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Conducting Polymer Based Electrochemical Biosensors

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x
www.rsc.org/

Conducting polymers (CPs) - based electrochemical biosensors have gained a great attention as such biosensor platforms are easy and cost-effective to fabricate, and provide a direct electrical readout for the presence of biological analytes with high sensitivity and selectivity. CPs material themselves are both a sensing element and a transducer of the biological recognition event at the same time, simplifying sensors designs. This review summarizes the advances in electrochemical biosensors based on CPs. Recognition probe immobilisation techniques, transduction mechanisms and detection of various target biomolecules have been discussed in detail. Efforts in miniaturisation of CP-based electrochemical biosensors and fabrication of sensor arrays are also briefly reviewed.

1. Introduction

A biosensor is an analytical device which consists of a biological recognition element and a transducer. Biosensors play an increasingly important role in healthcare, environmental monitoring, food quality monitoring and biosecurity. They enable detection of genetic abnormalities, pathogens, viruses, toxins and biological markers of disease [6]. When the recognition probe interacts with a target analyte, this interaction causes a signal which can be measured via the transduction of, for example, optical [10], electrochemical [11] or thermal [16] signals. *Electrochemical biosensors* have received significant attention as they can provide sensitive, selective, cost effective and rapid solutions. Examples of electrodes used in electrochemical biosensors are gold nanoparticles (Au), carbon (C), electrically conducting polymers (CPs) and carbon nanotubes (CNTs) [19]. Alternatively, composites of CPs with graphene[20] and metallic nano particles[21] have also been employed for biosensing. CPs differ from other materials due to their sensitivity towards chain conformation alterations, arising from their unique π orbital structure [22, 23]. Since the recognition element attachment and target hybridisation can cause perturbations in the chain conformation of CP films, a binding event can be converted to an electrical read-out providing fast, label free and sensitive measurements. This feature makes CPs excellent candidates as biosensors. This review focusses on CP-based electrochemical biosensors and will review probe immobilisation, transduction mechanisms and detection principles. In addition, miniaturisation of CP-based sensing elements is discussed, as well as fabrication of CP-based biosensor arrays.

2. Conducting Polymers

Conducting polymers (CPs) (also known as electrically conducting polymers (ECPs) or intrinsically conducting polymers (ICPs)) are materials which are organic in nature and yet electrically conductive[22]. Unlike conventional organic polymers, they hold unique properties such as electrical conductivity, high electron affinity and redox activity. Following their discovery, Alan G. MacDiarmid, Hideki Shirakawa and Alan J. Heeger were awarded the Nobel Prize in Chemistry in year 2000. The structures of the main conducting polymers poly(acetylene), poly(3,4-ethylenedioxythiophene) (PEDOT), poly(thiophene), poly(p-phenylene vinylene) (PPV), poly(pyrrole) and poly(aniline) are given in **Figure 1.**

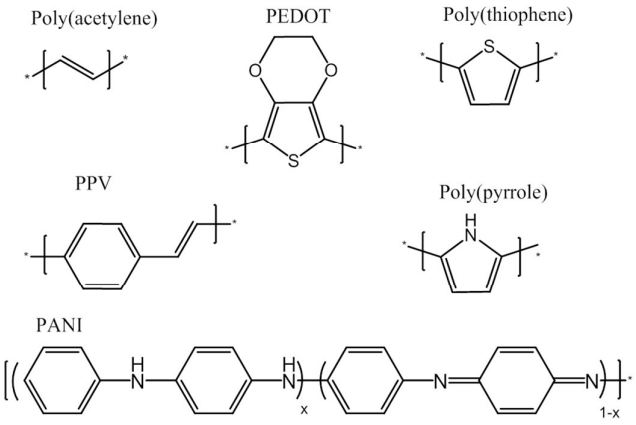

Figure 1: Structures of poly(acetylene), poly(3,4-ethylenedioxythiophene) (PEDOT), poly(thiophene) (PTh), poly(p-phenylene vinylene) (PPV), poly(pyrrole) (PPy) and

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poly(aniline) (PANI) [22].

Conducting polymers synthesis can be done chemically or electrochemically and has been reviewed elsewhere [24-26]. Oxidation of the CPs leads to p-doping and formation of self-localised charge carriers called positive polarons and positive bipolarons within the main polymer chain, whereas reduction leads to n-doping and formation of negative self-localised charge carriers called negative polarons and bipolarons [27].

In the last decades there has been a tremendous amount of research in applications of conducting polymers in supercapacitors [28, 29], light emitting diodes (LEDs) [30], field effect transistors (FETs) [31], solar cells [32, 33], actuators [34-38] and biosensors [6, 39, 40]. Such an abundance of applications is facilitated by the ease of tailoring CP properties. For example, both CP's monomers and the polymers themselves can be functionalized with various groups to tailor their properties. Addition of substituents not only allows easier processing and added functionality, but also may improve the electronic properties of the main polymer chain and increase the electrical stability [41]. Another approach to improve the processing of CPs is mixing them with charged and water soluble polyelectrolytes such as poly(4-styrenesulfonate) (PPS) [42]. A very well-known example of this is PEDOT-PSS which is essentially a macromolecular salt consisting of positively charged PEDOT and negatively charged PSS [43]. Functionalities on CPs, such as carboxylic acid (-COOH) and amine ($-NH_2$) have been utilised in biosensors based on CPs as these functionalities often serve to covalently attach biological molecules acting as recognition probes [40]. This and other biological probe immobilisation techniques are discussed in sections 3.1.

3. Conducting Polymers- Based Biosensors

In a CP based electrochemical biosensor, the recognition element is immobilised on the CP electrode. Common recognition elements are oligonucleotides (ONs), aptamers, antibodies and enzymes. Schematics of elements and architectures of CP based biosensors can be seen in **Figure 2.** Immobilisation procedures, target molecule types and measurement techniques are discussed in detail in the following sections.

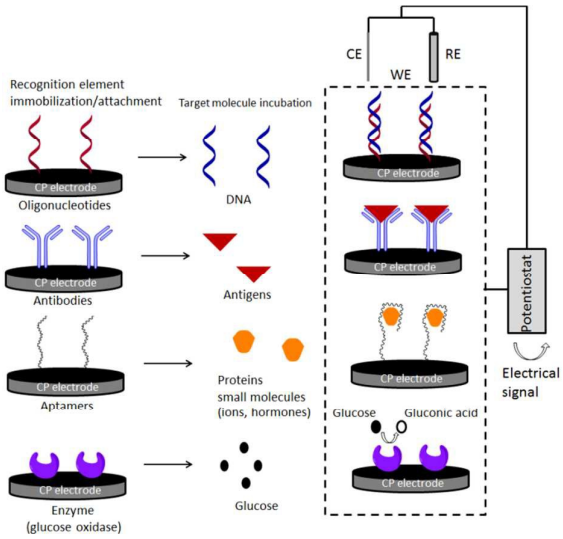

Figure 2: Recognition elements, target molecule types and general transducer architecture of CP-based biosensors.

3.1. Recognition element immobilisation techniques

Immobilisation of recognition elements is a central step for reliable and reproducible sensor fabrication. Choosing the right immobilisation technique requires in-depth knowledge of the properties of the recognition probe. Ideally, the immobilisation process should be efficient and simple and also not cause damage to the activity of the recognition probe. Electrochemical entrapment, covalent attachment, physical adsorption and affinity interactions are all commonly used methods to immobilise recognition elements on or within the CPs sensing films [44].

Physical adsorption exploits the interactions between the CP surface and the biomolecules. As the CPs can carry significant charge, electrostatic forces between the cationic CPs and anionic biomolecules (in particular ONs) play a central role. However, other interactions are also contributing, especially for adsorption of antibodies and other proteins. These interactions include hydrophobic forces and well as *Van der Waals'* forces [45] **(Figure 3A)**. Control parameters for an efficient adsorption include temperature, pH, solvent type and net charge of the bioprobe [46, 47]. Adsorption based CP biosensors were first introduced by Dicks et al. for the adsorption of glucose oxidase on PPy [48] and has later been used for immobilization of other enzymes [49-51] and ONs [52]. The main advantage of physical adsorption is that it does not require any functionalization of the monomers. However, due to the relatively weak forces involved in the adsorption of in particular DNA, probes may leach out from the interface over time [53]

To increase the binding efficiency, Umana & Waller [2] and Foulds & Lowe [54] pioneered the electrochemical entrapment technique whereby glucose oxidase was incorporated into PPy films via electropolymerisation of pyrrole in the presence of the enzyme. This resulted in a matrix of CP and glucose oxidase **(Figure 3B)**. PPy is often deemed the most suitable CP for this technique as it can be electropolymerised in aqueous solutions at neutral pH and by applying low potentials. In the following years, similar methods have been employed for the immobilisation of ONs [3], antibodies [55], other enzymes [56-58] and even cells [59-61] within sensor films. The technique provides straightforward and prolonged immobilisation

compared to physical adsorption. However, as the probes are, at least partially, buried within the bulk polymer film the target accessibility may not be efficient. Another disadvantage of entrapment is that, it is most suitable for water soluble monomers and thus not applicable for a wide range of CPs.

Covalent bonding of recognition probes to CP electrodes commonly utilises N-hydroxysuccinimide/1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (NHS/EDC) chemistry to couple carboxylic acid (–COOH) to amine (-NH_2) groups on the CP and probe respectively [62] **(Figure 3C)**. As the attachment is performed post-polymerisation, it is versatile, non-invasive and can be tuned according to the properties of the probe molecules. It provides robust binding between recognition probes and CPs and also increases the target accessibility as the immobilisation occurs at the CP surface. Importantly, the coupling procedure is performed in mild aqueous conditions, whereby the integrity of the probe can be preserved.

A range of enzymes, such as urease (urea detection) [63], pyruvate oxidase (phosphate ion detection) [64] and glucose oxidase (glucose detection) [65] have been covalently attached onto different CP films. Rajesh et al showed that a porous morphology of the CP film improved the enzyme loading and that the strong covalent linkage increased the stability of the enzyme immobilisation, giving a useful sensor life of up to 2 months [63]. This technique has also been utilised to attach ONs to carboxylic acid containing CPs such as 3-pyrrolylacrylic acid (PAA) [5, 9, 66, 67], 5-(3-pyrrolyl) 2,4-pentadienoic acid (PPDA) [68], and 3-pyrrolylpentanoic acid (PPA) [5, 68]. In these studies, the carboxylic acid functionalized monomers were co-polymerized with pyrrole, followed by attachment of the –NH_2 functionalized probe (ONs) via NHS/EDC chemistry. By using un-functionalized pyrrole as a spacer, the density of the probe ONs and resulting sensor responses can be optimized [67].

Affinity based attachment is an alternative to the covalent attachment. This also provides strong binding, while reducing the need for chemical reagents. Amongst various types of affinities, the avidin-biotin system has received a lot of interest due to its very specific and strong interaction [69]. Biotin can also bind with strepavidin and NeutrAvidin. The most commonly used attachment approach is called the biotin sandwich technique. The working mechanism is based on 1) electro-deposition of biotinylated monomers onto the electrodes, 2) introducing avidin and building avidin-biotinylated polymer bridges, 3) anchoring of biotinylated recognition probes onto avidin-biotinylated polymer composites **(Figure 3D)**. With the highest known non-covalent binding constant (K_d = 10^{-15} M) [70] the bond formation between biotin and avidin is not only straightforward but is also highly stable over a wide pH and temperature range and it is resistant to most organic solvents.Using affinity interactions, ONs [71, 72], antibodies [7], enzymes [73, 74], peptides [75] and aptamers [76] have all been successively immobilized onto CP electrodes. Several previously published reviews provide detailed mechanisms used in recogniton probe immobilisation methodologies [77, 78]

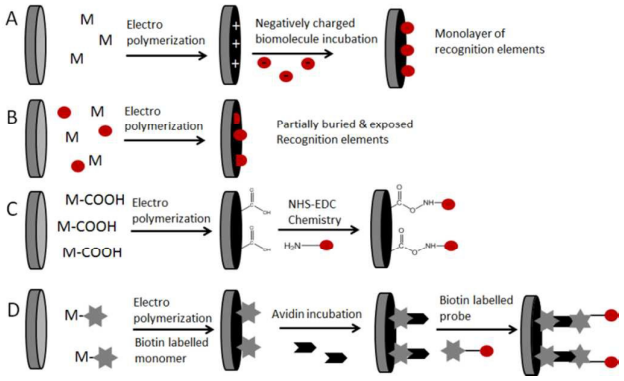

Figure 3: Schemes of bioprobe immobilisation strategies: A) physical adsorption, B) electrochemical entrapment, C) covalent attachment, D) avidin-biotin affinity interactions (M is monomer, M-COOH is carboxylic acid functionalized monomer, red dot is a probe (or recognition element, e.g. antibody, ON, enzyme) and star is biotin.

3.2. Transduction Mechanisms

In electrochemical biosensors, recognition events are converted into electrical read-out during the so-called transduction step. CPs are unique materials as they are actively involved in the transduction mechanism itself. When a CP sensor is introduced to a solution containing target molecules, specific binding between recognition element and target molecule alters the electrical and optical properties of the CP, which in turn can be monitored via electrical read-out techniques such as cyclic voltammetry (CV), amperometry or electrochemical impedance spectroscopy (EIS) [6, 19, 79]. The choice of measurement method mostly depends on the oxidation and reduction potentials of the CP used in the sensor. If the polymer is electroactive at low potentials like PPy, then EIS is a sensitive method to monitor the binding events (target to probe) in the presence of the redox indicators. The read-out can be based on oxidation/reduction of the CP electrode itself (direct signal) or accompanying redox indicators (indirect signal) such as potassium ferri cyanide/ potassium ferro cyanide $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$, methylene blue (MB) or ruthenium complexes.

3.2.1. Cyclic Voltammetry

Cyclic voltammetry probes a potential range at a determined scan rate, where oxidation/ reduction potentials and currents of CPs can be monitored in detail. Therefore, it can be used with a wide range of CPs and recognition elements in the presence or absence of redox indicators. In DNA sensing, both probe attachment and target sequence hybridisation alters the electron flow through the backbone of the polymer. For instance, Peng et al. co-polymerised pyrrole and 4-(3-pyrrolyl) butanoic acid (PBA) and covalently attached the –NH_2 substituted ONs onto the –COOH of poly(Py-*co*-PBA). The functionalised electrode was then introduced to different concentrations of complementary target [13] **(Figure 4 A-B)**, target hybridisation decreased the oxidation current dramatically and also resulted in a positive shift of the oxidation potential [80]. This phenomenon is attributed to the fact that an CP with such a rigid and bulky pendant groups such as double stranded DNA requires more energy to oxidize. Taleat et al. extended this approach by using a carboxyl-functionalized aniline, poly(*o*-aminobenzoic acid) (PABA), to detect MUC1, an antigen which has an important role in the tumorigenesis of several cancer types [81]. Following the PABA

deposition, MUC1 monoclonal mouse antibody (Ab1) was covalently attached to the surface via NHS-EDC chemistry. The obtained sensor was introduced to a MUC1 target solution where MUC1 selectively bound to the surface immobilised Ab1. In conventional biosensing experiments, the electrical read-out is obtained at this step (after the binding of target molecules). However, in this study, methylene blue (MB) was used as the electrochemical indicator where MB selectively binds to the guanine bases of DNA. Therefore, the PABA/Ab1/MUC1 electrode was incubated with a solution of an aptamer (APT) which was specifically designed to bind MUC1 antibody. Following the configuration of PABA/Ab1/MUC1/APT, electrode was exposed to methylene blue solution, which bound the aptamer. With increasing concentrations of MUC1 target, more aptamer bound to the surface and interacted with the MB. Thus, the oxidation signal of MB in the CV measurements increased as well **(Figure 4C)** [81].

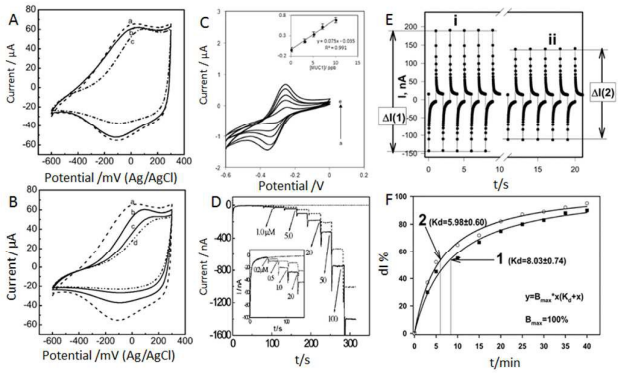

Figure 4: A) Cyclic voltammograms of poly(Py-*co*-PBA) based DNA sensor (a) after incubation in 880 nM of non-complementary ON solution, (b) in a solution of 3.5 nM complementary ON (c). (B) CV of poly(Py-*co*-PBA) (a) and after incubation in complementary ON at concentrations of 3.5 nM (b), 8.8 nM (c) and 87.6 nM (d). Scan rate: 100 mV/s in PBS [13]. *Reprinted with permission from Peng et al., Copyright 2005 Elsevier.* C) CVs of aptamer attached -PABA electrode (a) without MUC1 and after incubation with 3, 5, 7 and 10 ppb MUC1 concentrations (from b to e) [81]. *Reprinted with permission from Taleat et al., Copyright 2014 Elsevier.* D) Changes on the steady state current signal of GlOx/CP/Pt electrode before (solid line), after (dashed line) addition of glutamate with a concentration range of 0.2 µM to 100 µM [82]. *Reprinted with permission from Rahman et al., Copyright 2005 American Chemical Society.* E) Pulsed amperometric signals of ssDNA/Ppy-modified electrodes (*i*) before; (*ii*) after 30 min incubation of in the target DNA-containing samples. Potential pulse profile 1 s for 600 mV and 1 s for 0 mV vs Ag/AgCl. F) Normalized sensor response of ssDNA/PPy-modified electrodes 1) without mixing, 2) with mixing the 10 ngmL^{-1} target solution [83]. *Reprinted with permission from Ramanaviciene &Ramanavicius, Copyright 2004 Springer.*

3.2.2. Amperometry

Amperometry utilises applying a set potential for a period of time while recording the current. It has been widely employed for the detection of molecules such as proteins, biomarkers and particularly in enzymatic immunosensors [51, 54, 82, 84-86]. A noteworthy example is that by Rahman et al [82], where a functionalized conducting polymer of 5, 2':5',2''-terthiophene-3'-carboxylic acid was electrodeposited onto a Pt micro electrode (25 µm). Glutamate oxidase (GlOx) was immobilised to the CP micro electrodes via covalent attachment with the aim of detecting glutamate, the major excitatory transmitter in the human nervous system. The obtained sensor efficiently detected the glutamate down to 0.1 (±0.03) µM by monitoring of the oxidation of H_2O_2 which was generated by the GlOx at +0.45 V versus Ag/AgCl. The electrode signal in these experiments, was shown to reach steady state current in 10 seconds, and started to decrease gradually with the addition of glutamate **(Figure 4D)** [82]. Another important example of an amperometric sensor for DNA was introduced by Ramanaviciene et al. [83]. In this study, single stranded probe sequences were entrapped into PPy via in situ electrochemical polymerisation. Hybridisation of the target sequence was monitored via pulsed amperometric detection (PAD) **(Figure 4E)** with a detection limit of 0.37 ng mL^{-1} **(Figure 4F)**. For further details on amperometric biosensors based on CPs, the reader is referred to other comprehensive review articles, for example by Vidal et al. [87].

3.2.3. Electrochemical Impedance Spectroscopy

Electrochemical Impedance Spectroscopy (EIS) is a powerful technique to investigate electrical properties of interfaces such as charge transfer resistance and capacitance of conductors and semi-conductors [88]. EIS measures the current response of an electrode when a sinusoidal potential is applied in a wide frequency range [89]. The following relationships hold:

$$V_t = V_0.\sin(wt) \quad (1)$$
$$I_t = I_0.\sin(wt + \emptyset) \quad (2)$$

where V_t and I_t represent the potential and current at time *t*, V_0 and I_0 are amplitude of the potential and current whereas *w* is the radial frequency and Ø is the phase angle shift. In an expression similar to Ohms law, impedance is described as [90, 91]:

$$Z = \frac{V_t}{I_t} \qquad Z = \frac{[V_0.sin(wt)]}{[I_0.sin(wt + \emptyset)]} \quad (3)$$

Usually EIS measurements are carried out in a three terminal electrochemical cell consisting of a working electrode (WE), a counter electrode (CE) and a reference electrode (RE). The WE is either a semi-conductor or a conductor while the CE and RE are most commonly platinum (Pt) wire and Ag/AgCl$_{(aq)}$ electrodes respectively. Saturated Calomel Electrode (SCE), Standard Hydrogen Electrode (SHE) and Palladium Hydrogen Electrode (P-H) can also be used as reference electrodes. In some cases, two-terminal electrochemical cells can be employed, where a Pt wire is connected as both CE and pseudo-reference electrode. The applied potential, whether it is open circuit or direct bias potential, differs by the type of the WE, electrochemical cell arrangement and solution composition [92]. For instance, if the event to be measured is dependent on the solution composition, such as the presence of inhibitors in a metallic corrosion experiment [93] or a bacterial catalyst in a microbial fuel cell [94], and the event is reaching an equilibrium at the WE, impedance can be performed at the open circuit potential (OCP) in either a two [94] or three [93] terminal electrochemical cell. If the measurement is aiming to define dielectric properties of an electrode, then a constant dc potential bias can be applied [95]. In this type of measurement, redox couples such as O_2/OH^-, H_2/H_3O^+ [96] or $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ [97] can be employed and the impedance of the electrodes is measured through the reversible oxidation/reduction of the redox couple at the electrode surface. Potential oscillation, whether it is at OCP or constant bias potential, is generally very small (5-10 mV); thus, the electrical perturbation on the electrode is less than for the previously mentioned measurement techniques, and yet the results

are very informative. EIS provides information regarding the dielectric properties in addition to the charge and mass transport properties of the material in question.

When analysing impedance data of a semiconductor (i.e. CP), usually five major components are taken into consideration: solution resistance (R_s), charge transfer resistance (R_{ct}), double layer capacitance (C_{dl}), constant phase element (CPE) and Warburg impedance (W). One can either fit experimental data into an equivalent circuit diagram [98, 99] (i.e. Randle`s circuit) **(Figure 5A)** or calculate those elements from direct experimental graphs **(Figure 5B)**.

The capacitive element C_{dl} [100] can be calculated either via equivalent circuit diagrams or experimental graphs by using the relationship: $\omega = 1/(R_{ct}\ C_{dl})$ **(Figure 5B)**, where $\omega = 2\pi f$ and R_{ct} is the interfacial charge transfer resistance of the WE, f is the frequency at the top point of semicircle. It must be noted that such an idealised molecular organisation is not always possible. The capacitive behaviour of the double layer is quite sensitive and can easily deviate from ideal capacitance, owing to effects such as potential based perturbations, surface impurities, roughness of the electrode, ionic concentration, and the type of ions present [101]. Thus, the capacitive behaviour under such real conditions often deviates from the ideal C_{dl} and is represented as a constant phase element (*CPE*) [102]. *CPE* is differentiated from the C_{dl} with an exponent α ($0<\alpha<1$) **(Figure 5C)**. For $\alpha=1$ an electrode shows an ideal capacitive behaviour [103]. The Warburg impedance (*W*) is the resistance created by the diffusion of ionic species at the electrode surface [104]. *W* is a frequency-dependent element. At high frequencies, molecules do not have the chance to diffuse far and concentration of redox active species are replenished; thus the *W* impedance is small and the electrochemical reaction is charge-transfer controlled. On the other hand, at low frequencies, the redox active ions are depleted, thus *W* impedance increases and the reaction is dominated by mass transfer as the diffusing ions have to travel further. The charge transfer resistance (R_{ct}) is one of the most critical elements in EIS as it defines the impedance of the WE [104]. It is represented by the diameter of the full semicircle in a Nyquist plot. In equivalent circuit diagrams, the R_{ct} is generally in series with the *W* impedance and in parallel to the C_{dl} or *CPE* **(Figure 5C)**. It can also be calculated from the Nyquist diagram from the intersection of the real impedance with the x-axis **(Figure 5B).**

In the biosensing field, EIS has become a very popular technique to investigate binding events of biological molecules [105-107]. When a sinusoidal potential is applied to a biosensor, current flows through the transducer, sensing element, recognition probe and hybridized/bound target molecules respectively. This fact makes CPs excellent candidates for impedimetric biosensors as the recognition element immobilisation and subsequent target molecule binding/hybridisation alters the intrinsic properties such as charge transfer resistance and the capacitance of CP films. By using EIS these changes can be detected and utilised as an electrical read-out of the biorecognition event.

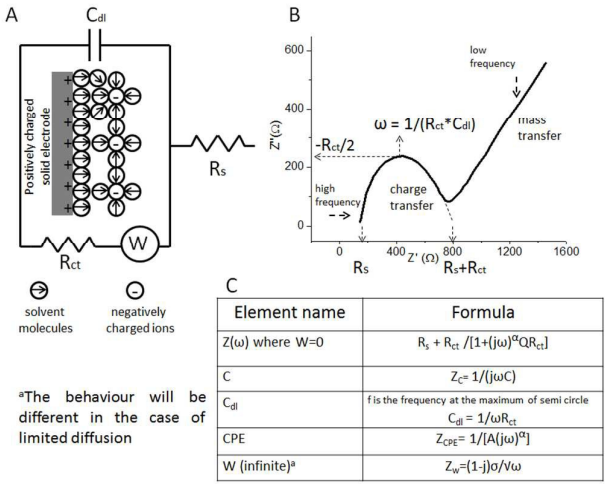

Figure 5: Analysing an EIS measurement with A) equivalent circuit diagrams [100], B) using experimental data, presented here in a Nyquist diagram [106]. C) Equations for experimental impedance and capacitance analysis [106].

For instance, Peng et al. introduced a gene sensor based on electro copolymerised 3-pyrrolylacrylic acid (PAA)/pyrrole (Py) [5]. Upon sensing element deposition a NH_2-functionalized ON probe was covalently attached to the acid functionalized CP. The sensor was then introduced to target DNA solutions with an increasing concentration range from 2×10^{-9} to 2×10^{-7} M. Impedance measurements were carried out in the presence of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The charge transfer resistance of the sensor was found to increase upon probe ON attachment and further upon complementary target hybridisation **(Figure 6A)**. Impedance results were fitted with a Randles equivalent circuit to calculate the interfacial R_{ct} and to evaluate the normalised changes in R_{ct} ($\Delta R_{ct}/R_0$) upon hybridisation, which were taken as the sensor responses (R_o is the charge transfer resistance of probe immobilised CP film). The detection limit was reported to be 0.98 nM. Subsequently, Booth et al also utilised poly(PAA-*co*-Py) to investigate the effect of probe/target length on the sensor performance [9]. In that study, a 23' mer probe ON was covalently attached to the CP electrode and the obtained sensors were incubated with 23', 50' and 113' mer target solutions with a concentration range of $2.5\times10^{-8} - 2.0\times10^{-3}$ M. It was found that the length of the target sequence has a significant effect on the sensor performance and that the sensor response ($\Delta R_{ct}/R_0$) was proportional to the target length. This effect was attributed to the extra charge accumulation coming from the longer strands **(Figure 6B)** [9].

Another noteworthy example of impedimetric biosensors was introduced by Darain et al. where a direct and label free immunosensor could be achieved using a tethered thiophene with carboxylic acid function (5, 2`:5`, 2``-terthiophene-3`-carboxylic acid). The resulting sensor was used to determine vitellogenin (Vtg), a female-specific protein, in *Carassius auratus* (gold fish) blood samples [15]. Upon electrodepositing the CP, immobilisation of the corresponding antibody, anti-Vtg, was carried out via NHS/EDC coupling **(Figure 6C)**. Then the response of CP/anti-Vtg-modified electrode was measured towards its specific antigen, Vtg, in the fish blood samples. EIS measurements were carried out for electrical

read-out at an open circuit potential, in PBS without any redox probe **(Figure 6D)**. Addition of Vtg led to a decrease in R_{ct} of the CP/anti-Vtg-modified sensor which was attributed to the alteration of the surface located charges due to the specific binding between antigen and antibody. Literature reports are however not always consistent regarding the impedance change upon protein binding. For instance, Ramanavicius et al. measured an increased charge transfer resistance upon antibody-antigen binding on a PPy based immunosensor [108]. These differences can be attributed to the fact that different proteins can have different net charge and orientation on the solid surface [109]. Several reviews focusing on impedimetric biosensors provide futher examples of this well-utilised readout methodology [105, 107]

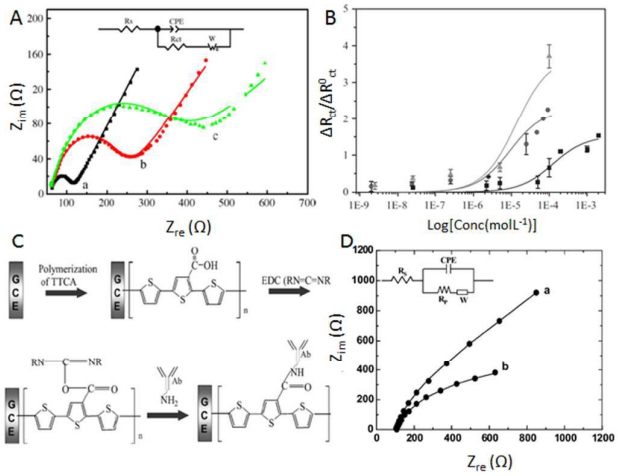

Figure 6: Nyquist diagrams of a poly(Py-*co*-PAA) electrode (a) before, (b) after immobilisation of probe ONs, (c) after hybridisation with 20.2 nM complementary target sequence.[5] *Reprinted with permission from Peng et al., Copyright 2007 Elsevier.* B) Sensor response ($\Delta R_{ct}/R_0$) versus logarithm of concentration for P(Py-*co*-PAA)/PSS film showing the effect of target sequence length (23-mer (black squares), 50-mer (dark grey circles), 113-mer (grey triangles))[9]. *Reprinted with permission from Booth et al., Copyright 2011 Elsevier.* Experiments in both A and B were performed in PBS solution (pH 7.4) containing 5.0 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$. C) Scheme representing the immobilisation of the receptive antibody, anti-Vtg, via NHS/EDC coupling to poly (5,2`:5`,2``-terthiophene-3`-carboxylic acid) [15]. D) Nyquist plot (a) before and (b) after 4.0 g/L addition of Vtg solution [15]. *Reprinted with permission from Darain et al., Copyright 2004 Elsevier.*

3.3. Detection of Different Types of Target Molecules

The aim of a biosensor is to detect biologically active species as target molecules. Thus, when the sensor is designed, the type of the target molecule should be taken into consideration. Using CPs as sensing films, proteins, DNA and sugars (e.g. glucose) can be detected via label free electrochemical read-out. In the following section, sensing of the main target molecule types is reviewed through discussion of selected studies.

3.3.1. Enzyme Based Hydrogen Peroxide Sensors

Glucose monitoring is essential for the management of diabetes, thus there has been a tremendous effort to develop non-invasive, fast and reliable glucose sensors over last three decades [110, 111]. CP-based glucose sensors provide promising solutions as they can accommodate the glucose oxidase (GOx) enzyme (which is the recognition probe of the glucose) and offer a fast and precise response via electrochemical measurement techniques. In a typical glucose sensor, surface immobilised GOx catalyses the oxidation of glucose in the presence of oxygen (O_2) to produce β-gluconic acid and hydrogen peroxide (H_2O_2) [2]:

$$\boldsymbol{\beta - glucose + O_2 + H_2O \longrightarrow \beta - gluconic\ acid + H_2O_2} \quad (4)$$

The rate of H_2O_2 production and O_2 consumption is proportional to the glucose present. Thus the amount of glucose can be determined via the electrochemical reduction of H_2O_2 at 0.8 V versus SCE:

$$\boldsymbol{H_2O_2 + 2H^+ \xrightarrow{2e^-\ (0.8\ V)} H_2O_2} \quad (5)$$

As an alternative measurement method, the amount of H_2O_2 (formed at 4) can be determined with Mo(IV) catalysed reduction of the H_2O_2 with iodide ions:

$$\boldsymbol{H_2O_2 + 2H^+ + 2I^- \xrightarrow{Mo(IV)} I_2 + 2H_2O} \quad (6)$$

Subsequently, I_2 is reduced at a potential of 0.2 V versus SCE and the electrical read-out is obtained:

$$\boldsymbol{I_2 + 2e^- \xrightarrow{(0.2V)} 2I^-} \quad (7)$$

An early example of such sensors was introduced by Umana and Waller [2] where GOx was immobilised in the PPy film via electrochemical entrapment. As PPy films were found to degrade above 0.8 V in the presence of H_2O_2 (eq.4), the authors instead utilised the indirect reduction of H_2O_2 presented in equation 7. In this case, amperometry was used as the measurement technique and an increase of the current was observed upon addition of glucose (Figure 7A). Despite the ease of fabrication and detection, this sensor had a one major drawback; the current response of the sensor decreased by 50% in 24 hours and completely diminished in two weeks. This behaviour was attributed to the leakage of GOx from the surface due to the lack of bonding between the polymer and enzyme. This prompted research into covalent attachment [112] and electrochemical adsorption [113] to increase the efficiency of the immobilisation. For instance, by using adsorption 80% of the current response was preserved for the first 50 hours [113], however without reports of long term shelf-life. Efforts to improve sensitivity have been made by increasing the electron transfer rate of the sensors and by incorporating nanoparticles such as Au [8] and Pt [17] within CPs. For instance, Kesik et al [8] synthesized an NH_2-functionalised monomer, 6-(4,7-bis(2,3-dihydrothieno [3,4-*b*][1,4]dioxin-5-yl)-2*H*-benzo[*d*] [1,2,3]triazol-2-yl)hexan-1-amine (BEDOA-6), to be used for covalent binding of GOx [8]. Upon electrodeposition of poly(BEDOA-6), mercaptopropionic acid (MPA) functionalized Au nanoparticles (Au NPs/MPA) and GOx were covalently attached onto the CP surface simultaneously (via NHS/EDC chemistry) (Figure 7B). The CP was utilised to provide an efficient conjugation between Au NPs/MPA and GOx as well as to transduce the electrical signal. AuNPs/MPA served to improve the

electron transfer from the active site of the enzyme to the CP. The detection limit of the sensor was reported to be $25x10^{-6}$ M (Figure 7C) which was in agreement with previous studies where composites of different materials were employed [114-116].

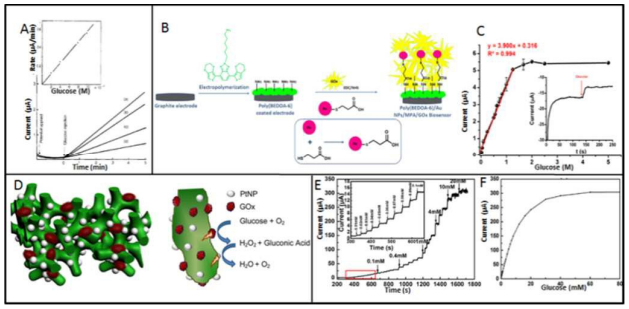

Figure 7: Amperometric signals arising from the PPy/GOx electrode upon addition of (a) 10^{-3}, (b) 8×10^{-4}, (c) 4×10^{-4}, (d) 2×10^{-4} M glucose. Inset represents the initial rate of glucose oxidation as a function of glucose concentration [2]. *Reprinted with permission from Umana & Waller, Copyright 1986 American Chemical Society.* B) Schematic of the working principle of poly(BEDOA-6)/Au NPs/MPA/GOx biosensor [8]. C) Calibration curve for poly(BEDOA-6)/Au NPs/MPA/GOx electrode after 0.025 mM to 1.25 mM glucose addition [8]. *Reprinted with permission from Kesik et al., Copyright 2013 Elsevier.* D) 3D heterostructure of the PtNP/PANI hydrogel, E) Amperometric signals and F) calibration curve of the PtNP/PANI electrode after addition of glucose with a concentration range of 1 µM - 80 mM [17] *Reprinted with permission from Zhai et al., Copyright 2013 American Chemical Society.*

Due to the stabile bond between GOx and CP provided by the covalent attachment, the authors observed no change of the current signal for up to three weeks. Another way of enhancing the sensor performance is to incorporate redox active molecules into the CPs. For instance, prussian blue was introduced within the poly[4(pyrrole-1-yl)-benzoic acid] [117] and PEDOT [118] films to increase electron transfer speed while the effective bonding of enzyme and CP was ensured by NHS-EDC coupling between the –COOH groups of the CPs and the $-NH_2$ groups of GOx. The obtained sensors displayed both a fast response time and selectively towards the enzymatically generated H_2O_2, with a detection limit of 10^{-5} M. In a recent study, a similar strategy was utilised by Krzyczmonik et al. to incorporate GOx within a composite layer of polyacrylic acid (PAA) and PEDOT [119] doped with poly(4-lithium styrenesulfonic acid) (PSSLi) as well as anthranilic acid (AA). PEDOT-PAA doped with PSSLi was proven to give the best results in terms of glucose oxidation current and stability, with a long shelf life (up to 20 days) [119].

Another immobilization approach was introduced by Welch et al., [120] who covalently tethered GOx onto a polymer brush of poly(glycidyl methacrylate) (PGMA) and poly(2-hydroxyethyl methacrylate) (PHEMA), which were grown from the PEDOT:PSS via ATRP. As the epoxy groups of PGMA can undergo a ring opening reaction in the presence of primary amines [121, 122], GOx was shown to be confined within these brushes with retained activity. PHEMA served as a swollen media for effective infusion of the GOx into the polymer matrix whereas PEDOT:PSS was employed as the transducer. The detection limit of this sensor was reported as 10^{-5} M with over 100 days of stability.

Another noteworthy example of increased sensitivity and stability was introduced by Zhai et al [17] by incorporating GOx into a PANI hydrogel/Pt nanoparticle (PtNP/PANI hydrogel) matrix (Figure 7D). GOx was crosslinked to the PtNP/PANI hydrogel matrix by glutaraldehyde where the PtNPs were employed to increase the electron transfer speed, similarly to the study where AuNPs were used [8]. The detection limit was reported to be $0.7x10^{-6}$ M which was the lowest reported detection limit to that point of time [123, 124].

Another oxidase enzyme that has been studied for H_2O_2 detection is horse radish peroxidase (HRP). Similar to GOx, incorporation of HRP within CP electrodes can be realised by either entrapment [125], physical adsorption [126] or covalent attachment [126, 127]. For instance, Kong et al covalently attached HRP to the poly (5,2':5',2"-terthiophene-3'-carboxylic acid) and used the obtained sensor for H_2O_2 detection [127]. The amperometric response of the sensor was monitored at -0.2V (vs Ag/AgCl) and steady state current values increased in a linear trend upon stepwise addition of H_2O_2 from 0.3 to 1.5 mM. The detection limit was reported to be 0.2 mM. In a relatively recent study, an improved detection limit was achieved by incorporating HRP within poly(N-[3-(trimethoxy silyl)propyl]aniline) on a gold nano-rod modified electrode. [128]. Both CV and amperometry were utilised to monitor the electrical read out arising from the H_2O_2 reduction. A remarkable detection limit was reported as 0.06 µM which was attributed to the increased surface area and electron transfer rate due to the gold nano-rods.

3.3.2. Protein Sensors

Immunosensors are extremely useful tools for environmental monitoring, food quality screening as well as disease control and they are based on detection of specific antigens [129]. Antigens can be defined as the foreign molecules that lead to the production of antibodies by the immune system [1]. By immobilizing the antibodies onto a solid surface, a specific antigen can be targeted. However orientation and surface density of the antibodies are crucial parameters to achieve consistent signals [130]. The commonly used immunoglobulin G (IgG) antibody consist of two main fragments; Fab_2 (two separate Fab) and Fc (Figure 8A). Only the Fab fragments have the antigen binding affinity whereas the Fc has antibody effector properties. Thus, for an active immobilised antibody, Fc should be connected to the sensor surface while Fab_2 should be facing towards the analyte solution [1, 131] (Figure 8A). Another important parameter in immunosensor fabrication is the prevention of non-specific antigen binding. Several methods have been explored to reduce non-specific binging, such as blocking using bovine serum albumin (BSA) [132],using antifouling materials, such as poly(ethylene oxide) based molecules [133] or antifouling polymer brush layers, for example poly(*N*-isopropyl acrylamide) [134].

CPs have been widely employed in immunosensors as they can be functionalized with different groups that can accommodate the

antibody and transduce the antigen-antibody binding event into electrical signals, in a similar manner as described for the enzymatic sensors. In an early example introduced by Ouerghi et al. the antibody was effectively attached onto the CP surface by biotin/avidin chemistry [7]. In constructing the sensor, 1) biotinylated pyrrole was electropolymerised onto the Au electrode, 2) the electrode was incubated with avidin solution, 3) the polypyrrole/biotin/avidin (PPy/Bi/Av) electrode was incubated with biotinylated antibody (anti-human IgG), 4) PPy/Bi/Av/AntiIgG sensor was incubated with related antigen (IgG). From PPy deposition to antigen incubation, each step was monitored with EIS in PBS (pH 7.4) at -1.4 V vs SCE without any redox probe. The binding events to the optimized sensor were monitored as an increase of the electrode impedance with a detection limit of 10 pg ml^{-1} (Figure 8B). The effect of the applied potential (from 0 V to −1.4 V vs SCE) during the EIS measurements on the sensor performance was studied by Hafaid et al. [14]. For a copolymer of Py and 3-N-hydroxyphtalimide pyrrole (Py-NHP) the optimum measurement potential was found to be -1.4 V, where the complete semi-circle was obtained. This behaviour was explained by the fact that at -1.4 V polypyrrole is completely insulating and at the same potential electrochemical reduction occurs of the NTA/copper complex which decreases the charge transfer resistance. In the following step, the poly(Py-*co*-PyNHP) electrode was functionalized with a Cu^{2+} containing organic complex to provide efficient attachment of a histidine tagged antibody. The obtained sensor was utilised for detection of an antigen (peptide conjugated to BSA) with varying concentrations from 100 pg/ml to 100 ng/ml (Figure 8C). The best sensor response and detection limit (21.4 pg mL^{-1}) was obtained at -1.4 V, as the redox process is the fastest at this potential.

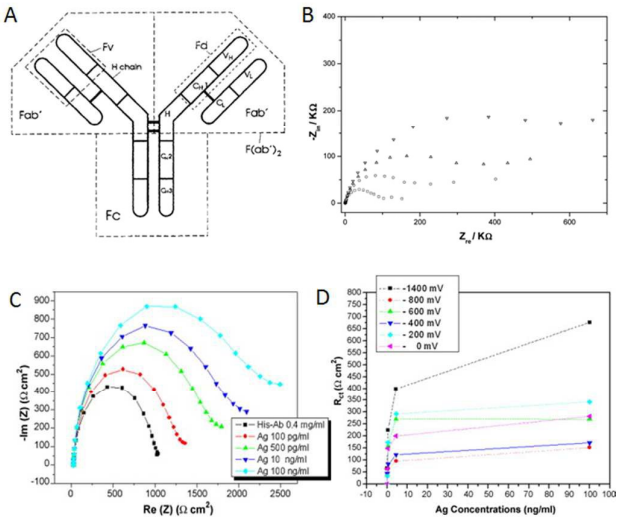

Figure 8: Segments of the immunoglobulin G (IgG) and schematic description of the antibody immobilisation leading to active, partially active and inactive antibody [1], *Reproduced with the permission from Lu et., Copyright 1996, The Royal Society of Chemistry (RSC).* B) Nyquist plots representing the impedance measurements of (□) antibody modified electrode, after addition of (○) 10 ng/ml, (^) 50 ng/ml and (ᵛ) 100 ng/ml antigen [7]. *Reprinted with permission from Ouerghi et al., Copyright 2002 Elsevier* C) Nyquist plots of an electrode containing histidine-tagged reduced antibody after addition of various concentrations of antigen [14], D) Effect of applied potential on the sensor response of the P(Py-PyNHP) films [14]. *Reprinted with permission from Hafaid et al., Copyright 2010 Elsevier.*

In a recent study, Wei et.al utilised creatinine functionalised PPy sensors to identify Allograft Dysfunction which is secondary to kidney transplant rejection [135]. Creatinine is a common marker for renal dysfunction and found at abnormally high levels in the kidney transplant patients. In this study, the authors employed a competitive binding strategy for the target detection. Creatinine, the target molecule itself, was embedded into the PPy matrix via electrochemical entrapment. Then, horse radish peroxide (HRP) conjugated creatinine antibodies (HRP-antibody) were introduced to the blood samples that were collected from the patients. When these samples were incubated with the electrodes, the HRP-antibody attached to both surface trapped and solution based creatinine. The current that was generated due to the reduction of H_2O_2, was measured via amperometric redox cycles. The sensitivity of the sensor was reported to be 0.46 mg/dL [135].

CP based immunosensors are not limited to PPy and its derivatives. For instance, Cui et al. developed poly(ethylene glycol) doped PEDOT sensors functionalised with tumour marker alpha fetoprotein (AFP) [136]. The PEDOT/PEG composite was decorated with Au nano particles (AuNPs) to increase the electron transfer rate as well as effective antibody binding. Upon the formation of PEDOT/PEG/AuNP/AFP, the sensor was incubated with different concentrations of antigen (from 10^{-19} g/ml to 10^{-5} g/ml) and the sensor performance monitored via EIS measurements with the detection limit reported to be as low as 0.0003 fg/ml.

In the light of the mentioned studies, there is a great potential for CP based immunosensors to detect diseases in a fast and cost-effective way. However, the net charge of the antigens and antibodies to be used should be investigated prior to sensor design to obtain the most reliable and efficient electrical read-out.

3.3.3. DNA sensors

CP based DNA sensors are attractive for applications such as forensic investigations, drug discovery and medical diagnosis. When constructing a CP based DNA sensor, there are three important parameters for consideration: 1) the probe ONs immobilisation technique should be tailored to retain the affinity of the probe for the complementary DNA sequence, 2) the CP should be hydrophilic enough to allow for efficient and fast electron transfer in aqueous media and, 3) the measurement technique employed should not lead to oxidative damage of the DNA. In this section, those parameters will be discussed through relevant studies.

Electrochemical entrapment is a simple and fast method to incorporate ONs into the CPs, where they are used as sole or secondary dopants during the electrochemical deposition of the CP. An early example of such an incorporation was introduced by Wang et al, who used single stranded ONs as the sole counter anions during the electrodeposition of PPy [3]. The PPy films were electrodeposited onto glassy carbon electrodes via CV (from 0.0 V to +0.70 V; 50 mV/s scan rate) in the presence of ($oligo(dG)_{20}$), ($oligo(dA)_{20}$) or KCl respectively (Figure 9A). It was found that a micromolar level of ON (100 µg/ml) was enough to obtain highly electroactive films while a considerably higher amount of KCl (1M) was required to achieve such electroactivity. The obtained sensors, $PPy/oligo(dG)_{20}$, $PPy/oligo(dA)_{20}$, and PPy/Cl (probe-free) were incubated with target DNA solutions and amperometric measurements were carried out to monitor DNA hybridisation events. Figure 9B shows the distinct, transient peaks following the hybridisation of complementary sequences. In the case of non-complementary target exposure, peaks with a reverse orientation

were obtained. No such peaks were observed in the case of the PPy/Cl electrode. In a subsequent study, [137] authors investigated the mechanism of PPy-ON complex formation. Electrochemical quartz crystal microbalance (EQCM) and voltammetry experiments revealed that the ONs are incorporated into the PPy network in a similar way to small inorganic anions and that the surface electrochemistry (in 1 M NaCl) is mostly dominated by the cation movement (eq. 8).

$$PPy(ODN^{n-})_X(Na^+)_{nX} \rightarrow PPy^{X+}(ODN^{n-})_X + nxNa^+ + nxe^- \quad (8)$$

In another example, an additional dopant was used to improve the conductivity and electrochemical activity of CP films where 0.1 M KCl was employed as the main dopant in the presence of ON as secondary dopant [83].

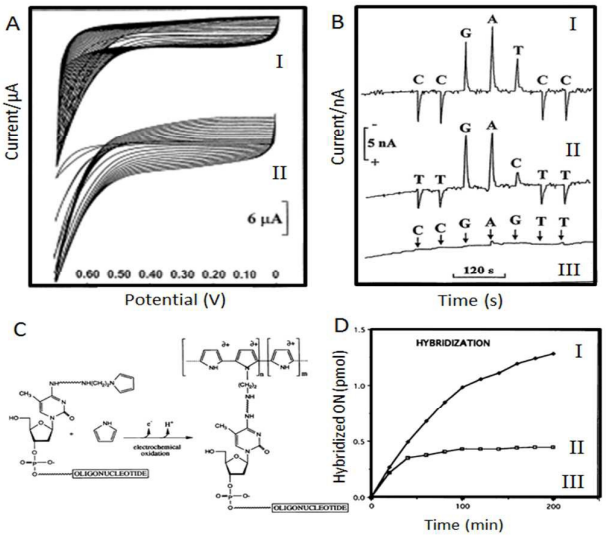

Figure 9: Electrodeposition of I) PPy with KCl as dopant, II) PPy-ON oligo(dG)$_{20}$ as dopant via cyclic voltammetry [3]. *Reprinted with permission from Wang et al., Copyright 1999 Elsevier.* B) Amperometric signals obtained upon incubation of I) PPy/oligo(dG)$_{20}$, II) PPy/oligo(dA)$_{20}$ and III) PPy/Cl$^-$ electrodes with target solutions (A= adenine, G=guanine, C=cytosine, T=thymine) [3]. C) Scheme representing the electrochemical copolymerisation of Py and Py-ON [12]. D) Hybridisation kinetics of I) 0.6x 10^{-6} M, II) 0.2x 10^{-6} M complementary and III) 0.2x 10^{-6} M non complementary target solutions on PPy-ON electrodes [12]. *Reprinted with permission from Livache et al., Copyright 1994Oxford University Press.*

Despite the straightforward immobilisation, lack of bonding between the ONs and CP commonly leads to leaking of the probe ONs from the CP film. To address this issue Livache et al. developed a technique based on the covalent attachment of ON probe sequences to the nitrogen atom of the pyrrole ring by phosphoramidite chemistry [12] before the electropolymerisation of the monomer (Figure 9C). Then, by electrocopolymerising of Py and Py-ON in the presence of 0.1 M $LiClO_4$, a matrix made of CP and ONs was achieved. Kinetic studies on the hybridisation of target sequence, as well as with non-complementary negative control, revealed that the hybridisation was completed within 60 minutes for 0.2 x 10^{-6} M target concentration. Obtained sensors had significant selectivity towards the target compared to the negative control where the electrode was incubated with 0.2 x 10^{-6} M non-complementary sequence (Figure 9D). Such PPy-ON matrices have also been utilised in several other studies where the DNA hybridisation was monitored via amperometry [12], photocurrent spectroscopy [138, 139] or EQCM [139].

Researchers have further explored the covalent attachment of –NH_2 or –COOH functionalized ONs with functionalized CPs to overcome the challenges of electrical entrapment [44]. For instance, Garnier et al [4] synthesised a functionalized pyrrole, 3-N hydroxyl phthalimide pyrrole carrying an ester leaving group [140]. After electro copolymerisation with 3-acetic acid pyrrole, –NH_2 functionalized probe ONs were attached to the poly(3-acetic acid pyrrole-*co*-3-*N*-hydroxy phtalimide pyrrole) by the chemical substitution of N-hydroxyphthalimide (Figure 10A). Hybridisation events were monitored by CV after incubation with 13 x 10^{-6}, 33 x 10^{-6} and 10^{-4} M target DNA and the detection limit was reported to be 2 x 10^{-6} M (Figure 10B).

In a study by Peng et al [13] a carboxylic acid carrying poly[pyrrole-*co*-4-(3-pyrrolyl) butanoic acid], (poly(Py-*co*-PBA), was electrochemically deposited onto GC electrodes and -NH_2 functionalized ONs were covalently attached to the CP via EDC coupling (Figure 10C). Target ON hybridisation was carried out, in ON concentration range from 3.5 x 10^{-9} to 87.6 x 10^{-9} M and monitored by CV (Figure 4B). To prevent the hydrolysis of the EDC and thereby improve the conjugation efficiency, the authors used NHS in conjuction with EDC in their subsequent studies [5, 9, 141].

One of the biggest advantages of covalent attachment compared to entrapment is that it is versatile, thus applications are not limited to the water soluble pyrroles. However, when choosing the sensing element, the nature of the CP must also be taken into consideration. It has been shown that, compared to pyrroles, thiophene based CPs have less electroactivity in aqueous media due to solvent induced microstructure collapse [142]. However, this issue can be mitigated, to some extent, by changing dopant from a small hydrophilic ion to a larger hydrophobic one [18, 143]. For example, the DNA sensing performance of poly(3-[3',3'''-bis(hydroxymethyl)-2':2'',5'':2'''-terthiophene)-3''-yl](E)acrylic acid), (PHTAA) was investigated using two films; one doped with $(CH_2)_4N(CF_3)_2(SO_2)_2$ (hydrophobic dopant)) and the other with $(C_2H_5)_4NBF_4$ (hydrophilic dopant) [18]. Following the polymerisation –NH_2 functionalized ONs were covalently attached onto both CP films and the sensors were incubated in PBS for 20 hours to test the stability in aqueous media before incubation with complementary target sequences (49 x 10^{-6} and 49 x 10^{-5} M) for 1 h each at 37 ^oC. Figure (10D-E) presents the impedance measurements carried out in a PBS solution containing 5 mM $Fe(CN)_6^{3-/4-}$. Variation in electrode capacitance revealed that the PHTAA electrode polymerized with the large hydrophobic dopant showed significant change in the case of complementary target hybridisation but no change was observed in the case of doping with a smaller hydrophilic dopant. The reason of this behaviour was explained by the fact that small ions such BF_4^- can easily exchange with the Cl^- ions in PBS during the electrochemical measurements and this can lead to microstructure collapse. This

study is a very important example of the effects of sensing element preparation on the sensor performance. Another good example is how the thickness of the CP film changes the sensor response and sensitivity [5]. In a study of poly(Py-*co*-PAA) with different thicknesses, the thinner films showed better sensor response due to larger surface to volume ratio, leading to faster and more efficient electron transfer [5].

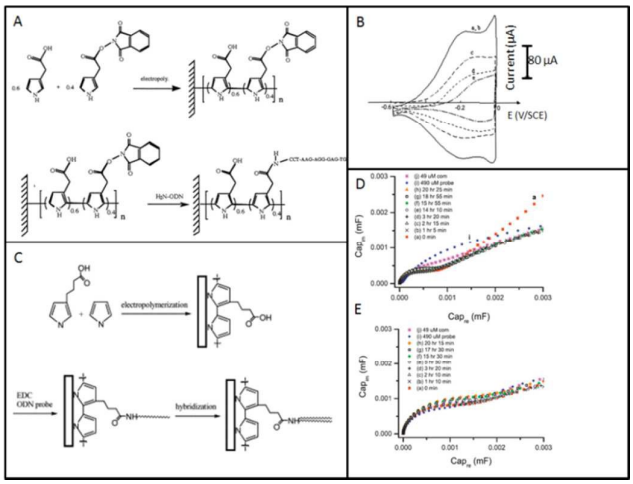

Figure 10: A) Reaction scheme describing the electrochemical deposition of poly(3-acetic acid pyrrole-*co*-3-*N*-hydroxy phtalimide pyrrole) and following chemical substitution of N-hydroxyphthalimide by ON [4]. B) Cyclic voltammetry measurement carried out a) before and after incubating the sensor with b) non complementary, c) $13x10^{-6}$M, d) $33x10^{-6}$M and e) 10^{-4} M target DNA [4]. *Reprinted with permission from Garnier et al., Copyright 1999 Elsevier.* C) Covalent attachment of NH_2 functionalized ONs onto –COOH functionalized poly(Py-*co*-PBA) via NHS-EDC chemistry [13]. *Reprinted with permission from Peng et al., Copyright 2005 Elsevier.* Complex capacitance measurements of PHTAA doped with D) $(CH_2)_4N(CF_3)_2(SO_2)_2$, E) $(C_2H_5)_4NBF_4$ (in both cases, stability of electrodes were tested with repetitive impedance measurements during 20 hours, followed by incubation with $49x10^{-6}$ and $49x10^{-5}$ M target solutions [18]. *Reprinted with permission from Spires et al., Copyright 2011 Elsevier.*

In a recent paper, Galan et. al utilised click chemistry to covalently bind acetylene terminated DNA probes onto azido-derivatised PEDOT electrodes [144]. The click reaction between probe and the CP was performed in 1:1 DMSO: H_20 in the presence of excess amount of Cu(I). Upon sensor preparation, the electrodes were incubated with target DNA sequences in a concentration range of 1 - 20 nM. The binding event between probes and targets was monitored via differential pulse voltammetry (DPV), with a reported detection limit of 0.13 nM.

3.4. Miniaturisation and Sensor Arrays

Miniaturization is a valid approach to increase the sensitivity of conductive polymer based electrochemical sensors. Nano-scale materials in general have the advantage of exhibiting intrinsically high surface area, which is a prerequisite for high sensitivity. A fast development is occurring in both the fabrication of nanomaterials, and their application in sensing devices, with silicon nanowires, carbon nanotubes and graphene emerging as particularly promising materials. Conductive polymer nanowires (CPNWs) offer high sensitivity and the possibility of fabricating array sensing devices, similarly to other nanomaterials. [145-149] The fabrication of CPNWs is effective and simple compared to other nanomaterials. There are a range of methods available to synthesise conducting polymer nanomaterials. In-situ polymerization, through direct electrochemical synthesis is of particular interest, as the nanowires can be made directly where and when they are needed. [145, 150-153] Another promising fabrication route is that of template assisted synthesis (methods are available that do not require subsequent template removal), and the large amount of material that can be produced in this way widens the scope of the type of sensors that can be constructed. [54, 59, 154, 155] Alternatives such as electrospinning [156], dip-pen [157] or direct writing of CP nanomaterials also exist [158, 159]. To build up the sensor, the CPNWs are functionalized with biomolecules after the fabrication, using protocols already discussed for the standard electrochemical CP sensors. [160] The specific biomolecule binding can be detected in a label-free manner via electrical readouts, similarly to the macroscopic sensors already described. The sensitivity and selectivity achieved with the CPNWs is generally very good and competes well with other types of sensors. Challenges of CPNWs have been their environmental stability and the consistency of fabrication methods, areas where progress is continuing to be made. We have recently reviewed this field [109], and a full account is outside the scope of this review.

Miniaturization of sensors also has several other advantages, such as the ability to use very small sample volumes and providing the possibility of arraying formats. Gene microarrays, which are widely used in diagnostics, provide evidence for the power of the arraying technology where the expression level of thousands of genes can be measured simultaneously [161-163]. This technology relies on the binding of surface anchored DNA probes to complementary analyte oligonucleotide fragments. Current gene microarray sensor technology requires processes to convert samples to a form suitable for detection on the microarray. There are also issues as limited tagging efficiency and bleaching of fluorescent labels. To improve this type of technology, it is desirable with simplified and label-free detection and increased speed, portability and reduced cost. The direct electronic transduction of binding events to CPs, as discussed in this review, lends itself well to an improved arraying technology. By creating a sensor array, signals can be collected and processed from several different sensors, or for several different samples, simultaneously. Successful examples of CP-based arrayed biosensors demonstrate sensing of DNA targets [164-166] as well as protein [167, 168]. One limiting factor however, is the need for post-fabrication immobilisation of probes, which poses restrictions on production time. To functionalise the CP *monomer* with an oligonucleotide probe *prior* to electropolymerisation of the

monomer onto an electrode is more amendable to mass manufacture, thus affording direct immobilisation of the probe onto the sensing element *simultaneously* with the creation of the sensing element. Livache at al. have demonstrated such an approach to create arrays of addressable CP microelectrodes [169-171] and utilized mainly optical detection mechanisms.

A new generation of CP-based biosensor devices can be expected to integrate on-chip microfluidics with an array of miniaturized parallel sensors with a panel of probes relevant for example for a particular disease or risk profile.

3.4. Summary and Outlook

This review outlines the types, and main elements, of conducting polymer-based electrochemical biosensors. CP biosensors generally report very high sensitivity, and offer label-free electrochemical detection. In this review, methods of signal transduction, and the electrochemical read out techniques such as amperometry, cyclic voltammetry and electrochemical impedance spectroscopy were discussed. Particular emphasis has been devoted to reviewing the techniques available for recognition probe immobilization. To advance these sensors, improvements to the stabilization of the CP-recognition element complexes is desired, as well a further increase of the sensitivity. This can be achieved by improving the electroactivity of the CP[68], immobilizing the recognition elements more efficiently[44, 172], amplifying the transduced signal [173] and enhancing the nanoporosity of the polymer film [174]. When constructing a CP biosensor, the type of the target molecule must of course be considered, as well as the appropriate recognition element. Once the target/probe pair is identified, the immobilization techniques and recognition signal transduction and amplification methodologies must be assessed. The signal transduction arises from effects on the charge mobility of the CP from the binding events, or from effects on polymer conformation. Although the signal transduction is rather well understood for simple biomolecules, such as DNA, more work is needed to fully understand the signals arising from protein or pathogen binding. Ideally, a biosensor exhibits not only high sensitivity but also high selectivity and stability. Furthermore, portability, and stability against degradation are desirable. For conductive polymer based biosensors, factors such as film thickness [5], surface-to-volume ratio [174], wettability [175] and surface chemistry all need optimisation. A high selectivity is of particular importance to enable the use of real samples such as blood, milk or urine with high levels of interfering compounds. Improvements of the sensor selectivity may be targeted by focussing on parameters such as pH and ionic strength. Going forward, the incorporation of successful miniaturized sensor designs into arrays to build up sensors chips able to detect a panel of relevant targets is expected to fully capitalise on the promising developments achieved so far in application of conducting polymers in biosensing.

Acknowledgements

The University of Auckland and the MacDiarmid Institute for Advanced Materials and Nanotechnology are gratefully acknowledged for the on-going financial support

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