Snoek

Scott's

Omega 3&6

Fish paste

Lipstick

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WESTERN CAPE

Figure 70: Fish and fish products used as sample for immunosensor application.

7.2 Sample preparation

Approximately 5g of each sample was weighed into a beaker and 20 ml of phosphate buffer was added. They were then heated to boil for 20 minutes and cooled to room temperature. The mother liquor was decanted into a vessel and it was analysed using EIS.

7.3 Results and discussion

In order to compare the selectivity of flourene derivative immunosensor and xanthene derivative immunosensor for parvalbumin in real samples EIS was performed by using these two immunosensors with fish and fish products extracts. The samples were analysed in duplicate. Two fish species and five fish products were examined. For each sample measurement new immunosensor was developed. Snoek fish mostly contain a white muscle and the white muscle is known to have a high content of parvalbumin. Lopata et al and co-

workers have reported IgE cross-reactivity among fish species consumed predominantly snoek fish was one of them. They have established that there are two allergenic isoforms of snoek parvalbumin and that indicated that the commercial antibody used was not effective in detecting all isoforms present in fish species [86]. A parvalbumin content of 1.34 pg/ml was observed using flourene derivative immunosensor and 1.41 pg/ml using xanthenes derivative immunosensor. Scott's emulsion consists of cod liver oil and cod fish is known to have high parvalbumin content. The concentration of parvalbumin in scott's emulsion was estimated to be 9.10 pg/ml using flourene derivative and 2.30 pg/ml using xanthenes derivative. Omega 3&6 contains salmon oil and the salmon is famous for its salmon pink colour. It showed no reaction for both immunosensors indicating that it does not contain traces of parvalbumin or the amount of parvalbumin is too small to be detected.

Lipstick contains fish scales predominantly from herring fish. It has shown to have parvalbumin content of 6.53 pg/ml from flourene derivative immunosensor and 5.15 pg/ml from xanthenes derivative immunosensor. Eye shadow was analysed and the parvalbumin content was estimated to be 1.40 pg/ml using flourene derivative immunosensor and 1.31 pg/ml using xanthene derivative imunosensor. Tuna fish consists of pink to dark red muscle tissue. Dark muscles are known to contain small traces of parvalbumin. Tuna fish showed no reaction for both immunosensors indicating that the parvalbumin content is too low to be detected. Fish paste contains a combination of herring and anchovies, the parvalbumin content have been estimated to be 8.23 pg/ml using flourene derivative and 7.40 pg/ml using xanthene derivative.The results are summarized in table 9. Both immunosensors are in agreement of the quantity of parvalbumin present in the sample, the results are slightly different except for the Scott's whereby xanthene derivative immunosensor detected a very low quantity than flourene derivative.

Sample	Flourene derivative immunosensor pg/ml. Ave, n=2	Xanthene derivative immunosensor pg/ml. Ave, n = 2
Snoek	1.34	1.41
Scott's	9.10	2.30
Omega 3&6	No traces of parvalbumin	No traces of parvalbumin
Lipstick	6.53	5.15
Eye shadow	1.4	1.31
Tuna	No traces of parvalbumin	No traces of parvalbumin
Fish paste	8.23	7.40

Table 9: Summary of results obtained from EIS for samples.

Skin prick test was performed by Hilger and co-workers [87] on patients with fish allergy. One of the patients showed allergic symptom with a concentration of 0.07 µg/ml parvalbumin. Even at low concentration people who are allergic to fish can react (show symptoms). The lowest detection limit was found to be 0.05 ng/ml parvalbumin [66]. Both xanthene derivative immunosensor and flourene derivative immunosensor were able to measure significantly lower concentrations (2.42 pg/ml and 1.50 pg/ml) than in the previous studies [66; 87]. Immumosensors prepared were reproducible, repeatable and stable and retained the bioactivity of the biomolecule. All samples were in the linear range of both immunosensors. Flourene derivative and xanthene derivative, their porosity facilitated antibody immobilization through non site region allowing the binding site to be available for antigen binding.

Chapter 8

Conclusion and thesis summary

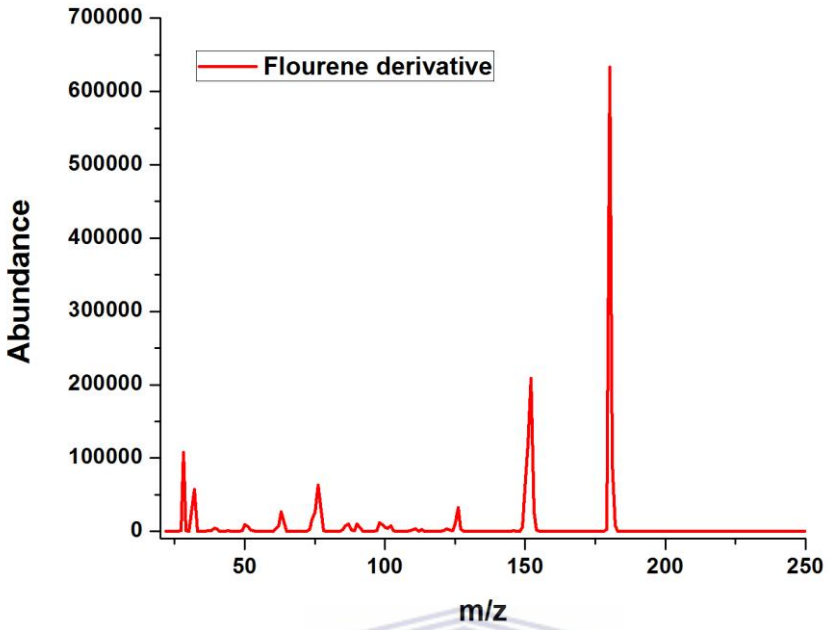
Two noncyclic organic compounds namely 9,9'-(Ethyne-1,2-diyl)bis(Flouren-9-ol) and 9-(4-methoxyphenyl)-9H-xanthen-9-ol were synthesized successfully and immunosensor developed on these two compound platforms by immobilization of an antibody, was prepared in a simple and quick manner. Both flourene derivative and xanthene derivative prepared as thin films at GC electrode were characterized successfully using DSC, FTIR, UV/Vis, Flourescence, CV, SWV and PXRD. FTIR confirmed their structures. CV and SWV confirmed the presence of reversible redox couple and SWV was used to determine the formal potential which was found to be -134 mV for flourene derivative and -73 mV for xanthene derivative vs Ag/AgCl. The formal potential was used for monitoring the binding between the analytes and the immunosensors using EIS. For both compounds a yellow solution was observed with flourene derivative more intense in colour. From flourescence results a small stokes shifts was observed for both compounds. These two compounds behaved in a similar manner due to their π-π^* transitions, structural rigidity and bulkiness. Flourene derivative has a rigid planar biphenyl unit and xanthene derivative has an hydroxyl moiety as well as a pyranyl oxygen.

Capacitance, as measured by EIS was quantitatively related to the concentration dependent binding of analyte to the immunosensor. Flourene derivative immunsensor showed a high sensitivity for the analyte with a detection limit of 1.50 pg/ml. Xanthene derivative immunosensor also showed a high sensitivity of the analyte with detection limit of 2.42 pg/ml. The sensitivity and the detection limit were determined sing EIS. Flourene derivative immunosensor was found to be more sensitive than xanthene derivative immunosensor, with good repeatability and reproducibility. The detection limit of these immunosensors for the selected analyte was much lower than the detection limit of other analytical methods [65; 66]. UV/Vis absorption spectroscopy was used to confirm the binding of the analyte to the native antibody in solution, by measuring the response as a function of concentration. Both immunosensors were successfully applied in fish species and fish products analysis. Tuna and omega 3&6 showed no traces of parvalbumin. Low concentrations of parvalbumin in snoek, lipstick, eyeshadow, scott's and fish paste were determined successfully. In

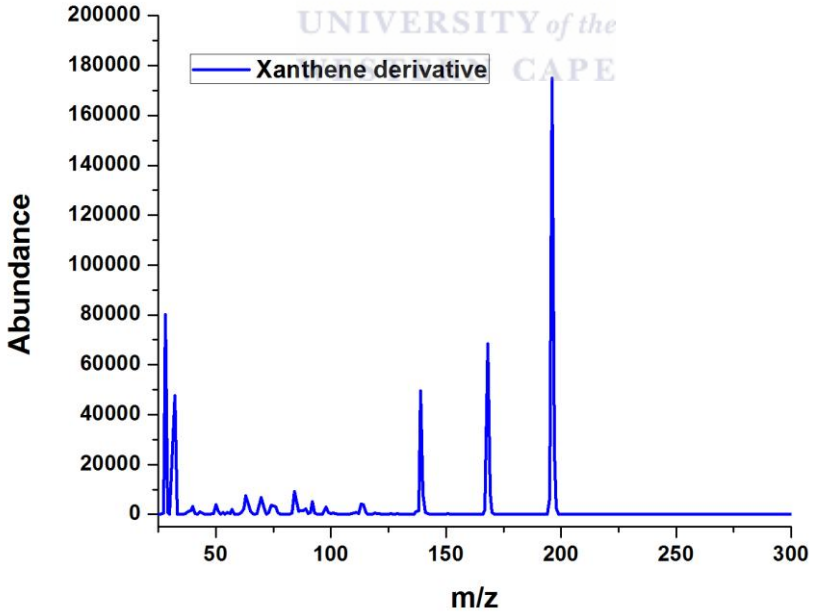
comparison, the EIS was found to be more sensitive than ELISA method. ELISA is time consuming compared to the EIS.

Supramolecular structures are constructed by non-covalent interactions and self-assembly and their stoichiometry and topology depend on the phenomenon of molecular recognition which occurs between the host and guest molecules and the resultant structure can in principle explain its reactivity and stability. Hydrogen bonding is the most important intermolecular interaction that influences molecular recognition. Morphological and physical properties of flourene derivative immunosensor and xanthene derivative immunosensor make them very versatile sensing platforms with high sensitivity to the presence of biochemical species which penetrate inside the pores. New novel platform immunosensor has been successfully developed. The synthesis was quick, easy and done inhouse (UWC sensorlab). Both immunosensors were developed using a non-labelled antibody which was directly attached to the platform. Unlike in ELISA there was no blocking or washing step while developing the immunosensors. The higher sensitivity shows that there was a better antibody orientation. This indicates enhanced molecular recognition. The antibody was attached via nonsite directed approach allowing binding sites to be available for analyte binding. These platforms are effective for biomolecule immobilization and retain bioactivity. They are capable of directly detecting antibody/antigen interactions. It was established that the mechanism of adsorption is controlled by physical forces. Real sample analysis was performed efficiently in real time with minimum sample preparation.

Appendix 1



Appendix 1a: Mass spectrum of fourene derivative.



Appendix 1b: Mass spectrum of xanthene derivative.

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