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Polymers in biosensors

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1. Introduction

This chapter will be devoted to the incorporation of polymers within biosensors, beginning with a history and descriptions of basic sensor formats - while concentrating on optical and electrochemical sensors. Initially the chapter will discuss the incorporation of polymers as simple coatings for biosensors. These coatings are typically used (i) to improve selectivity (by preventing interferents from reaching the active parts of the sensors) and (ii) to improve the biocompatibility of biosensors. Similar coatings which are used as anchors for biomolecules in various techniques will also be discussed. Following this is a section on polymers which have a more active role. Conducting polymers will be discussed and their structures and use in biosensors will be described. A section follows on redox active polymers and their use to 'wire' biological moieties to electrodes. Finally we will discuss molecularly imprinted polymers and their potential to replace biological molecules as active components within biosensors.

2 History and format of biosensors

A biosensor is a device that measures the presence or concentration of biological molecules, by translating a biochemical interaction at the sensor surface into a quantifiable physical response; this is usually optical or electrochemical in nature. Most sensors consist of three principle components, as described below and detailed in figure 1:

i. The first of these includes a receptor species, which is usually biological in origin such as an enzyme, antibody or DNA strand capable of recognising the analyte of interest with a high degree of selectivity; this is usually concurrent with a binding event between a receptor and the analyte. However receptor species for biological molecules which are themselves artificial in nature can also be utilised.

ii. The second component that must be present is a transducer, enabling the translation of the binding event into a measurable physical change; possible events include the generation of electrons, protons, an electrochemically active chemical species such as hydrogen peroxide - or simple physical changes such as a change in conductivity, optical absorbance or fluorescence.

iii. Thirdly there must be inclusion of a method for measuring the change detected at the transducer and converting this into useful information.

Usually biological molecules are utilised as the active recognition entity within a sensor. These display unsurpassed selectivities; for example glucose oxidase will interact with glucose and no other sugar, and in this way will act as a highly selective receptor. In the case of glucose oxidase, the electrochemically inactive substrate

glucose is oxidised to form gluconolactone along with the concurrent generation of the electroactive species hydrogen peroxide. Enzymes also generally display rapid turnover rates and this is often essential to (a) avoid saturation and (b) to allow sufficient generation of the active species in order to be detectable.

Antibodies bind solely to their antigens and achieve specificity via a complex series of multiple non-covalent bonds. Since the principle of immunoassays were first published by Yalow and Berson (1959), there has been an exponential growth in both the range of analytes to which the technique has been successfully applied and the number of novel assay designs that have been reported. Development of enzyme-labelled immunoanalytical techniques e.g. Enzyme-Linked Immuno Sorbent Assay (ELISA) has provided analytical tests without the safety risks associated with radiolabelling-based techniques.

The rapid measurement of analytes of clinical significance e.g. towards various disease markers would permit earlier intervention, which in a medical setting is frequently of utmost importance. There has been much research directed towards the development of direct immunosensors that do not rely on the use of a detectable label. Such a system will lead to simpler assay formats and ideally shorter detection times. A reusable and rapid detection system would, moreover, allow for continuous real-time measurement, so helping to maintain optimal homeostatic conditions.

Unfortunately there are also some disadvantages related to the construction and use of biosensors. Often the biological species can either be extremely expensive or difficult to isolate in sufficient purity. Immobilisation of these species can lead to loss of

activity and the presence of various chemical species in the test solution can also cause loss of activity, (for example enzymes can be easily poisoned by heavy metals). In biological samples such as blood or saliva, there can also be solutes that are electrochemically active and interfere with determinations of the target species. Again in physiological fluids such as blood, various species may be present which bind to the surface so causing fouling and loss of sensor response.

A series of extensive reviews on biosensors and their history have been published elsewhere (Hall 1990, Eggins 1996, Wang 2001) and therefore only a brief history will be given here. Easily the most intensively researched area has been towards the development of glucose biosensors (Wang 2001, Newman *et al* 2004). The reason for this is the prevalence of diabetes which has become a world-wide public health problem. The incidence of diabetes is continuing to increase with at the time of writing 170 million sufferers diagnosed world-wide (World Health Organisation, www.who.org), with this number being estimated to reach 300 million by 2045 (Newman *et al* 2004). Diabetes is related to a number of factors such as obesity and heart disease - all of which make this disease one of the leading causes of death and disability in the world. The world market for biosensors is approximately $5bn with approximately 85% of the world commercial market for biosensors currently being for blood glucose monitoring (Newman *et al* 2004).

These factors have lead to the development of a number of inexpensive disposable electrochemical biosensors for glucose, incorporating glucose oxidase (GOD) immobilised at various electrodes. They are generally amperometric sensors, with

electrodes polarised at a set potential; the oxidation or reduction of a chosen electroactive species at the surface will then lead to generation of a detectable current.

3. Polymer membranes in biosensors.

Two major problems which can affect the performance of a biosensor are the presence of interferents and also biofouling. Interference from electroactive substances is especially problematic when electrochemical measurements are being made on physiological materials such as blood. For example, glucose sensors can be affected by the presence of species such as ascorbate or acetaminophen (paracetamol), both of which can be oxidised at electrode surfaces. Physiological fluids, especially blood, also have a tendency to deposit materials such as proteins, usually irreversibly, onto solid surfaces. This biofouling process can diminish the response of sensors – and in some cases can passivate them completely. This is especially a problem for sensors that we wish to utilise more than once or for sensors that are implanted *in vivo*. A detailed review on enhancing blood compatibility has been recently published elsewhere (Gavalas *et al* 2006).

Application of a permselective coating to the sensor can prevent or minimise the access of interfering compounds to the sensor surface, thereby minimising interference from electroactive species. Polymeric materials have led the way, with two of the earliest and most commonly utilised being the fluorinated ionomer Nafion (Turner and Sherwood 1994) - and cellulose acetate (Maines *et al* 1997). A beneficial side-effect is that these materials can also confer a degree of biocompatibility. The structures of both materials are shown in figure 2.

Cellulose acetate has been widely utilised both as a selective barrier as well as for enhancing biocompatibility within electrochemical sensors (Maines *et al* 1997). The cellulose acetate layer permits only small molecules, such as hydrogen peroxide to reach the electrode, eliminating many electrochemically-active compounds that could interfere with the measurement.

Nafion has also been widely utilised as a coating material, as reviewed here (Wisniewski and Reichart 2000). The polymer displays the advantages of being chemically inert and easily cast from solution. As shown in figure 2b, the polymer is anionic and upon casting forms a structure with hydrophilic channels contained within a hydrophobic matrix. Films formed from this material are reasonably robust, show strong exclusion of anionic interferents and display enhanced biocompatibility (Moussy and Harrison 1994, Moussy *et al* 1994). For example Nafion coated electrodes show a much lower rate of signal attenuation when implanted *in vivo* for two weeks compared with untreated electrodes (Moussy and Harrison 1994). Coating a glucose oxidase based biosensor with Nafion was found to help screen out interference from urea and ascorbate (Moussy *et al* 1994).

A range of other polymers have also been utilised in the attempted prevention of biofouling as described in the extensive review by Kingshott and Griesser (1999). Some of the most popular materials have been ones based on polyethylene glycol/oxide (PEG/PEO) (Kingschott and Grieser 1999). The reasons why a PEG/PEO surface should resist biofouling so well, is a topic for a complete review article in itself, however, it is widely reported that a very low adsorption of proteins occurs at the surface of these materials (Kingschott and Grieser 1999). PEG/PEO

chains are usually highly solvated in aqueous system meaning that any incoming protein molecules will experience a surface that is largely composed of water, so mimicking the typical conditions found within biological systems. This is thought to be a major contributor to their biocompatibility.

Simple physical adsorption of Pluronic (Figure 2c) surfactants which consist of PEO-polypropylene oxide-PEO block terpolymers (Green *et al* 1998) has been utilised to treat a variety of materials. The polypropylene oxide (PPO) section of the chain is more hydrophobic and therefore is absorbed onto the substrate being treated. This leaves the hydrophilic PEO blocks stretching out from the surface into the aqueous phase. The makeup of the surfactant materials affects their biocompatibilities; for example - increasing the length of the PPO section, leads to enhanced protein repulsion compared to increasing the length of the PEO section. Possibly only short PEO chains are necessary for effective protein repulsion - and increasing the PPO section leads to better anchoring of the surfactant, thereby preventing either leaching of the coating into the aqueous phase or displacement by protein molecules (Green *et al* 1998).

Hydrogels have also been investigated as coatings for use within sensors. A hydrogel is usually based on polymers such as poly(vinyl alcohol) or poly(acrylic acid), which would normally be soluble in water, but either during or after the polymer synthesis, the linear polymer chains become crosslinked into a polymer network. The resultant network has a high affinity for water but does not dissolve, but rather it is capable of adsorbing water with consequent swelling of the polymer matrix. Due to their high water content, hydrogels often show high biocompatibility. Typical materials involve

crosslinked polyethylene oxide or polyhydroxyethyl methacrylate (Fig. 2d). They are attractive materials not only because of their biocompatibility but also because water soluble analytes are capable of diffusing quickly through the water-swollen polymer. The swelling behaviour can be easily controlled by the amount of crosslinking; a network with few crosslinks will adsorb large amounts of water with a high degree of swelling. Less hydrophilic monomers, incorporation of hydrophobic co-monomers or a high degree of crosslinking all act to reduce water adsorption, usually leading to a firmer, more rigid firmer gel.

Hydrogels have been shown to act as stabilising layers when applied to sensors; for example crosslinked PEO has been used to stabilise an implanted glucose sensor (Csoeregi *et al* 1994). A more widely utilised application for hydrogels however has been as enzyme stabilising agents. Enzymes can often denature and lose their efficiency; however this effect can be mitigated by encapsulating it inside a hydrogel. A swollen hydrogel of high water content mimics an aqueous environment and helps to prevent denaturing. For example, glucose oxidase could be incorporated within a crosslinked PHEMA membrane and enabled formation of a glucose sensor which only showed a 20% loss in activity after 3 months continuous operation (Doretti *et al* 1996). Other polymers have also been utilised (Gibson and Woodward 1992, Gibson *et al* 1992); for example a variety of enzymes have been stabilised using cationic polymers such as diethylamino modified dextran. Alcohol oxidase retained 100% of its efficiency after 2 months at 37^{o}C when stabilisers were utilised (Gibson *et al* 1992).

Other polymers have also been studied as biocompatible agents. A study of protein deposition on membranes made from polyvinylchloride, polyurethane and silicone rubber based materials, for utilisation in solid state ion sensors (Cha *et al* 1991), found that polyurethane and silicone based membranes exhibited less protein adsorption following exposure to blood. More recent research utilises silicone modified polyurethanes and showed enhanced biocompatibility compared to polyurethane and polyvinylchloride (Berrocal *et al* 2001). The modification of polyvinyl chloride membranes with anionic surfactants also improves biocompatibility and has been utilised in the development of amperometric enzyme electrodes (Reddy *et al* 1997).

Cell membranes consist mainly of phospholipids and therefore attempts have been made to synthesise polymers that contain phospholipid type headgroups. Polymers, for example, based on the phospholipid polar group, such as 2-methacryloyloxyethyl phosphorylcholine (Fig. 3a), copolymerised with other methacrylate monomers have been pioneered by Ueda (1992). By correct selection of the monomers, materials were formulated and found to greatly minimise adsorption of blood proteins onto surfaces. Poly (2-methacroylethyl phosphorylcholine), Figure 3b, could be plasma deposited onto silicone rubber and the adhesion of albumin was found to be minimised by factors of up to 80 (Hsuie *et al* 1998). These materials have been successfully applied to the outer membranes of ion-selective electrodes (Berrocal *et al* 2002, Ysjima *et al* 2002) and show enhanced biocompatibility. Glucose biosensors also showed enhanced *in vivo* lifetimes compared with unmodified sensors (Ishihara *et al* 1998) with a subcutaneously implanted probe showing lifetimes of up to 14 days. Other "natural" species can also be grafted onto polymer films, for instance heparin is found

on the inside of vascular walls and can be grafted onto polyvinyl alcohol based coatings (Brinkman *et al* 1991).

An alternative approach has been to electropolymerise suitable monomers to form protective coatings. 1,2-diaminobenzene (Mylker *et al* 1997), for example, when deposited at the bioelectrode surface serves to both stabilise the electrode due to its inherent high biocompatibility whilst also imparting selective exclusion of interferents such as ascorbate. Similar results were also obtained using polypyrrole (Vidal *et al* 1999).

Plasma polymers have been the subject of recent interest. Basically many chemical species if irradiated in a glow discharge or RF device will form a reactive plasma and deposit as a polymer on almost any surface (Muguruma and Karube 1999). One advantage of this method is that it will often give pinhole free, highly crosslinked films which are extremely even over substrates of almost any shape. There has been some work on utilising these materials in biosensors. Ethylenediamine was plasma polymerised onto a QCM chip to give a polymer surface that was very suitable for immobilisation of antibodies (Nakanishi *et al* 1995). Plasma polymers are also especially suitable for use in microfluidic type devices due to their even deposition even over complex shapes (Hiratsuka *et al* 2004). Deposition onto surface plasmon resonance chips has also been studied (Mugurama *et al* 2000).

This process has also been utilised for the development of immunosensors; for example butylamine plasma polymer films have been utilised for the electrostatic

adsorption of anti-ceruloplasmin antibody (Wang *et al* 2004). This system gave immunosensors capable of detecting 0.15 μg ml^{-1} of antigen.

4. Commercial coatings

Coatings have also been used elsewhere in the biosensing world. The two examples given below are commercial variations on the theme of sensors, although they tend to be used more as research tools rather than as widespread applications.

Surface Plasmon resonance (SPR) is a technique widely used for probing immunological interactions. Commercial SPR systems are widely available with Biacore AB being the major systems provider at the time of writing (Karlsson 2004). SPR is a method that combines optical and electrochemical phenomena at a metal surface and is capable of measuring real-time label free biomolecular interactions. The nature of the surface of the SPR chip can affect the nature of any interactions. Polymer coatings are often utilised, usually to minimise non-specific interactions. Most commercial chips are coated with carboxymethylated dextran or substituted variants (Karlsson 2004).

One of the major biotechnology success stories of recent times has been the sequencing of the human genome. The detection of specific DNA sequences has been a major issue in the field of biological sciences for many years. Early methods were intensively laborious, expensive and time consuming and have now been superseded by the appearance of DNA arrays, permitting multiple sequence detection with high specificity and rapid response times. DNA microarrays are constructed by spotting a

variety of known oligonucleotides onto precisely defined locations on a solid substrate, usually a glass microscope slide. Immobilisation of the DNA is often electrostatic, usually by means of a cationic polymer such as polylysine. A wide variety of precoated slides are now commercially available.

5. Conducting polymers in biosensors

Conducting polymers are especially suitable for immobilisation of enzymes at electrode surfaces and this process has reviewed in detail elsewhere (Gerard *et al* 2002, Barisci *et al* 1996). A variety of monomers can be electropolymerised on an electrode surface and under correct conditions form stable conductive films. Typical conductive polymers include polyaniline, polypyrrole and polythiophenes (figure 4). Polymer of these types generally contain a highly conjugated backbone and display properties such as electrical conductivity, low energy optical transitions and a high affinity for electrons. If during the electrochemical polymerisation process, biological molecules are present in the solution they can be entrapped within the film during the deposition process (Cosnier 2003, Geetha *et al* 2006). Alternatively a polymeric film can be deposited electrochemically and then the biological species can be adsorbed onto - or be chemically grafted to the film (Gerard *et al* 2002, Barisci *et al* 1996). This leads to a close association between the conductive polymer and the biomolecule which could potentially facilitate rapid electron transfer between the active species and an electrode surface. Alternatively should the active species interact in some way with the environment, this could lead to a change in the properties of the conductive film. For example, if an antibody is included in the film and binds its antigen, the resultant conformational changes could affect the film. This then may lead to a measurable change in its electrochemical or optical properties. Therefore the

conductive polymer can be thought in someway to be acting as the transducer element within the biosensor (Figure 1).

One of the simplest methods involves the entrapment of enzymes such as glucose oxidase within polyaniline films (Cooper and Hall 1992). Aniline was electrochemically polymerised from a solution containing 3 mg/ml glucose oxidase onto platinum electrodes. Exposure of these electrodes to glucose solution led to the formation of hydrogen peroxide which could be measured electrochemically. The advantages of this method is that it allows the controlled deposition of biological molecules onto electrodes of just about any size and composition. Further work utilising this system (Skinner and Hall 1997) utilised an AC impedance detection technique and showed that not only could the hydrogen peroxide produced by oxidation of glucose be detected - but even in anaerobic conditions the presence of glucose could be detected by the enzyme electrode.

A variety of electrodeposited polymers have been studied as hosts for enzymes (Cosnier 2003, Geetha *et al* 2006). Polyaniline has been used as a host for, amongst others, enzymes such as glucose oxidase (Ramanathan *et al* 1996), lactate dehydrogenease (Chaubey *et al* 2000) and horseradish peroxidase (Yang and Shaolin 1997). Polypyrrole is also a popular material since, like polyaniline, it can be deposited in a variety of oxidation states as well as charged or uncharged, conducting or insulating forms. For example urease or glutamate dehydrogenase were co-deposited with polypyrrole or physically adsorbed onto preformed films (Gambhir *et al* 2002). Polypyrrole films have also been used as hosts for cholesterol oxidase for use as cholesterol biosensors (Kajiya *et al* 1991, Kumar *et al* 2001, Vidal *et al* 2004).

Cholesterol biosensors have also been constructed using polyaniline (Wang and Mu 1999, Singh *et al* 2006). Pyruvate oxidase has been incorporated within a copolymer of a modified pyrrole monomer with thiophene and used to detect pyruvate (Gajovic *et al* 1999). A platinum microelectrode was used as the substrate for deposition of polypyrrole containing a three enzyme mixture (xanthine oxidase, purine nucleoside phosphorylase and adenosine deaminase), with the resultant sensor being capable of detecting adenosine at concentrations down to 100 nM (Llaudet *et al* 2003).

Our group has taken this process further, utilising both non-conductive and conductive polymers to fabricate arrays of conductive microelectrodes with entrapped biological molecules such as glucose oxidase (Barton *et al* 2004). Basically an insulating film of polydiaminobenzene is electrochemically deposited on an electrode and then ablated with to create an array of pores. Conductive polyaniline containing enzymes is then deposited within the pores as shown schematically within figure 5a-c. Scanning electron microscopy clearly demonstrates formation of pores within the film and mushroom-like protrusions of polyaniline (figure 5d, e). This technique has been used to develop sensors for pesticides based on acetylcholineesterase immobilised within polyaniline. These sensors allowed determination of pesticide concentrations as low as 10^{-17} M (Law and Higson 2005, Pritchard *et al* 2004).

Enzymes are not the only biomolecules that can be immobilised within electrodeposited films. There has been a sustained effort into the development of conducting polymer based immunosensors as recently reviewed by Cosnier (2005). A wide variety of biomolecules that have been attached to or co-deposited with

conducting polymers have been detailed within other reviews (Cosnier 2003, Geetha *et al* 2006) - a few of which will be described here.

One of the earliest uses of these techniques was for the entrapment of anti-human serum albumin (anti-HSA) within polypyrrole (John *et al* 1991). The resultant films were studied by AC voltammetry and shown to respond to the presence of HSA. Polypyrrole containing cyano groups could be electrodeposited and the resultant films utilised for the electrostatic binding of anti-rabbit IgG (Ouerghi *et al* 2001) with the heavy chains of the antibodies being preferentially bound to the film, thereby orientating the antibody on the surface. The resultant films when studied by AC impedance were found to be capable of detecting rabbit IgG at levels of 10 ng/ml. Polypyrrole could also be used as the host for anti-HSA and when interrogated with pulsed electrometry could detect HSA at levels of 25 pg/ml (Sargent *et al* 1990). Antibodies for bovine serum albumin (BSA) and digoxin could also be incorporated into polypyrrole (Grant *et al* 2003). Moreover, the use of radiolabelled antibodies allowed accurate quantification of the levels of antibody incorporation within the films and also the optimum method for antibody incorporation to be determined. Similar films containing anti-BSA when combined with AC impedance measurements could detect the antigen with a linear response from 0 to 75 ppm (Grant *et al* 2003).

An alternative method involved depositing biotin functionalised polypyrrole and then utilising the strong biotin-avidin to deposit first a layer of avidin followed by a layer of biotinylated anti-human IgG (Ouerghi *et al* 2002). Many other groups have also utilised the biotin-avidin interaction for binding biomolecules to conducting polymers (Cosnier 2005). Electrostatic interactions have also been utilised since often

conductive polymers are charged. Antibodies for species such as digoxin and hepatitis B have been deposited on polypyrrole (Purvis *et al* 2003), leading to development of a potentiometric biosensor with detection limits down to pg/ml levels and good stability. Many conducting polymer films can also be generated which contain reactive species such as n-hydroxy succinimide which can then react with groups such as amines (contained within many enzymes and antibodies), thereby covalently immobilising them on the polymer surface (Cosnier 2005).

Oligonucleotides have also been widely investigated in conjunction with conducting polymer films (Davis and Higson 2005). Early approaches used simple adsorption of oligonucleotides onto polypyrrole (Mineban *et al* 1994). This was later found to be highly dependent on the oxidation state and therefore the number of positive charges that are available within the polypyrrole film (Mineban *et al* 2001). Co-deposition of DNA stands with conducting polymers has also been widely utilised; for example short single-stranded oligonucleotides could be incorporated within polypyrrole (Wang *et al* 1999) to allow the electrochemical detection of their counterstrands. Polyaniline and polydiaminobenzene have also been successfully utilised as hosts for DNA (Davis *et al* 2004). When polypyrrole and a single stranded oligonucleotide were co-deposited onto carbon nanotube modified electrodes, the resultant biosensor could detect 10^{-6} mol l^{-1} of the counterstrand - and was also found to be capable of differentiating between the counterstrands and other oligonucleotides with one, two and three base mismatches (Cai *et al* 2003). Other methods such as use of the avidin-biotin pair and chemical grafting have also been utilised to attach oligonucleotides to conducting polymers (Cosnier 2005).

The majority of the sensors constructed using conductive polymers are electrochemical in nature, however, some alternative methods have been utilised. Various oligonucleotides were synthesised with a pyrrole unit on one end. These were then electropolymerised as copolymers with pyrrole onto individual gold microelectrodes of a 128 electrode array (Livache *et al* 1998). Detection of a DNA target could then be determined by fluorescence measurements. Other work involved taking an indium tin oxide coated optical fibre and electrochemically depositing a biotin substituted polypyrrole layer (Konry *et al* 2003). This layer was then used to attach first avidin and then biotinylated cholera toxin. The resultant sensor was capable of detecting anti-cholera toxin antibodies using a luminol based assay, with negligible response to other antibodies. Similarly, a pyrrole-benzophenone copolymer was electrodeposited on optical fibres and the HCV-E2 envelope protein antigen immobilised photochemically (Konry *et al* 2005) to generate an optical biosensor capable of selectively detecting anti-E2 antibodies.

6. Redox-active polymers in biosensors

The earliest electrochemical glucose biosensors relied on detection of either oxygen (Clark and Lyons 1962) or hydrogen peroxide at an electrode surface. Unfortunately this leads to the possibilities of interference by electroactive species such as ascorbate. Also the active site of the enzyme may be insulated from the electrode by the surrounding protein shell. These problems can be circumvented by utilising an artificial electron charge transfer moiety known as a mediator. Use of mediators lead to the development of so called 'second generation biosensors' with a typical example being shown in figure 6 where a ferrocene compound is utilised to “shuttle” electrons between the enzyme and the electrode (Cass *et al* 1984). As an alternative to the use

of mediators, it has been proposed that a suitable polymer could "wire" the enzyme to the electrode. Conducting polymers have been utilised for this purpose, although another possible method that has been studied utilises a polymer that does not conduct electrons along the polymer backbone but rather shuttles electrons between electroactive groups bound along the polymer chain.

One of the earliest proposed methods was that of Heller in 1990 who suggested the use of a composite material containing polypyridine and osmium 2,2-bipyridine (Figure 7a). The resultant substituted polymer was deposited at an electrode surface as an electrostatic complex with glucose oxidase and shown to respond to glucose in the physiological range. Polymers of a similar type containing reactive groups such as succinimide were used to covalently immobilise enzymes (Heller 1990). This gave rise to the construction of films of up to 1 μm thick which gave a strong electrochemical response to glucose. Similar polymers were used to immobilise horseradish peroxidase on glassy carbon for the measurement of hydrogen peroxide at much lower potentials than normally required (Yang *et al* 1995). Pyruvate sensors have also been constructed using these systems (Gajovic *et al* 2000).

An alternative system was developed based on osmium modified polyvinyl imidazole (Figure 7b) which when mixed with a polyethylene glycol based crosslinker, could be used to immobilise glucose or lactase oxidase onto electrodes. Again this lead to the formation of sensors for their respective substrates (Ohara *et* al 1994). The performance of these sensors could be improved by adding a second polymer, Nafion - and allowed constructions of sensors with linear ranges of 6-30 mM (glucose) and 4-7 mM (lactate). In both cases only a negligible response was observed to common

interferents (Ohara *et* al 1994). Similar polymers were used to immobilise glutamate oxidase and horseradish peroxidase - and in conjunction with a HPLC technique were used to determine levels of the neurotoxin N-oxalyl-diamino propionic acid (Belay *et al* 1997). Using oligosaccaride dehydrogenase as the enzyme, electrodes capable of detecting a range of sugars and saccarides were developed (Tessema *et al* 1997).

This technique is a highly versatile one, where the behaviour of the polymers can be fine-tuned by variation of their substituents. For example, using a layered enzyme electrode where both glucose oxidase and bilirubin oxidase are "wired" by polyvinyl pyridine/osmium polymers to a glassy carbon electrode, concentrations of glucose as low as 2 fM were detected in the presence of atmospheric oxygen (Mano and Heller 2005). In a similar way, single stranded DNA was complexed with a redox polymer and bound at an electrode surface. Hybridisation of this strand with a probe DNA which then had horseradish peroxidase attached, allowed detection of DNA down to levels of just 3000 copies (Zhang *et al* 2003).

Hydrogels containing redox active groups could be generated by the photochemically initiated polymerisation of polyethylene glycol dimethacrylate and vinyl ferrocene. These materials were utilised to immobilise glucose oxidase on gold electrodes (Sirkar and Pishko 1998) with the resulting glucose sensors showing good linearity between 2-20 mM. It was also possible to produce patterned sensors using these materials and photolithographic techniques. Vinyl ferrocene could also plasma polymerised onto a needle-type electrode to give a redox layer onto which further plasma processes could be used to deposit acetonitrile to give a hydrophilic surface,

suitable for the immobilisation of glucose oxidase and construction of a glucose sensor (Hiratsuka *et al* 2005).

7. Molecularly imprinted polymers in biosensors

The use of biological molecules within sensors can lead to problems. The molecules can be difficult to purify, can be expensive and often display limited stability. One possibility to try to address this problem is to make artificial systems that mimic the behaviour of biologicals such as enzymes or antibodies. Molecularly imprinted polymers (MIPs) represent a possibly solution (Whitcombe and Vulfson 2001, Hillberg *et al* 2005, Alexander *et al* 2006). Basically a template molecule, which can be biological in nature - is mixed in solution with a variety of polymerisable monomers, some of which will interact with it. The monomers are then polymerised and crosslinked to create a network with the template complexed within it. If the template is then washed out, a "pocket" remains and this could then potentially selectively entrap more template molecule. This is summarised in figure 8.

Although sensors which contain MIPs are not biosensors in the classical sense i.e. there is no biological molecule contained within the polymer, they can be synthesised containing recognition sites for biological molecules by using a biological template - although not as selectively as their biological counterparts. MIPs have shown some promise as sensors for biological molecules. For example, an inorganic polymer film containing glucose was deposited on a quartz crystal microbalance by a sol-gel process. If the template is removed this allowed the resultant film to act as a sensor, giving a change in mass when exposed to aqueous glucose (Lee and Kunitake 2001). Vanillylmandelic acid, which can be a marker for some tumours, was incorporated as

a template into a crosslinked methacrylic acid polymer film, cast on an electrode (Blanco-Lopez *et al* 2003) and washed. Voltametric measurements were then made on the system when immersed into solutions of vanillylmandelic acid. The resultant sensor was capable of detecting the analyte at concentrations between 1×10^{-4} and 1.7×10^{-3} M. Later work refined this to permit a linear response between 5×10^{-8} and 1×10^{-5} M (Dineiro *et al* 2005). An amperometric detector for fructosylamine utilising a polyvinyl imidazole based MIP has also been described (Sode *et al* 2003).

Electrochemical generated polymers have also been used and if deposited in the presence of a template which is later removed - have allowed the resultant film to detect a target analyte. For example poly *o*-phenylene diamine could be electrochemically deposited from a glucose solutions onto a QCM chip. When washed, the resultant chip showed a sensitivity for glucose (Malitesta *et al* 1999). Also sensors for atropine (with a linear range between 8×10^{-6} and 4×10^{-3} M (Peng *et al* 2000)) and sorbitol, (which is thought to cause complications for diabetes patients (Feng *et al* 2004)) have been developed, both being based on poly *o*-phenylene diamine. The sorbitol sensor had a range of 0-16 mM and was selective with respect to other sugars.

An interesting development of this technology is that MIPs have been used to sense cells as well as molecules. Coating a QCM with a crosslinked polymer into which yeast cells had been impressed, gave a selective sensor for yeast over other bacterial strains (Dickert and Hayden 2002). Sensors for enzymes and viruses could also be obtained by this method (Hayden *et al* 2003) and could be applied successfully to the detection of viruses in tobacco plant sap (Dickert *et al* 2004).

8. Conclusions

We have within this chapter surveyed some of the applications of polymers in biosensors. It is obvious that this is a field which will command much interest over the years to come. The versatility of polymers available, conductive or insulating, hydrophobic or hydrophilic, rigid or flexible, impervious to water or swellable - gives rise to a wide range of potential applications. This is aided by their processability, the wide range of synthetic and deposition methods and the ability to fine-tune their properties by changes in the chemical and physical structures of the polymers.

The most relevant fields for polymer research in the future in the field of biosensors, we feel, are those focussed towards designing “smart” polymers. We have seen attempts to replace the biological components of biosensors with MIPs, which has the potential for eliminating the problems of stability and supply of biological molecules. The use of conducting polymers will also be of great interest, whether of the conjugated type e.g. polypyrrole - or the redox hydrogel polymers developed by Heller and others, to directly “wire” biomolecules to electrode.

Finally, the vast majority of these biosensors will be required to deal with physiological samples such as blood - or potentially to be implanted as functional components within *in vivo* devices. It is obvious that such sensors, unless they are designed for single use only, must show biocompatibility and stability over extended periods of time.

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Figure 1. Schematic of biosensor components.

Figure 2. Structures of polymers used within biosensors: (a) cellulose acetate (b) Nafion (c) pluronic type surfactants (d) polyhydroxyethyl methacrylate.

Figure 3. Structure of methacroyl phosphoryl choline (a) monomer (b) polymer.

Figure 4. Structures of conducting polymers: (a) polypyrrole (b) polythiophene (c) polyaniline.

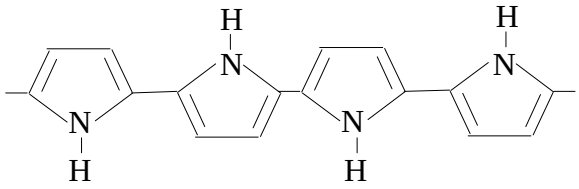
Figure 5. (a) deposition of insulating layer (b) sonochemical formation of pores (c) polymerisation of aniline, SEM pictures of (d) pores (e) polyaniline 'mushroom' protrusions.

Figure 6. The oxidation of glucose at an electrode, mediated by a ferrocene derivative.

Figure 7. Structures of redox active polymers based on osmium bipyridyl complexes substituted onto (a) polyvinyl pyridine (b) polyvinyl imidazole.

Figure 8. Schematic representation of the imprinting process: A template is complexed, either covalently or noncovalently with functional monomers. The complex is polymerized with an excess of crosslinker to form a rigid porous shell around the template. Removal of the template creates a recognition site or cavity capable of reversibly rebinding the template. Reproduced with permission from the author (Whitcombe and Vulfson 2001) and Wiley-VCH.

a.



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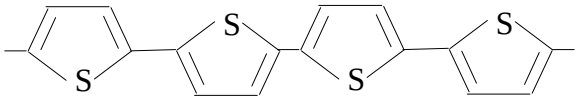

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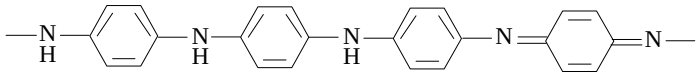

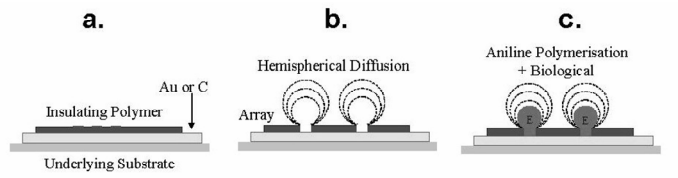
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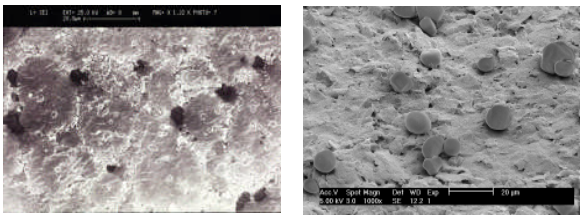
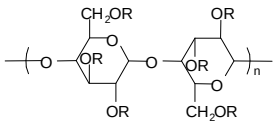
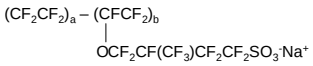


d. e.



a. R = $COCH_3$

d. R = $-(CH_2)_2OH$



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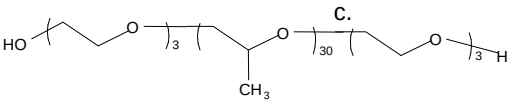

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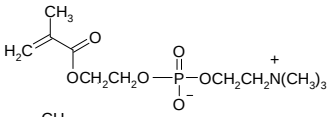

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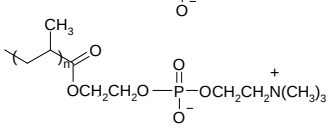

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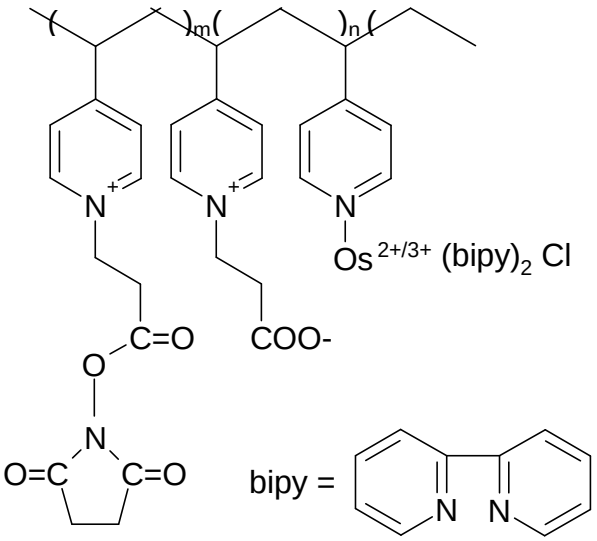
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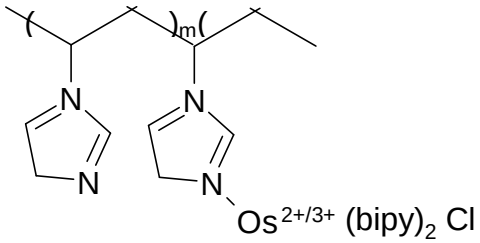
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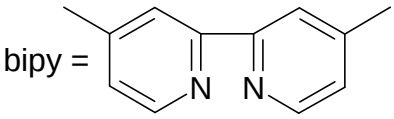
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Polymers in biosensors.

Davis, Frank

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Frank Davis and Seamus P. J. Higson, Polymers in biosensors. In: Biomedical Polymers, Woodhead Publishing (6 Aug 2007), By M. Jenkins (Author, Editor) ISBN 1845690702
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