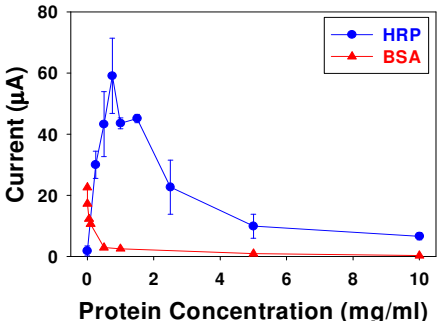
ELUCIDATION OF THE MODE OF ACTION OF A CONDUCTIVE POLYMER-BASED ELECTROCHEMICAL IMMUNOSENSOR

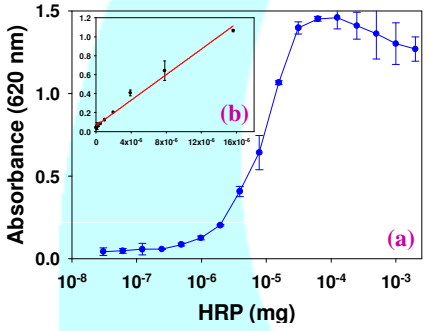
Aoife Morrin, Anthony J. Killard and Malcolm R. Smyth

School of Chemical Sciences, National Centre for Sensor Research (NCSR), Dublin City University, Dublin 9, Ireland

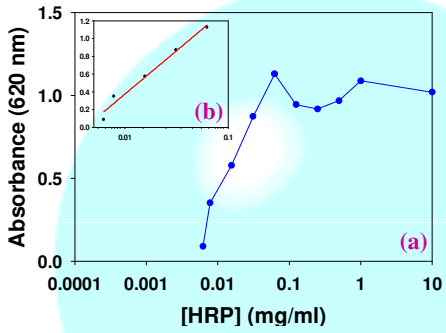
An amperometric biosensor has been developed, incorporating the electroactive polymer, polyaniline (PANI), which undergoes redox cycling, and can couple electrons directly from the enzyme active site, to the electrode surface. Construction of this sensor was achieved by electropolymerisation of polyvinylsulphonate-doped aniline onto the surface of a screen-printed carbon-paste electrode. Biomolecules could then be doped onto the surface of the polymer by electrostatic interactions with the polymer backbone. A key component in a biosensor is the recognition molecule and its immobilisation. This study investigates this process of protein immobilisation using amperometric and colorimetric techniques. Immobilisation of protein (enzyme or antibody) onto the transducer is achieved by electrostatic interactions. By applying bovine serum albumin (BSA) electrostatically at the electrode, efficient blocking of the electrode surface from the bulk solution was achieved above approximately 0.75 mg/ml. When horseradish peroxidase was immobilised on the electrode surface at various concentrations, optimal amperometric responses were achieved at approximately the same protein concentration. Determination of the number of molecules of protein immobilised on the surface of the electrode at this concentration was done using a colorimetric enzyme assay. It was found that under optimal immobilisation conditions, a protein monolayer was formed at the electrode surface. In the case of enzymes such as horseradish peroxidase (HRP), this provides simultaneous blocking of the electrode surface from bulk solution interactions as well as yielding optimal electron transfer properties.



Sensor response vs. [protein] used for immobilisation. Current response increased as a function of [HRP] up to 0.75 mg/ml (n=3). Above this, responses decreased. [BSA] immobilised was found to be inversely proportional to the current responses from HRP and H_2O_2 in bulk solution.



Colorimetric HRP calibration assay (n=3).
(a) Absorbance vs. Mass of HRP.
(b) Linear region of assay from 0 to 1.6×10^{-5} mg ($y = 6.7 \times 10^{4}x + 6.1 \times 10^{-2}$, $r^2 = 0.9871$).



Colorimetric assay of HRP modified electrodes.
(a) Absorbance of HRP modified electrodes.
(b) Linear region of assay from 6.25×10^{-3} mg/ml to 6.3×10^{-1} mg/ml ($y = 9.8 \times 10^{-1}\text{Log}x + 2.3$, $r^2 = 0.9777$).

Absorbances corresponding to [HRP] used during immobilisation could be related to the absolute mass of HRP present on the electrode surface. This equation was calculated to be :

$$\text{Mass of HRP immobilised} = 1.4521\times10^{-5}[\text{HRP}] + 3.375\times10^{-5}$$

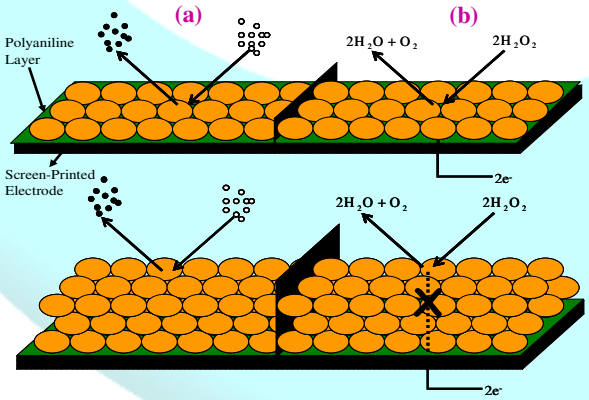
Theoretical number of molecules of HRP required to form an 'ideal' monolayer on the surface of electrode:

4.237 x 10^{11} molecules

Number of molecules of HRP immobilised on surface of electrode at optimum concentration (by deduction):

4.3618 x 10^{11} molecules

Correlation of these figures suggests formation of a protein monolayer. Diminished current responses above optimum concentration (0.75 mg/ml) of immobilised HRP may be due to inhibited substrate diffusion to enzyme that is in electronic communication with the electrode surface, or impeded electron transfer.



Behaviour of the HRP modified electrode for the colorimetric assay (a) and the amperometric assay (b). The case for a monolayer is depicted (*top*), when maximum absorbance and optimum current response, respectively are observed. When multiple layers of protein are immobilised (*bottom*), little change in response for the colorimetric assay occurs, however it results in a significant decrease in current response.

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