Performance Enhancement of Electrochemical Biosensors by Exploitation of Nanoparticles

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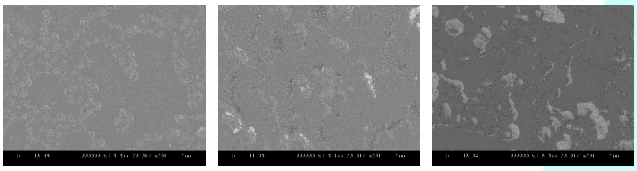
The unique chemical and physical properties of nanoparticles make them extremely suitable for designing new and improved sensing devices, in particular electrochemical sensors and biosensors. Nanoparticles can play numerous advantageous roles in biosensor platforms that can serve to improve protein immobilisation and can augment charge transfer. This work examines some novel nanoparticle strategies for enhancing performance of a conducting polymer-based biosensor.

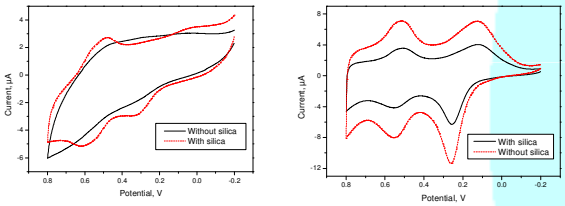
One approach was to manipulate the structure of a conducting polymer film, polyaniline (PANI), used as the diffusionless mediator in a biosensor, in order to gain more control and stability over immobilised protein. By incorporating silica nanoparticles into the films, the amount and stability of subsequently immobilised enzyme was greatly enhanced when compared to films without silica. The proposed biosensor exhibited enhanced sensitivity and stability. A study is also being carried out where the immobilised silica nanoparticles are dissolved from the films so as to obtain controlled nanostructured PANI films. It is expected that this approach could be used to prepare biosensors with excellent charge transfer capabilities.

Utilising nanoparticles for signal amplification in an electrochemical immunosensor platform where both gold and silica nanoparticles are applied as conjugate labels to the sensing species is being investigated. A bienzyme-based immunosensor platform that uses an enzyme channeling system (based on horseradish peroxidase (HRP) and glucose oxidase (GOD)) for generating electrochemical catalytic signal is presented. The slow rate of turnover of GOD by glucose compared to that of HRP by H_2O_2 was shown to be a severe limitation in terms of sensitivity. This work demonstrates how the use of nanoparticles, having an excellent ability to bind proteins, can be exploited to increase the local concentration of GOD near the sensing surface yielding, remarkable amplifications in catalytic signal.

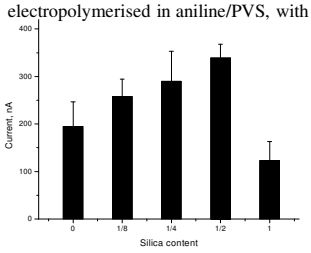
Silica/PANI/PVS-based biosensor



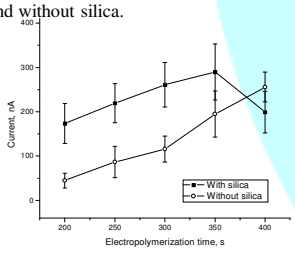
SEM of electrodes modified with silica particles (left) and covered with PANI electropolymerized for 200s (middle) and 400s (right).



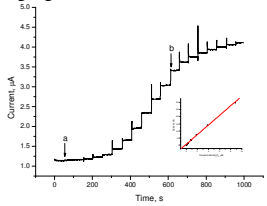
CVs of prepared PANI modified-electrodes with and without silica particles. Left, electrodes were prepared by dipping the electrodes into aniline for 2 min and electropolymerised in solution containing HCl and PVS. Right, electrodes electropolymerised in aniline/PVS, with and without silica.



Effect of silica content on the biosensor response to 10 µM H_2O_2 at – 0.1 V vs. Ag/AgCl.

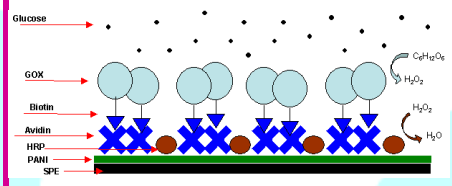


Effect of electropolymerisation time on biosensor prepared with and without silica particles. Response to 10 µM H_2O_2 at – 0.1 V vs Ag/AgCl.

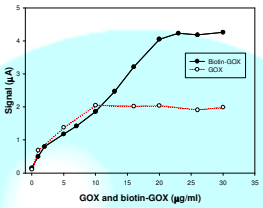


Response curve of the biosensor. a-b, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0, 500.0 µM, after b, each addition 0.5 mM. Inset, linear region of the response.

Enzyme-Channeling System In Flow-Injection Analysis

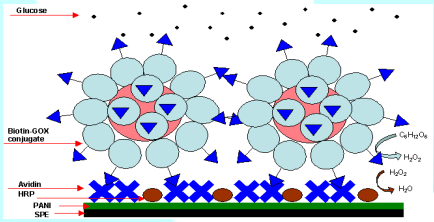


Enzyme-channeling biosensor - Avidin and HRP immobilised to PANI/PVS electrode surface. Biotin-GOX specifically binding to avidin via biotin-avidin interaction. The two enzymatic reactions occurring are shown.

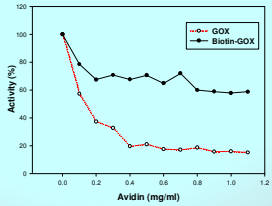


Responses from GOX and biotin-GOX at fixed avidin amount on the electrode surface (0.8 mg/ml).

Signal Amplification Approach

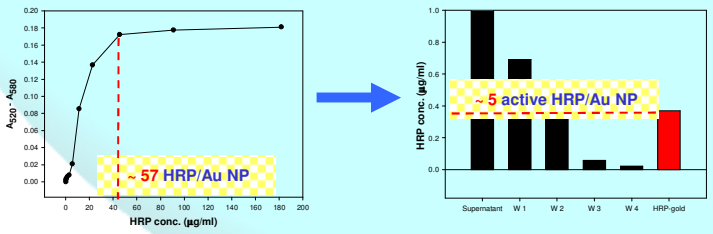


Enzyme-channeling biosensor - Biotin-GOX molecules carried by nanoparticles (e.g. gold). A higher number of conjugates binds to the surface via avidin-biotin interaction resulting in a signal amplification.



GOX and biotin-GOX (20 µg/ml) responses varying the avidin amount on the electrode surface.

Spectrophotometric Conjugation Study



Spectrophotometric characterisation of HRP/Au NP. Conjugation to Au NP (diameter:17 nm) resulted in a loss of activity of the HRP. This loss may be due to large diameter of the gold nanoparticle (low level of curvature), causing deformation of the protein structure.

Conclusions

The use of silica nanoparticles incorporated in the PANI/PVS polymer structure resulted in enhanced biosensor performance. This may be due to an increased surface area available for enzyme attachment. Silica is known to be a good platform for protein adsorption. Given that the polyaniline preferentially formed on the silica, it may be that the HRP can interact indirectly with the silica by adsorbing onto the PANI-modified silica surfaces. This is, therefore, an efficient way to improve enzyme immobilisation, without reducing the charge transfer capabilities of the system. The exploitation of conducting nanoparticles in the immunosensor platform for signal enhancement is in the exploratory stages. Conjugation of proteins to gold (diameter:17 nm) resulted in a loss of biomolecule activity. This phenomenon may be related to the conformational change of the protein structure due to the low level of curvature of the gold nanoparticles. Modifications to the conjugation process are under investigation in order to achieve the goal of signal amplification in the immunosensor system.

NCSR National Centre for Sensor Research