DEVELOPMENT OF BIOSENSORS FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN ENVIRONMENTAL MONITORING OF WATER

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Development of Biosensors for the Determination of Polycyclic Aromatic Hydrocarbons in Environmental Monitoring of Water

by

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TO MY ***MOTHER***

MY BROTHERS AND SISTERS

MY FIANCEE

AND SPECIAL GRATITUTE TO MY LATE ***FATHER***

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Malaysia, wait for my return...

"If I fail, I try again, and again, and again. If you fail, are you going to try again? The human spirit can handle much worse than we realise. It matters how you are going to finish. Are you going to finish strong?"

- Nick Vujicic -

Declaration

I hereby declare that this thesis is my own work, in partial fulfilment of the requirements of the Doctor of Philosophy degree. It is based on research carried out in the Tyndall National Institute, University College Cork, Ireland between May 2008 and February 2012.

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Date:

Abstract

The work presented in this thesis described the development of the biosensors with optical and electrochemical detections for determination of polycyclic aromatic hydrocarbons (PAHs) in environmental monitoring of water. This research employs the enzyme linked immunosorbent-assay (ELISA) principle, in which an alkaline phosphatase (AP) and a horseradish peroxidase (HRP) were used as the enzyme labels. The influence of several parameters such as the concentration of the biocomponents, the suitable organic solvents and the working pH was tested in order to achieve maximal binding of the antigen-antibody. It was found that DMSO was the most suitable organic solvents to dilute PAHs. The most promising pH for diethanolamine (DEA) buffer was pH 9.5, due to the fact that the alkaline phosphatase works in alkaline environment. An amperometric immunosensor was developed, consisting of a three electrode system biochip with gold as the working electrode. The electrochemical behaviour of the biochips was investigated by cyclic voltammetry and impedance spectroscopy using ferrocyanide/ferricyanide redox pair. The open-circuit potential measurement of Ag/AgCl reference electrode showed that it was stable for 5 hours in 1 M KCl, with a small potential drift of 13 mV observed for the first 10 minutes of operation.

Two haptens were synthesised, involving the conjugation of bovine serum albumin (BSA) with phenanthrene and pyrene. The BSA-phenanthrene molecule produced a better signal in capture assay compared to the BSA-pyrene coating conjugate. Various ELISA formats were employed for the detection of PAHs. It was found that the indirect competitive assay showed the best performance for benzo[a]pyrene detection, with a commercial monoclonal antibody (4D5) used as a primary antibody that was raised specifically for benzo[a]pyrene. The limit of detection was found at 1.42 ng ml^{-1} using bare gold electrode. However, the sensitivity of the sensor was increased with the modification of the gold electrode with 11-mercaptoundecanoic self-assembled monolayer (0.56 ng ml^{-1}) with an IC_{50} value of 6.8 ng ml^{-1}. The biochip coated with coating conjugate was found to be stable for 8 days at 4 °C. For validation studies, two methods based on cell-based biosensors and chromatographic techniques were performed for PAH determination.