APPLICATION OF NANOPARTICULATE CONDUCTING POLYANILINE IN NANOFILM BIOSENSOR TECHNOLOGY

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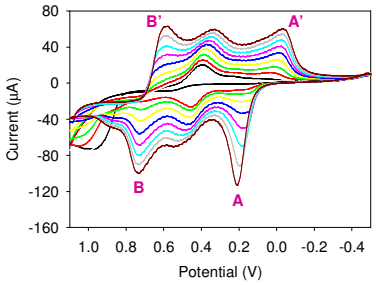
This biosensor uses a novel aqueous-based nanoparticulate polyaniline (PANI), synthesised using dodecylbenzenesulphonic acid (DBSA) and aniline as starting material.[1] These polymer nanoparticles have been electrodeposited onto the surface of carbon electrodes resulting in conductive nano-films, which were examined by electrochemistry, scanning electron microscopy (SEM), atomic force microscopy (AFM), profilometry and spectroelectrochemistry. Biomolecules were then electrostatically adsorbed onto this surface and physical techniques have shown that the nanofilm possesses properties which allow for uniform adsorption of protein to take place. This effective biosensor format has been characterised using a horseradish peroxidase (HRP) and H_2O_2 format. This sensor exhibits higher signal/noise (S/N) ratios and quicker response times than previous PANI biosensor formats developed by our group, due to its nanofilm characteristics.

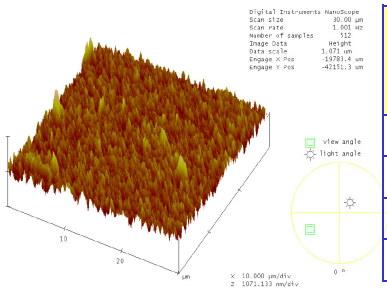
Characterisation of PANI-DBSA nanoparticulate films

PANI-DBSA nanoscale particles help to overcome processability issues associated with other PANI polymers:

- They are readily dispersed in aqueous media for electrodeposition onto electrodes to form nanofilms.
- They offer bulk solution handling characteristics with nanoscale material control.
- They can be combined with biomolecular species at this point, or subsequent to their fabrication on a sensor surface.



PANI–DBSA nanoparticles were electrodeposited onto glassy carbon (GC) surfaces by potentiodynamic cycling, resulting in conductive nanofilms with characteristic PANI behaviour. Peaks **A** and **B** are the transformation of leucoemeraldine base (LB) to emeraldine salt (ES), and ES to peringraniline salt (PS), respectively. Peaks **B'** and **A'** represent conversion of PS to ES, and ES to LB, respectively.

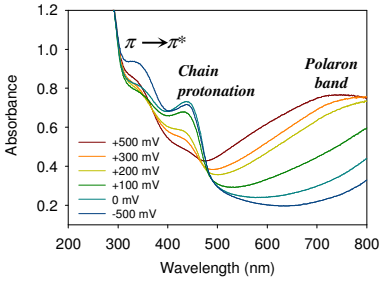


AFM 3-D image of a PANI-DBSA nanofilm electrodeposited on an electrode surface.

No. of deposition cycles of PANI-DBSA on GC	*Root-Mean-Square (nm)*
10	24.74
20	83.19
30	115.55
40	86.66

Highest surface roughness at 30 cycles.

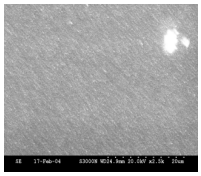
Profilometery studies estimated the thickness of the PANI-DBSA films to be 350 nm (for 30 electrodeposition cycles on GC), proving the nanoscale of the film. This thickness is about 100 times thinner than polyaniline films electropolymerised from monomer, previously synthesised in this group. Thinner films have the advantage of more rapid electrochemical switching speeds and even higher conductivity, which could be exploited for an effective biosensor format.

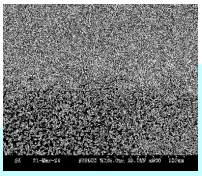


Spectroelectrochemistry of PANI-DBSA film electrodeposited on ITO glass. UV-Vis scans were carried out in phosphate buffer, pH 6.8 while the potential was held constant. The UV-Vis absorption spectra contained three characteristic absorption bands of polyaniline, observed at about 330 nm, 420 nm and 750 nm.

Immobilisation of protein onto nanoparticulate films

Protein was immobilised onto the PANI-DBSA nanofilms by means of electrostatic adsorption using 0.1 mg.ml^{-1} protein. This was characterised in terms of SEM and colorimetric assays to determine absolute mass.

(A) 

(B) 

SEM images of the bare nanofilm **(A)**, and with immobilised gold-labelled β-HCG antibody (silver-enhanced) **(B)**. This shows that protein can be uniformly distributed on the surface by means of electrostatic adsorption and that no clustering is evident.

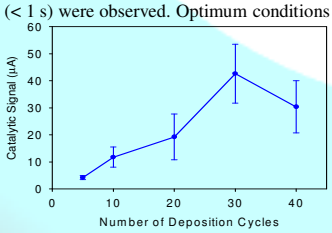
In order to assess the absolute mass loading of protein, a colorimetric assay was carried out where the absorbance of the HRP-modified PANI-DBSA surface was measured, and related to the absolute mass of HRP present on the electrode surface.[2] Calculations show that the mass of immobilised protein on the surface approximates a monolayer:

***Theoretical* amount of HRP for monolayer formation** → **4.71×10^{10} molecules.mm^{-2}**

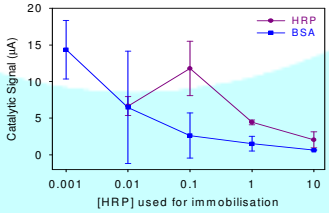
***Calculated* amount of HRP present** → **7.11×10^{10} molecules.mm^{-2}**

Application of nanoparticulate films in a biosensor

Optimisation of the catalytic response of the HRP-modified PANI-DBSA/GC electrode was carried out using a H_2O_2 format in batch set-up. High S/N (3 times greater than previous PANI-based biosensor formats developed in our group), and fast response times (< 1 s) were observed. Optimum conditions are shown below.



Optimum catalytic response obtained with 30 deposition cycles of nanoparticles. This corresponds to the roughest surface, as calculated by AFM.



Optimum catalytic response and blocking obtained using 0.1 mg.ml^{-1} enzyme for the electrostatic immobilisation – corresponding to monolayer formation.

Conclusions and future work

- It has been demonstrated that that PANI-DBSA nanoparticles can be effectively electrodeposited on glassy carbon to form nanothin films, capable of electrocatalysis in a biosensor. This effectvive biosensor format exhibits low background noise, fast response times and a high S/N ratio.
- Future work will involve alternative ways of depositing the nanofilm, and integrating this nanofilm in an immunosensor format.

[1] Moulton, S., Innis, P., Kane-Maguire, L., Ngamna, O., Wallace, G., *Curr. Appl. Phy.*, 4 (2004) 404-406.
[2] Morrin, A., Guzman, A., Pingarron, J., Killard, A.J., Smyth, M.R., *Biosens. Bioelec.*, 18 (2003) 715-720

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