The Use of Conducting Polymers for Enhanced Electrochemical Determination of Hydrogen Peroxide

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The role of hydrogen peroxide in a wide range of biological processes has led to a steady increase in research into hydrogen peroxide determination in recent years, and conducting polymers have attracted much interest in electrochemistry as promising materials in this area. We present an overview of electrochemical devices for hydrogen peroxide determination using conducting polymers, either as a target or as a byproduct of redox reactions. We describe different combinations of electrode modifications through the incorporation of conducting polymers as the main component along with other materials or nanomaterials. We critically compare the analytical performances cited and highlight some of the future challenges for the feasible application of such devices.

Keywords**:** hydrogen peroxide; conducting polymers; amperometric determination; organic electrochemical transistors

Introduction

Hydrogen peroxide (H_2O_2) is a key marker in biological processes because it is involved in signaling paths such as cellular growth, senescence[1] and apoptosis[2], and can be generated by means of different stimuli[3]. It may be related to some neurological disorders such as Parkinson's, rheumatoid arthritis and Alzheimer's together with other reactive oxygen species[4]. In addition, it plays a crucial role in many other sectors including chemical, pharmaceutical and food manufacturing[5]. It is also important in wastewater treatment and wood and paper bleaching[6]. Moreover, it is a byproduct of many biochemical reactions involving oxidase enzymes such as glucose, cholesterol and lactate oxidase, among many others[7]; therefore, detecting H_2O_2 can indirectly detect those biomolecules in different fluids. For these reasons, and due to their wide range of applications, the development of H_2O_2 sensors has recently been the focus of extensive research.

Many different techniques have been developed to determine H_2O_2 including fluorimetry[8,9], chemiluminescence[10,11], chromatography[12,13] and spectrometry[14]. Nevertheless, the implementation of some of these techniques requires complex or expensive instrumentation as well as qualified personnel. Of all the techniques, electrochemical approaches measure H_2O_2 relatively simply and with better sensing parameters, including high sensitivity and fast response time[15]. The ease of miniaturization[16,17] and the broad range of electrode modification possibilities make electrochemical devices suitable for *in situ* H_2O_2 determination. In spite of all the advantages, electrochemical devices have limitations, such as inefficient electron transfer from the recognition element to the substrate, only moderate selectivity with some real samples and lack of measurement reproducibility. The body of research into electrode modification has been continuously expanding in recent years in an effort to overcome these drawbacks while still encompassing essential electrode requirements: conductivity, chemical stability and appropriate surface area and properties.

Conducting polymers (CPs) are interesting candidates as sensing and transducer materials due to their electrical properties, and offer an alternative to metallic and inorganic semiconductors[18]. Conducting polymers are organic materials capable of conducting electricity along their backbones due to the conjugated π-electron or C=C conjugated bonds. Conducting polymers have been extensively studied in the thirty years since they were first discovered by Hegger, McDiarmid and Shikarawa[19]. Nevertheless, and in spite of reaching high conductivity values, early conducting polymers were unstable in air and were considered difficult to prepare[20]. Further research on the polymerization of polyanilines, polypyrroles and polythiophenes improved the preparation methods and led to a drastic increase in conductivity and stability under electrical and thermal conditions. The structures of the most commonly used conducting polymers in sensing applications, polyaniline (PANI), polypyrrole (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT), are shown in Figure 1.

[Figure 1 here]

CPs offer unusual electronic properties such as low ionization potential and high electron affinity, and they have been successfully used as sensing elements, immobilization matrices and even as semi-permeable membranes for electrodes[21]. The ability to change their electrical conductivity by several orders of magnitude and their electron affinity depending on their redox state make CPs especially attractive for enhancing sensing performance[22]. Many factors can influence the properties of conducting polymer film, such as the deposition method, the solution pH and temperature, the dopant and doping level and the surface conditions of the electrode[20]. Controlling the polymerization method and how the polymer is deposited on the electrode surface are key factors in tailoring CP properties. Polymerization can be either chemical or electrochemical, and the thickness and conductivity of the film can vary depending on the technique used (e.g. potentiostatic or galvanostatic[23]). The deposition method defines the adhesion of the conducting polymer to the electrode surface, which is mainly established by weak physical interactions. Roughening the electrode surface and covalently attaching the polymer to the substrates are the most frequently used ways to improve adhesion and avoid delamination or cracking of the coating film when the electrodes are exposed to wet conditions or mechanical stress[24]. Doping is based on the insertion of molecules that modify the electronic structure via the formation of local excitations in the way of polarons and bipolarons as delocalized charge carriers that allow the electrons to move along the polymer backbone[25,26]. Depending on the nature of the dopant, the polymer can undergo oxidation (*p*-doping) and have a positive charge, or reduction (*n*-doping) and have a negative charge. The size of the dopant is also variable. The doping process not only modulates the final conductivity of the film, but also its bulk properties such as density, volume, porosity, solubility and color, and its ultimate electrochemical stability. In addition, the doping process is reversible and the polymer can switch from the insulator to the conducting redox state by means of the incorporation or release of the dopant by applying potential to the polymer[27]. The ability to tailor the characteristics of conducting polymers expands their range of application to include, for instance, field-effect transistors, supercapacitors, solar cells and biosensors[18]. However, the intrinsic limitations of conducting polymers can hinder their application in these fields. The over-oxidation mechanism of conducting polymer is not yet fully understood and it is a considerable barrier for advancing polymer-based devices towards real-world applications: It irreversibly changes the structure of the polymer by reducing the conjugation lengths and inducing chain scission, leading to a decrease in or loss of conductivity. Over-oxidation is influenced by pH, and the properties of conducting polymers are also affected and modulated by pH, temperature, humidity and sensitivity to O_2 and CO_2. To overcome some of these inherent limitations, conducting polymers have been hybridized with other suitable materials.

Nanostructured and carbon-based materials such as metal nanoparticles, nanowires, carbon nanotubes and graphene sheets are very attractive components for use in sensor development.

Their surface can be easily modified and they have a large surface-area-to-volume ratio, which enhances the transduction mechanism, thereby providing higher sensitivity towards the target analytes. The insertion of graphene or carbon nanotubes into the structure of conducting polymers by covalent functionalization and π-π or electrostatic interactions improves the order of the CP backbone and the delocalization of the charge carriers, leading to a higher conductivity[28]. Although the applications of hybrid composites combining nanomaterials and CPs have recently been reviewed for sensing and, especially, biosensing purposes[22,29,30], we focus on the use of CPs for the development of electrochemical sensors for the detection of H_2O_2. This review includes reports from the last five years and highlights those which have addressed the analytical performance for H_2O_2 determination in real samples such as body fluids or beverages, among others. We have divided the review into two sections based on the electrochemical techniques used to determine H_2O_2: amperometry and organic electrochemical transistors (OECTs), as depicted in scheme 1.

[Scheme 1 here]

Determination of hydrogen peroxide

Amperometric H_2O_2 determination

In general, H_2O_2 can undergo electrochemical oxidation and reduction (equations 1.a and 1.b) depending on a variety of factors, from the type of substrate to the experimental conditions[31]. Electrochemical H_2O_2 sensors turn chemical information into electrical information, enabling the specific quantification of the target analyte within a complex matrix.

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \qquad (1.a)$$

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^- \quad (1.b)$$

Most of the electrochemical sensors developed to detect H_2O_2 are voltammetric sensors, which take advantage of the redox reaction that occurs upon the application of a time-dependent potential to the working electrode (changing its potential relative to the fixed potential of the reference electrode), which produces a measurable current that flows between the working and auxiliary electrodes. Among the voltammetric techniques, amperometry, in which a constant potential is applied to the working electrode and the current is measured as a function of time, uses Faraday's law to calculate the amount of analyte, making this method the most commonly used tool for detecting H_2O_2 with high sensitivity and with low limits of detection. The following scheme shows the most frequently used combinations of sensor elements within the developed amperometric sensors, from the simplest, with only two components, to the most elaborate configuration combining up to four elements:

[Scheme 2 here]

In this review, most of the sensors mentioned mainly use glassy carbon electrodes (GCE) and screen-printed electrodes (SPE) as their electrode substrates, while the CPs are basically those shown in Figure 1. We have classified the sensors based on their H_2O_2 detection strategy: direct detection as the main target, or indirect detection as a byproduct of a redox reaction. We can also differentiate the sensors by the additional components they incorporate in their membranes to improve selectivity, and other analytical parameters such as polymeric coatings (e.g. Nafion), nanomaterials like nanoparticles or nanofibers, and enzymes, among others.

Direct amperometric H_2O_2 determination

Among the amperometric sensors that solely use CPs for the detection of H_2O_2, Agrisuelas et al.[32] have recently achieved direct H_2O_2 detection in commercial hair lighteners and antiseptics with recoveries of between 98.9 and 100.8% by electropolymerizing poly(azureA) on disposable screen-printed carbon electrodes (SPCEs), together with sodium dodecyl sulfate. The amperometric response to H_2O_2 was measured at 0.5 V with sensitivities of 72.4 nA μM^{-1} cm^{-2} in a concentration range from 5 μM to 3 mM. Ethanol, sodium citrate, glucose, caffeine and L-dehydroascorbic acid were tested as interfering species in order to assess the potential applicability of the sensor in real samples. None of these substances interfered with the H_2O_2 response.

Direct H_2O_2 determination can also be achieved using catalyzers or cofactors such as cytochrome C, hemoglobin and peroxidase enzymes. The most frequently used enzyme to decompose H_2O_2 is horseradish peroxidase (HRP) which can be easily isolated from plant radish, *E. coli* or yeast, and is classified as oxidoreductase (1.11.1.7). HRP catalyzes the oxidation of a substrate using H_2O_2 as the oxidizing agent, allowing for direct electron transfer through the electrode. In these cases, the presence of the conducting polymer improves the performance of the sensor due to its intrinsic conductivity and its mechanical function as a matrix for the immobilization of the enzyme. For example, very low detection limits, 0.03 nM, were achieved by Zhang et al.[33] by preparing a hybrid composite via electrostatic interactions with PEDOT:PSS and chitosan micelles on top of a GCE surface. HRP was entrapped between the conducting polymer and a layer of Nafion by the drop-casting technique, and exhibited an excellent electrocatalytic activity towards H_2O_2, while the electron transfer was enhanced by the presence of the conducting polymer composite.

Figure 2 shows the differential normal pulse voltammograms and calibration curves, with a linear range of between 0.1 nM and 0.01 mM. The wide detection range was attributed to the large surface area of the hybrid film due to the ability to immobilize a large amount of enzyme. In addition, this large surface area facilitated the signal transduction from the enzyme to the electrode. The sensor was tested in real samples, testing the applicability to detect H_2O_2 in a commercial disinfectant and it was validated via the potassium permanganate titration method with satisfactory results and a relative standard deviation (RSD) from 3.1% to 4.8%.

[Figure 2 here]

One-dimensional ordered conducting polymers (especially CP nanowires) have also been successfully used on H_2O_2 sensors. The use of these one-dimensional ordered conducting polymers enhances, for example, the electron transfer through the electrode, not only because of the intrinsic conductivity of the conducting polymer, but also because of the increased surface area, thus obtaining more effective modified electrodes. Rizarullah et al.[34] demonstrated this effect by characterizing carbon paste electrodes with and without PANI nanofibers. The final performance of these modified electrodes (HRP/glutaraldehyde/PANI nanofibers/carbon paste electrodes) resulted in a linear range between 0.1 and 0.3 mM and a sensitivity of 12104 nA μM^{-1} cm^{-2}. Nevertheless, the optimal conditions for these electrodes were pH 7 (PBS) and a working temperature of 50 °C. Although this temperature does not denature the immobilized enzyme, it can represent a substantial drawback when measuring real samples.

As mentioned above, nanomaterials have attracted much attention in the exploration of surface modifications due to the different physical properties in nanoscale compared to their respective properties in bulk. The incorporation of different nanostructures into a system that already contains CP films generally improves the interaction and the electrocatalytic activity with the analyte in a synergistic way, which leads to amplified output.

For example, the addition of Prussian Blue (PB) NPs on PEDOT [35] resulted in a modified GCE able to detect H_2O_2 with a limit of detection of 0.16 µM, within a linear range of 0.5 to 839 µM and a time response within 5 s. No interference was observed due to the presence of DA, AA, UA or lactose, and real milk samples were evaluated by the standard addition method, with recoveries between 98% and 102.7% and RSD between 2.4% and 3.8%, demonstrating the viability of the sensor for use in real sample analyses.

Mercante et al.[36] achieved considerably high sensitivity (677 nA μM^{-1} cm^{-2}) using a ternary-graphene-based composite with reduced-graphene oxide (rGO), PEDOT:PSS and gold nanoparticles (AuNPs). HRP was immobilized on a screen-printed gold electrode by drop-casting and with the addition of a cross-linker to enhance the electron transfer between the *heme* group of HRP and the electrode surface. The electrocatalytic reduction of H_2O_2 was evaluated at -0.4 V and the obtained linear range, from 0.5 to 400 µM, was suitable for determining H_2O_2 in real tap water samples and bovine milk. Actually, the use of hydrogen peroxide as a pre-oxidant in municipal water treatment is well documented and has been practiced for over 20 years, and its use for the activation of the lactoperoxidase system in the preservation of milk has proven effective against both gram-positive and gram-negative bacteria[37,38]. Different electrode configurations were characterized to prove the synergistic effect between PEDOT:PSS and AuNPs, as well as the reduction in peak separation, which led to faster electron transfer with the whole electrode modification. However, all electrochemical measurements were performed under N_2-saturated solutions in order to avoid other reduced interferences. Thus, their real-world application in field measurements, for example, may be limited.

Kumat et al.[39] used a polymer nanocomposite to obtain synergistic effects from the mixture of polymer, nanoparticles and graphene, and achieved a composite with superior conducting properties. Silver nanoparticles (AgNPs) were incorporated into the previously synthesized PANI-rGO system by means of a self-assembly method. The final electrode was assembled onto a glassy carbon surface by drop-casting. Nafion solution was also deposited to enhance the adherence of the complex. The quantitative determination of H_2O_2 was performed amperometrically at -0.4 V in phosphate buffer. Results showed a linear relationship in the range of 0.01 and 1000 μM with a limit of detection (LOD) of 50 nM and a sensitivity of 14.7 $\mu A\ mM^{-1}\ cm^{-2}$. The authors demonstrated the synergistic effect of the system, with PANI being responsible for the electron transfer during the catalytic reaction occurring between H_2O_2 and AgNPs. The electrode modified with the conducting polymer composite exhibited a greater electroactive surface area (resistance of charge transfer, $R_{ct}=9\ \Omega$ with PANI versus $R_{ct}=27.5\ \Omega$ without PANI) and facilitated the electron transport through the electrode. However, and in spite of having checked for interference species, similarly to the previous example of Mercante et al.[36], amperometric measurements were also done under N_2, which may represent a drawback for electrode implementation in terms of time and cost effectiveness.

Systems incorporating one-dimensional ordered conducting polymers and nanostructured materials exhibit better characteristics in terms of the individual performance of these components, demonstrating their synergistic effects. For instance, Yang et al.[40] presented a 3D-macroporous PEDOT prepared by electrodeposition on a GCE surface where PB NPs were incorporated into the system by immersing the electrode in an electrolyte solution containing PB, generating the spontaneous growth of NPs on top of the polymer film. In this case, the use of the conducting polymer had two functions: first, it reduced the iron to initiate PB NP growth; and second, it provided a larger surface area on which to capture more NPs. Chronoamperometric and CV experiments showed improved H_2O_2 catalysis. Amperometric measurements were taken under 0.11 V at pH 3 in a 0.1 KCl solution, yielding a linear range from 0.17 µM to 0.257 mM with an LOD of 80 nM for 3D-PEDOT-PB/GCE compared to the two linear ranges obtained from PB/GCE (from 12 µM to 0.57 mM and from 0.57 mM to 1.57 mM) with an LOD of 3 µM obtained based on the first linear range**.** Figure 3 shows the clear synergy between the components of the conducting polymer-modified electrode, which clearly shows an enhancement of the analytical parameters.

[Figure 3 here]

Additionally, interference studies have also been conducted using UA, DA, glucose, highly concentrated sodium chloride (NaCl) and AA with negligible responses which did not interfere with H_2O_2 determination. 95% of the signal was also retained after a month, suggesting the potential of this hybrid composite for use in bioelectrochemical sensors and devices.

Baghayeri et al.[41] used a PPy nanocomposite made up of poly (styrene-alt-maleic anhydride) (PMSA) grafted with 4-aminobenzenesulfonate (4ABS) (PMSA-g-4ABS) with functional groups to improve the interaction with the redox protein hemoglobin (Hb). Hb has one electroactive iron *heme* group that allows for direct electron transfer from the protein to the electrode (GCE). The conducting polymer nanocomposite-based electrode demonstrated a fast response towards H_2O_2 detection of 4 s, a linear range of 0.8 to 460 µM and an LOD of 0.32 µM. With these characteristics, the sensor applicability was evaluated by detecting H_2O_2 in rainwater by means of the standard addition method, and human serum samples using 10-fold dilution and spiking known H_2O_2 concentrations. The overall recoveries of the sensor were between 97.8 and 103.5%.

Amperometric H_2O_2 detection as a byproduct of redox reactions

Apart from developing sensors to directly detect H_2O_2, sensors for the detection of H_2O_2 as a byproduct of the oxidation reaction are an essential component in the development of biosensors. The primary group of these biomolecules is made up of molecules such as glucose, cholesterol and xanthine, which undergo oxidation by an oxidase enzyme and produce H_2O_2 as a detectable byproduct. An electron cascade is generated from the oxidation of the main component to the final reduction of H_2O_2, which is detected by the electrode. Of all the electrochemical sensors detecting H_2O_2 as a byproduct, glucose sensors are undoubtedly the most frequently used and studied because of the ease of operation and the efficiency of their specific enzyme (glucose oxidase, GOx) and the global market coming from the diabetic population. General equations of the electrochemical glucose determination are given below:

$$glucose + O_2 \xrightarrow{Glucose\ Oxidase} gluconic\ acid + H_2O_2 \quad (2)$$

$$H_2O_2 \xrightarrow{\qquad} O_2 + 2H^+ + 2e^- \quad (3)$$

Kausaite-Minkstimiene et al.[42] demonstrated glucose detection in 10-fold diluted human serum samples using solely CP-based sensors. They described an environmentally friendly synthesis of poly (pyrrole-2-carboxylic acid) particles (PCPy) by means of chemical oxidative polymerization using H_2O_2 as the initiator of the polymerization. They took advantage of the carboxylic groups from the particles to attach the GOx, forming a covalent link between the conducting polymer and the enzyme. Thus, they achieved measurement recoveries in real samples of between 99.1 and 106%, with a linear range from 0.1 to 15 mM and an LOD of 39 μM.

Functionalization of the conducting polymer was also reported by Tekbaşoğlu et al.[43], who described direct glucose detection in commercial beverages (cola and juice samples) with recoveries of between 96.8% and 99.6% with 87% of the initial response retained after eight weeks. Their graphite electrode modification consisted of the oxidative polymerization of EDOT and bis(2-pyrdylimino) isoindolato-palladium complex, which had been shown to mimic catalytic enzymes which decompose H_2O_2 into O_2 and H_2O. Further immobilization of GOx was achieved by cross-linking the enzyme with the free amino functional groups of the polymeric composite. Despite attaining good analytical performance, real-world applications of this sensor for glucose detection may be diminished due to the narrow linear range capable of measuring glucose, which ranges from 0.25 to 2.5 mM.

Glucose content in diluted grape juice and honey was also determined by electrochemical synthesized PEDOT-modified electrodes with polyacrylic acid and poly(4-lithium styrenesulfonic acid) [44]. The amperometric glucose measurements were validated with a reference enzymatic method, and the sensor presented a linear range from 0.96 to 30 mM, with an LOD of 0.29 mM and proved stable for 30 days. Other recent examples involving the polymerization of the conducting polymer together with other electron mediator dopants are described, for instance, by Vagin et al. [45] and Wannapob et al. [46]. However, apart from testing some interference species such as AA and UA, none of them have reported glucose determination in real samples.

Hybrid composites using nanomaterials are also used to quantify glucose through H_2O_2 detection. Screen-printed carbon electrodes were modified with PEDOT microspheres and platinum nanoparticles (PtNPs) by Liu et al.[47]. Polymeric microspheres were obtained using calcium carbonate template-assisted polymerization by first preparing the $CaCO_3$ template particles, and then applying mild and advanced chemical oxidation. The colloid suspension was mixed with K_2PtCl_4 to obtain PtNPs on the surface of the conducting polymer microspheres. In this case, PtNPs worked as a catalyst for H_2O_2 oxidation, PEDOT-microspheres functioned as high-surface area support for the deposition of the NPs, while both together facilitated the fast electron transfer during the process. Absorption of GOx was achieved by incubation for 12h on an appropriate solution. The whole mixture was drop-casted onto the surface of screen-printed carbon electrodes followed by the drop-casting of a Nafion layer in order to protect the enzyme-hybrid composite. Amperometric measurements were taken at 0.6 V in a 10 mM PBS (pH 7.4) solution. Notably good results were obtained in terms of both linear range (from 0.1 to 10 mM, which includes main glucose blood levels in diabetic patients) and sensitivity (116.25 nA μM^{-1}), which is attributable to the morphology of the sensing layer, where a large inter-particle space helped the diffusion of the substances in between. Moreover, UA and AA were tested as interfering species at their corresponding concentrations in human serum with no interference in H_2O_2 measurements. Despite the good performance of this type of enzymatic sensor combining biocomponents, inorganic and organic materials (enzymes, metal NPs and conducting polymer), the authors did not present the analysis of glucose in any biological fluid, although they envisioned developing 'advanced functional bio-conductive inks' for future successful approaches to biosensor manufacturing.

Another hybrid composite using metal nanoparticles was characterized by Gokoglan et al.[48] using a distinct conducting polymer (poly(9,9-di-(2-ethylhexyl)-fluorenyl-2,7-diyl) end capped with 2,5-diphenyl-1,2,4-oxadiazole (PFLO)). Glutaraldehyde cross-linking agent was used to immobilize the GOx-AuNPs solution on the polymer surface. The final graphene/PFLO/AuNPs-GOx electrode showed a sensitivity of 7.35 nA/μM^{-1} cm^{-2}, within a linear range of 0.1 to 1.5 mM and an LOD of 81 μM. Different mono- and disaccharides, such as fructose, galactose and mannose, as well as AA and urea, were tested as interference species with no significant response. Commercial lemon soda and milk glucose content were evaluated and compared to the glucose concentrations reported on the product label with relative errors below 3.6%, pointing to the real-world applicability of such a sensor. Moreover, the authors claim that this constitutes the development of a portable and cheap biosensor due to the use of graphene-paper as substrate. Although substrate materials and portable devices are beyond the scope of this review, it is worth highlighting the importance of developing simple, robust, portable and cheap devices for glucose detection for the point-of-care market[49,50] .

More complex hybrid composites include the incorporation of ionic liquid into the system. Ionic liquids are characterized by their wide potential window, high ionic conductivity and electrochemical stability and high biocompatibility, which makes them suitable for use in biosensing. For instance, brominated 1-decyl-3-methyl imidazole ([Denim]Br) was used by

Zhu et al.[51] in combination with a PANI-TNT composite (titanium oxide nanotubes) for an effective glucose amperometric determination with 177.16 nA μM^{-1} cm^{-2} sensitivity and a linear range from 0.01 to 2.5 mM. In spite of the insignificant response of the examined interference species (UA, AA and acetaminophen), experiments with real samples were not reported. Therefore, the real-world applicability of this hybrid composite has not been satisfactorily demonstrated. Conversely, Zhou et al.[52] prepared a hybrid composite with ionic liquid for the accurate determination of glucose levels in pre-treated and diluted animal serum and human urine and serum with recoveries of between 96.8 and 101.2%. The composite was made up of PEDOT, multi-wall carbon nanotubes functionalized with carboxyl groups (MWCNT-COOH) and the ionic liquid $BminPF_6$, which was obtained by on-step potentiostatical polymerization on GCE. GOx immobilization was done by COOH activation leading to covalent bonds between the enzyme and the polymer composite. After pH, temperature and working potential optimization, the electrodes showed a linear range of 0.6 μM to 2 mM with an LOD of 0.015 μM, with reproducibility RSD of 0.73% and repeatability RSD of 1.01%. The electrodes maintained 98.3% of their response capability after 30 days within an RSD of 0.54%.

A comparative study of the analytical parameters reported in the articles reviewed that described amperometric sensors tested in real samples is listed in table 1. Good performance was obtained in these reports particularly concerning stability, reproducibility and limits of detection. Most of them have tested the hydrogen peroxide/glucose conducting polymer-based amperometric sensor against other electroactive species that could interfere in sensor selectivity and sensitivity, such as ascorbic acid, glucose and uric acid, among others, which are mainly present in human body fluid and food samples. However, only a few of them really focused their work on testing the electrodes in real relevant samples, such as hair lighteners, disinfectants and milk, in which peroxide is used as a preservative and sterilizing additive[53,54] , or in beverages and human body fluids, where glucose is present. The studies show characterization information to demonstrate the superiority of conducting polymers in terms of sensing parameters. Indeed, they demonstrate an increase in peak currents and a decrease in peak separation during cyclic voltammetries in different electrode configurations in order to highlight the role of the CPs, which finally accelerate the electron transfer between the analyte or mediators and the electrode surface. This information is also consistent with most of the electrochemical impedance spectroscopy spectra done to characterize the electrode surfaces as well. In general, the measured resistances are much lower when a CP is part of the system, indicating the higher conductivity achieved was due to the CP, which is related to the larger active surface area obtained when working with conducting polymers, and even larger when dealing with nanostructured conducting polymers. Nevertheless, only one of the mentioned articles [40] demonstrates and characterizes the contribution of the CP to the final analytical parameters of the sensor, such as sensitivity, linear range and limit of detection: noticeably, the LOD improved by two orders of magnitude to the respective control-modified electrode. Although characterization steps are crucial in electrode configuration, and in all examples, the use of CPs demonstrated better characteristics than in their respective controls, the quantification of the enhancement of H_2O_2 determination performance due to the incorporation of CPs still needs to be addressed.

[Table 1 here]

Organic electrochemical transistors (OECTs)

Since OECTs based on polypyrrole were first reported by Wrighton et al.[55], organic electrochemical transistors have attracted much attention due to the low working voltages required and their stability to operate in aqueous media. The working principle of OECTs is based on a three-electrode system where the current flowing along the organic semiconductor connecting two of these electrodes (source and drain) changes as a function of the polarization of the third electrode (gate). Therefore, the potential is applied on the gate electrode in contact with the electrolyte and it modulates the ion motion in solution through the organic semiconductive channel, which leads to a change in the conductivity of the channel. Scheme 3 shows a schematic representation of an OECT electrode configuration and its possible functionalizations.

[Scheme 3 here]

The most often used configuration, as reported later in this review, consists of using the conducting polymer as the channel component, connecting the source and drain electrodes. Therefore, the conducting polymers act as the sensing and transducer material, since it changes its electrical properties as a function of the analyte concentration. Moreover, conducting polymers can also be placed on the gate electrode, either acting as the transducer or as an immobilization matrix where other biomolecules, such as enzymes or polymeric coatings, will be incorporated on the same gate electrode. Different configurations are also reported in this review, considering, for example, the incorporation of the recognition site in the conducting polymer along the channel between source and drain, or for instance, the manufacturing of the three electrodes of the OECT with the same conducting polymer.

PEDOT:PSS has been successfully used as the active layer bridging source and drain electrodes, and is the most used conducting polymer in OECTs due to its high conductivity, broad pH range of operation and electrochemical stability attributable to the bridging of the dioxyethylene group across the 3 and 4- positions of the heteroring. PEDOT:PSS goes from the conductive (oxidized) $PEDOT^+$ state to the semiconducting (neutral) $PEDOT^0$ state due to the doping or de-doping caused by the oxidation/reduction process:

$$PEDOT^+ : PSS^- + M^+ + e^- \leftrightarrow PEDOT^0 + M^+ : PSS^- \tag{4}$$

where M^+ is a cation in the electrolyte medium and e^- an electron from the source electrode. The migration of cations into the polymer causes compensation with the sulfonate groups of PSS, thus changing the electronic structure of the polymer, leading to a decrease in the current along the conductive channel.

Therefore, glucose sensors have been developed using OECTs based on the fact that the oxidation of glucose oxidase, and consequently, the production of H_2O_2, modifies the gate electrode voltage, which, in accordance with the working principles of OECTs, ultimately modifies the current along the channel as a function of glucose concentration. The working voltage on the gate is chosen depending on the gate modification materials as well as the change in conductivity it produces on the channel; the greater the difference in current before and after applying the corresponding voltage, the more sensitive the channel is.

The earliest CP-based transistors for glucose detection through GOx incorporation were based on the sensitivity of the polymers to pH changes, such as PANI[56] and the change in the redox state of the conducting polymer due to the oxidase reaction[57]. H_2O_2 involvement in such transistors was first demonstrated by Malliaras et al.[58,59] who used PEDOT:PSS as a sensing layer and Pt wire as gate electrodes where the H_2O_2 produced was oxidized. The mechanism behind the detection was suggested to be due to the reduction of the polymer after the H_2O_2 oxidation at the gate to maintain the charge balance, or due to the redistribution of the potential at the solution/conducting polymer interface after the H_2O_2 production. A better understanding of the sensing mechanism of those simple glucose sensors was reported afterwards[60], attributing it to the Faradaic current at the gate electrode generated by H_2O_2 production. Since then, different configurations and functionalizations of the channel and gate electrodes have been studied to improve sensing characteristics. Even transistors in which the channel, source, drain and gate electrodes were made from conducting polymer[61,62] were able to detect glucose down to micromolar concentrations. More sophisticated systems have emerged from the incorporation of nanomaterials or ionic liquid on gate electrode functionalization, which has greatly improved the sensitivity of such devices and has broadened the ranges of detection of different analytes determining the course for OECT implementation.

For example, Liao et al.[63] prepared gate electrodes by anodization of a Ti wire forming TiO_2 nanotube arrays (TiNTAs). PtNPs were then electrodeposited on the surface. The electrode was immersed in a GOx solution and then covered by a layer of Nafion. NP and enzyme loading was higher than in a conventional Pt gate electrode due to the porosity of the TiNTAs. PEDOT:PSS was used to bridge the source and drain electrodes. Figure 4 shows the current response of the OECT device to successive additions of a) H_2O_2 and b) glucose. The insets show the normalized current responses (NCR, which represents the drain current before and after the addition of H_2O_2/glucose at the concentration of interest) as a function of their respective concentrations. A linear range was obtained from 1 to 500 μM of H_2O_2 with a detection limit of 1 μM. The same trend was observed when GOx and Nafion layers were incorporated to use glucose as the target. A wider linear range was obtained (from 100 nM to 5 mM) with a sensitivity of 0.009 NCR decade^{-1}, with a lower detection limit of 100 nM. With the enzyme-Nafion configuration, selectivity tests were conducted by adding AA and UA as the main interferences in human body fluids without producing any significant interference.

[Figure 4 here]

The same electrode configuration was tested amperometrically in order to compare the two different configurations. OECT resulted in better sensitivity and detection limits (100 μM amperometrically versus 100 nM with OECT). Real samples were also tested by diluting human serum and were validated against a hospital-used blood sugar instrument, achieving a relative error of less than 4%.

The simultaneous detection of glucose and lactate was achieved by Ji et al.[64] (Figure 5), who combined two transistor systems with two different oxidase enzymes in one microfluidic chip with a dual channel. The H_2O_2 produced from each oxidase reaction did not diffuse and there was no crosstalk between the two sensors. While PEDOT:PSS was spin-coated in both transistors channels, the gate modifications consisted of the deposition of poly (N-vinyl-2-pyrrolidone)-capped PtNPs with the subsequent drop-casting of a Nafion layer and enzyme immobilization using a chitosan matrix, both for GOx and lactate oxidase (LOx). The microfluidic chip had a detection time of around 1 min and an LOD of 1 μM for glucose and 10 μM for lactate. It was used to determine salivary glucose concentrations for both non-diabetic and diabetic patients in a 10-fold dilution. Glucose results were compared to those obtained by their portable prototype for real-time glucose determination, which was successfully validated. Although neither the validation of the lab results nor the prototype results compared to standard glucose determination methods were reported, the integration of the microfluidic transistor into a portable device linked to a smartphone via Bluetooth was considered to have great potential for real-time, non-invasive glucose sensing applications.

[Figure 5 here]

Apart from being the key component on the bridging source and the drain channel, PEDOT:PSS can also be included in gate modification as the transducer element, as Pappa et al. showed [65]. A multianalyte biosensing platform was built by functionalizing the gate electrode by mixing PEDOT:PSS with polyvinyl alcohol (PVA) in order to introduce hydroxyl groups where the corresponding oxidase enzyme (either GOx, LOx or cholesterol oxidase (ChOx)) was covalently attached. A ferrocene-chitosan hybrid electron mediator was added to the gate electrode to improve the efficiency of the electron transfer. The final Au/PEDOT/oxidase/ferrocene-chitosan gate, together with the spin-cast PEDOT:PSS on the channel, provide LODs of 10 µM, 50 µM and 10 µM for glucose, lactate and cholesterol respectively, and linear ranges going from 0.02 to 1 mM for glucose, from 0.1 to 2 mM for lactate and from 10 to 700 µM for cholesterol. The multianalyte platform consisted of a transistor microarray for the selective determination of the abovementioned three components, integrated together with a "finger-powered" microfluidic system, and was used to simultaneously determine glucose, lactate and cholesterol content in human saliva. As in the previous example, although the concentrations obtained were not validated against other conventional methods, the OECT array with finger-powered microfluidics resulted in a potential platform for use as a non-invasive, portable multianalyte device for point-of-care diagnostics (Figure 6).

[Figure 6 here]

Another example in which PEDOT:PSS was used both for gate and channel modification is in the study conducted by Welch et al. [66]. PEDOT:PSS was spin-coated onto the Pt gate electrode and the surface activated by plasma oxidation. Poly(glycidyl methacrylate and poly(2-hydroxyethylmethacrylate) (PGMA:PHEMA) brushes were immediately polymerized on the PEDOT:PSS surface by means of the “grafting” method using an atom transfer radical polymerization technique. GOx was covalently attached to the brushes. Electrochemical measurements were taken using a phosphate buffer (0.12 M). The device exhibited a strong response at low concentrations and an even higher response at concentrations ranging from 3.84 to 100 mM. This range covers the physiological and pathological glucose levels in human blood, saliva and brain tissue.

Furthermore, Liao et al.[67] used two different conducting polymers in the same OECT device to detect uric acid, cholesterol and glucose through H_2O_2 production by their respective oxidase enzymes. PEDOT:PSS was deposited as the sensitive part of the channel and PANI worked as the transducer element included in the gate modification. The platinum gate electrode was modified by a bilayer made of graphene oxide sheets (GO) and Nafion with graphene flakes. The gate modification was already useful for repelling positively charged molecules by the protonated conducting polymer and the anionic electroactive species by the acidic sulfonic groups of Nafion. In addition, selectivity towards the targets was achieved with the subsequent enzyme immobilization (either uricase (UOx), CHOx or GOx) by means of a glutaraldehyde cross-linker. UA sensors showed an LOD of 10 nM with a sensitivity of 147 mV decade^{-1} and a linear range of 100 nM to 500 μM. Comparatively, amperometric measurements with the same electrode configuration reached a limit of detection of around

3 μM, which was two orders of magnitude higher than the OECT configuration. The cholesterol sensors showed a limit of detection of 100 nM and glucose sensors showed an LOD of 30 nM. UA and glucose were also tested by adding saliva samples to PBS solution, and achieved consistent results for potential real-world applications.

A comparative study of the analytical parameters mentioned in the reported OECTs is listed in Table 2. In general, OECTs can achieve lower limits of detection than amperometric methods due to the fact that a small change in the gate voltage of an OECT can be reflected as a significant variation in the channel current, making these devices highly sensitive biosensors. However, as we have concluded from the studies reported in this review, most transistors are fabricated based on thermal evaporation and photolithographic techniques. Additionally, some of them require UV or plasma treatment, which must be taken into consideration in terms of a final application, as some of these techniques are expensive and time-consuming. The fabrication steps may therefore hamper the practicality of using OECT in low-cost, portable devices. This impracticality is also reflected in the number of studies that have tested their devices in real samples for real-world applications. Most of the devices listed in Table 2 were analyzed as proof-of-concept for the detection of glucose (or other molecules) by means of highly sensitive sensors using an H_2O_2 determination strategy, and wider applications have been suggested using other enzymes by modifying the gate electrode with the incorporation of other (nano)materials. Nevertheless, the demand for small portable sensor devices has driven the quest for other suitable substrates, such as fabrics or PET [68], and simpler fabrication techniques, such as screen printing or inkjet-printing[69] to allow for the low-cost mass fabrication of OECTs.

Summary and conclusions

The main advantage of using conducting polymers as charge-transfer media is that they offer the possibility of tailoring their characteristics to adapt to the surrounding conditions. The chemical or electrochemical polymerization or the media in which they operate can help modulate the final performance of target detection and define the adhesion of the film to the electrode surface, even becoming a serious limitation for sensor development. In most of the works cited in this review, modified electrodes were compared to bare electrodes. The main electrochemical techniques used to characterize the electroactivity of the electrode surface were cyclic voltammetry and electrochemical impedance spectroscopy. In most cases, the incorporation of the conducting polymer improved the electrocatalytic activity towards hydrogen peroxide in comparison to bare electrodes, or in comparison to other modifications without the presence of the conducting polymer. Consequently, the main contribution found in the use of conducting polymers in electrode modification is the increase in redox peak currents, leading to a faster electron transfer from the analyte to the electrode surface. The use of conducting polymers as a transducer material in hydrogen peroxide sensors provides an additional conduction path to the already conductive electrode surface, which theoretically results in enhanced H_2O_2 determination. Nevertheless, their use as a sensing material has not yet been demonstrated, since all the above mentioned examples report conducting polymers as the transducer part to facilitate or accelerate the electron transfer propagation in the devices, while the catalytic activity towards H_2O_2 is produced by the incorporated mediators or materials taking part in the blends and composites. The characteristics of conducting polymers increase the output signal in response to the chemical reaction taking place at the electrode: the determination of hydrogen peroxide is never due to the change in the polymer redox properties, but rather to the electrode redox properties. In most cases, the comparison of analytical parameters for H_2O_2 detection with and without conducting polymers has not been reported, although for OECTs this comparison would not be meaningful since it is the basic component of the channel. Thus, even if the increase in the sensitivities obtained for those sensors or the wider linear ranges reached have been demonstrated, the quantification of this improvement has yet to be addressed.

In general, although a great number of works have focused on the use of conducting polymers for sensing applications, their sensing mechanisms are not yet fully understood. Some potentiometric and chemiresistor studies have also been found in the literature[70–72], but the need to control the redox state as well as the acid-base and ionic equilibria simultaneously has paved the way for the progress of CP-based sensors towards amperometric devices or OECTs, since they operate by amplifying the signal due to the catalytic properties, or as immobilizing agents due to their biocompatibility[73]. Some of the intrinsic limitations of CPs, such as pH dependence, over-oxidation and influence of the electrolyte nature, must be addressed in order for CP-based sensors to become practical in real-world applications and to overcome the challenging issues of market access.

The applications of each conducting polymer also pose some limitations due to their intrinsic properties. For instance, PPy conductivity is irreversibly destroyed upon exposure to H_2O_2 [74,75], which hinders its use where hydrogen peroxide is present in the vicinity the sensor. Conversely, PANI does not react with H_2O_2, which facilitates its application in the field, although its electrochemical activity decreases at pH above 5 [74]. However, some of these limitations have been overcome over time by the use of conducting polymer derivatives and by tailoring their characteristics through the use of composite films [75] which extend, for instance, the operational pH range. It is not surprising, therefore, that the majority of the amperometric electrodes and organic electrochemical transistors, as mentioned in this review, incorporate PEDOT in their systems because of its environmental and thermal stability, its high electroconductivity and good film-forming properties. It is worth mentioning that PEDOT:PSS has also attracted attention due to its biocompatibility for the immobilization of biomolecules, as demonstrated by Richardon-Burn et al.[76]. It is for this reason that PEDOT is mainly used in combination with oxidase enzymes in most sensor configurations to achieve highly sensitive transistors.

Although hydrogen peroxide plays an important role in many different areas, as mentioned at the beginning of this review, most of the reported H_2O_2 sensors focus on healthcare applications, and especially on glucose determination. The increasing prevalence of diabetes mellitus, which an estimated 8.4% of the world's population currently suffers from [77], and the growing demand for means of monitoring and controlling glucose levels, has obviously driven the analytical field towards glucose detection in order to generate simple, low-cost, sensitive and reliable sensors. Although the advantages of the incorporation of CPs for the electrochemical detection of hydrogen peroxide have been significantly demonstrated, the implementation of CPs may require their integration in a portable, low-cost substrate such as paper[78] in order to make their contribution a real breakthrough in the field.

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Declaration of interest statement

Conflict of interest The authors declare that they have no conflict of interest.

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Tables

Table 1. Comparison of analytical performance of some H_2O_2 conducting polymer-based amperometric sensors used in real samples.
R = recovery; RSD= relative standard deviation

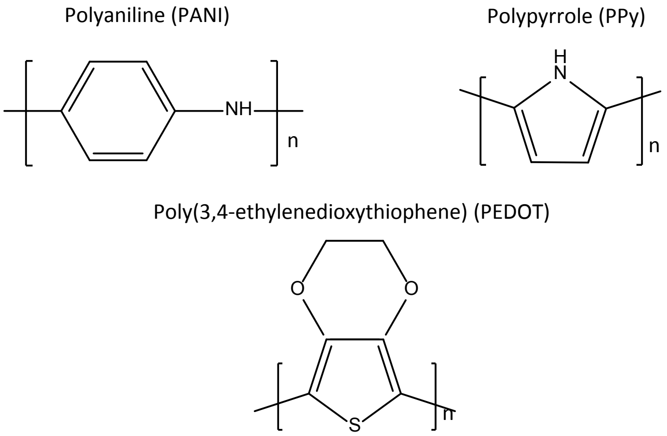
Working Electrode	**Linear Range (mM)**	**Sensitivity (nA μM^{-1} cm^{-2})**	**Limit of detection (μM)**	**Sample validation**	**Accuracy**	**Ref.**
H_2O_2 as target						
SPCE/PAA(DS)	0.005 – 3	72.4	1.43	commercial hair lightener and antiseptic. R= 98-100 %	reproducibility RSD=6.2 %, repeatability RSD=3.4 %	[32]
GCE/Cs micelle/PEDOT:PSS/HRP/Nafion	0.0000001 – 0.01		0.00003	commercial disinfector diluted 10.000.000-fold	70 % signal after 4 weeks reproducibility RSD=3 %	[33]
GCE/PEDOT/PBNPs	0.0005 – 0.839		0.16	milk R=98-102.7 %	90.8 % signal after 4 weeks reproducibility RSD=4.5 %	[35]
SPGE/PEDOT:PSS/ rGO/AuNPs/HRP	0.0005 – 0.4	677	0.08	tap water and bovine milk R=99 %	94 % signal after 1 week reproducibility R=7.8 %	[36]
GCE/Ppy-PMSA-g-4ABS	0.0008 – 0.46		0.32	rainwater and diluted human serum R=97.8-103.5 %	92 % signal after 4 weeks repeatability RSD=3.2 % reproducibility RSD=4.34 %	[41]
H_2O_2 as byproduct						
Graphite/GA/PCPy-Gox	0.1 – 15		39	human serum 10-fold dilution R=99.17-106 %	95.3 % signal after 4 weeks reproducibility RSD=5.21 %, repeatability RSD=1.83 %	[42]
ITO/(PEDOT-PdBI-co-HKCN)/Gox	0.25 – 2.5		176	coke and juice R=96-99 %	87 % signal after 8 weeks	[43]
Pt/PEDOT/PAA/Gox	0.96 – 30		290	diluted grape juice and honey R=5 %	30 days stability	[44]
Graphene/PFLO/AuNPs-Gox	0.1 – 1.5	7.357	81	commercial lemon soda and milk relative errors 2.1 and 3.6	repeatability RSD=3.35 %	[48]
GCE/ILs/PEDOTM-MWCNT-COOH/GOx	0.00006 - 2	0.0895	0.015	diluted animal serum and human serum and urine R=96.8-101.2 %	98.3 % signal after 4 weeks reproducibility RSD=0.73 % repeatability RSD=1.01 %	[52]

Table 1. Comparison of analytical performance of selected conducting polymer-based OECT based on H_2O_2 detection for different targets.

CP on electrode	Additional materials	Linear Range (µM)	Limit of detection (µM)	Sample validation	Observations	Ref.
PEDOT:PSS on channel	PtNPs/GOx/Nafion	0.1 to 5000	0.1	diluted human serum RSD <4%	90 % signal after 10 days. Reproducibility RSD=4.1 %	[63]
PEDOT:PSS on channel	PtNPs/Nafion/GOx-chitosan		1	simultaneous detection by 10-fold diluted human saliva	validated portable glucose prototype	[64]
	PtNPs/Nafion/LOx-chitosan		10			
PEDOT:PSS on channel	PEDOT:PSS/chitosan-ferrocene/GOx	20 to 1000	10	Simultaneous detection in human saliva	microfluidic device	[65]
	PEDOT:PSS/chitosan-ferrocene/LOx	100 to 2000	50			
	PEDOT:PSS/chitosan-ferrocene/ChOx	10 to 700	10			
PEDOT:PSS on channel and gate	PGMA:PHEMA brushes/GOx	--	0.95		100% signal after 100 days	[66]
PEDOT:PSS on channel. PANI on gate	UOx-GO/Nafion-graphene		0.01	Saliva in standard addition method		[67]
	CHOx-GO/Nafion-graphene		0.1			
	GOx-GO/Nafion-graphene		0.03	Saliva in standard addition method		

Figures

Figure 1.



Polyaniline (PANI)

Polypyrrole (PPy)

Poly(3,4-ethylenedioxythiophene) (PEDOT)

Figure 2.

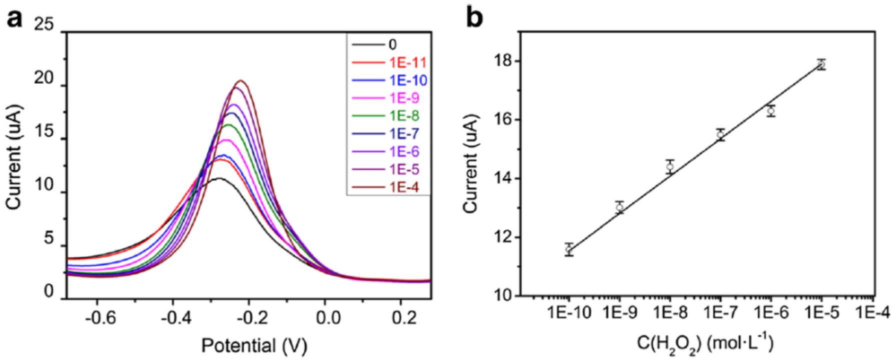
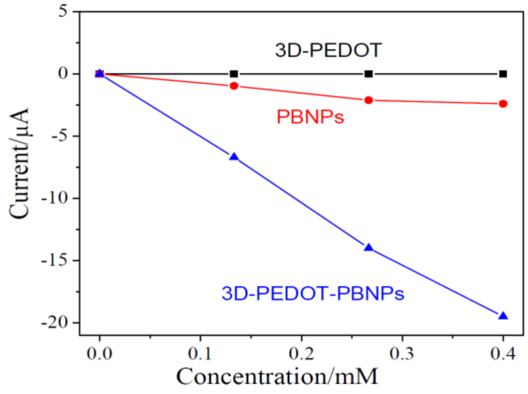


Figure 3.



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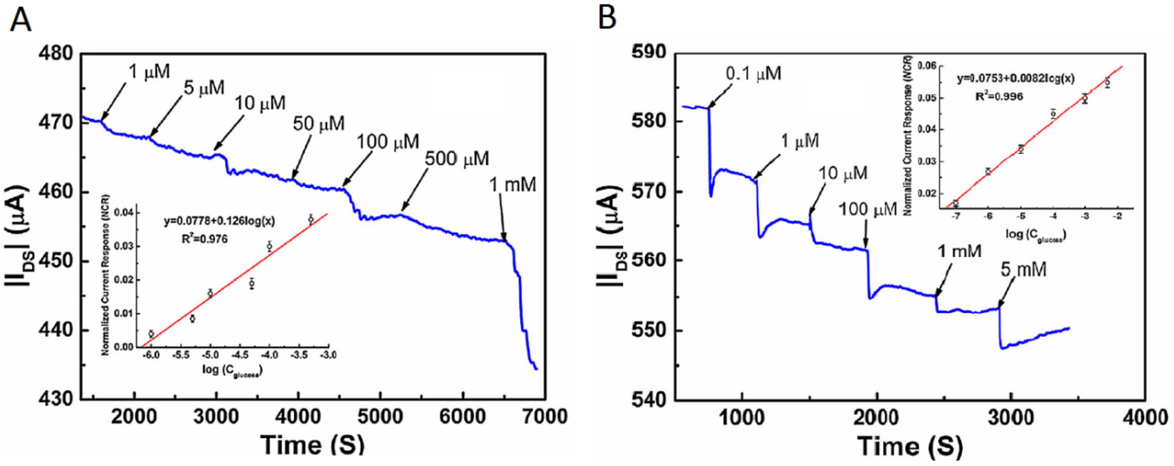


Figure 5.

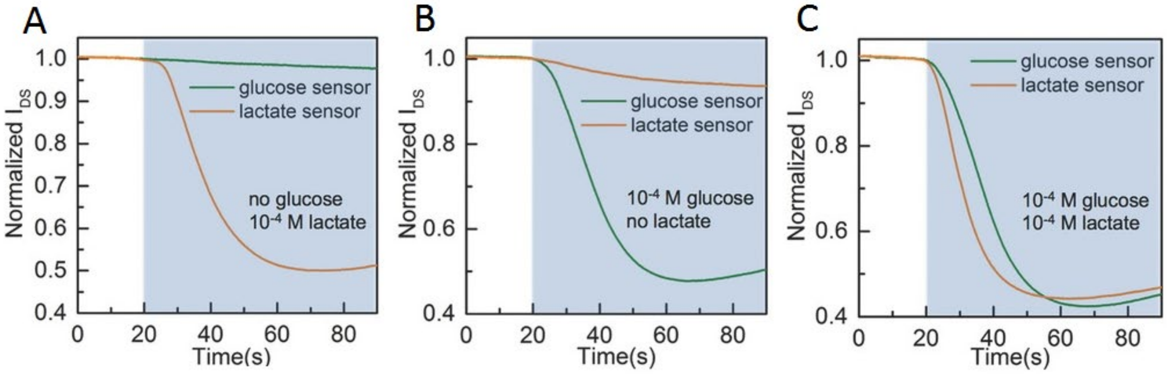


Figure 6.

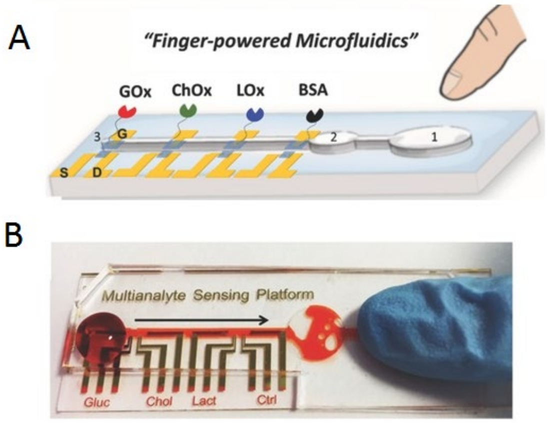


Figure 1. Structures of polyaniline, polypyrrole and poly(3, 4-ethylenedioxythiophene).

Figure 2. A) Differential normal pulse voltammograms for Nafion/HRP/PEDOT:PSS/CS micelle/GCE in 0.1 M PBS (pH 7) with different concentrations of H_2O_2. **B)** Calibration curves corresponding to the response recorded on the Nafion/HRP/PEDOT:PSS/CS micelle/GCE biosensor versus the concentration of H_2O_2. Reproduced with permission from ref. [33].

Figure 3. Peak current responses for 0, 0.13, 0.26 and 0.4 mM H_2O_2 on the indicated modified electrodes. Adapted from ref. [40].

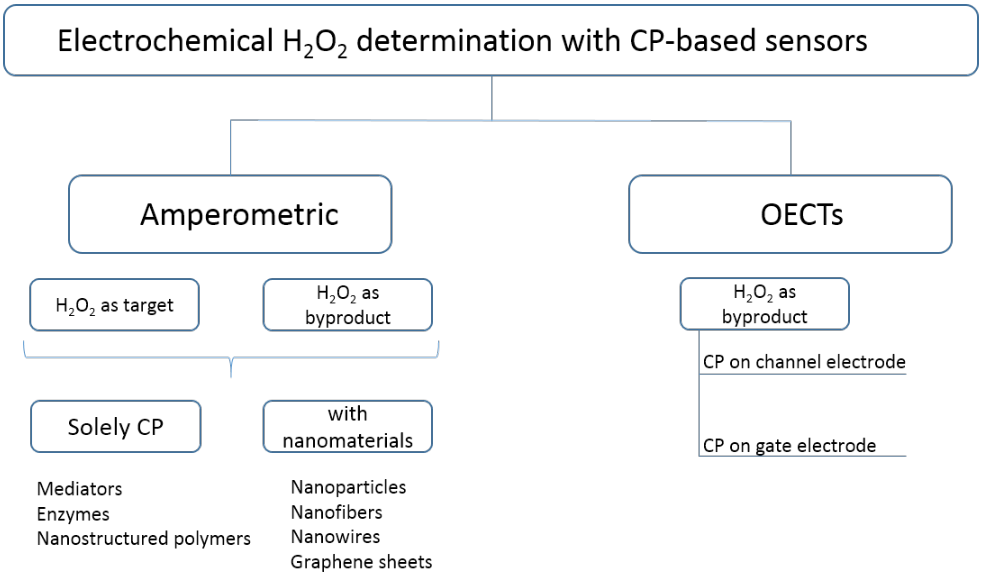
Figure 4. Current responses of the OECT to successive additions of **A)** H_2O_2 and **B)** glucose. Insets: NCR as a function of analyte concentration. Adapted with permission from ref. [63].

Figure 5. Normalized current response after addition of analyte **A)** 10^{-4} M lactate, **B)** 10^{-4} M glucose and **C)** 10^{-4} M lactate and 10^{-4} M glucose simultaneously, at V_{DS} -0.2 V and V_G 0.5 V. Adapted with permission from ref. [64].

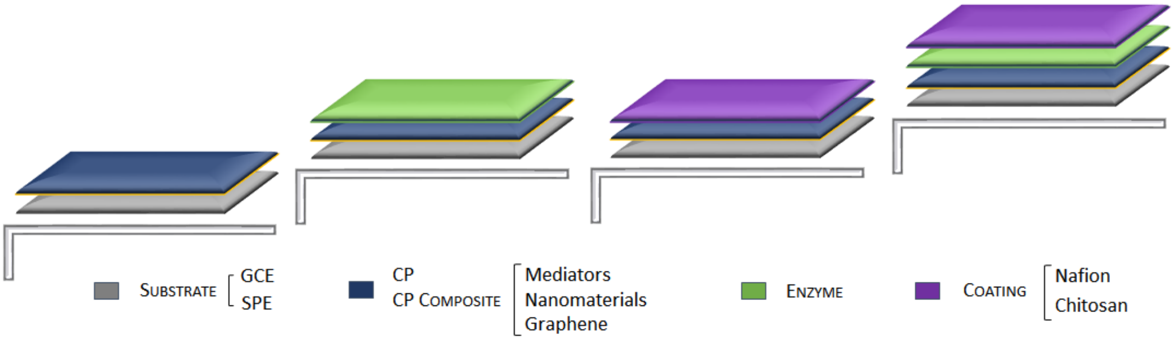
Figure 6. **A)** Schematic illustration of the embedded "finger-powered" microfluidic biosensing platform **B)** Photograph of the device, showing the red solution that was pressure-driven from the inlet through the sensing areas, as indicated by the arrow. Adapted with permission from ref. [65].

Schemes

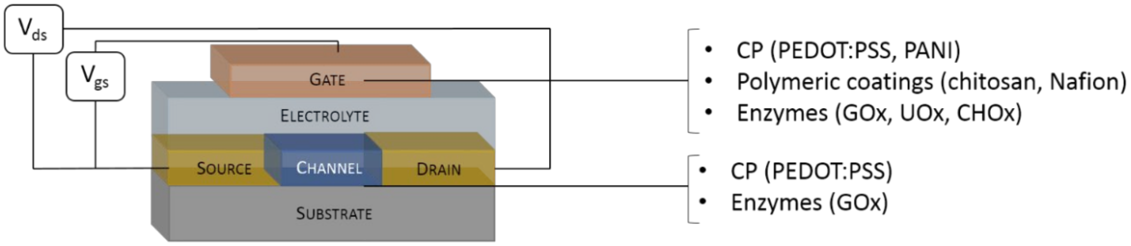
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 1. The main electrochemical detection of H_2O_2 using CPs as a component of the device.

Scheme 2. The main combinations of components of amperometric sensors: substrate, CP-composite, enzyme and coating layer.

Scheme 3. A schematic of an OECT configuration