

The development of a 'labelless' reversible immunosensor for the detection of *L. monocytogenes* cell surface protein, Internalin B.

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Abstract

Electrochemical impedance spectroscopy (EIS) is a common technique used to probe bioaffinity interactions on electrically conducting polymer surfaces. EIS methods can be employed to investigate 'labelless' detection of analytes via impedimetric transduction. This paper describes the development of a direct immunosensor for the detection of a cell-surface protein on *Listeria monocytogenes*, an extremely important food-borne pathogen. *Listeria monocytogenes* are facultative anaerobic, non-spore-forming, Gram-positive, motile rods that employ the surface bound protein, Internalin B (InlB), to promote invasion into host cells. A recombinant form of InlB was previously cloned and expressed in *E. coli*. and a panel of antibodies and antibody fragments directed against the protein were also produced. Here, we describe how a portion of the recombinant InlB protein, the F3 fragment, and an anti-InlB polyclonal antibody, were used to develop a platform for the labelless immunosensing of InlB.

Sensors were fabricated by electropolymerisation of planar screen-printed carbon electrodes with polyaniline (PANI), to produce a conductive substrate. Polyclonal anti-InlB antibody was subsequently incorporated onto the PANI layer using a biotin-avidin system for site-specific immobilisation. The sensors were then probed with

varying concentrations of InlB antigen and the impedimetric response at each concentration was recorded. An anti-IgG antibody was immobilised at the electrode surface, as a control and subsequently exposed to the same concentrations of InlB. Impedimetric data for the control sensors were also recorded. Upon exposure to a range of concentrations of antigen, complex plane impedance analyses were used to relate the differing redox states of the polymer layer, to the possible charge transfer at the surface, with respect to the related mechanisms between the antibody and the polymer. These effects were subsequently monitored to assess the impedance of the polymer thereby determining the amount of bound antigen at the sensor surface. Calibration profiles for both sample (InlB) and control (IgG) sensors were constructed. A limit of detection of 1ng/ml were achieved for Internalin B.

1. Introduction

Immunosensing devices transduce antigen-antibody interactions into directly measurable signals and allow simple, rapid and sensitive quantification of analytes in biologically complex samples [1]. There are two main types of immunosensors, direct and indirect. Indirect immunosensors employ labels (fluorescent tags, enzymes, radioisotopes, etc.), whereas direct methods are 'label-free'. The use of labels can often mean longer analysis times, with extra steps required, thus increasing the complexity of the procedure. The direct detection of bioaffinity reactions can be performed with piezoelectric, optical, and electrochemical transduction methods [2-6].

Electrochemical immunosensors are used extensively for the direct detection of antibody-antigen interactions. Their main advantages are that they are non-invasive, require little sample pre-treatment and are often reversible [7]. Electrochemical immunosensors are concerned with the formation of a recognition complex between the sensing biomaterial and the analyte under investigation in a monolayer or thin-film configuration, on an electronic transducer [8]. The formation of a complex on a conductive or semiconductive surface may alter the capacitance and the resistance at the surface-electrolyte interface [9], and this can be exploited to determine the concentration of the required analyte.

Impedance spectroscopy is a powerful tool for the analysis of interfacial property changes of modified electrodes, upon the occurrence of a biorecognition event such as antibody-antigen binding, at the modified surfaces [10]. Kinetics and mechanisms of electron-transfer processes that correspond to the biocatalytic reaction occurring at modified electrodes can also be derived from Faradaic impedance spectroscopy (FIS). Therefore, impedance spectroscopy is a useful electrochemical tool for the characterisation of biological thin-films involved in the transduction of biomolecular interactions at electrode surfaces [11 & 12].

Conductive polymers are excellent platforms for the immobilisation of biomolecules at electrodes. Sergeant *et al.* [12] have investigated the binding mechanisms of antibody-antigen interactions on conducting polypyrrole films using impedance spectroscopy. Concentration-dependent impedance measurements were calculated based on the theory of charge generation and transportation at the polymer interface.

This was based on the fact that proteins in solution act as polyelectrolytes and hence, as an antibody is a protein, its electrical charge will be affected on binding an antigen [13]. As antigen concentrations are increased, the impedance of the system increases, especially at low frequencies. The impedimetric response of an antibody-loaded film may be represented as either a Bode or Nyquist plot. A Bode plot shows the total impedance of the system vs. frequency and is indicative of the overall result. A Nyquist plot illustrates the faradaic (real) impedance of the system vs. the capacitive (imaginary) components [14]. Nyquist plots show detailed effects of antigen concentration on the real (Z'') and imaginary (Z') components of antibody-loaded polymer films [15].

Prior to the addition of specific antigen, the real and imaginary components of the Nyquist plots follow the traditional shape. However, once antigen is introduced to the system and binding occurs at the polymer interface the plots become more difficult to interpret. Both the faradaic and capacitive components tend to increase as antigen concentration increases, however, the increases in the real component dominates the total increase in impedance, i.e. the faradaic component of the impedance increases more dramatically and is frequency independent [11]. This principle forms the basis of the 'labelless'-immunosensor described here.

The chosen target for this 'labelless' reversible immunosensor was Internalin B, which is a cell surface protein of *Listeria monocytogenes*, an important food borne pathogen with an extremely high mortality rate of approximately 30%. *Listeria monocytogenes* can be readily isolated from food, water, soil and silage and grows between 1 - 45°C [16]. It is responsible for most food-borne outbreaks of listeriosis and belongs to the bacterial genus *Listeria*. Listeriosis is most prevalent in immunocompromised patients, with the risk of infection up 1000 times more likely than that of the general public [17]. In most cases of infection, symptoms present as those commonly associated with food poisoning and include fever, vomiting, diarrhoea and headache. However, in more severe cases meningitis, septicaemia and spontaneous abortion can occur. Therefore a rapid method for detection is vital. Labelless affinity sensors are a new generation of biosensors and address a number of problems associated with the rapid diagnosis of food borne pathogens by allowing cost-effective, specific and sensitive detection of bacterial pathogens for use at point of care.

A range of novel antibodies and antibody fragments directed against important cell surface proteins on *L. monocytogenes* were previously developed and characterised [18-20]. An anti-InlB polyclonal antibody was specifically immobilised at the electrode via a series of surface modification steps. The sensors were then probed with varying concentrations of InlB antigen (F3 fragment) and the impedimetric responses recorded. An anti-IgG antibody was also immobilised, as a control and subsequently exposed to the same concentrations of InlB antigen. The concepts described by Grant *et al.* [7], were applied to obtain affinity binding and signal transduction processing for the electrode platform assemblies. The planar electrodes and impedimetric detection methods were subjected to reproducibility and control analysis studies and tested in buffered samples.

2. Experimental

2.1 Reagents

Sodium di-hydrogen orthophosphate, di-sodium hydrogen orthophosphate, sodium chloride, (all 'AnalaR' grade), were purchased from BDH lab supplies (Poole, Dorset, UK.) Aniline hydrochloride, albumin bovine (BSA), biotinamido hexanoic acid-3-sulfo NHS and immunopure biotinylation kit (BK101) were purchased from Sigma-Aldrich Co, Ltd (Gillingham, Dorset, UK.) Potassium hydrogen phosphate was purchased from Fisher Chemicals (Loughborough, Leicester, UK). Neutravidin and biotin-sulfo-NHS were purchased from Pierce Biotech (Rockford, IL, USA)

2.2 Buffers and solutions

Phosphate buffered saline (PBS), comprising of 0.13 mM NaH_2PO_4 , 0.528 mM Na_2HPO_4 and 0.51 mM NaCl, pH 7.4 was prepared using distilled deionised water. 0.2M aniline hydrochloride monomer solution was prepared in aniline buffer (0.5 M KCl and 0.3 M HCl at pH 1.0) using distilled deionised water. Antibodies against InlB were purified from polyclonal serum, reconstituted in PBS, pH 7.4 (as above) and frozen in 125 μl working aliquots at 1mg/ml. Internalin B antigen test solutions were prepared in PBS, pH 7.4 (as above) at a range of concentrations from 1ng-100ng for impedimetric exposure analysis.

2.3 Materials

Carbon screen-printed electrodes assemblies (Microarray Ltd. Manchester, UK) were used as the biosensing platforms in this research and are shown in Figure 1.

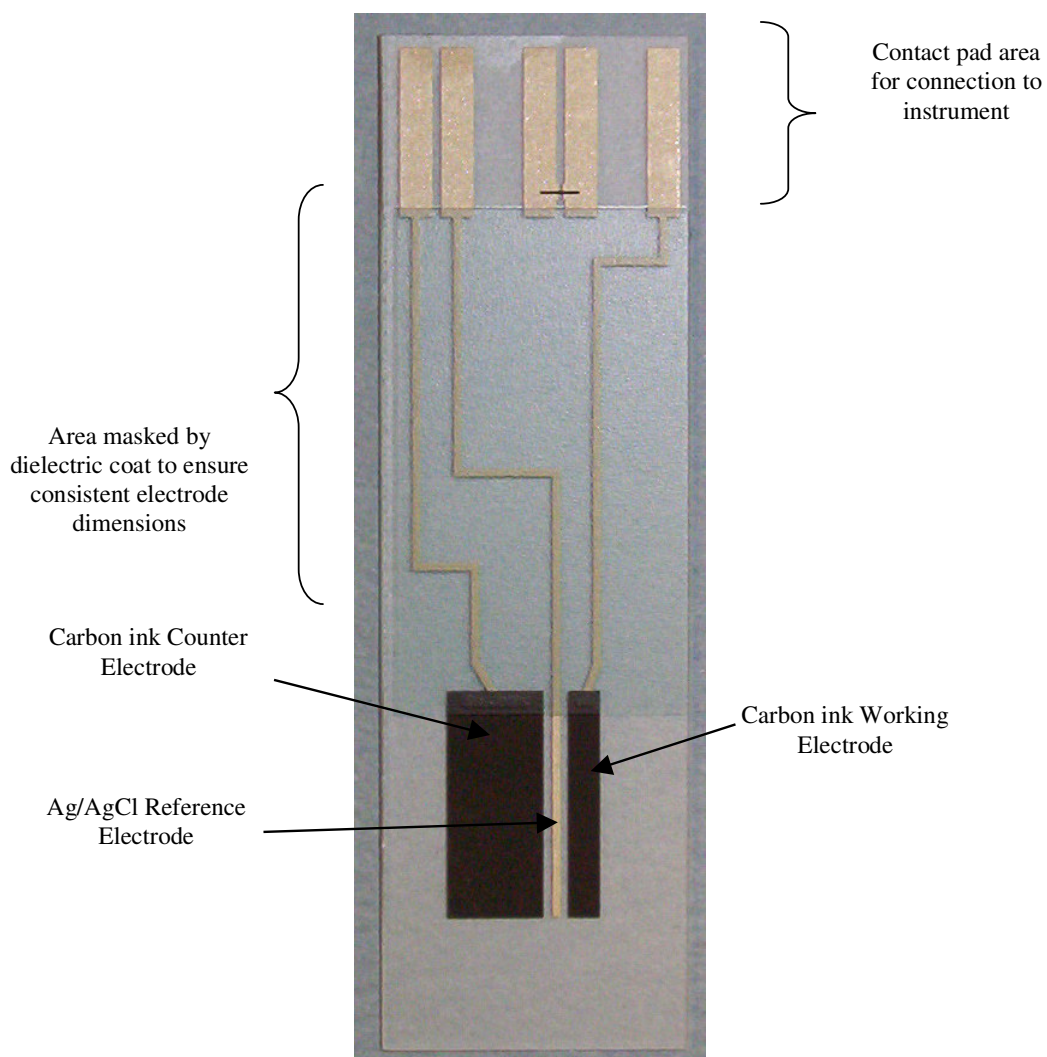


Fig. 1 *Single screen-printed planar carbon electrode.*

2.4 Instrumentation

A Sycopel PCI-100 MK3 Potentiostat computer interface was used in conjunction with a 'Ministat Potentiostat' for all electrochemical studies. All current/charge transients were recorded using a compatible PC and Sycopel software. AC Impedance measurements were performed using an ACM Auto AC DSP frequency response analyser. The test frequency varied from 10000Hz to 1Hz a.c. and a 5mV amplitude perturbation was used. The potentiostat was linked to compatible PC utilising ACM software for data acquisition.

2.5 Carbon Electrode fabrication

2.5.1 Electrochemical insulation of carbon working electrode.

The monomer solution used was thoroughly purged with N₂ for 20 minutes in a sealed cell to provide an oxygen free atmosphere. The cycle number and potentiodynamic sweep rates were determined for each set of electrodes prior to deposition (data not shown). The insulation of planar carbon electrodes was achieved by sequentially scanning the working electrode potential from -200mV to 800mV (vs. Ag/AgCl) and back to the starting potential, at a scan rate of 50mV/s.

2.5.2 Electrodeposition of polyaniline (PANI) onto the planar carbon working electrode

The potentiodynamic electrodeposition of PANI onto the planar carbon working electrodes was achieved electrochemically by sequentially cycling the working electrode potential from -200mV through to 800mV (vs. Ag/ AgCl) and back to the starting potential at a scan rate of 50 mV/s. A 0.2M aniline hydrochloride solution was prepared in a 0.5 M KCl, pH 1.0. The conducting films increased in thickness, as more charge was passed with each potential sweep. The optimisation of cycle number and the polymerisation time was dependent on the quality of the monomer solution and previous studies have shown that 15 cycles was sufficient for optimal electrodeposition of the polymer thin-films [7].

2.6 Sensor modification

Potentiodynamic cyclic voltammetry was employed for the electrodeposition of PANI as a planar thin-film over the working electrode of the carbon sensor. After 15 cycles the sensor was subjected to a single linear sweep to 800mV, removed from the set-up,

rinsed in aniline buffer, pH1.0, and left to air-dry overnight. Water-soluble biotin-sulfo-NHS (30µl), at a concentration of 10 mg/ml was placed on the polymer-coated working electrode surface for 24 hours. The sensors were then rinsed with deionised water. Neutravidin (30µl), at a concentration of 10µg/ml was then placed on the working electrode for 1 hour. Finally, the sensor surface was thoroughly rinsed with de-ionised water.

2.7 Site-specific coupling of antibody within PANI films

2.7.1 Biotinylation of antibodies

Antibodies were biotinylated to facilitate immobilisation onto the neutravidin-modified polymer films with biotinamidohexanoic acid-3-sulfo-N-hydroxysuccinimide (BAC-sulfo-NHS), using the procedure outlined in the Sigma Immunoprobe biotinylation kit (BK101). The strong affinity between avidin and biotin (affinity constant $K_a = 10^{15} \text{ M}^{-1}$) allows direct coupling of the biotinylated antibodies to the modified electrode. The extended spacer arm from the hexanoic acid improved the interaction between avidin and the biotinylated antibody by overcoming steric hindrances present at the biotin binding sites. A molar ratio of 10:1 biotin to antibody was used to ensure efficient labelling and the antibody was subjected to fast gel filtration post-biotinylation to remove any unreacted biotin. An avidin-HABA (4-Hydroxyazobenzene-2-carboxylic acid) assay was then performed to determine the degree of biotinylation; dye/protein ratio and biotin-antibody conjugate concentration [21]. Biotinylated antibodies were diluted in PBS, pH 7.4, and stored at -20° C at a concentration of 1 mg/ml.

2.7.2 Immobilisation of biotinylated antibodies on modified sensor surfaces

Biotinylated antibody (100µl of a 1mg/ml solution) was added to the sensor surface. This solution was left to incorporate at the working electrode for 1 hour at room temperature and any unbound material rinsed off with deionised water. Finally, any non-specific interactions were blocked by adding 10^{-6} M bovine serum albumin (BSA) in PBS, pH 7.4 (as before) to the sensors for 1 hour at room temperature. The modified sensors were washed with deionised water and left in PBS, pH 7.4 overnight at 4° C .

2.8 Electrochemical Impedance Spectroscopy (EIS).

A Gill AC DSP Frequency Response Analyser was used to perform electrochemical impedance spectroscopy (EIS). Data processing and interpretation of the electrochemical impedance measurements were analysed, over a frequency range from 10,000Hz to 1Hz, (5mV amplitude perturbation). Following the immobilisation of antibody molecules at the sensor surfaces, EIS was performed to interrogate the electrodes. A baseline trace was recorded initially in PBS, pH 7.4, alone. Antibody-loaded sensors (electropolymerised carbon thin-film), were then exposed for 30 minute time periods to increasing concentrations of antigen by serial addition. After 30 minutes exposure to a single concentration, the working electrode was flushed with PBS, pH 7.4 and the impedance trace was then recorded in PBS, pH 7.4 alone between 10,000Hz to 1Hz. This process was then repeated for the full range of antigen concentrations from 1ng/ml-100ng/ml.

Complex plane impedance analyses were used to relate the changes in sensor output to the antibody/antigen binding events at the electrode-polymer interface. These changes included the differing redox states of the polymer, the faradaic charge transfers and also the capacitive transduction events. Results were plotted in representative Nyquist and Bode forms as well as a percentage change in impedance from the baseline response.

3. Results & Discussion

Impedimetric studies focusing on the detection and quantification of InlB antigen-F3 were performed on planar carbon electrodes whereby the antibody was immobilised within a conductive thin-film of electrodeposited polyaniline (PANI) via avidin-biotin interactions. Results are shown for both sample and control sensors, incorporating anti-InlB antibodies and anti-IgG antibodies respectively. Calibration profiles are given, representing both the impedimetric and non-faradaic response of the anti-InlB-doped polyaniline-coated carbon electrodes against varying concentrations of InlB.

3.1 Evaluation of polyaniline deposition at sensor surface

The electrochemical formation of polymer layers of controlled thickness at electrode surfaces via potentiometric deposition gives rise to a reproducible method for biosensor fabrication. A number of polymers, such as polyacetylene, polythiophene, polyaniline, polyindole and polypyrrole are suitable for this application due to their ease in electropolymerisation and conductive nature [22 & 23]. In this case, polyaniline (PANI) was used, as it can be easily electropolymerised from an aqueous solution of aniline monomer. Potentiodynamic cyclic voltammetry was employed and PANI was laid down as a planar thin-film over the working electrode of a carbon sensor. A typical cyclic voltammogram for the electrodeposition of polyaniline (PANI) as a thin-film on a planar carbon electrode is shown in figure 2.

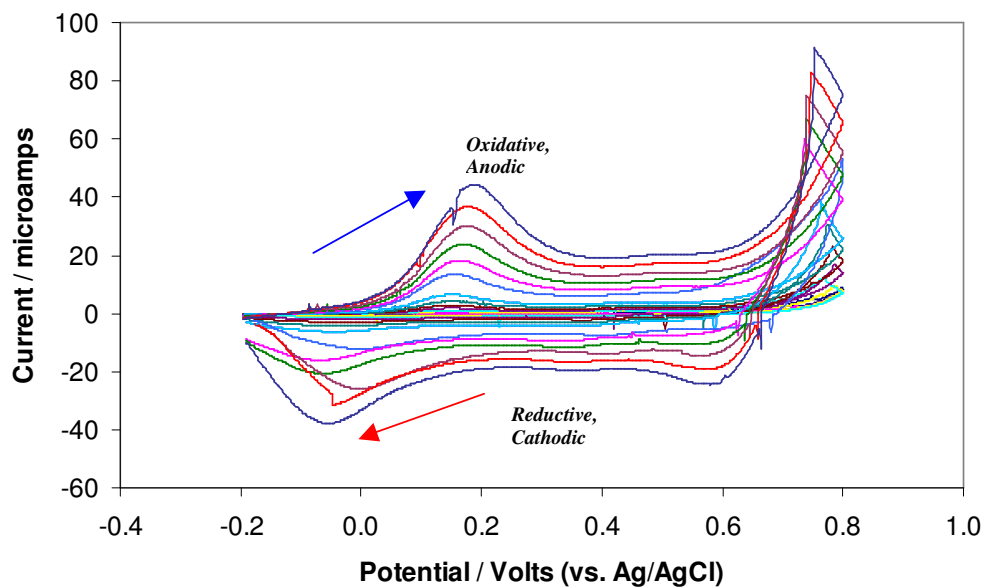


Fig. 2 Cyclic voltammogram for the potentiodynamic electrodeposition of polyaniline (PANI) as a thin film on planar carbon screen-printed electrodes.

The working electrode potential was scanned from -200mV to $+800\text{mV}$ (vs. Ag/AgCl) at a sweep rate of 50 mV/s . The cycle number and polymerisation time (~ 10 mins) were investigated to determine the most efficient and reproducible polymerisation of the PANI films. There were no further peak increases in both anodic and cathodic current transients after 15 cycles, indicating that optimum conductive deposition had occurred.

The controlled potential electrolysis of an aqueous solution containing monomers produces a suitable surface for biomolecule immobilisation. The main advantage of electrochemical polymerisation is that films can be prepared easily in a rapid one-step procedure. Furthermore, this method enables exact control of the thickness of the polymer layer based on the measurement of the electrical charge passed, during the electrochemical polymerisation.

3.2 Evaluation of impedimetric sensor activity

Following electropolymerisation of the sensor surfaces with PANI, sample and control antibodies were site-specifically coupled to the sensors. Subsequent probing of the modified sensors with antigen (InlB F3 fragment), demonstrated the principle of a labelless and reversible detection method for *L. monocytogenes* cell surface protein InlB. Initial examination of the total impedance of each of the films at various concentrations of InlB, allowed construction of a calibration curve for InlB. Total impedances were recorded across an InlB concentration range of 1-100ng/ml for the anti-InlB loaded PANI-coated electrode at 1 Hz. Three replicate measurements were recorded on separate electrodes for each concentration and CVs of less than 10% were determined (Fig. 3).

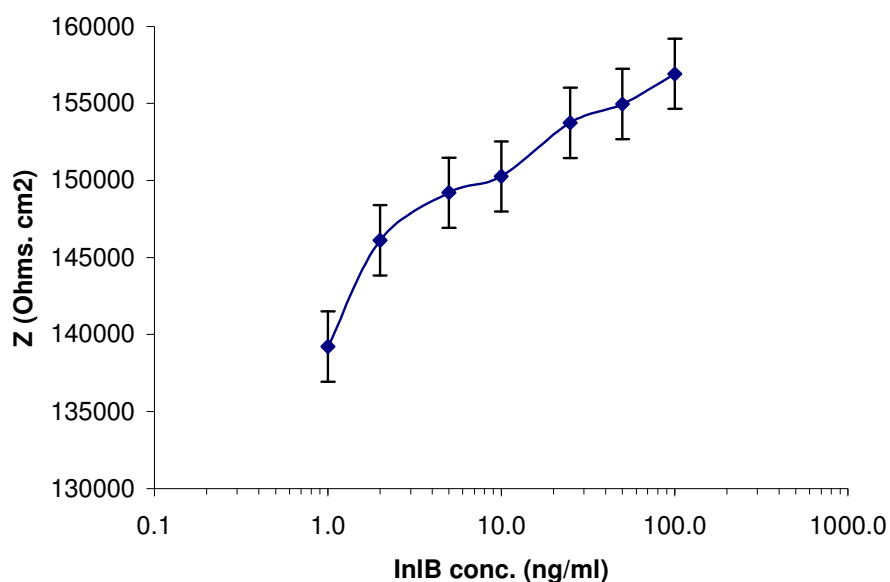


Fig. 3 Calibration curve of InlB concentration at 1Hz. Calibration curve generated as a result of exposure to of an anti-InlB loaded PANI film to increasing concentrations of InlB. The curve displays total impedance (Z) against InlB concentration.

3.2.1 Electrochemical impedance interrogation of InlB-doped PANI thin-film electrodes exposed to increasing InlB concentrations.

Impedimetric data was collected for the InlB-doped sensor between 10,000Hz -1Hz in PBS, pH 7.4, i.e. buffer containing no antigen, as a baseline trace. Three sensors were used for each experiment and each sensor was interrogated in triplicate for reproducibility, (nine replicates in total). After 30 minutes exposure to buffer alone (baseline trace), the sensor was flushed thoroughly with 50ml of PBS, pH 7.4 before the impedance spectra was recorded in PBS. This sensor was then exposed to an increasing antigen concentration, (1ng/ml-100ng/ml) and the process was repeated for the full range of antigen concentrations. 9-matched sensor pairings (sample & control) were evaluated at each concentration and the corrected response plotted.

Figure 4 shows the impedimetric data collected, presented in the form of a Nyquist plot depicting the faradaic (Z') and capacitive (Z'') components of the ac impedance analysis of the anti-InlB-doped PANI films following exposure to InlB antigen (1ng/ml – 100ng/ml).

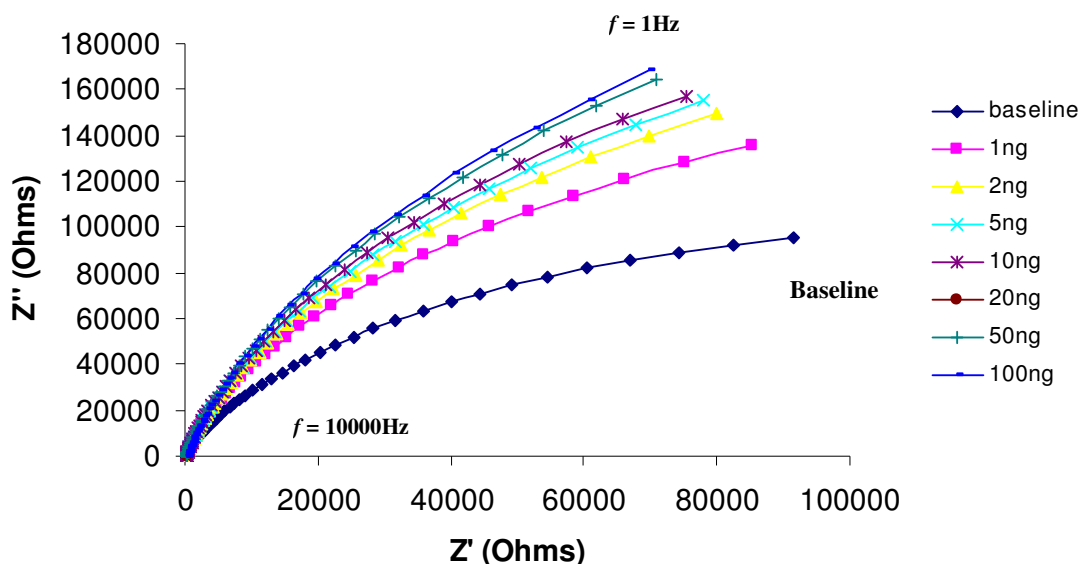


Fig. 4 Nyquist plot showing faradaic component (Z') against capacitive component (Z'') for anti-InlB-doped PANI films on carbon following exposure to various concentrations of InlB.

From Figure 4 it is apparent that the Z' component and the Z'' component of impedance both increase with decreasing frequency from the baseline trace. It can be seen from Figure 4 that there was a measurable signal response for the faradic component (Z') and the capacitive component (Z'') of the Nyquist Plot for increasing concentrations of InIB exposed to anti-InIB -doped PANI film carbon sensors. It can also be deduced that a charge-transfer mechanism between the antibody/antigen has occurred, since a slight increase in the resistive (Z') component of the total impedance is observed. The capacitive resistance (Z'') increases to a larger extent at greater antigen concentrations (towards 100ng). However, it is worth noting that there is better evidence of signal differentiation observed at the lower frequencies (towards 1 Hz).

The total impedance (Z' & Z'') can be represented in the form of a Bode plot (vs. log frequency) as shown in Figure 5. The data shown here is plotted for the low frequency region (10Hz to 1Hz) where the differences in the spectra obtained are greatest. The normalised impedimetric response (Z/Z_0) was obtained by dividing the impedimetric response at each InIB concentration (Z) by the impedimetric response at zero InIB concentration (Z_0), i.e. the baseline signal, to give a normalised response that can be plotted against frequency in the form of a Bode plot.

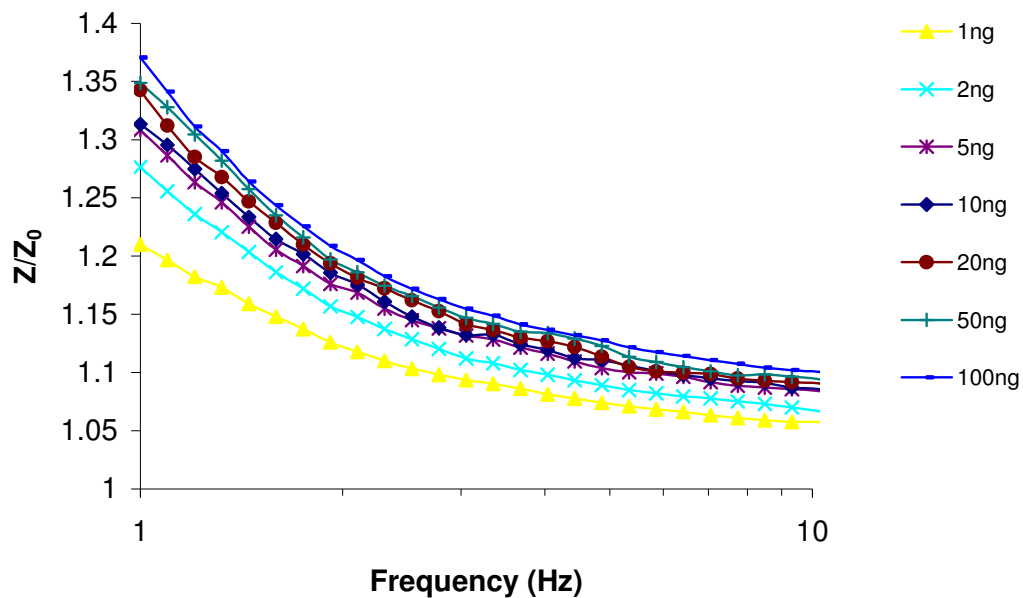


Fig. 5 Bode plot showing faradaic and non-faradaic changes in total impedance for anti-InIB-doped PANI films on carbon electrodes following exposure to various concentrations of InIB, normalised from baseline trace (Z/Z_0).

3.2.2 Calibration curves showing faradaic and non-faradaic responses of anti-InIB-doped PANI-coated electrodes against increasing concentrations of InIB.

The total impedance of the system increases with exposure to increasing InIB antigen concentration from the baseline trace up to 100ng/ml as shown in figure 3. It is apparent from the data shown in figure 3, that the greatest difference from the baseline trace is observed at the lowest frequency of 1Hz. The percentage impedance change from the baseline at 1Hz can be used to form the basis of a calibration profile for differentiation of various concentrations of InIB applied to anti-InIB antibody-doped PANI films on carbon planar electrodes. Figure 6 shows the faradaic calibration profile obtained at 1Hz where the greatest impedance increases, at each concentration are noted and normalised against zero antigen (Z/Z_0).

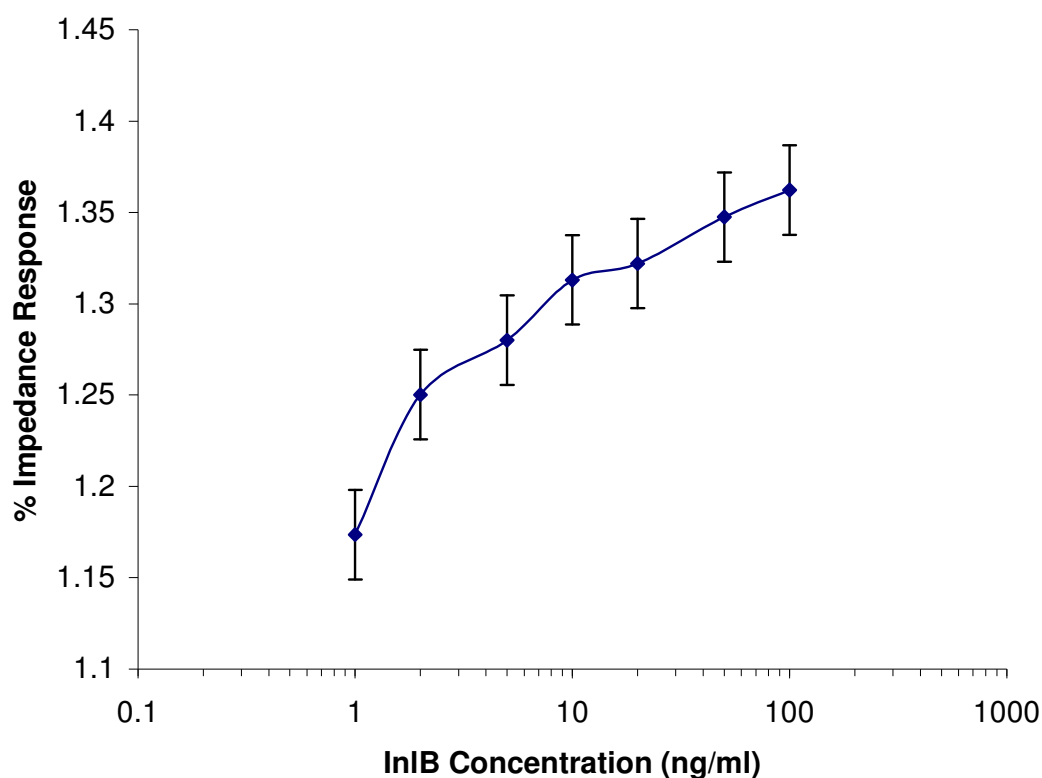


Fig.6 Calibration curve of InIB concentration at 1Hz. The curve generated as a result of exposure of anti-InIB-loaded PANI-film to increasing concentrations of InIB at 1 Hz. The curve displays the % impedance response against InIB concentration (ng/ml), normalised against the baseline response (zero antigen).

The calibration curve indicates that this technique can be used for the labelless impedimetric detection and subsequent quantification of InIB antigen F3.

An alternative method of representing the response of the anti-InIB doped polyaniline coated electrodes to increasing concentrations of InIB, is by observing the non-faradaic or capacitive response. The capacitive response can be calculated from the Z'' value at the characteristic frequency by using the following equation;

$$\text{Equation 1} \quad C = 1 / (2\pi f Z'')$$

The characteristic frequency (f) is determined as the frequency (Hz) where the phase angle is closest to 90° , indicating that the system exhibits near ideal capacitor behaviour [24]. For this set of impedimetric data, the characteristic frequency was calculated to be 28Hz and therefore the non-faradaic response of the immunosensor was determined and a calibration curve constructed based on that value. Figure 7 shows the calibration curve for the non-faradaic capacitive response against InIB concentration for the anti-InIB-doped PANI electrodes at the characteristic frequency of 28Hz.

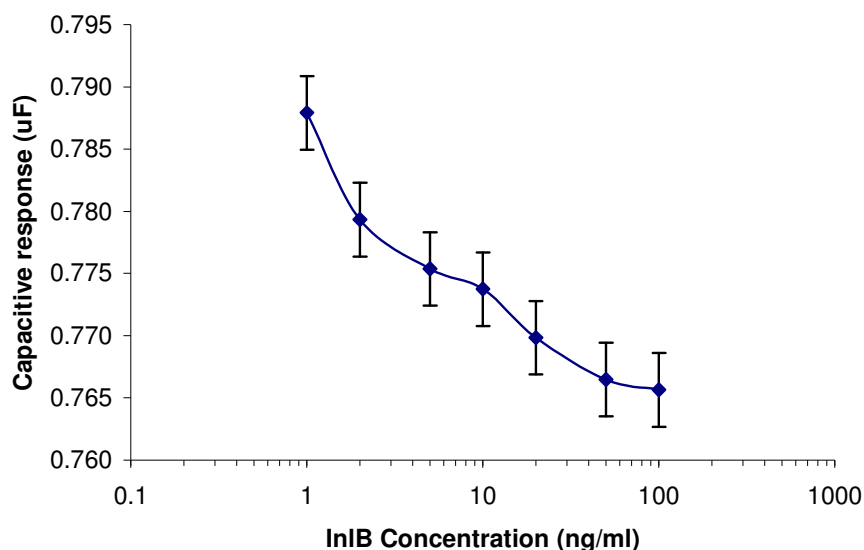


Fig.7 Calibration curve of non-faradaic capacitive response against InIB concentration (ng/ml) for anti-InIB-doped PANI-coated carbon electrodes at the characteristic frequency of 28Hz where the phase angle is closest to zero.

3.2.3 IgG-doped polyaniline thin-film electrode exposed to increasing InIB concentrations – control plots

Control experiments using anti-IgG-doped electrodes were used to validate the specificity of the observed impedance responses for the anti-InIB-doped electrodes. Alfonta *et al.* [25] employed this method of using a non-specific antibody for control sensors. Control sensors were fabricated in exactly the same way as the anti-InIB antibody-doped sensors and were exposed to increasing concentrations of InIB under the same experimental conditions. The impedimetric responses were then compared and specific impedance changes reported. Figure 8 shows the spectra obtained from the control sensors, represented in the form of a Nyquist plot. This graph depicts the faradic and capacitive components of the ac impedance of the control anti-IgG-doped PANI-films following exposure to the same concentration range of InIB antigen (1ng-100ng/ml).

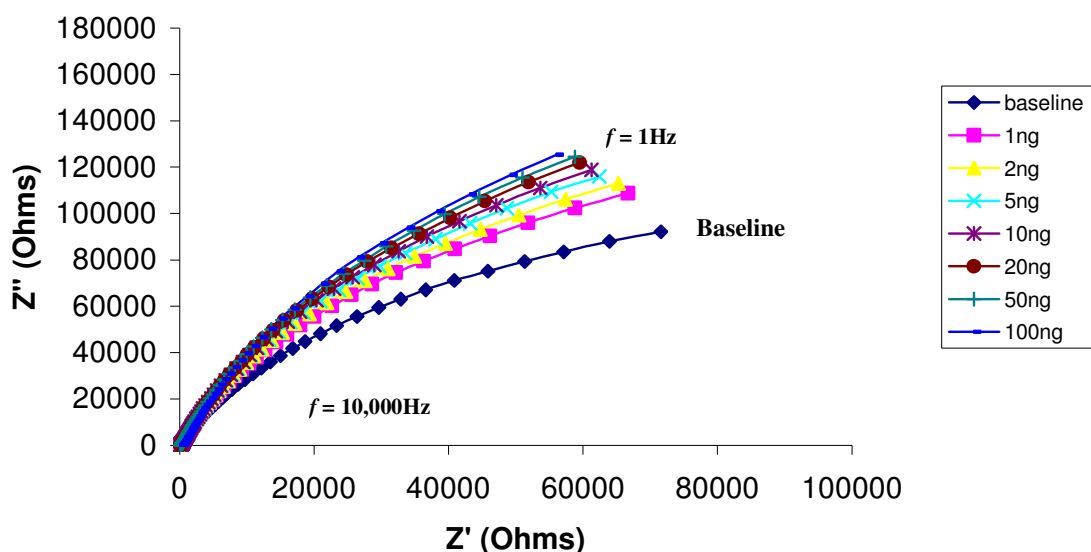


Fig. 8 Nyquist plot (Z' vs. Z'') for anti-IgG-doped (control) PANI thin-film following exposure to increasing InIB concentrations.

It can be seen from Figure 8 that there is a measurable increase in signal response for the faradic component of the Nyquist Plot (Z') for increasing concentrations of

InIB exposed to the control sensors. However, the signal responses are much lower than that of the specific response of Internalin B in the sample plots

Figure 9 shows the normalised impedance changes from the baseline trace for the lower frequencies (1Hz to 10Hz) for the control sensors. This figure for IgG recognition can be directly compared to Figure 5 for InIB recognition.

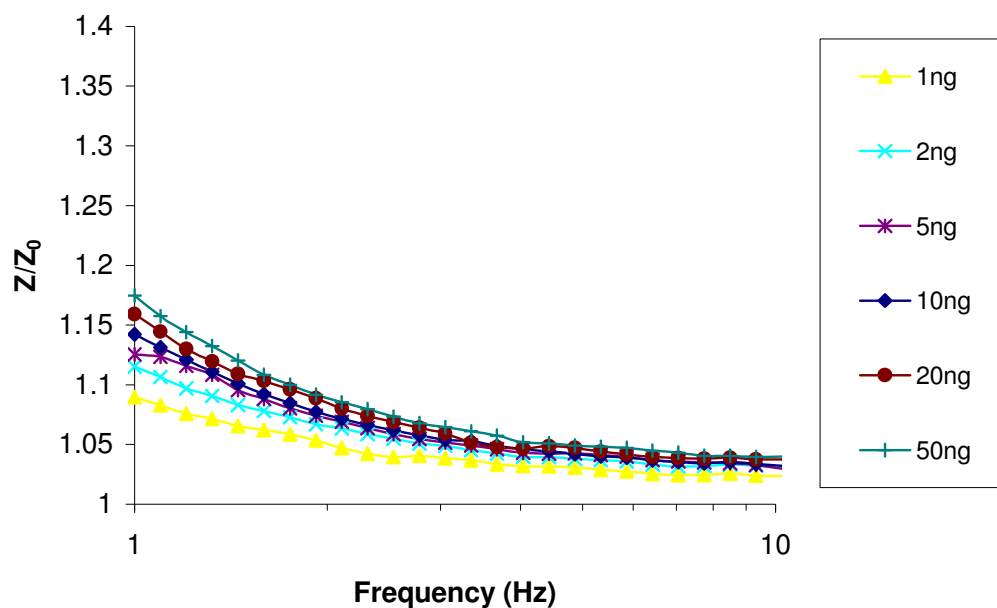


Fig. 9 Bode plot showing faradaic and non-faradaic changes in total impedance for anti-IgG-doped PANI films on carbon electrodes following exposure to various concentrations of InIB, normalised from baseline trace (Z/Z_0).

Comparing the Bode and Nyquist responses for both the sample (InIB) and the control (IgG) experiments, we can see that although there is a definite impedimetric response observed for the control (IgG-doped) spectra, they are significantly lower than the specific sample responses (InIB-doped). Figure 10 shows that although anti-IgG doped PANI thin-films show a degree of interaction with InIB antigen, it is sufficiently lower to allow deduction of sample signal and construction of a calibration curve for the detection of InIB

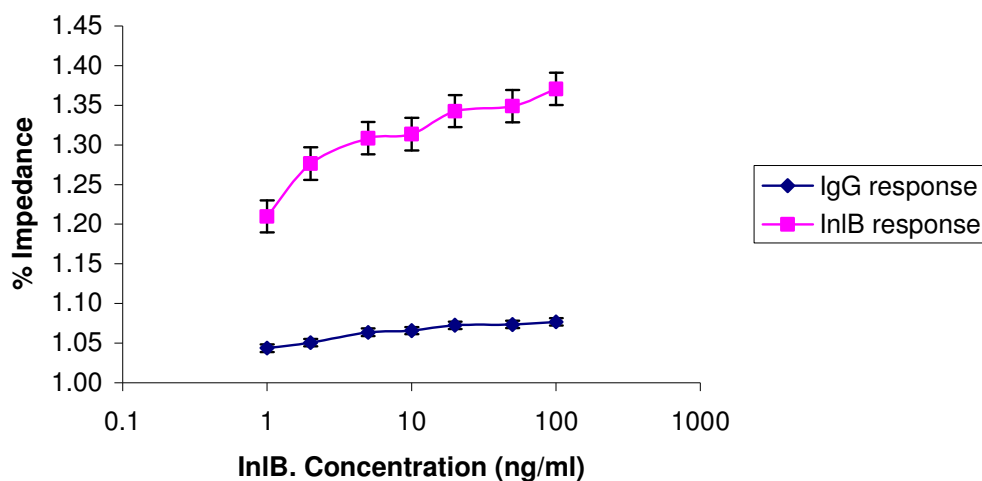


Fig.10 Calibration curve showing the percentage impedance change from the baseline trace for increasing InIB antigen concentrations, at a frequency of 1Hz (5mV a.c) for both sample and control doped sensors.

4. Conclusions

An electrochemical immunosensor for direct detection of the 23kDa protein InlB F3 at nanogram level was developed. Polyaniline was successfully laid down as a conductive thin-film layer on planar carbon electrode surfaces. This facilitated the immobilisation of antibody within the conducting thin film at the electrode surface. Sensor modification with neutravidin and biotin allowed site specific coupling of the antibody to the polyaniline matrix. Impedimetric interrogation of the thin-film carbon sensors yielded positive results with more rigorous, reproducible data collected at the lower end of the frequency range (1-10Hz). The specific interactions and resultant complexes formed gave rise to an overall increase in terms of impedance change from the baseline response at the electrode / solution interface. Plots of the real (Z') and imaginary (Z'') components of the impedance versus frequency provided information for construction of calibration curves, recorded by multiple interrogations of both sample and control sensors (9 replicates), with InlB-F3 antigen. A dynamic range 0-100 ng/ml was derived. The proposed immunosensor provides a promising tool for the labelless detection of InlB a cell surface protein of *Listeria monocytogenes*.

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6. References

1. Byfield, M.P., Abuknesha, A., 'Biochemical aspects of biosensors', *Biosens. Bioelectron.* **1994**, *9*, 373-400.
2. Homola, J., Yee, S.S., Gauglitz, G., 'Surface plasmon resonance sensors: review', *Sens. Actuators* **1999**, *B54*, 3-15.
3. O'Sullivan, C.K., Vaughan, R., Guilbault, G.G., 'Piezoelectric immunosensors - Theory and applications', *Anal. Lett.*, **1999**, *32*, 2353-2377.
4. Allen, S., Chen, X., Davies, J., Davies, M.C., Dawkes, A.C., Edwards, J.C., Roberts, C.J., Sefton, J., Tendler, S.J.B., Williams, P.M., 'Detection of antigen-antibody binding events with the atomic force microscope', *Biochem.* **1997**, *36*, 7457-7463.
5. Perrin, A., Lanet, V., Theretz, A., 'Quantification of specific immunological reactions by atomic force microscopy', *Langmuir*, **1997**, *13*, 2557-2563.
6. Dong, Y., Shannon, C., 'Heterogeneous immunosensing using antigen and antibody on gold surfaces with electrochemical and scanning probe detection', *Anal. Chem.* **2000**, *72*, 2371-2376.
7. Grant, S., Davis, F., Pritchard, J.A., Law, K.A., Higson, S.P.J., Gibson, T.D., 'Labelless and reversible immunosensor assay based upon an electrochemical current-transient protocol.' *Anal. Chem. Act.* **2003**, *495*, 21-32.
8. Ouerghi, O., Touhami, A., Jaffrezic-Renault, N., Martelet, C., Ben Ouada, H., Cosnier, S., 'Impedimetric immunosensors using avidin-biotin for antibody immobilisation', *Bioelectrochemistry*, **2004**, *56*, 131-133.
9. Katz, E., Willner, I., 'Probing biomolecular interactions at conductive and semi-conductive surfaces by impedance spectroscopy: routes to impedimetric immunosensors, DNA sensors and enzyme biosensors.' *Electroanalysis*, **2003**, *15*, 913-947.
10. Guan, J.G., Miao, Y.Q., Zhang, Q.J., 'Impedimetric Biosensors: review', *J. Biosci. & Bioeng.* **2004**, *4*, 219-226.
11. Ameer, S., Maupas, H., Martelet, C., Jaffrezic-Renault, N., Ben Ouada, H., Cosnier, S., Labbe, P., 'Impedimetric measurements on polarized functionalized platinum electrodes: Application to direct immunosensing', *Mater. Sci. and Eng.* **1997**, *c5*, 111-119.

12. Sargent, A., Sadik, O.A., 'Monitoring antibody-antigen reactions at conducting polymer-based immunosensors using impedance spectroscopy', *Electrochim. Acta* **1999**, *44*, 4667-4675.
13. Prasad, B., Lal, R., 'A capacitive immunosensor measurement system with a lock-in amplifier and potentiostatic control by software', *Meas. Sci. Technol.* **1999**, *10*, 1097-1104.
14. Kharitonov, A.B., Alfonta, L., Katz, E., Willner, I., 'Probing of bioaffinity interactions at interfaces using impedance spectroscopy and chronopotentiometry', *J. Electroanal. Chem.* **2000**, *487*, 133-141.
15. Katz, E., Alfonta, L., Willner, I., 'Chronopotentiometry and faradaic impedance spectroscopy as methods for signal transduction in immunosensors', *Sens. Actuators B*, **2001**, *76*, 134-141.
16. Jones, D., Seeliger, H., 'The genus *Listeria*', p1595-1616, in *The Prokaryotes* 2nd Edn., **1992** Balows, C.A., Truper, H.G., Dworkin, M., Harder, W., Schleur, K-H. (eds.), Springer Verlag, Heidelberg, Germany.
17. Jensen, A., Frederiksen, W., and Gerner-Smidt, P., 'Risk factors for Listeriosis in Denmark, 1989-1990', *Scand. J. Infect. Dis.*, **1994**, *26*, 171-178.
18. Leonard, P., Hearty, S., Quinn, J., O'Kennedy, R., 'A generic approach for the detection of whole *Listeria monocytogenes* cells in contaminated samples using surface plasmon resonance', *Biosens. Bioelectron.* **2004**, *19*, 1331-1335.
19. Hearty, S., Leonard, P., Quinn, J., O'Kennedy, R., 'Production, characterisation and potential application of a novel monoclonal antibody for rapid identification of virulent *Listeria monocytogenes*', *J. Microbiol. Methods.*, **2006**, *66*, 294-312.
20. Tully, E., Hearty, S., Leonard, P., O'Kennedy, R., 'The development of rapid fluorescence-based immunoassays, using quantum dot-labelled antibodies for the detection of *Listeria monocytogenes* cell surface proteins', *Int. J. Biolog. Macromol.*, **2006**, *39*, 127-134.
21. Green, N.M., 'A spectrophotometric assay for avidin and biotin based on binding of dyes by avidin', *Biochem J.*, **1965**, *94*, 23c.

22. Zotti, G., Cattarin, S., Comisso, N., 'Cyclic potential sweep electropolymerisation of aniline. The role of anions in the polymerization mechanism.' *J. Eletroanal. Chem.* **1988**, 239, 387-396.
23. Lillie, G., Payne, P.A., Vadgama, P., 'Electrochemical impedance spectroscopy as a platform for reagentless bioaffinity sensing', *Sens. Actuators B*, **2001**, 78, 249-256.
24. Berney, H., West, E., Haeefele, J.E., Alderman, J., Lane, W., Collins, J.K., 'A DNA diagnostic biosensor: development, characterisation and performance', *Sens. Actuators B*, **200**, 68, 100-108.
25. Alfonta, L., Bardea, A., Khersonsky, O., Katz, E., Willner, I., 'Chronopotentiometry and faradaic impedance spectroscopy as signal transduction methods for the biocatalytic precipitation of an insoluble product on electrode supports: routes for enzyme sensors, immunosensors and DNA sensors', *Biosens. Bioelectron.* **2001**, 16, 675-687.