New Generation of Immunosensors Based on Electroactive Conducting Polymers.

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An immunosensor consists of a specific bio-receptor (biological molecules or antigens) which able to interact specifically with i) antibodies and ii) transducer layer which converts the molecular interaction into a physical signal, e.g. an electric signal, allowing to quantify the information. Several techniques, including radiolabelling, use of impedance spectroscopy[1-2] or incorporation of an antibody into polypyrrole[3], have been proposed to analyze the antibody-antigen interaction (Ab-Ag). Our earlier experience has demonstrated that the electrochemical polymerization of a polypyrrole precursor, functionalized by an easy leaving group (N-hydroxyphthalimide), gives an easy and powerful route to electroactive polymer films for the recognition of biological molecules (enzyme[4] and DNA[5]).

Here we report on the development of novel modified electrodes based on a conjugated polymer, e.g. polypyrrole fuctionalized with a polycarboxylic acid poly(Pyrrole-NTA) for the specific recognition of antibodies. Thus the grafting of polycarboxylic chelates onto a conducting polymer allows to bind a cation, e.g Cu^{2+},Co^{2+}, Ni^{2+}, which enables a further complexation with (poly)histidine i.e. Tag (6x His) attached to an antigen.

Thus upon complexation with Cu^{2+}, Poly(Py-NTA) shows a stable and reversible redox wave characteristic of Cu^{+}/Cu^{2+}. This poly(Py-NTA-Cu^{2+}) film is then immersed in a PBS buffer solution containing 5 nmole of Antigen RH 24, bearing histidine, The electrochemical response shows the slight decrease of the anodic oxidation peak Epa, together with a decrease of current intensity of the Cu^{2+} electrochemical signal which confirms the grafting of the antigen RH24. These poly(Py-NTA-Cu2+-Antigen) electrodes are then incubated in PBS buffer solution containing 0.6 nmole Antibody 13B5. The modified electrochemical response confirms the recognition of the antibody by this an antigen functionalized on polypyrrole conducting film.

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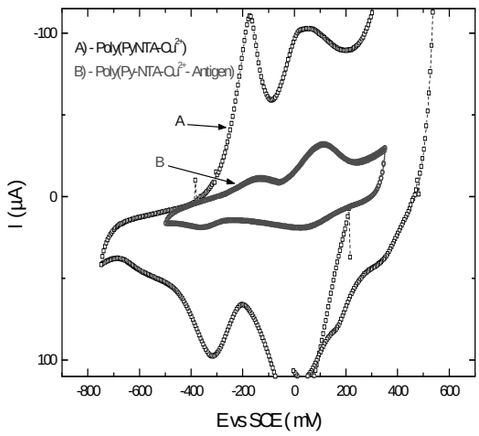

Figure 1 : Cyclic voltammograms of Poly(Py-NTA) film in aqueous solution containing 0.5M NaCl (scan rate of 20 mV s^{-1})
A) Poly (Pyrrole-NTA-Cu^{2+}) film.
B) Poly(Pyrrole-NTA-Cu^{2+}-Antigen RH24) Antigen RH24 5 nmole.

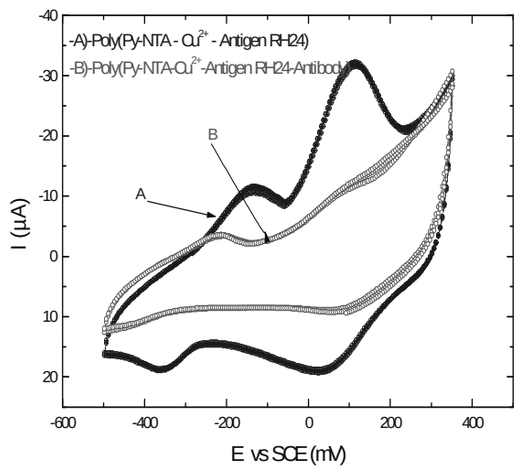

Figure 2: Cyclic voltammograms of Antigen RH 24 film in aqueous medium containing 0.5M NaCl (scan rate of 20 mV s^{-1}) in the following conditions

A) Poly(Pyrrole-NTA-Cu^{2+}-Antigen)
Antigen RH24 : 5 nmole
B) Poly(Pyrrole-NTA-Cu^{2+}-Antigen RH24-Antibody)
Antibody 13B5: 0.6nmole.