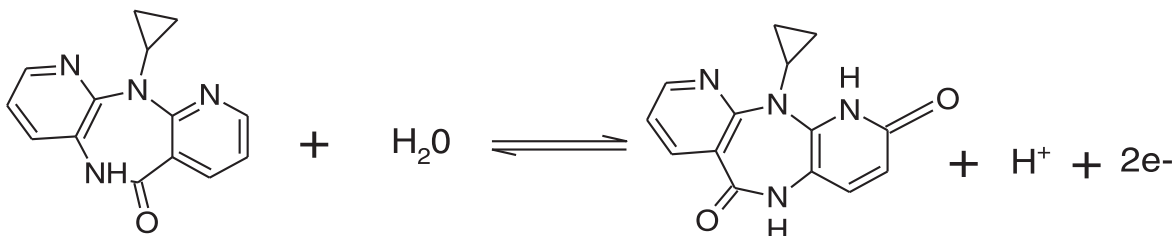
**The MIP/CP electrodes.**—Massumi and co-workers constructed non-imprinted polymer and molecular imprinted polymer modified carbon paste electrode (CPE) choosing square wave voltammetry (SWV) for their study.<sup>36</sup> The oxidation signal of NVP on the surface of MIP/CPE was well defined and remarkable compared to the weak and broad peak of the NIP/CPE with a potential at 0.65 V. The LOD and LOQ were 38 nM and 10 nM, respectively. The developed sensor exhibited high sensitivity and better performance towards the detection of NVP in real samples. The proposed mechanism in this study in Scheme 3 concluded that the same numbers of  $\text{H}^+$  and  $\text{e}^-$  of the secondary ring nitrogen participate in the redox reaction of the drug. The nitrogen in the secondary ring of the NVP oxidized to form a radical cation which occurred with the transfer of one

electron meanwhile the next was step was deprotonation of the radical as seen in Scheme 2.

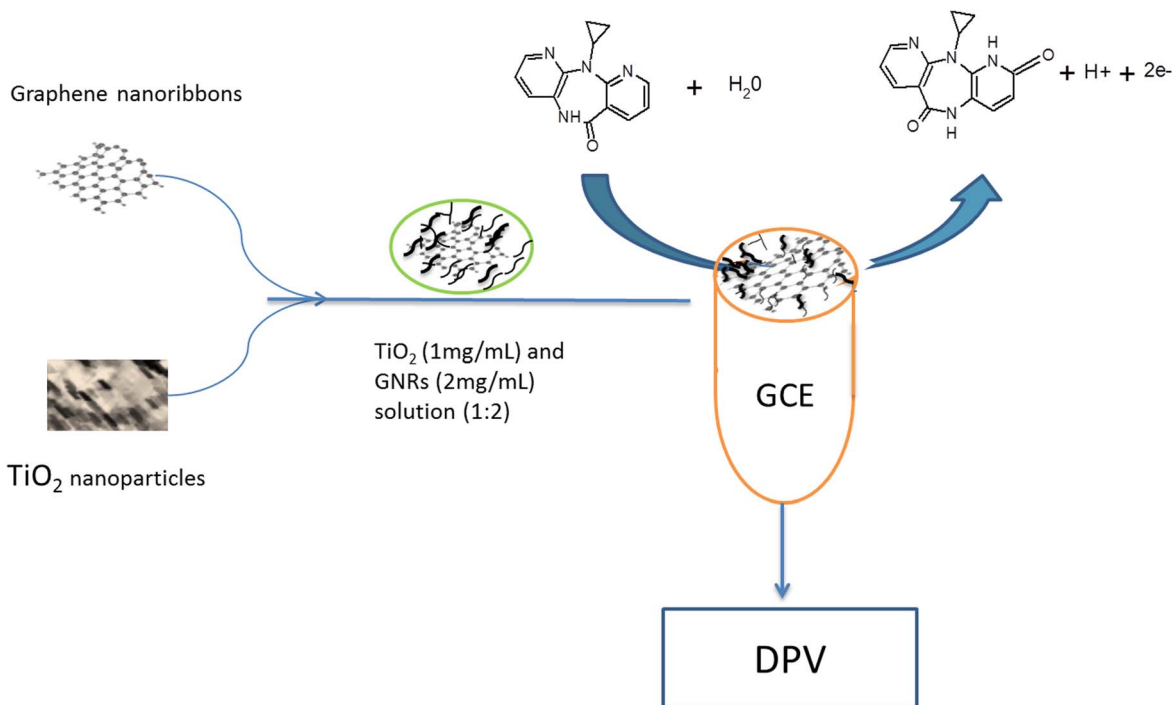
**GCE/MUHC-AgNPs/c-MWCNTs.**—Ahmadi et al. (2019) designed a sensor based on carboxylate multi-walled carbon nanotubes as a mediator modified GCE surface and prepared silver nanoparticles decorated with 11-mercaptopundecanoyl hydrazine carbothioamide (MUHC-AgNPs/c-MWCNTs/GCE).<sup>46</sup> The NVP signal at the modified electrode using the amperometry method increased the current response in the range of 50.0 nM–1.0  $\mu\text{M}$  to 1.0–21.0  $\mu\text{M}$ . The LOD of 14 nM, LOQ of 46.7 nM, and a linear response were achieved over-optimized conditions in 0.01 M NaOH solution. The research demonstrated effectiveness and reproducibility of the system and the resistance to interference. This method was applied to assay NVP in spiked biological samples and tablets.

**GCE/ $\text{TiO}_2$ /GNR.**—Apath et al. (2020) reported GCE as a working electrode modified with graphene nanoribbons decorated with  $\text{TiO}_2$  nanoparticles for analysing the electrochemical behaviour of NVP using CV, EIS, CA, and DPV in 100 mM PBS, pH 11.0.<sup>37</sup> CV data confirmed irreversible electroactivity with an anodic peak at 0.4 V. Furthermore, they applied the DPV technique to quantify NVP drug in pharmaceutical formulation, which showed an increase in peak response at potential 0.4 V, which indicates the applicability of DPV. They mentioned that the obtained  $\text{TiO}_2$ /GNR/GCE nanocomposite resulted in good reproducibility during analysis and the proposed procedure was successful. DPV gave linear calibration in the range 0.02–0.14  $\mu\text{M}$  a LOD calculated to be 43  $\mu\text{M}$ . The fabrication procedure, as well as the reaction mechanism on the surface of the electrode, is shown in Scheme 4 below.

**Scheme 2.** The mechanism of NVP oxidation.<sup>33</sup>



**Scheme 3.** The reaction mechanism of NVP oxidation.<sup>36</sup>



**Scheme 4.** The electrochemical oxidation mechanism of NVP at CuO-CNP-GCE.<sup>41</sup>

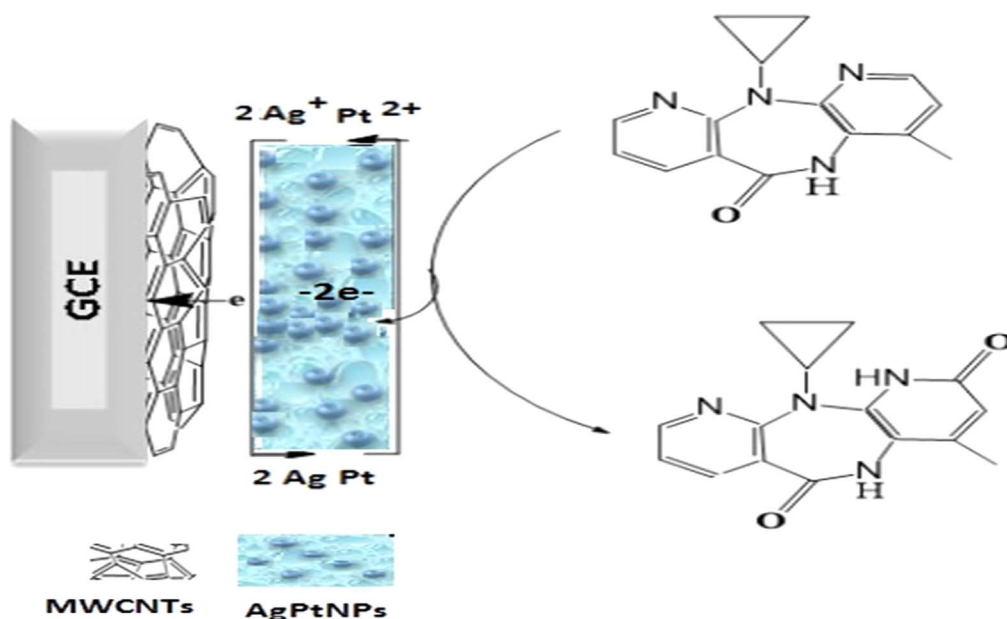
**GCE/MWCNT/Ag Pt.**—Bimetallic nanoparticles are formed from a mixture of metals and have superior properties than monometallic.<sup>35</sup> Okumu and co-workers previously reported the synthesis of the first bimetallic noble metal sensor at different ratios for the detection of NVP.<sup>38</sup> The electrochemical properties of the film on the surface of GCE resulted in an unstable signal. In the year 2020, the group develop unique strategies for enhancing stability and LOD by supporting the noble metal Ag-Pt bimetallic sensor with multi-walled carbon nanotubes (MWCNTs).<sup>47</sup> With the added MWCNTs as support for the bimetallic nanoparticles, the nano-sensor exhibited greater sensitivity attributed to an increase in surface area. The oxidation of NVP by GCE/MWCNTs/Ag-PtNPs was seen at potential 0.59 V and 0.54 V (CV & DPV respectively) in 0.01 M NaOH as electrolytes solution. The best results with the LOD and LOQ were obtained at 0.021 and 0.070  $\mu\text{M}$ , respectively. This also resulted in high sensitivity of 10.25  $\mu\text{A}\mu\text{M}^{-1}$  and good linearity. The developed nanosensor was applied for the NVP analysis in real samples with mechanism shown in Scheme 5.

**CPE-Bi<sub>2</sub>O<sub>3</sub>.**—Electrodes based on bismuth film have been used for electro-analysis as a substitute for poisonous mercury electrodes as it is more sustainable, safer than mercury and insensitive to dissolved oxygen.<sup>48</sup> The choice of bismuth for trace analysis is advantageous because of its capability to bind many heavy metals by forming multi-element alloys. The preparation of the active materials using the combustion method and detection of NVP was done by Terada et al. (2015) using bismuth oxide (Bi<sub>2</sub>O<sub>3</sub>) adjusted carbon

paste electrode (CPE).<sup>49</sup> The next step was the electro-reduction of CPE-ErBi<sub>2</sub>O<sub>3</sub> by cycling the CPE-Bi<sub>2</sub>O<sub>3</sub> in 0.1 M KOH in the range of 1.3–0 V. The resultant material was suggested to be ErBi<sub>2</sub>O<sub>3</sub>, a good boost for electron transfer between NVP and electrode surface.<sup>49</sup>

NVP resulted in an irreversible anodic peak in both 0.2 M PB, pH 8, and 0.04 M Britton and Robinson buffer. However, an enhanced anodic response was achieved in the PB solution. An enhanced signal was observed in CPE/ErBi<sub>2</sub>O<sub>3</sub> in comparison to CPE/Bi<sub>2</sub>O<sub>3</sub>. The reaction for this study is the same as the one in Scheme 1.<sup>31</sup> At optimal conditions, the DPV gave linearity in the range of 50–50 mM with detection and quantification limits of 33.09 and 110 nM, respectively. The constructed sensor was applied in real samples as seen in Table II.

**GCE/Pd@rGO decorated with MoS<sub>2</sub>QDs.**—Tiwari and co-workers took an advantage of the interesting performance of quantum dots to obtain sensor characterized by higher sensitivity.<sup>39</sup> In this case, Pd@rGO decorated with MoS<sub>2</sub> QDs modified electrodes was developed to obtain the rapid electrochemical detection of NVP. The NVP oxidation peak was identified at potential 0.65 V vs Ag/AgCl much lower than modified Pd@rGO/GCE or MoS<sub>2</sub> QDs/GCE. The sensor showed stability and sensitivity in the range 0.1–80 mM with better LOD of 50 nM. The sensor was suitable for NVP detection in complex system under adjusted conditions. The mechanism proposed revealed the interaction of NVP with the modified electrode in the secondary ring nitrogen with



**Scheme 5.** The electrochemical oxidation mechanism of NVP on GCE/MWCNTs/Ag-PtNPs.<sup>47</sup>

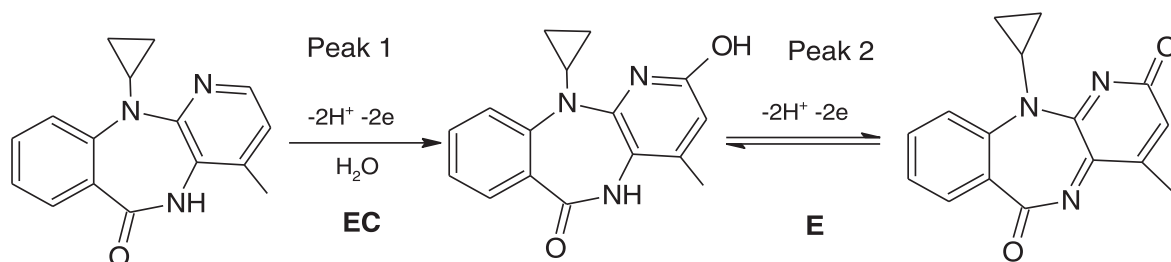
an electron loss and formation of a radical- cation which later forms another radical after deprotonation in agreement with the mechanism proposed by Teradal and co-workers.<sup>51</sup> Then, a dimerized product was formed after combination of these two radicals.

**GE/AuNPs/p (MB)/f-MWCNTs.**—Gold nanoparticles have interesting catalytic, electrochemical and structural properties.<sup>50</sup> Polymerization of organic dyes displays capability of reversible properties and so can be employed as mediators.<sup>40</sup> Carbon-supported nanoparticles are well documented for the reduction of surface area which enhances the properties to a large extent. Gholivand et al. (2017) offered an approach for evaluating NVP by combining the materials mentioned above.<sup>40</sup> A GE/AuNPs/p(MB)/f-MWCNTs revealed excellent stability, repeatability, and reproducibility using the DPASV technique. The discovered results established that the anodic intensity increased linearly by adding more NVP concentrations in the range of 0.1–50 nM and the LOD of 53 nM was obtained. The constructed electrode platform (AuNPs/p(MB)/f-MWCNTs/GE) was successfully applied for the detection of NVP in real samples.

**GCE/CuO-CNP.**—Metallic oxides are remarkable surfaces that are chemically rich with OH groups that can be easily modified by several surfaces adoring molecules but have some limitation of wide bandgap energy. The catalytic properties of metallic oxides can be enhanced by modifying the energy bandgap, improving the absorption sensitization, or scaling down the dimension of the catalyst. Carbon nanoparticles were used to fulfil what has been mentioned above. Shahrokhian co-workers reported the use of CuO-CNP/GCE

for the detection of the NEV.<sup>41</sup> The technique that was used for the study was linear sweep which exhibited an oxidation peak towards NVP with enhancement of 276-fold and 350-fold compared to CuO-GCE. Their work described carbon nanoparticle-supported metallic oxide has reduced surface area which enhanced the properties to a large extent. The CV behaviour of NVP showed enhanced well-defined irreversible anodic peak at potential of 0.785 V. An irreversible oxidation peak was observed with a reduction peak at more negative potentials in the reverse sweep in the first scan. Later, two new redox couples were seen, which were then assigned to redox activity of the NEV oxidation product i.e., the first redox peak pair and the second redox couple associated to the new compound formed during the reaction. According to these shreds of evidence, a nature of the electron transfer-chemical reaction-electron transfer (ECE) mechanism was proposed to explain the electrooxidation of NEV as seen in Scheme 4. The sensor exhibited a dynamic linear range in three concentration intervals (0.1–0.8, 1–10, and 10–100 mM) with a limit of detection of 66 nM. The proposed nanocomposite was described as a superb material to construct a sensor for the determination of NEV in real samples with complex matrices such as human blood.

**CPE/Ura.**—Zhang and co-workers employed a novel uracil incorporated carbon paste electrode.<sup>43</sup> 0.1 M NaOH was used as an electrolyte solution to perform CV measurements on a bare and uracil modified CPE with and without 100  $\mu$ M NVP. The reaction mechanism is shown in Scheme 7. In CV measurements without NVP, redox peaks were observed in of 0.0–1.0 V range in both CPE and Ura/CPE. When the concentrations of NVP were added into



**Scheme 6.** The mechanism of uracil grafted on CPE (I) and the electrochemical oxidation mechanism of nevirapine on modified electrode (II).<sup>43</sup>

sodium hydroxide, a wide oxidation peak was noticed on the unmodified electrode at 0.67 V while the Ura/CPE showed signal shifted negatively at potential 0.54 V which enhanced almost 10-fold. Under optimized conditions, the DPV showed the linearity in the range of 0.1–70.0  $\mu\text{M}$  for the anodic peak current as function of NVP concentrations. The LOD was calculated to be 0.05  $\mu\text{M}$ . The constructed modified CPE sensor was suitable for NVP determination in real samples.

**GCE/HgF.**—According to research conducted by Castro and co-workers, a thin Hg film was formed in a  $10^{-2}$  M Hg ( $\text{NO}_3$ )<sub>2</sub> to determine NVP at the sub nanomolar concentration.<sup>51</sup> Hg plating was carried over on a GCE for 5 min at  $-0.9$  V. The sensor showed a response to NVP yielding cyclic peak at  $-0.55$  V with no oxidation peak noticed in the first scan. The electrolytes used for the experiment were  $2.0 \times 10^{-3}$  mol  $\text{L}^{-1}$  NaOH with linearity over the range 0.01–0.14 ppm and a LOD of 3 nM were estimated from detection of 0.02 ppm after a 6 min accumulation. The proposed modified and unmodified sensors comparing their detection limits reported in literature for NVP detection are shown in Table II. As shown in Table II the sensor base on MIP/ErGO recently developed by Pour et al. (2021)<sup>33</sup> showed a higher limit of detection and concentration range than other reported sensors followed by HgF.<sup>44</sup> Although the best results were found in HgF but due to its toxicity, this kind of sensor has been discontinued.

**Pt/CoPc-cou-f-MWCNTs/Naf-5.**—The paper published recently by Kantize and co-workers<sup>52</sup> reported a Pt-modified electrode (CoPc-cou-f-MWCNTs/Naf-5/Pt) fabricated by sequential drop casting. Under optimized conditions, linear sweep voltammetry (LSV) method was used for the voltammetric determination of the NVP with a linear range of 0.6 nM to 30  $\mu\text{M}$  and an estimated detection limit of 0.2 nM. In the same study chronoamperometry was used over two concentration ranges. The obtained two linear ranges were 2.5  $\mu\text{M}$  to 30  $\mu\text{M}$  and 4 nM to 312.5 nM with a detection limit of 0.21 nM. The sensor was suitable for NVP detection at pH 12. The mechanism proposed agrees with the mechanism proposed by Masumi and co-workers.<sup>36</sup>

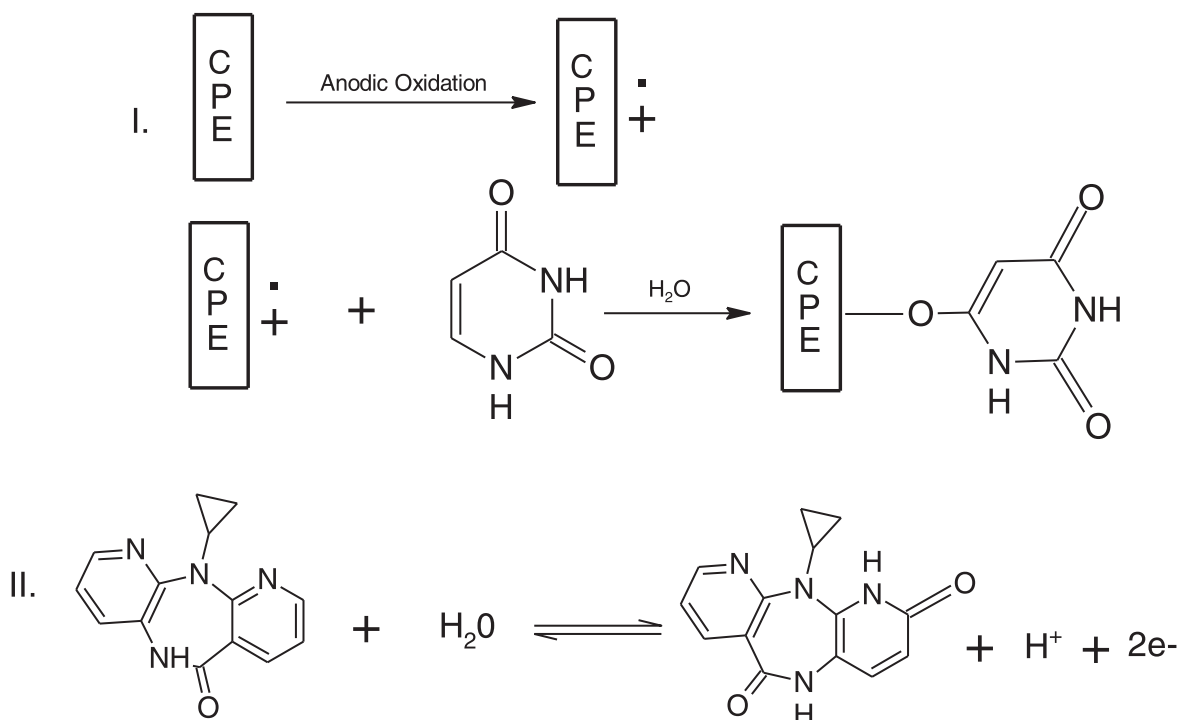
### Electrochemical Detection of Efavirenz (EFV)

EFV, (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one, with a brand name sustiva or stocrin, is specific, nonnucleoside reverse transcriptase inhibitor for HIV-1. EFV was recommended for use on the 21st of September 1998, leading it the 14th approved ARV drug. The drug is ineffective against HIV-2, due to different structure of the pocket of the HIV-2.<sup>44</sup>

**Efavirenz detection at unmodified electrodes.—PGE.**—Several sensors including pencil graphite electrode (PGE), GCE and BDDE were investigate by Topal and co-workers using CV and DPV in Britton–Robinson and acetate buffers at different pH values to choose the best one for the determination of EFV.<sup>45</sup> The best results were obtained in PGE; therefore, further measurements were continued with this sensor. The CV of the 30ppm EFV in Britton–Robinson buffer at pH 3.0 at a sweep rate of 100  $\text{mVs}^{-1}$  on the PGE showed an anodic peak at potential 1.40 V. A DPV method was validated for the quantification of EFV in poly (*n*-butyl cyanoacrylate) (PBCA) nanoparticles. The linearity was presented in the range 0.05–8.11  $\mu\text{M}$  with the LOD and LOQ that were established at 13.8 and 42 nM, respectively.

**Efavirenz detection at modified electrodes.—EPPG/ErGO-Pt/Nafion.**—Raj and co-workers continued their effort to develop and validate an SWV method based on a novel, electrochemically reduced graphene oxide (ErGO), integrated with platinum nanoparticles and ErGO-Pt/Nafion on the surface of edge plane pyrolytic graphite for the determination of the EVF.<sup>53</sup> CV showed an anodic peak at potentials 1.16 V in PB, pH 7.2 added 50 mM EFV. SWV showed a well-defined peak at approximately 1.11 V and can be related to studies in literature.<sup>53</sup> The coated GCE exhibited sensitive detection capability giving an excellent signal for EFV in the linear range of 0.05–150 mM. The LOD and LOQ were 1.8 and 6.0 nM, respectively. The proposed method has been successfully applied to determine EFV in human urine, plasma, and tablet samples.

**GCE/NiO–ZrO<sub>2</sub>.**—Thapliyal and co-workers constructed the GCE coated with the oxide of nickel and zircon mixture to develop



**Scheme 7.** Proposed electro-oxidation mechanism of EFV.<sup>53,54</sup>

**Table III. Summary of the analytical performances of the recent literature on EFV analysis by electrochemical sensors.**

| Sensor                   | Method Analysis | LOD (nm) | Linear range ( $\mu\text{M}$ ) | Electrolyte solution            | Real sample           | References         |
|--------------------------|-----------------|----------|--------------------------------|---------------------------------|-----------------------|--------------------|
| PGE                      | DPASV           | 13.8     | 0.05–8.11                      | Britton–Robinson buffer, pH 3.0 | Tablet                | <a href="#">45</a> |
| GCE/HgF                  | DPASV           | 3.0      | 0.03–0.79                      | NaOH                            | —                     | <a href="#">44</a> |
| ErGO-Pt/Nafion/EPPG      | SWV             | 1.8      | 0.05–150                       | PBS, pH 7.2                     | Urine, Plasma, tablet | <a href="#">52</a> |
| GCE/NiO-ZrO <sub>2</sub> | CV              | 1.4      | 0.01–10 mM                     | PBS, pH 7.2                     | Urine, tablet         | <a href="#">53</a> |

GCE/NiO-ZrO<sub>2</sub>.<sup>53</sup> The cyclic voltammetric method has been used for the analysis of EFV drug. At physiological, pH of 7.2, the developed sensor showed a fast response of 1.1 V in concentration of 10 mM EFV. GCE/NiO-ZrO<sub>2</sub> showed a linear range between 0.01 to 10 mM and a LOD of 1.36 nM. The analytical performance of GCE/NiO-ZrO<sub>2</sub> was evaluated for the analysis of EFV drug in tablets and urine. The mechanism proposed is shown in Scheme 7.

**GCE/HgF.**—An adsorptive stripping voltammetric method was also proposed for EFV analysis at GCE modified with mercury film (GCE/HgF) in a  $2.0 \times 10^{-3}$  mol L<sup>-1</sup> NaOH solution under optimized.<sup>44</sup> The obtained limits of detection expressed as concentration in mol L<sup>-1</sup> was  $3.0 \times 10^{-9}$  in a linear range 0.01–0.25 ppm. A vital point of difference with respect to this sensor for electro-analytical work on efavirenz is that the detection is observed in the cathodic region (−0.38 V), instead of the anodic peak around (1.0 V) that has been observed by other sensors. A comparison of the electrochemical sensors reported to quantify EFV is depicted in Table III indicates that the proposed sensors are superior with GCE/NiO-ZrO<sub>2</sub> being the most superior to other sensors, particularly in terms of the detection limit.

### Conclusion, Challenges, and Future Perspective

Early and accurate detection of anti-HIV drugs is important from the perspective of the electrochemical sensor field to prevent their lethal influence on the body or to effectively treat the disease at an early stage. The review discusses a thorough, literature survey of the electrochemical sensors used to determine the first generation of anti-HIV drugs using electrochemical methods, and their pros and cons. Electrochemistry is a well-established and rapidly growing area with several possible applications in the pharmaceutical field. The continuous advances in sensor designs will greatly assist in the development of simultaneous determinations of target molecules. The construction of an effective and specific nano framework will be the future of the detection of these drugs. Nowadays, the search for finding cheap, reliable, and simple methods is still ongoing. Introducing better materials for electrochemical sensors, a variety of modifiers have been researched to date. There are both merits and demerits of using nanoparticles as modifiers in any sensor construction route. The combination of different materials with different characteristics to develop a sensor with better electrochemical performance may sustain an improvement in the detection of anti-HIV drugs, but complexity in the mechanism of the process can emerge. Hence, it is vital to have a physicochemical basis for the constructed sensor with a clear understanding of the role of each added material. Several pieces of the literature revealed more studies on NVP, whereas for EFV only a few studies have been mentioned. Although this research is promising on paper and researchers are proud of major advances, there is still a long way to go. Further developments are encouraged to bring these sensors into the commercial market and for routine use. On the other hand, there are many challenges facing this technology as well as their modifiers, like the integration of these materials on the electrode surface, fouling of the active layer on the surface, and the aggregation of nanoparticles on the surface of the electrode. Another tricky challenge is agglomeration which reduces their effectiveness as an electrode material and their catalytic ability is also a big problem. There is no information that was found on nanoparticle-based electrochemical sensors for the detection of DLV at the time of presenting this review. Therefore, further investigation is recommended to address this issue. For understanding the redox reaction mechanism that took place at these electrodes, CV and LSV techniques were used. Of all the published methods, the DPV method is the most frequently studied one which is used for the detection of real samples. These modified electrodes showed a higher detection limit and wider concentration range than the reported bare sensors in this review. Future perspectives can include a combination of these techniques with spectroscopic methods to

enrich the electrochemical information that can assist to solve the puzzle involved in sophisticated electrochemistry processes and confined chemical reactivities. Another pressing matter that needs to be addressed is the missing and poorly discussed information on stability and interferences studies in most of these articles.

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