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Short communication

Strategic issues in reliable sensing

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Abstract

Amperometric enzyme biosensors must possess two important characteristics if they are to be successfully utilised as reliable monitoring devices. They must exhibit linearity over concentrations relevant to the target analyte, and they must avoid contamination, adverse reactions with the sample matrix response to interferents that react directly at the polarised working electrode surface.

Covering polymeric membranes have provided a useful route to overcoming these problems. This report summarises successful modulation of membrane bulk as well as surface properties using surfactant-loaded diffusion limiting PVC and phenolic membranes, and the possible exploitation of direct response conducting poly (pyrrole) membrane loaded with affinity molecules through impedance spectroscopy.

Keywords: PVC; Surfactant; Poly (pyrrole); Biosensor; Electropolymerisation

1. Introduction

The successful long-term development of amperometric enzyme electrodes in ''real'' samples demands materials interfaces for these devices that control both the level of access of substrate/cosubstrate to the reactive enzymic layer and also the extent of surface biofilm formation from sample colloids and cells. The latter presents an especially intractable problem since no material surface is immune from some degree of surface coating when exposed to a biomatrix (e.g. blood, tissue) [1].

The failure pathway to loss of response is especially complex in vivo and extends from plasma protein deposition, fibrin formation and cellular recognition/attachment through to complement activation and immuno-response to fibrous capsule growth and ultimate calcification.

Single homogenous, porous polymer membranes such as Nafion® have been the focus of special attention as membrane barriers for sensors. However, dual component phases have proved to be especially valuable. Here, a model system based on PVC has been used to variously support lipid (isopropyl myristate, IPM) and surfactants (Triton X-100, pluronics F68, taurocholic acid, methyl trialkyl chloride) as plasticisers. The base PVC has 8–10% crystallinity and it was the intention to use differentially hydrophobic and lipophilic plasticisers to control the diffusion of enzyme substrates and products [2] via control of the inter-polymer chain void volume and chain flexibility. Surfactants were also co-entrapped in electropolymerised non-conducting phenolic films to vary selectivity and any surface fouling.

As an extension of the work, conducting poly (pyrrole) films were used as cationic phases to entrap or adsorb ionic receptor protein molecules (avidin, antibody to lutenising hormone) for a possible impedimetric route to detection of ligand binding interactions [3]. Electrochemical impedance spectroscopy enables charge transfer to be followed and any possible conformational changes in the polymer chains due to steric effects of ligand binding can be followed.

2. Experimental

PVC membranes were prepared by solvent casting. A PVC polymer solution in THF with added plasticiser surfactant or IPM was layered on a planer glass surface and allowed to precipitate as a film by slow solvent

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evaporation over 1 to 2 days at room temperature and slow solvent venting. To reduce variable thickness and membrane density, constant revolution (1000 rpm) spin coating was also employed.

For phenolic electropolymerisation, various phenols (phenol, phenol red, disperse blue, rosolic acid, new fuschin) were either directly electropolymerised at a Pt surface at fixed anodic voltage in stirred solution (+0.85 V vs. Ag/AgCl) using a two electrode cell or deposited via voltammetric cycling between 0 and +0.9 V vs. Ag/AgCl in a three-electrode cell using an Ecochemie μAutolab type II. This was accomplished as required in solutions containing surfactant. Poly (pyrrole) electropolymerisation at interdigitated gold electrodes (Southampton University Microelectronics Centre, Southampton, UK) was achieved by cyclic voltammetry between −0.9 and +0.9 V vs. Ag/AgCl. Films were deposited over 15 cycles in the presence of a receptor protein and electrochemical impedance spectroscopy (EIS) analysis performed on a HP 4192A LF impedance analyzer using a frequency range from 5 Hz to 13 MHz in phosphate buffer.

Enzyme electrodes for oxidases (e.g. lactate) were produced by crosslinking of enzyme using conventional glutaraldehyde and bovine serum albumin (BSA)—this was done between Cuprophan dialysis membranes (Gambro, Lund, Sweden) to permit ready substitution of barrier PVC membranes.

All chemicals used were purchased from Sigma-Aldrich (Poole, Dorset, UK).

3. Results and discussion

Calibration curves of oxidase enzyme electrodes obtained using PVC barrier layers demonstrated the substrate impermeability of unplasticised PVC, and this switched to lipophilic solutes using IPM incorporation; thus at 15% w/v IPM, >100:1 catechol to ascorbate selectivity was observed. Catechol is an electrochemically detected end product of a glucose-6-phosphatase-diaphorase reaction couple usable as a label in immunoassay. This reporter molecule is generated in the bulk sample, but napthoqunione sulphonic acid could instead be localised behind such a PVC membrane.

Triton X-100-loaded PVC at various concentrations (20–90% w/v) helped permeabilise PVC in a controlled manner, whereby the effective K_m of the relevant oxidase enzyme could be extended to the full clinical range.

Electropolymerised phenol reduced surface fouling of a Pt electrode as evidenced by the response to various electrochemically active compounds. Phenol also had the effect of partially rejecting interference where H_2O_2 was the enzymic product desired for selective detection.

The other phenolics demonstrated similar selectivity properties despite differences in monomer molecular weight and the presence of different side groups. The exception was poly (rosolic acid), which totally excluded anionic interferents. However, selectivity of phenolics was enhanced when electropolymerisation occurred at pH 9. The degree of fouling in blood was reduced by the incorporation of any of the surfactants (Fig. 1). This was possibly the result of surface-exposed surfactant chains providing a mobile polymer–sample interface less liable to static colloid attachment.

Bode plots of poly (pyrrole) films loaded with avidin showed no significant change in either impedance or phase angle on exposure to biotin. However, when a subsequent redox cycle was performed, a biotin-dependent reduction in the phase angle peak was seen (Fig. 2). The same observation was made for the binding of lutenising hormone to antibody-loaded films. The development of a binding

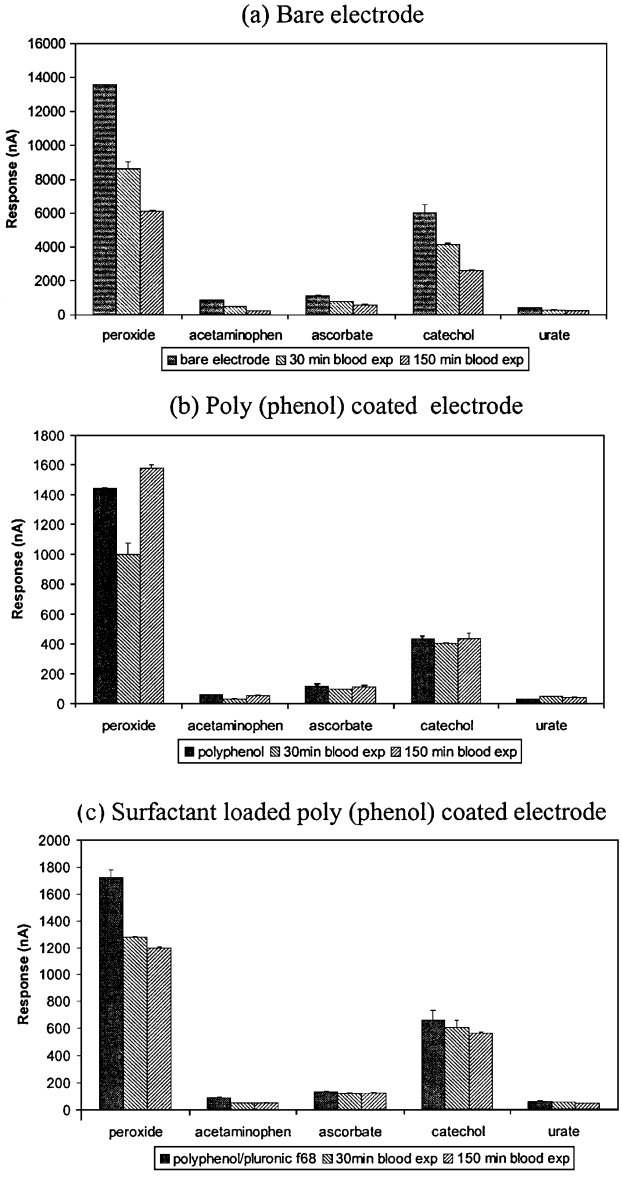

Fig. 1. Heparinised whole blood exposure of bare and phenol-coated Pt electrodes. Electrode rinsed in isotonic buffer before response determination in each analyte solution (5 mM phosphate buffer, pH 7.4).

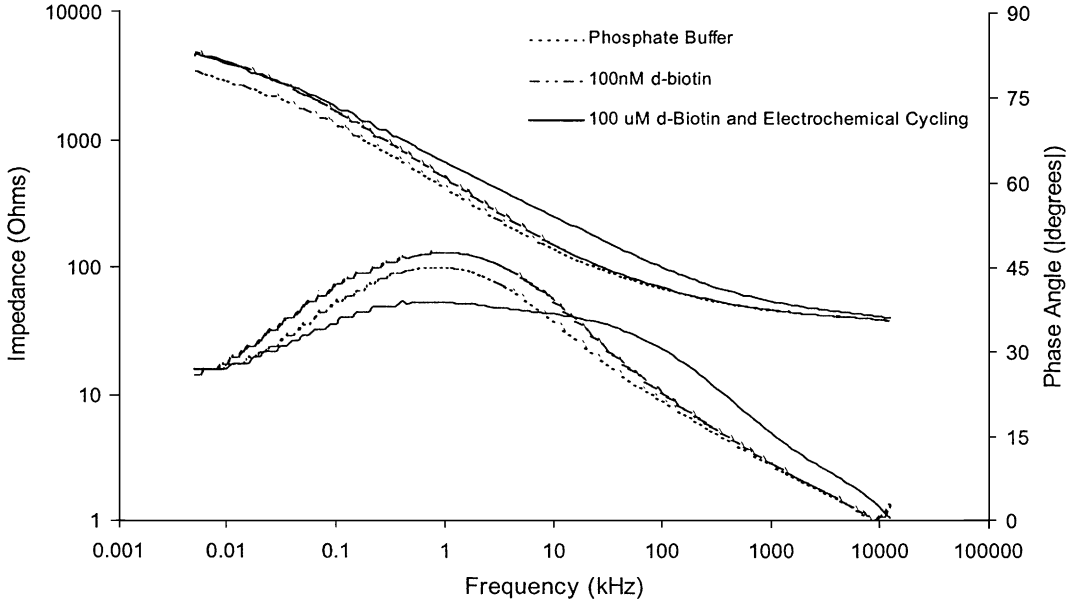

Fig. 2. EIS response of avidin-loaded poly (pyrrole) film exposed to biotin for 30 min followed by a −0.9 to +0.9 V (vs. Ag/AgCl) electrochemical cycle.

response only after voltage cycling suggests that conducting polymer film oxidation/reduction could possibly permit chain rearrangement around bound complexes.

In the case of in vivo monitoring, it appears that a static biomaterial/biosensor implanted interface is not sufficient to allow for the maintenance of a stable interface, let alone one that does not foul. It is likely that the surface reactions that matter occur in the first few seconds and that sensor implant geometry is as relevant as sensor surface chemistry. In a new technique termed Open Microflow, a partially implanted biosensor with tissue negative pressure-driven fluid flow from an external reservoir has shown the potential to create a constantly renewing interface and continuously modified sample (subcutaneous tissue) matrix. The functional outcome appears to be enhanced biosensor stability in vivo [4].

4. Conclusions

Thick PVC membranes, phenolic films and functionally responsive poly (pyrrole) layers offer different ways of altering the response of a working electrode surface whilst ensuring that any electrochemical voltage regimen for selectivity or stability is kept relatively simple. This materials selection has distinct advantages for biosensing.

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