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Electrochemical Biosensors for Point-of-care Applications

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ABSTRACT

Biosensor refers to powerful and innovative analytical tool involving biological sensing element and transducer with broad range of applications, such as diagnosis, drug discovery, biomedicine, food safety and processing, environmental monitoring, security and defense. Recent advances in the field of biotechnology, microelectronics, and nanotechnology have improved the development of biosensors. Glucometers utilizing the electrochemical determination of oxygen or hydrogen peroxide employing immobilised glucose oxidase electrode seeded the discovery and development of biosensors. Molecular recognition based on geometry and forces of interaction play an important role in the biosensor development. The advent of nanotechnology led to highly efficient and sensitive biosensors. They also provide an effective immobilisation matrix for the various bioreceptors. Enzymatic and their mimetic (metalloporphyrin)-based biosensors for reactive oxygen, nitrogen species and cytochrome *c* will also be discussed. The role of antibodies and their applications in immunosensors development for cytochrome *c* and superoxide dismutase will be highlighted. The electrochemical biosensors are less expensive, miniaturised and used for point-of-care applications. Further, the fabrication of labVIEW based virtual biosensor instrumentation and microcontroller based portable biosensor for wide variety of applications also devices will be presented.

Keywords: Superoxide dismutase; Cytochrome *c*; Polypyrrole; Nitrate Reductase; Simultaneous determination; Nanoparticles; Biosensors; Point-of-care

1. INTRODUCTION

This review is an attempt to describe the recent advancements in biosensing technology for point-of-care applications. A biosensor is an analytical tool used to find analytes, It consists of three parts: (i) the bioreceptor, (ii) the transducer or the detector portion, and (iii) the reader. A biomolecule that recognizes the target analyte is a bioreceptor or biorecognition element[1]. The biomarker serves as predictor of a regular biological pathogenic process. A biomarker indicates a clear physical trait or a biologically induced observable improvement in the body that is related to a particular disease or health condition. Consequently, quantification of various biomarkers can be great importance in the therapeutic research and clinical diagnosis.

To estimate biomarker proteins various existing techniques such as enzyme-linked immunosorbent assays (ELISA), Western blot, high performance liquid chromatography (HPLC), flow cytometry and spectrophotometry were used. Due to longer analysis time, expensive tools and the expertise needed for operation, the implementation of these techniques at POC application is limited. Hence notable efforts are being made to overcome these challenges, to develop electrochemical biosensing technologies for quick, precise, sensitive and selective finding of biomarker proteins. So, we have reviewed here the point-of-care biosensors for various diseases, especially hypoxia, oxidative stress and apoptosis biomarkers proteins. Also, the design and fabrication of virtual biosensor instrumentation and microcontroller based portable and cost effective biosensor devices will be reviewed. Such biosensors that would be of interest to biologist and therapists to obtain informatics required in real time to assess the development of diseases progression, therapeutics and also applications for POC.

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2. ENZYMATIC BIOSENSOR FOR CYT C

Cyt c is a significant biomarker of apoptosis. The identification of cyt c release is therefore critically significant as this not only offers useful information about the existence and nature of apoptosis but also act as a preclinical marker of various pathologies, therapeutic treatment and medical diagnostics. The apoptosis/mitochondrial and DNA damage have been implicated in disease that are connected to oxidative stress and hypoxia[2]. This Cyt c used to measure cell death in hypoxia/oxidative stress. Cyt c present in an oxidized (ferric) or reduced (ferrous) form. However the structures of the two kinds of cyt c are identical, the difference in oxidation states does make major difference in binding and biochemical properties. Cyt c oxidase (CcO) based cyt c

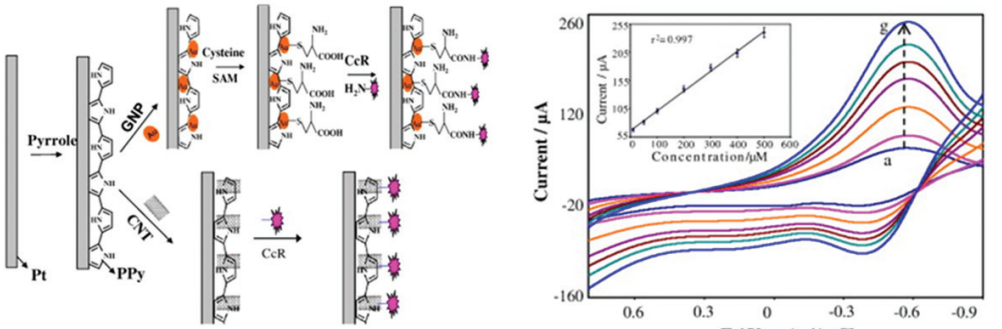
biosensors have been reported[3]. This cyt c oxidase biosensor lack of specificity or quantify only the non-apoptotic form of cyt c (Fe (II)).

In this review, authors reported using CcR (Cytochrome *c* reductase) immobilised on nanoparticles decorated electrodes to detect the oxidized type of cyt c (Fe^{3+})[4]. For the construction of biosensors, two different kinds of nanomaterial decorated biosensor sites were used (a) integrated polypyrrole (PPy) matrix carbon nanotubes (CNT) modified on Pt electrode and (b) Gold nanoparticles functionalised self-assembled monolayer (SAM) (GNP) in PPy-Pt. The integration of CNT/GNP into the PPy modified electrode showed nanoporous structures with a huge effective surface area for CcR immobilisation and enhanced the biocatalytic enzyme activity for responsive determination of apoptosis biomarker protein cyt c. Both the biosensors cyclic voltammograms showed reversible redox peaks at −0.45 and −0.34 V vs Ag/AgCl, characteristic of CcR. The CcR-CNT biosensor, in contrast, yielded a limit of detection 0.5±0.03 µM cyt c, which was 4-fold better than the CcR-GNP biosensor (2±0.03 µM). Furthemore, the CcR-CNT biosensor achieved a much broder linear range (1–1000 µM) over the CcR-GNP biosensor (5–600 µM) with 2-fold better sensitivity. The schematic representation of architectures for cyt c biosensors is shown in Fig. 1(A).

The current responses to cyt c obtained with the CcR-CNT-PPy-Pt biosensor are shown in Fig. 1(B). Moreover, the CcR-CNT was used quantify the cytosolic cyt c released from the mitochondria of apoptotic human lung carcinoma A549 cells and the findings were validated using the standard western blot analysis[4].

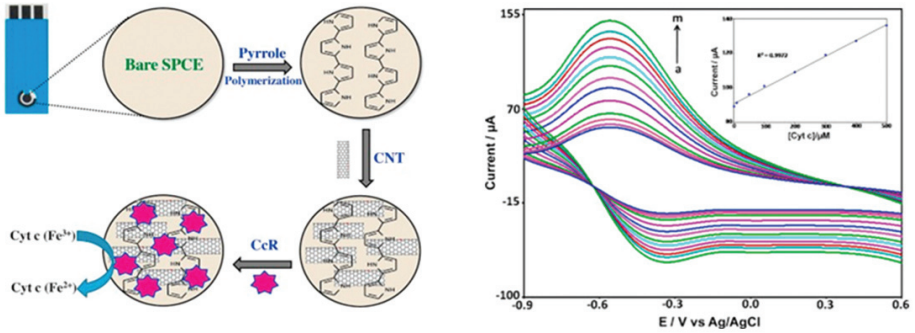
Furthermore, substitution of traditional electrochemical cells by screen printed electrodes (SPEs) linked with portable potentiostats is the main trend in the transfer of electrochemical laboratory equipment to handheld field analysers[5]. The most significant advantage of this miniaturised electrochemical biosensor based on SPE is its compatibility with microelectronics, which enables laboratory prototypes to be commercialisation into future potential bioanalytical devices. Our group reported, a portable, cost-effective electrochemical assay to detect cyt c release quickly, sensitively and quantitatively[6]. The manufactured tools includes CcR-CNT-PPy nanocomposite modified SPE interfaced with a cost-effective PIC microcontroller based data acquisition unit performing cyclic voltammetry for the determination of cyt c Fig. 2(A).

The linear response range of the CcR-CNT-PPy-SPE modified biosensor to the cyt c concentration was under optimal conditions from 10 nM to 500 µM with a correlation



(A) (B)

Figure 1. (A) Schematic description of the CcR based biosensors working mechanism (B) Characteristic CV responses of the CcR-CNT-PPy-Pt electrode in 0.1 M PBS containing 0.5 mM HQ, without (a) and with 50 to 500 µM of cyt c (b–g) measured at scan rate of 50 mVs^{-1}. A linear plot of calibration of cathodic peak currents against cyt c concentrations (Fig. 5 Inset). Every point reflects the three measurements mean (±0.03 SD. (Reproduced with permission from (Pandiaraj[4], *et al.*) © 2013b Elsevier).



(A) (B)

Figure 2. (A) Schematic diagram of the miniaturised cyt c biosensor assay (B) Typical cyclic voltammograms obtained with the cyt c biosensor assay for increasing concentrations of cyt c (a) 0 nM, (b) 50 nM, (c) 100 nM, (d) 200 nM, (e)500 nM, (f) 1 µM, (g) 10 µM, (h) 50 µM, (i) 100 µM, (j) 200 µM, (k) 300 µM, (l)400 µM, and (m) 500 µM. (Inset: calibration curve for cyt c concentrations from1 µM to 500 µM). (Reproduced with permission from (Pandiaraj[6], *et al.*) © 2014a Elsevier).

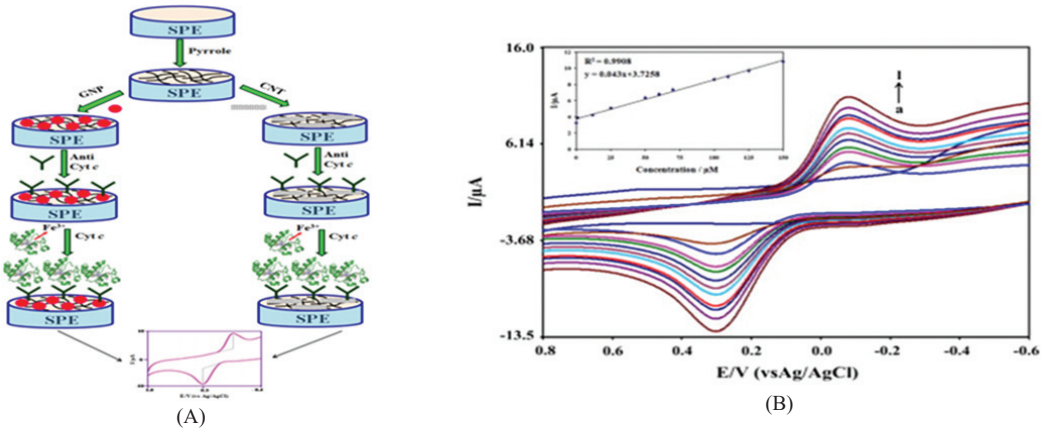
coefficient of 0.9972 and a sensitivity of 0.102 ± 0.005 μA μM^{-1} cm^{-2} (n = 3) Fig. 2(B). An estimated detection limit was 10 ± 0.05 nM. Compared to the traditional Pt electrode the analytical parameters of the present miniaturised biosensor have been remarkably improved. Furthermore, this assay was applied successfully to measure the release of cyt c from cardiomyocytes and the results were well correlated with standard ELISA.

3. LABEL FREE ELECTROCHEMICAL IMMUNOSENSORS FOR CYT C AND CU/ZN SOD1

For the cyt c detection, the particular cyt c monoclonal anti-cyt c was immobilised on the electrode to capture cyt c, and then the cyt c adsorbed electrode measured by cyt c heme (Fe(III)/Fe(II)) by its electrochemical activity. This direct redox response was effectively used here to calculate apoptosis biomarker cyt c quantitatively without usage of any redox probe or enzymatic label. Moreover, the direct electron transfer of cyt c with the electrode surface was performed by modifying the electrode surfaces with nanostructures. Our group reported two kinds of nano-architectured immunosensor platforms designed to better the direct electroactivity of cyt c[7]. The first configuration concerned the SAM modification on GNP in PPy tailored SPE. Second architecture features an efficient incorporation of CNT using nafion on PPy modified SPE Fig. 3(A). The characteristic cyclic voltammetric anodic and cathodic peak current response and the concentration of cyt c are shown in Fig. 3(B). However, the whole analytical efficeny of GNP based immunosensors (limit of detection 2 nM; linear range: 2 nM - 150 μM; sensitivity: 154 nA nM^{-1}) was enhanced than that of the CNT-PPy (detection limit 10 nM; linear variance : 10 nM - 50 μM; sensitivity :122 nA nM^{-1}). The anti-cyt c/SAM/GNP/PPy immunosensors greater analytical output is due to the thickly and distributed uniformly GNP that very much enhanced the active surface area for cysteine SAM formation. The self assembled monolayer on GNP/PPy nanocomposite has also facilitated the formation of a steady and covalent immobilisation of great number of anti-cyt c which could further enhance the linear range and sensitivity of the immunosensor[8].

SOD1 is an important biomarker of oxidative stress. Superoxide dismutase (SOD) is a family of important antioxidant metalloenzymes that serve as first line of protection against the toxic effects of ROS in living cells. The oxidative stress in cells is the main source of ROS which can cause many human diseases, including cancer. Superoxide dismutase maintains the proper balance of ROS by catalyzing dismutation of $O_2^{\bullet -}$ to O_2 and H_2O_2[9]. Therefore, the quantitative finding of small amounts of SOD1 availability in biological samples is of great significance to preclinical diagnosis of certain diseases. So, in the case of electrochemical immunosensors, the biospecific interaction is electrochemically transformed into an electrical signal. Specifically, SOD1 can amplify the interaction signal, which produces electroactive products on a continuous basis[10]. We have developed and reported a label-free immunosensor for oxidative stress biomarker protein SOD1[10]. The reported immunosensor was comprised of a screen-printed carbon electrode (SPCE) modified with self-assembled monolayers (SAMs) of gold nanoparticles (GNPs) on electropolymerised polypyrrole (PPy) and biofunctionalised with monoclonal anti-SOD1 antibody Fig. 4(A). Due to the inherent nitrite oxidase activity of SOD1, the nanostructured immunosensor was indirectly used to check the SOD1 levels through electrocatalytic oxidation of nitrite Fig. 4(B) represents the characteristic CV responses of the anti-SOD1/SAM/GNP/PPy/SPCE after the immunochemical incubation of SOD1 in 0.1M PBS (pH 7.0) containing constant (100 μM) nitrite solution[10]. The experimental voltammograms exhibited prominent anodic peaks at 0.8 V due to the nitrite oxidase activity with enhanced current response for increasing SOD1 levels.

The SOD1 immunosensor revealed a linear response in range of concentration 0.5 nM – 5 μM (r^2 =0.995 and n=3) with a limit of detection 0.5 nM and sensitivity of 46.6 ± 3.5 nA nM^{-1}. These findings demonstrated the potential ability of the establised immunosensor to detect SOD1 levels commonly found in the cytosol of plasma, cells, serum and blood.



(A) (B)

Figure 3. (A) Schematic description of step-wise construction of the label-free cyt c immunosensors (B) Characteristic CV responses of the anti-cyt c/SAM/GNP/PPy/SPE in 0.1 M PBS (pH 7.0) containing 1 to 150 mM of cyt c (b-l) measured at scan rate of 50 mV/s. A linear calibration plot of cathodic peak currents against cyt c concentrations (inset). The RSD is 3.8 % (n=5). (Reproduced with permission from (Pandiaraj[7], *et al.*) ©2014b Elsevier).

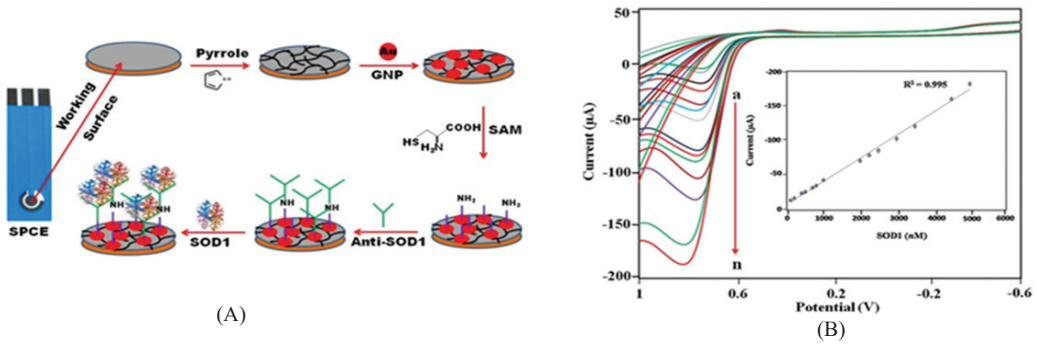


Figure 4. (A) Schematic representation of the fabrication of label-free SOD1 immunosensor. (B) Typical CV responses (a - n) of the anti-SOD1/SAM/GNP/PPy/SPCE after the immunochemical incubation of 100 to 5000 nM of SOD1 in 0.1M PBS (pH 7.0) containing constant (100 µM) nitrite solution and measured at a scan rate of 50 mV s−1. (Reproduced with permission from (Santharaman[10], *et al.*) © 2016 Elsevier).

4. SIMULTANEOUS ELECTROCHEMICAL DETERMINATION OF SUPEROXIDE ANION RADICAL AND NITRITE USING SOD1

Since, $O_2^{\bullet-}$ selectively scavenged by SOD1, it is broadly used for the finding of $O_2^{\bullet-}$ compared to other proteins, enzymes and hemin. Thus, SOD1, a particular enzyme for $O_2^{\bullet-}$ dismutation, offers a best potential for the sensitive and selective measurement of $O_2^{\bullet-}$ in electrochemical biosensors[11]. Prior, first generation SOD biosensors for determination of superoxide radicals have been created by immobilisation of superoxide dismutase within the gelatine (G) on a Platinum electrode surface[12]. Herein SOD1 is also used for the electrocatalytic oxidation of NO_2^-, because of its active site channel is narrow that it can allow selectively only little substrates (Fig. 5). Rajesh[12], *et al*., developed a very high sensitive biosensor for the direct and simultaneous determination of NO_2^- and $O_2^{\bullet-}$ by merging of CNT solubilised in nafion in PPy matrix on Platinum electrode followed by immobilisation of SOD1 using glutaraldehyde as cross-linking agent on it. The biosensor showed a linear result over the concentration range from 0.1 to 750 µM, with a detection limit of 0.1 ± 0.03 µM for $O_2^{\bullet-}$ and a with its related linear range of 0.5–2000 µM, with a detection limit of 0.5 ± 0.025 µM for NO_2^-. This modified electrode is fairly useful not only in detecting $O_2^{\bullet-}$ and NO_2^- autonomously but also in determining the concentration of $O_2^{\bullet-}$ and NO_2^- simultaneously *in vitro* and from cancer cells.

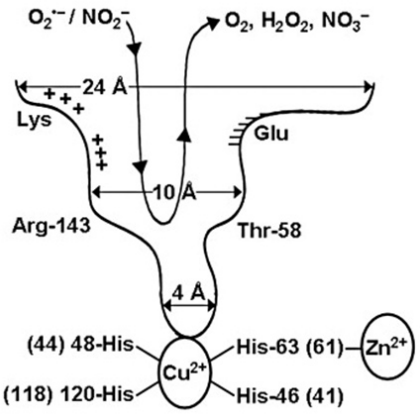


Figure 5. Schematic description of active site channel of SOD1. (Reproduced with permission from (Rajesh[12], *et al.*) © 2010 Elsevier)

4.1 Electrochemical Response to $O_2^{\bullet-}$ and NO_2^-

Figure 6(A) compares the CV measured at the SOD1–CNT–PPy–Pt and CNT–PPy–Pt electrodes in 0.1M PBS (pH 7.0) in the presence and absence of $O_2^{\bullet-}$. Herein, KO_2 was used for production $O_2^{\bullet-}$. The electroreductive reactions of NO_2^- in these biosensors gave NO as a product causing interfering by its reaction with the oxygen. Therefore, the anodic NO_2^- oxidation method using the nitrite oxidase activity of SOD1 was used here for determining NO_2^-. The nitrite oxidase function exhibited by SOD1 is as shown in Fig. 6(B).

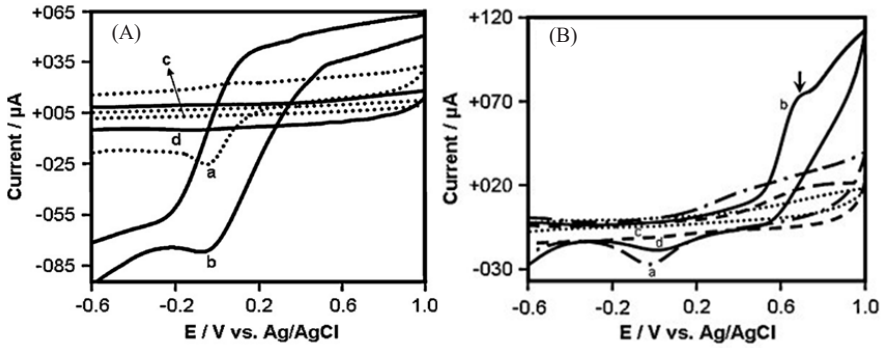


Figure 6. (A) CVs obtained at SOD1–CNT–PPy–Pt and CNT–PPy–Pt electrodes (dotted) in the absence (a and c); (solid) in the presence (b and d) of 500 µM KO_2 solution in 0.1M PBS (pH 7.0) containing 100 µM DTPA; scan rate: 50mVs^{-1}. (B) Typical CV response of SOD1–CNT–PPy–Pt and CNT–PPy–Pt electrodes (dotted) in the absence of (a) and (c); (solid) in the presence of (b) and (d) of 250 µM NO_2^- solution in 0.1M PBS (pH 7.0) containing 100 µM DTPA; scan rate: 50mVs^{-1} vs. Ag/AgCl. (Reproduced with permission from (Rajesh[12], *et al.*) © 2010 Elsevier).

5. SOD1 AND NaR BASED BIENZYMATIC AND BIOMIMETIC COPPER(II) CHLOROPHYLLIN (CuCP) BIOSENSORS FOR NITRITE AND NITRATE DETERMINATION

Nitric oxide (NO) and its metabolites (viz., NO_2− and NO_3−) are broadly used as biomarkers of a variety of pathological conditions viz., hypoxia, ischemia, cancer, respiratory diseases and so on. Nitrate is one of the important stable metabolites of NO. Therefore, the measurement of nitrate is a biomarker of NO generation in biological systems[13]. Madasamy[14], *et al.*, reported a new bienzymatic biosensor for the simultaneous determination of NO_2^- and NO_3^- ions using SOD1 and nitrate reductase (NaR) coimmobilised on carbon nanotubes (CNT) – PPy nanocomposite modified on Platinum electrode. The electrocatalytic function of SOD1 towards NO_2^- oxidation found at +0.8 V was linear from 50 nM to 1mM with a detection limit of 50 nM and sensitivity of 98.5 ± 1.7 nA μM^{-1} cm^{-2}. Alike, the coimmobilised NaR showed its electrocatalytic function towards NO_3^- reduction at -0.76 V showed a linear response from 200 nM to 10 mM NO_3^- with a detection limit of 200 nM and sensitivity of 84.5 ± 1.56 nA μM^{-1} cm^{-2}. Also, the bienzymatic biosensor covered layer with cellulose acetate membrane for the deletion of non-specific proteins was successfully used for the sensitive and selective determinations of NO_2^- and NO_3^- present herein human plasma, whole blood and saliva samples. The above generated bienzymatic biosensor is showed in Fig. 7.

5.1 Simultaneous Determination of NO_2^- and NO_3^-

Figure 8(A) shows the CVs measured for the bienzymatic biosensor by raising the concentration of NO_2^- from 500 nM to 300 µM and NO_3^- from 700 µM to 400 µM at a scan rate of 50 mV s^{-1} in 0.1 M PBS (pH 7.0). The obtained results shows the increase of well-distinguished anodic peak at +0.8 V and cathodic peak at -0.76 V. The calibration curve for the simultaneous measurement of NO_2^- (r^2 =0.999) and NO_3^- (r^2 =0.995) is represented in Fig. 8(B). These results represent that the NaR–SOD1–CNT–PPy–Pt electrode can be successfully used for the simultaneous measurement of NO_2^- and NO_3^-.

Our previous research concentrated on the enzymatic determination of NO, NO_2^- using copper, zinc superoxide dismutase (SOD1), and NO_3^- using nitrate reductase (NaR) modified Platinum electrodes. Herein, copper(II) chlorophyllin (CuCP) contains copper at the core of the porphyrin with excellent electrocatalytic redox behaviour alike SOD1. Balamurugan, *et al.* reported a new electrochemical assay for the combined measurement of nitric oxide (NO) and its metabolites nitrite (NO_2^-) and nitrate (NO_3^-) in volume miniaturised model at cost-effective using copper(II) chlorophyllin (CuCP) modified sensor electrode. This biosensor electrode exhibited a broad linear response over the concentration range from 200 nM to 500 µM with a detection limit of 100 nM and sensitivity of 85.4 nA µM-1. Also, NO_2^- measurement exhibited linearity

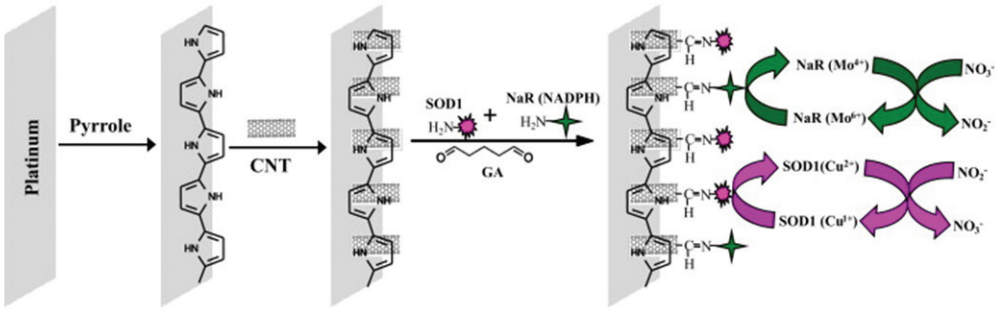
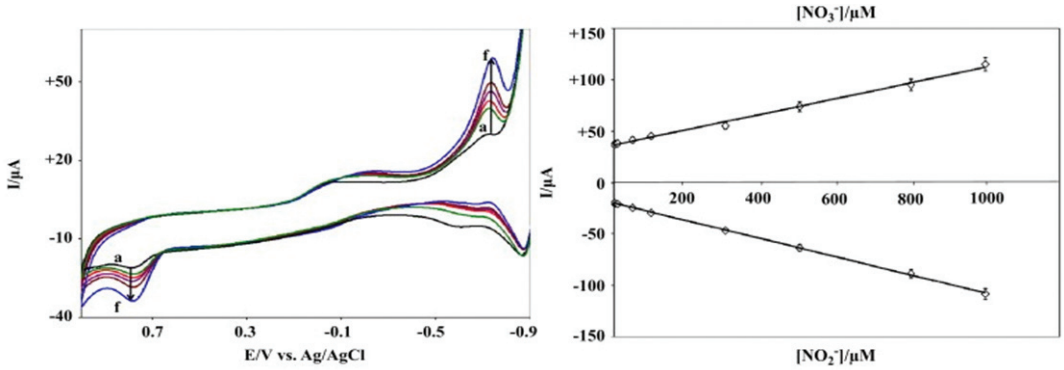


Figure 7. Schematic illustration of the construction of NaR–SOD1–CNT–PPy–Pt electrode and representation of reactions during the simultaneous determination of NO_2^- and NO_3^- (Reproduced with permission from (Madasmay[14], *et al.*) © 2014 Elsevier).



(A) (B)

Figure 8. (A) Typical CV responses obtained for the NaR–SOD1–CNT–PPy–Pt electrode in (a) 500 nM NO_2^- +700 nM NO_3^-, (b) 10 µM NO_2^- + 30 µM NO_3^-, (c) 30 µM NO_2^- + 50 µM NO_3^-, (d) 50 mM NO_2^- + 100 mM NO_3^-, (e) 100 mM NO_2^- + 200 mM NO_3^- and (f) 300 mM NO_2^- + 400 mM NO_3^- solution at scan rate: 50 mV s^{-1} vs. Ag/AgCl. (B) A linear calibration plot of anodic peak currents against NO_2^- concentrations (y= 0.087x - 19.96 and r^2=0.999) and cathodic peak currents against NO_3^- concentrations (y= 0.075x + 36.77 and r^2 =0.995). (Reproduced with permission from (Madasmay[14], *et al.*) © 2014 Elsevier).

of 100 nM to 1 mM with a detection limit of 100 nM for NO_2^- and 96.4 nA μM^{-1} sensitivity. Using this electrochemical assay kit, the concentrations of NO and its metabolites NO_2^- and NO_2^- present in human plasma samples were estimated and agreed with standard Griess method. The schematic illustration of the fabrication of CuCP–ZnO–SPCE is shown in Fig. 9.

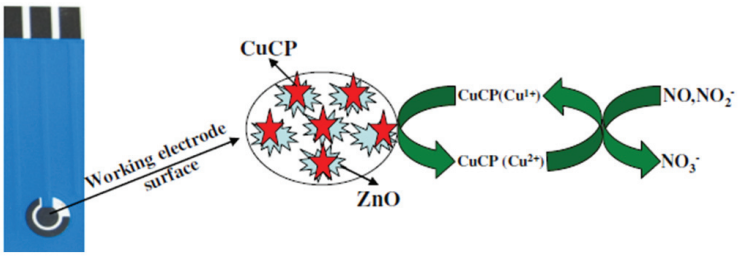


Figure 9. Schematic illustration of the fabrication of CuCP-ZnO-SPCE and the description of the biochemical reaction occurring at the biosensors surface during the detection of NO, NO_2^-. (Reproduced with permission from (Balamurugan[15], *et al.*) © 2015 Elsevier).

6. PORTABLE ELECTROCHEMICAL BIOSENSOR

An important electrochemical technique cyclic voltammetry is very important used to learn the mechanism of charge transfer reaction of redox species, in particular to determine the concentration of biomolecules using its oxidation/reduction reactions. During cyclic voltammetry studies, the biasing voltage across the working and reference electrodes varied linearly, at the same time the resultant current across the working and counter electrodes measured throughout the electrochemical process in a three electrode system. We designed and programmed a portable electrochemical device to perform cyclic voltammetry technique for analysis[6].

6.1 Virtual Electrochemical NO Analyser

Our group have developed user friendly and cost-effective virtual electrochemical biosensors using a home-made potentiostat for the determination of NO in exhaled breath and also hydrogen peroxide from stimulated endothelial cells[16,17]. In this, (NIMyDAQ) based data acquisition system was used to collect the data from the electrocatalytic oxidation of NO by copper, zinc superoxide dismutase (Cu, ZnSOD). The GUI (graphical user-interface software) electrochemical control programs were developed using LabVIEW10.0 to sweep the potential, obtain the current response and process the obtained current signal as ilustrated in Figs. 10(a) and 10(b). The SOD1 immobilised on the carbon nanotubes in polypyrrole matrix modified platinum electrode was employed as the NO biosensor. The electrochemical studies using the SOD1 modified sensing electrode exhibited the characteristic quasi-reversible redox signal at the potential, +0.06 V *vs* Ag/AgCl. The interferences due to biological substrates were removed by coating nafion on SOD1 electrode, and NO was hence measured selectively. Furthermore, this biosensor exhibited broad linearity, high sensitivity, better reproducibility and demonstrated long term sustainability. The electroanalytical efficiency of the effectively developed virtual electrochemical NO analyser was compared to well with the physical instrument CV and concluded that it is suitable for the biological applications.

6.2 USB Interface based Electrochemical Analyser for cyt c Assay

Here for a quick, sensitive, and quantitative determinaion of cytochrome c (cyt c) release, a compact, low cost portable electrochemical assay is presented[6]. In the developed cyt c biosensors comprise of two parts: (i) a miniaturised electrochemical biosensor based on functionalised screen printed electrodes (SPE) of the cytochrome c reductase (CcR); (ii) a data acquisition unit using microcontroller integrated with potentiostant circuit capable of carrying out cyclic voltammetry for the assay studies. The working electrode surface of SPE was incorporated with polypyrrole (PPy)-carbon nanotubes (CNT) nanocomposite for an improved immobilisation of the enzyme, CcR. The data acquired from biosensor are processed digitally by the microcontroller and further transferred for analysis to a PC via USB port as ilustrated in Fig. 10(c). Implemented here GUI based interface makes the

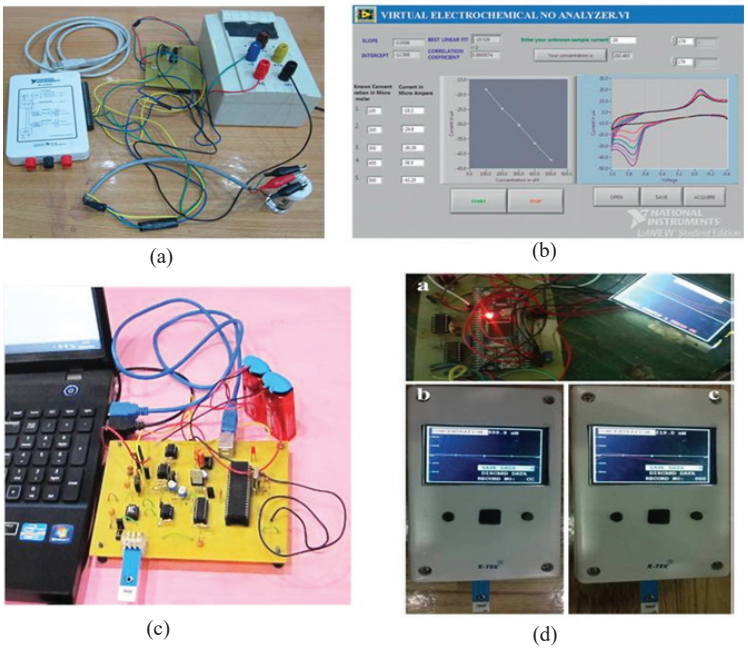


Figure 10. (a) The photograph of the constructed potentiostat circuit connected with NI MyDAQ. (Reproduced with permission from (Balamurugan[13], *et al.*) © 2018 Elsevier). (b) Front panel of the virtual electrochemical NO analyser shows the typical cyclic voltammetry (Reproduced with permission from (Madasamy[16], *et al.*) © 2012 Elsevier). (c) Photograph of the PIC microcontroller and USB based electrochemical analyser (Reproduced with permission from (Pandiaraj[6], *et al.*) © 2014 Elsevier). (d) ARM controller based portable biosensing device was used here for the determination of nitrite levels (Right) (Reproduced with permission from (Santharaman[18], *et al.*) © 2017 Elsevier).

analyser simple to perform. Under optimal conditions, the CcR-CNT-PPy-SPE in linear the concentration range of cyt c from 10 nM to 500 µM. Moreover, this assay was effectively applied to determine the release of cyt c from cardiomyocytes and the results were well correlated with standard ELISA. By combining the cost-effective instrument based on the microcontroller with the SPE miniaturised nanocomposite, this tool could be used as an alternative assay for detecting cyt c in realtime.

6.3 ARM Microcontroller based Portable Biosensing Device using Cytochrome c Reductase

Nitrite (NO_2^-) supplementation limits the oxidative stress that is caused by hypoxia- and activates the alternative NO pathway, that may partially account for the nitrite-mediated cardio defense. So, because of its essential function in human pathophysiology, sensitive and selective biosensors with point-of-care tools need to be explored to detect the physiological nitrite level. In this investigation, cytochrome c reductase (CcR) conjugated to self assembled monolayer (SAM) on gold nanoparticles (GNPs) in polypyrrole (PPy) nanocomposite matrix on the screen printed carbon electrode (SPCE) was used as a biosensor for the measurement of nitrite based on its electrochemical catalytic properties[18]. CcR was covalently conjugated with SAM layers on GNPs by using glutaraldehyde as cross-linking agent. The CcR modified electrode showed a couple of well defined and nearly reversible cyclic voltammetric peaks at−0.34 and−0.45 vs.Ag/AgCl. Under optimal conditions, the biosensor could be used for the determination of NO_2^- with a linear range from 0.1–1600 µm and a detection limit of 60 nM with a sensitivity of 0.172 µA $\mu M^{-1} cm^{-2}$. Further, we have designed and developed a new and cost effective portable electrochemical analyser using ARM microcontroller for the measurement of NO_2^- in hypoxia induced H9c2 cardiac cells. The data obtained here employing the fabricated point-of-care electrochemical nitrite analyser were comparable with the standard cyclic voltammetry instrument. Thus, the biofunctionalised CcR and ARM microcontroller based electrochemical nitrite analyser provided an enhanced stability, efficient strategy and a new promising potenial for nitrite measurement in biological samples and the development of point-of care applications as shown in Fig. 10(d).

7. CONCLUSIONS

Biosensors are very important tools for the detection of biomarkers. We have developed miniaturised screen printed biosensing electrodes coupled with portable biosensor devices for the detection and determination of biomarkers for various diseases, including hypoxia, oxidative stress (SOD1) and apoptosis (cyt c) and reported. A disposable cost-effective volume miniaturised electrochemical biosensors and label free immunosensors for fast, sensitive, and quantitative detection of cyt c and SOD1 were presented. Also, new electrochemical assay methods developed for selective simultaneous determination of NO and its metabolites NO_2^- and NO_3^- & super oxide anion radical were reviewed. Further, we have fabricated the virtual electrochemical analyser instrumentation, PIC microcontroller with USB interface based electrochemical analyser and ARM microcontroller based portable devices. Such developed POC device would be simple to handle, need low- power to work, less-expensive, useful for detection in-field.

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