

Flexible Ultra Thin PolyDVB/EVB Composite Membranes for the Optimisation of a Lactate Sensor

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ABSTRACT

An ultrathin composite membrane has been developed as the outer covering barrier in a model amperometric lactate oxidase enzyme electrode. The membrane was formed by cathodic electropolymerisation of divinylbenzene/ethylvinylbenzene at the surface of a gold coated polyester support membrane. Permeability coefficients were determined for O₂ and lactate across membranes with a range of polymer thicknesses. Anionic interferents (such as ascorbate) were screened from the working electrode by the composite membrane. The composite enzyme electrode showed an increased working concentration range and extended linearity of responses in comparison to an uncoated enzyme electrode.

Keywords: cathodic electropolymerisation, composite membrane, amperometric enzyme electrode, polydivinylbenzene, lactate.

1. Introduction

Biosensor research represents an ever growing field of interest, with increasing numbers of sensors being marketed commercially over the last 15 years [1-7]. Further developments in membrane technology have helped to maintain a sustained interest in the area of amperometric membrane based sensors [8-10].

When measuring whole blood samples, two problems must be overcome: Firstly interferents that may be present in the blood (such as ascorbate) must be prevented from reaching the electrode since they can be oxidised and give rise to erroneous readings. Secondly 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. One approach is to employ two separate functional membranes [11]. An outer membrane is provided to act as a substrate diffusion limiting barrier as well as to provide favourable biocompatibility [12]. An underlying (inner) membrane is also employed as a permselective barrier [13] and is typically positioned immediately over the working electrode to screen out interferents. Unfortunately both membranes act as additional diffusional barriers through which the enzyme substrates and/or H_2O_2 must traverse, so hindering their passage to the enzyme or working electrode - this frequently increases the overall response time.

This has led to research towards the development of single multi-functional ultra thin film composite membranes [14,15]. Membranes of this type have been shown to be capable of serving as both highly substrate diffusion limiting barriers towards enzyme substrates, whilst also effectively screening anionic interferents from the working electrode via a charge exclusion principle [16]. The usual method for formation of composite membranes of this type is to deposit a homogeneous film (ideally 100nm thickness) of a biocompatible polymer onto a highly porous support membrane, which offers mechanical support to the structure. Amperometric glucose oxidase laminate enzyme electrodes employing such biocompatible composite membranes have been found to display shortened response times [17].

It should be noted in this context that the separational performance of a homogeneous polymer film membrane relies on its chemical properties and not on its thickness *per se*, whereas the rate at which the separation can occur is dependent on

the thickness of the membrane. It therefore follows that the thinner a membrane is, the better will be its performance also. On the other hand it should be appreciated that if a membrane is made too thin, it will become excessively fragile and thus be rendered useless. A membrane of substantially reduced thickness which could offer the required separational performance, whilst still possessing the mechanical strength of a conventional contemporary membrane, would therefore clearly be highly advantageous.

Various methods exist for the manufacture of membranes of this type, with one of the most applicable being that of electrodeposition of either a conducting or non-conducting polymer, the use of which within biosensors has been reviewed [18]. Electrodeposition affords the possibility of accurate control of the location and thickness of a deposited polymer film. The active species e.g. an enzyme is incorporated within the polymeric film [18].

Previous work within our group combined the use of an active layer such as a chemically crosslinked film of glucose oxidase - which is either (i) directly deposited onto or (ii) laminated to a supported electro-deposited membrane at an electrode surface. Once deposited, this active layer is then covered with the permselective composite membrane which both screens out interferents and offers favourable biocompatibility. Our work initially utilised highly porous commercial alumina membranes as supports for ultra-thin polydivinylbenzene/ethylvinylbenzene (DVB/EVB) or polyacrylonitrile films [19, 20]. The DVB/EVB system was found to be especially suitable, giving increased linearity of glucose response, good exclusion of ascorbate and favourable biocompatibility [19]. The alumina support membranes were, however, somewhat inflexible and brittle. The next phase of this work was focussed towards developing this sensor further by increasing its mechanical flexibility and so increasing the possible scope of application within sensors for practical applications. A sensor that can withstand greater manipulation would in particular allow for the production of different future cell designs. This led to the development of a glucose sensor containing a permselective membrane based on commercial polyester membranes [21] - which besides their improved physical properties displayed improved biocompatibility, stability and accuracy in responses in comparison to similar enzyme electrodes employing alumina membranes.

Ever since La Roche produced the first lactate sensor, (the “Lactate Analyser LA640”) in 1975, research has continued in this area. Within clinical analysis and biotechnology, glucose and lactate are two of the enzyme substrates that are most often determined at this present time [22].

Lactate is widely distributed within blood, muscle tissue and body fluids and is an important parameter within both sports medicine and clinical care [23]. For example, the monitoring of lactate levels is useful in high dependence care units and during surgery, since the determination of lactate levels may provide early detection of clinical shock and therefore this approach helps to minimise metabolic stress [24]. The monitoring of patient lactate levels is particularly important when conditions such as myocardial infarction, congestive heart failure, respiratory failure, pulmonary edema, septacemia or haemorrhage have been diagnosed [25]. These conditions result in an inadequate cardiac output, causing a decrease in blood pressure. The limited blood flow then causes the circulatory system to deteriorate, which in turn reduces the cardiac output further, causing a vicious cycle that leads to 85% of patients who develop cardiogenic shock not surviving [26]. The poor available supply of oxygen to shocked tissue diminishes the oxidative metabolism and results in acidosis. When this occurs the cells must obtain their energy by the anaerobic process of glycolysis, which causes excessive quantities of *lactic acid* to be released into the bloodstream. In addition to this, the poor blood flow through the tissues limits the removal of carbon dioxide from the blood, which reacts with water to form very high concentrations of intracellular carbonic acid, and so the condition *acidosis*. Early detection of these excessive levels of blood lactate may therefore prove crucial to a patient’s survival. Normal physiological measurements of lactate within whole blood range between 0-2 mM, whilst levels of lactate found in a patient suffering from this type of shock rarely exceed 20 mM [27].

The use of lactate sensors within sports medicine is also widely used to monitor fitness as well as to detect injury to tissues and even thrombosis [28]. Lactate sensors also allow an athlete to monitor the success of their training by determining the muscles’ anaerobic threshold during physical exercise [23]. Actively contracting muscles obtain energy in the form of Adenosine Triphosphate (ATP) from glucose stored in the bloodstream and the breakdown of glycogen stored in the muscles. The breakdown of glucose initially produces pyruvic acid, which, when mixed with oxygen is converted to carbon dioxide, water and ATP. If the muscles are contracting

vigorously for long periods of time, however, the circulatory system begins to become diminished of oxygen. Under such anaerobic conditions, the pyruvic acid produced via the breakdown of glucose is converted to lactic acid, which leaks out of the muscles into the blood stream and is carried around the body.

During normal cellular respiration, with only moderate physical exertion, the enzyme lactate oxidase (LOD) acts as a catalyst for the oxidation of lactate back to pyruvate and the flow of oxygen to muscle cells is generally sufficient to prevent this build up of lactate and so prevent cramp. If, however, the oxygen supply remains diminished, the functioning of the body becomes impaired and the muscles fatigue very quickly.

As soon as oxygen becomes available again, the lactic acid is reconverted to pyruvic and then into carbon dioxide, water and ATP. Given that high levels of lactate within the bloodstream are detrimental to an athlete's performance, many undergo endurance training in an attempt to enable the body to perform at a greater pace with a minimal amount of lactate production. This kind of training requires the monitoring of lactate levels in blood up to concentrations of 22 mM [23, 28].

Lactate analysis is also widely used within the food industry. It is useful for evaluating food freshness and stability of milk, dairy products, fruits, vegetables, sausages and wines [28]. Lactate is a major metabolite of bacterial energy metabolism and is produced during the anaerobic fermentation of glucose and other carbohydrates [29]. The conversion of pyruvate to lactate by bacteria within milk produces yoghurt and many cheeses and also helps create pickled foods, soy sauce, dough breads and even chocolate.

A sensor utilising the flexible ultra-thin polyDVB/EVB-coated membranes described earlier [21] could produce reliable results for all types of lactate sensor described above. The advantages of this system over many of the other types of sensors reported are the control of membrane thickness conferred by electrochemical deposition and the flexibility of the resultant glucose sensor. These membranes have already been shown to exclude ascorbate interference and when utilised to measure glucose in whole blood samples, to confer excellent haemocompatibility. Results from these sensors have been shown to correlate with standard hospital tests for glucose with a high degree of accuracy [21]. The flexibility of the composite membranes, however, would be most advantageous towards the development of a flexible sensor for use in sport science. As described earlier, the flexibility of the sensor could allow

a lactate sensor to be produced in the form of a sticking plaster, to determine lactate levels within sweat upon the surface of an athlete's skin.

This paper therefore describes the fabrication of a range of polyDVB/EVB-coated membranes (coated via 1, 2, 3, 5 and 10 voltammetric cycles) and their incorporation within a lactate oxidase sensor. The permeability coefficients for the range of polymer-coated membranes towards the enzyme substrates were investigated, along with the membranes' effects on response linearity and screening of electroactive interferents.

2. Experimental

2.1 Reagents and Membranes

N,N-dimethylformamide, tetrabutylammonium perchlorate (electrochemical grade) and divinylbenzene (70-85% DVB, 15-30% 3- and 4- EVB, containing 0.1% polymerisation inhibitor: 4-tert-butylcatechol) were all purchased from Fluka Chemicals (Dorset, England).

Disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4) and sodium chloride (NaCl) were all obtained from BDH (Poole, Dorset). Lactate oxidase from *Pedicoccus species* (20-40 units/mg solid), L-lactic acid (lithium salt) bovine serum albumin (fraction V) and glutaraldehyde (grade II, 25% aqueous solution) were all purchased from the Sigma Chemical Company (Poole Dorset, UK).

Aluminium oxide Chromatography Grade was obtained from F.S.A. Laboratory Supplies (Loughborough, UK). Granular calcium hydride was purchased from Fisher Scientific (Leicestershire, UK).

Electrolube® silver conductive paint (Electrolube® Ltd, Berkshire) was purchased from Maplins Electronics, Manchester, UK and used for the fabrication of Au counter electrodes.

Cyclopore™ 0.4 μm polyester membranes, were purchased from Whatman International, Maidstone, UK. 0.015 μm pore diameter polycarbonate membranes (Poretics Corporation, Livermore, USA) were used as the lower membranes within enzyme/membrane laminates [17].

2.2 Buffers and Solutions

A phosphate buffer (pH 7.4) comprising 5.28×10^{-2} M Na_2HPO_4 , 1.3×10^{-3} M NaH_2PO_4 and 5.1×10^{-3} M NaCl in deionised water was used for the preparation of all solutions for diffusion chamber mass transport investigations and lactate calibration measurements.

2.3 Preparation of Conductive faced Membrane Electrodes and Counter Electrodes

In order to electropolymerise the DVB/EVB monomer onto the host membranes, the uppermost surface was first rendered electrically conductive by Au sputter coating the membranes with an Edwards S150 B sputter coater in a similar manner as used for electron microscopy sample preparation [16]. The gold-coated membranes were then attached to multicore wires using fluxless solder and were then used as working electrode assemblies for the electrochemical preparation of thin polymer film composite membranes.

Gold counter electrodes were constructed by coating glass slides in a similar manner and attaching multicore wires via the application of ‘*Electrolube*’ silver conductive paint. A further coating of epoxy resin served to insulate the wire/electrode joint, whilst also enhancing the mechanical strength of the electrode.

2.4 Removal of Polymerisation Inhibitor present in as-received Divinylbenzene

Prior to the polymerisation of the divinylbenzene, the polymerisation inhibitor (4-tert butylcatechol) was removed from the as-received monomer as previously described [19]. It must be noted, however, that the monomer preparation consisted of a mixture of 70-85% divinylbenzene plus 0.1% polymerisation inhibitor with the rest of solution constituting 3- and 4-ethylvinylbenzene. Since no attempt was made to separate the DVB from the EVB, the resulting polymer films obtained are copolymers of the two styrenic monomers.

The as-received DVB/EVB was extracted 10 times with 5% aqueous sodium hydroxide to remove the 4-tert-butylcatechol. The extract produced was washed six times with high purity de-ionised water and then passed through a column of neutral

activated aluminium oxide onto granular calcium hydride. The inhibitor free divinylbenzene was then stored at 4°C over calcium hydride prior to use.

2.5 Electrodeposition of polyDVB/EVB onto Polyester Host Support Membranes

Solutions of monomer (70% v/v) in *N,N*-dimethylformamide containing 0.12M electrolyte tetra-*n*-butyl ammonium perchlorate were degassed for 20 minutes using purified nitrogen prior to polymerisation. The electropolymerisation of DVB/EVB onto gold-coated polyester membranes was achieved via the same method described earlier [21]: *i.e.* by cyclically scanning the working electrode potential from 0 V through to -4.0 V and back to the starting potential (vs. Ag) at a scan rate of 50 mVs⁻¹. Polymer-coated membranes were produced via 1, 2, 3, 5 and 10 potential cycles.

2.6 Enzyme Laminate Fabrication

Lactate oxidase was immobilised within an enzyme laminate for the detection of lactate concentrations in phosphate buffer. The laminate was prepared as follows: Lactate oxidase (40 units ml⁻¹) and bovine serum albumin (0.1mg ml⁻¹) were dissolved in (pH 7.4) phosphate buffer to form a LOD/BSA solution. 6µl of this solution was then added to 3µl glutaraldehyde (0.5% v/v in buffer) mixed rapidly and placed on a 1cm² portion of (0.015µm pore) polycarbonate membrane. A 1cm² portion of the thin-polymer-film composite membrane (polymer film uppermost) was then placed on top of the enzyme/albumin matrix and the enzyme membrane laminate compressed under gentle finger pressure for approximately 5 minutes to allow crosslinking of the protein and thus immobilisation of the enzyme. The enzyme laminate was then positioned over the working electrode of a modified Rank oxygen electrode [13] such that the polymer-coated surface faced uppermost (Figure 1b).

2.7 Enzyme electrode glass cell for lactate determinations

A bespoke glass electrochemical cell was utilised as before [19,20], Figure 1c, for enzyme electrode lactate determinations. This glass cell consisted of two halves clamped together. The base was constructed with a flat circular surface, at the centre of which was a platinum working electrode of 2mm diameter. The polymer coated

composite membrane laminate was positioned over the platinum electrode of the cell (polarised at +650mV vs. Ag/AgCl) and sealed in place with an 'O-ring' to follow the detection of H₂O₂. The upper half of the cell acted as a chamber into which solutions and reference and counter electrodes were introduced. A circular hole in the base of this served to expose the underlying working electrode to the solution. A further circular indentation was made in the underside surface of the solution well to house the O-ring. A silver electrode was placed vertically into the main chamber of the cell to act as a combined counter and reference electrode. The enzyme laminate was positioned over the Pt working electrode with the polymer-coated surface positioned uppermost.

2.8 Investigation into Solute Permeability Coefficients

The mass transport, and therefore permeability coefficients, of both oxygen and lactate across a range of ultra-thin polymer composite membranes were determined using conventional diffusion chamber apparatus as previously described [13]. A Rank Oxygen Electrode assembly (as previously described [13]) was used for the determination of oxygen levels within solution, whilst a lactate enzyme electrode (as described above), was fabricated for the determination of lactate levels.

3. Results

3.1 The Electrodeposition of polyDVB/EVB onto Polyester Host Support Membranes

The electropolymerisation of DVB/EVB onto gold-coated polyester membranes was achieved via the same method described earlier [21]. Polymer-coated membranes were produced via 1, 2, 3, 5 and 10 potential cycles.

The surface topography of polyDVB/EVB-coated polyester membranes was investigated and described in our earlier paper [21]. Membranes were found to exhibit a 'pitted' topography following the first few potential cycles, due to the critical gel-point being reached earlier than on the surface, as the concentration of soluble polymer was present in the pores was greater. Since the membranes incorporated within this lactate oxidase sensor are identical to those employed within the glucose oxidase sensor described earlier [21], the film thickness has been calculated via charge integration to be approximately 50 nm for a 10 layer film.

3.2. Enzyme Immobilisation

The prototype sensor described in this chapter was designed to electrochemically quantify levels of lactate concentrations via the amperometric detection of hydrogen peroxide produced by the enzyme lactate oxidase (LOD):



LOD has previously been shown to remain active for up to 21 days (from an initial activity of 42.4 $\mu\text{M H}_2\text{O}_2/\text{min/mg}$ to approx. 5 $\mu\text{M H}_2\text{O}_2/\text{min/mg}$), when immobilised within a crosslinked within a gel of bovine serum albumin with glutaraldehyde [22]. The rigid matrix formed in this way also adheres to both underlying and upper membranes to produce an enzyme laminate. The LOD enzyme described in the chapter was therefore immobilised in this way.

3.3. The Determination of Permeability Coefficients

The Permeability coefficients, (P), for lactate and oxygen through polymer-coated polyester membranes were obtained as described above. Figure 2a depicts the permeability coefficients for lactate across a membrane coated with polyDVB/EVB via 1, 2, 3, 5 and 10 potential cycles, whilst Figure 2b depicts the P coefficients for oxygen.

The P values for lactate across polyDVB/EVB-coated polyester membranes (Figure 2a) decreased most markedly following the first potential cycle, after which point they tend towards a plateau. This would indicate that the membrane pores become blocked at this point, however, this may not be the case since the P values for oxygen indicate that it is after the second potential that the mass transport of oxygen molecules through the membrane is most greatly hindered (Figure 2b).

The oxygen P values were found to decrease as each successive polymer was applied and the underlying membrane became increasingly coated with layers of polymer. The greatest decrease in the mass transport of oxygen through the membrane

was found to occur following the initial first and second potential cycles, after which point the P values decrease at a reduced rate.

The difference in P values between lactate and oxygen can initially be explained by the larger lactate molecule meeting increased resistance to the diminishing pore size following the first potential cycle. Our earlier papers [19-21] describe how the smaller oxygen molecule may still be able to diffuse through any small pinhole areas of the pores that remain unblocked after the first cycle, whereas the lactate begins to partition through the polymer film itself. Following the application of the second potential, however, both substrates begin to use partitioning as their mode of transport.

Once all membrane pores are blocked, however, molecular size may no longer be important and both substrates must partition across the polymer barrier. We have already seen that the O_2 permeability decreases significantly after the second initial polymer layer has been deposited and then continues to decrease gradually further with each successive layer deposited, implying that partitioning becomes the increasing mode of mass transport for O_2 across the membrane, Figure 2b. The greatest decrease in lactate permeability on the other hand, occurs following the first polymer coatings, Figure 2a; at this point the lactate must now also partition through the composite membrane. Following 10 potential cycles, the lactate permeability coefficient reaches a minimum as the polymer film at this point is at its thickest. The gradual decrease in both lactate and oxygen permeability as each successive coating is applied may be due to either a) an increased resistance being offered to a larger molecule as it partitions across the polymer, or b) a possible residual 'pin-hole effect'.

Earlier SEM studies [21] suggested that, although not visible at the surface, the vast majority of the membrane pores have some amount of polymer formed within or across them after 2 potential cycles. Since it is following these first few potential cycles that membrane pores become blocked, successive potential cycles therefore contribute to the cumulative thickness of solid polymer formed, resulting in increasingly lowered P values.

Previous studies employing thin-polymer-film composite membranes as the upper covering membrane within glucose sensors have shown that, in this context, membranes exhibiting lower glucose/oxygen P ratios are associated with extended sensor response linearity ranges [19-21]. It therefore follows that lowered

lactate/oxygen P ratios would also contribute to greater linearity of response and so these values were calculated and are depicted in Figure 2c.

P ratio values are seen to decrease slightly following the first potential cycle, since the larger lactate molecule first meets an initial increased resistance as the first electrodeposition of polyDVB/EVB decreases the pore diameter. P ratio values are then seen to rise again slightly, with the second potential cycle. Individual P values for both substrates have indicated that it is within the first two cycles that the polymer blocks the membrane pores. Following the second potential cycle, however, the P ratio values continue to decrease gradually with each successive polymer coating applied.

Figure 2c shows that lactate/O₂ P ratios fall for the membrane coated via 5 potential cycles to values approaching those seen at membranes coated with one or no polymer layers. The permeability coefficients, of course, for both solutes, however, are lower. The P ratios are seen to increase following the tenth potential cycle, however, a characteristic also observed for glucose/oxygen P ratios in our previous work on glucose sensors [21]. This was attributed to a slight deterioration in the uniformity of the polymer-coated membrane surface following multiple potential cycles [21]. For this reason, the prototype lactate oxidase enzyme electrode was constructed by utilising a polyDVB/EVB-coated composite membrane coated via 5 potential cycles as the upper covering barrier.

3.4. Calibration and Comparison of a Range of Lactate Sensors Incorporating Flexible Thin-Film PolyDVB/EVB Composite Membranes

A range of lactate oxidase enzyme electrodes was constructed, with each electrode employing a flexible composite membrane produced via 1, 2, 3, 5 or 10 potential cycles. Calibration curves were obtained over a concentration range of 0mM to 25mM lactate in phosphate buffer (Figure 3). All membranes served as effective limiting barriers to the diffusion of both the enzyme substrates and the responses to normal physiological concentrations found in human whole blood (0-2mM) were found to be linear for all membranes. With each polymer coating deposited, the amperometric responses became increasingly linearised and response times for all sensors were found to be <1 minute.

3.5. *Exclusion of Electroactive Interferents Present In Human Whole Blood*

An ultra-thin polyDVB/EVB composite membrane coated via 5 potential cycles was employed as the upper covering membrane in the enzyme electrode. The sensor described within this chapter is initially aimed towards the detection of lactate within whole blood for use within clinical emergency situations, *e.g.* to monitor the conditions of shock experienced by a patient. The possibility of utilising the ultra-thin-composite membranes within a sensor designed for in-vivo analyses or within whole blood in vitro, requires that the membranes permit effective screening of electroactive interferents that may be present in blood. In the same way, that the effect of anionic interferents such as ascorbate were investigated for the glucose sensors described earlier [19-21], the lactate sensor produced was also assessed for its possible capabilities for screening interferents. What is of interest is that the exclusion cannot be solely on the basis of charge exclusion or lactate itself would be unable to cross the composite membrane. Our previous work [21] has shown that glucose passes more slowly through these membranes than the much smaller oxygen molecule, therefore it appears that a combination of size and charge exclusion minimises the passage of ascorbate. Also for interference to occur, ascorbate would have to cross the entire membrane to be reduced at the electrode whereas lactate will be oxidised within the membrane and produce hydrogen peroxide which is uncharged.

The enzyme electrode was therefore evaluated for its ability to screen anionic interferents as described earlier [19-21] and was exposed to lactate both in the absence and presence of 1mM ascorbate. It was found that the enzyme electrode response was not significantly affected by the presence of ascorbate until a concentration of 20mM lactate was exceeded. The response was found to increase by 10nA following the addition of 20mM lactate with ascorbate and 10nA for 30mM lactate with ascorbate. This increase in response may be due to the production at high lactate concentrations of high levels of pyruvic acid and hydrogen peroxide by the enzyme. Previous researchers [30] have reported the facile reaction between pyruvate and hydrogen peroxide to give acetate, water and carbon dioxide, which is acidic ($pK_a = 6.1$) and in sufficiently large quantities could lower the enzyme electrode's net anionic charge and allow anionic interferents to reach the working electrode more freely. Since the typical concentration range of lactate found within blood is between

0mM and 2mM, these results therefore demonstrate that for physiological lactate analyses, the sensor proves to be effective at screening electroactive interferents.

The membrane described here for use within a lactate sensor employed the same membranes described earlier [21] and SEM images depicting the surfaces of both the bare flexible polyester membrane, and the polyDVB/EVB-coated membrane following exposure to whole human blood for 2 hours, showed that both coated and uncoated surfaces offered favourable haemo/biocompatibility [21].

4. Conclusions

Flexible ultra-thin-polyDVB/EVB composite membranes have been electrochemically fabricated and successfully employed as the outer covering barrier in a model lactate oxidase sensor for the determination of lactate.

Cyclically scanning the working electrode potential between 0 V and -4.0 V (vs. Ag) via 1, 2, 3, 5 and 10 cycles produced a range of composite membranes. Diffusion chamber experiments were performed to determine the permeability coefficients of lactate and oxygen across the range of membranes. As the pores of the underlying polyester membranes became increasingly blocked by polymer, the lactate and O_2 P values decreased as both solutes begin to partition through the polymer film to reach the enzyme matrix.

The composite membranes fabricated were employed as the outer covering barrier in model lactate oxidase sensors for the determination of lactate. Lactate calibration curves were produced for all enzyme electrodes and were found to display increasingly substrate diffusion limiting properties with increasing polymer coatings.

The sensors also effectively screened against electroactive interferents via a charge exclusion mechanism. All composite membranes produced linear responses for normal physiological concentrations in human whole blood (0-2mM) and linearity of sensor responses was also achieved up to 25mM in some cases. The sensor response time was found to be <1 minute. The underlying polyester membrane proved to act as a suitably biocompatible support for the polymer film, which also displayed favourable biocompatibility, thereby helping minimise surface biofouling effects.

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LEGENDS FOR FIGURES.

Figure 1. Schematic of (a) a polycarbonate-DVB/EVB composite membrane (b) side view of an enzyme laminate placed over the working electrode of the modified Rank Cell (c) electrochemical cell used for enzyme electrode measurements.

Figure 2. Permeability coefficients across polyDVB/EVB coated thin polymer film composite membranes for (a); lactate, (b); oxygen and (c); the lactate/oxygen permeability ratios across polyDVB/EVB coated membranes.

Figure 3. Lactate oxidase enzyme electrode calibrations employing membranes coated via 0 ($\square$), 1 ($\times$), 2 ($\blacklozenge$), 3 ($\bullet$), 5 ($\blacksquare$), and 10 (+) potential cycles



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