Characterisation Of An Amperometric Immunosensor Surface By Scanning Electron Microscopy

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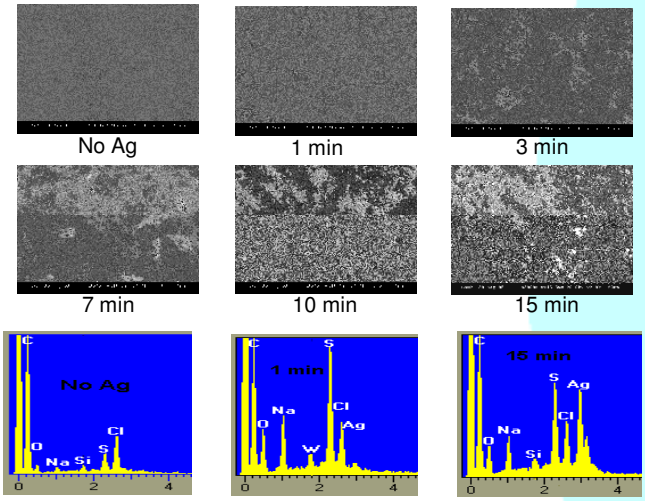
This effective polyaniline-based amperometric immunosensor platform has been developed over several years in the NCSR at Dublin City University. Construction of this sensor is achieved by electropolymerisation of polyvinylsulphonate-doped polyaniline (PANI/PVS) onto the surface of a disposable screen-printed carbon-paste electrode. Antibodies are then doped onto the surface of the polymer to yield an immunosensor capable of rapid, single-step, separation-free assays for real-time monitoring.

Although scanning electron microscopy (SEM) can provide adequate surface information as to the overall topography of protein-modified PANI/PVS films, the resolution of individual proteins or groups of proteins was not possible. However, through the use of proteins conjugated to non-fading, electron-dense particles such as gold, it was possible to indirectly visualise the distribution of proteins. A colloidal gold-labelled anti-goat antibody (Ab) was used for the visualisation of two immunosensor platforms where both anti-atrazine single chain Ab and anti-biotin Ab were immobilised on the polymer surface. Various incubation and washing steps were carried out to allow specific interactions between the immobilised Ab and anti-goat-gold Ab to occur. A silver enhancement treatment was optimised in order to improve the visualisation of the gold label. The silver enhancement caused the reduction of silver ions, resulting in the precipitation of metallic silver around the gold colloids, so as to enlarge the gold particles for enhanced visualisation. Energy Dispersive X-ray (EDX) analysis was also performed to assess the amount of silver on the surface.

Silver Enhancement Optimisation

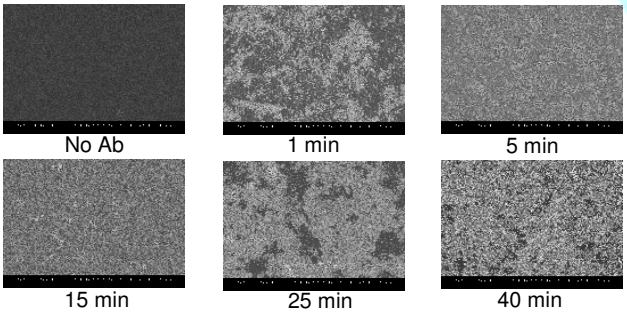
Anti-goat-gold was directly immobilised on PANI/PVS modified screen-printed electrodes and then treated with silver enhancement solution for 1 to 15 min.



EDX spectra. Silver was shown to increase with increasing treatment time.

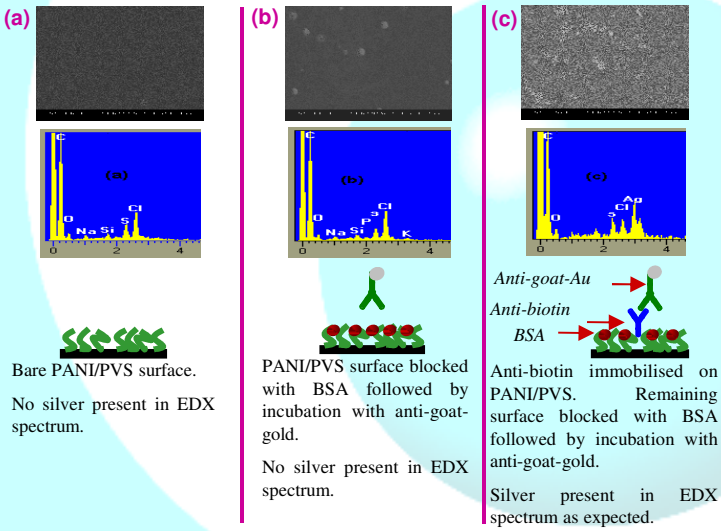
Antibody Immobilisation Time Study

Electrostatic adsorption was used to immobilise Ab to the immunosensor surface using a potential of –500 mV vs. Ag/AgCl. A range of immobilisation times were used for binding colloidal gold-labelled anti-goat Ab to the PANI/PVS modified screen-printed electrode. The surfaces were then silver enhanced for 10 min.



Anti-Biotin Immunosensor Surface

In order to visualise immobilised anti-biotin antibody, anti-biotin (developed in goat) was electrostatically adsorbed to the PANI/PVS surface for 25 min. The surface was then incubated with BSA, followed by anti-goat-gold. Silver enhancement was then carried out of the gold label **(c)**.



Bare PANI/PVS surface.

No silver present in EDX spectrum.

PANI/PVS surface blocked with BSA followed by incubation with anti-goat-gold.

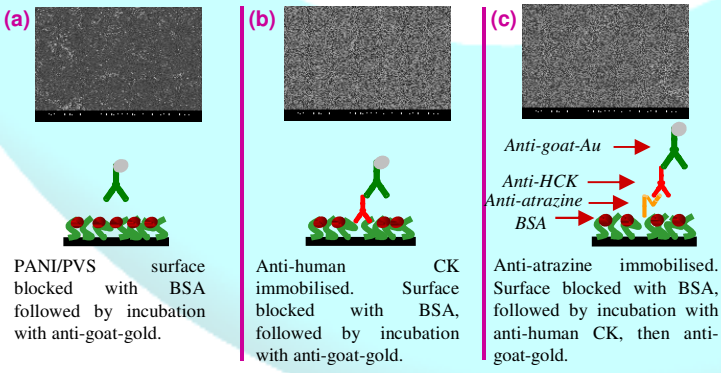
No silver present in EDX spectrum.

Anti-biotin immobilised on PANI/PVS. Remaining surface blocked with BSA followed by incubation with anti-goat-gold.

Silver present in EDX spectrum as expected.

Anti-Atrazine Immunosensor Surface

Similar work was carried out for an anti-atrazine immunosensor surface.



PANI/PVS surface blocked with BSA followed by incubation with anti-goat-gold.

Anti-human CK immobilised. Surface blocked with BSA, followed by incubation with anti-goat-gold.

Anti-atrazine immobilised. Surface blocked with BSA, followed by incubation with anti-human CK, then anti-goat-gold.

Conclusions

SEM coupled with EDX analysis is a valid technique to characterise this immunosensor as it supplies useful information about protein distribution on the electrode surface. It can also be exploited to prove specific immuno-interactions. Electrochemical techniques have, to date, been the main tool used to characterise this immunosensor platform. However, this research shows that SEM/EDX analysis can provide topological imagery that compliments electrochemical data.

National Centre for Sensor Research