The Pharma Innovation Journal 2022; SP-11(11): 31-34

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(11): 31-34

www.thepharmajournal.com
Received: 01-09-2022
Accepted: 06-10-2022

Sruthi PS
Research Scholar, National Institute of Food Technology, Entrepreneurship and Management, Pudukkottai, Road, Thanjavur, Tamil Nadu, India

S Shanmugasundaram
Professor and Head, National Institute of Food Technology, Entrepreneurship and Management, Pudukkottai, Road, Thanjavur, Tamil Nadu, India

Corresponding Author:
S Shanmugasundaram
Professor and Head, National Institute of Food Technology, Entrepreneurship and Management, Pudukkottai, Road, Thanjavur, Tamil Nadu, India

Immobilization and characterisation of glassy carbon electrode based biosensor for acrylamide detection

Sruthi PS and S Shanmugasundaram

Abstract
This present work describes a rapid detection method by biosensors to detect acrylamide, which is well known as a probable human carcinogen. Immobilization plays an important role in the biosensors, as it is the heart of any sensing technique developed. Here, the study is about the immobilization of silver nanoparticles and haemoglobin on the working electrode surface of the glassy carbon electrode. Furthermore, the immobilization was analysed electrochemically using cyclic voltammetry. Surface morphology of the working electrode was well studied using scanning electron microscopy and its proves the immobilization has done uniformly. The immobilized electrode shown a calibration plot for the acrylamide detection with an R^2 of 0.99, which signifies the effective immobilization.

Keywords: Biosensors, acrylamide, glassy carbon electrode, immobilization, silver nanoparticle, haemoglobin

1. Introduction
Acrylamide (AA) is a chemical compound, which is frequently used in the production of polyacrylamide and is very useful in papermaking, wastewater treatment and oil recovery process. Apart from all these uses, it is remarkable that acrylamide (AA) can cause high neurotoxicity, potential carcinogenicity and genotoxicity in human population [1]. Acrylamide is commonly found in foods with high starch content (Potato chips, banana chips, bread) when subjected to high temperature treatment. It is significant that even lower quantity of high starch foods can contain higher levels of AA [2]. All the above-mentioned harmful health effects of AA make its necessity to detect the presence of it in the foodstuffs.

Some of the common instrumental techniques applied to detect AA are Gas chromatography (GC), High-pressure liquid chromatography (HPLC) coupled with mass spectrometry [3, 4]. The main drawback of these techniques are time consumption, high cost and requirement of trained person to operate.

Electrochemical biosensors have the advantage of rapid detection, portability and simplicity, which increased the attention of researchers to employ this for the AA detection [5]. Several studies proved that haemoglobin (Hb) have the property of forming adducts with acrylamide due to the chemical reaction between α-NH2 group of the *N*-terminal valine of Hb [6, 7]. Hence, Hb can be utilized as a bio recognition element to detect the AA. Direct adsorption of biomolecules like Hb on bare electrode surface may result in denaturation and loss of its bioactivity, therefore adsorption of this kind of biomolecules onto the interface of nanoparticle can preserve its bioactivity due to the improved biocompatibility of nanoparticles [8].

Application of nanomaterials in biosensor construction will improve its sensitivity and surface enhancement for immobilization. It also improves the catalytic properties of electrochemical sensor [9, 10]. There are many nanoparticles used in the construction of biosensors. Some of the widely used nanomaterials in electrochemical biosensors are gold [11], Si [12], activated nanocarbon [13] and platinum nanoparticles [14]. Apart from this, silver nanoparticles (AgNps) have gained its significance in biosensor modification due to its ability to readily transfer the photo-induced electrons and low cost [15, 16].

Immobilization is a technique, be either physical or chemical, which bring out an interaction between bio recognition elements to the transducer surface. Choosing of an appropriate immobilization technique is very crucial in biosensor development, because selecting the unsuitable method can inactivate the bio recognition element.

This research work mainly focuses on the immobilization of silver nanoparticles (AgNps) and haemoglobin (Hb) on the surface of glassy carbon electrode for the construction of acrylamide biosensor and its characterisation using cyclic voltammetry (CV) and Scanning electron

microscopy (SEM) and plotted a linearity plot for the developed biosensor.

2. Materials and Methods

2.1 Experimental materials and Apparatus

All chemicals required for conducting experiments were procured from Sigma Aldrich, India. Acrylamide standard, human haemoglobin (lyophilized powder), Phosphate buffer, Silver Nitrate, Trisodium Citrate. Three-electrode system with working electrode as glassy carbon, reference electrode as Ag/Ag Cl, platinum as the counter electrode and the potentiostat Sensit BT: SNS configuration potentiostat (Palmsens 4) was brought from M/s Class One Systems, New Delhi.

2.2 Modification of glassy carbon based working electrode

Initially, the glassy carbon electrode was polished with alumina powder slurry of 0.5µm and then it was subsequently washed with water and ethanol. Then the working electrode was dried at room temperature. Further, 5µl of AgNps was drop casted on the electrode and was kept for incubation at 8 °C for 30 minutes, then 5µl of 5mg/ml Hb was casted above AgNps on the working electrode and incubated for 15 minutes in ambient condition. The immobilization or modification on the electrode was based on the ionic interactions.

2.3 Electrochemical measurement for immobilization

The glassy carbon electrode was characterized using cyclic voltammetry (CV) before and after the modification of electrode. The measurement was conducted in 5mM ferric cyanide solution with 0.1M phosphate buffer dissolved with 5mM of acrylamide standard solution. Potential range for the CV was from -0.75 to 0.75V at a scan rate of 0.5V/s.

2.4 Morphological Characterisation for immobilization

The surface morphology of the bare glassy carbon electrode and AgNps/Hb immobilized electrode was examined under Scanning electron microscopy (SEM, Vega3 Tescan) with a secondary electron detector using an accelerating voltage of 5kV, 50 to 5000X magnification and the working distance was kept at 25mm. Vega 3 control software (version 4.2.28.0) was used for image processing.

2.5 Detection of acrylamide using amperometric method

Various concentration of acrylamide standard ranging from 5mM to 75mM were spiked with 10 ml of 0.1M phosphate buffer. This solution was taken as electrolyte. Furthermore, the three-electrode system was immersed in to the electrolyte and amperometric technique was applied with a dc potential of 0.28V to detect the analyte dissolved in the electrolyte. The response signal obtained was in the form of current and it was recorded.

3. Results and discussion

3.1 Cyclic voltammetry measurement for immobilisation

The figure 1 depicts that the peak current in the voltammogram is high for electrode immobilized with AgNps and Hb, when compared to the bare glassy carbon electrode. This hike in peak current is an evident that the immobilization has done successfully. Increased conductivity of the AgNps on the electrode surface results in the increased peak potential after immobilization. This enhances the response simultaneously. The opposite charge interactions between the AgNps (-Ve) and Hb (+ve) makes the immobilization effective. Similar results was reported by Asnaashari and her co-workers [17].

3.2 Change in Morphological characteristics of the working electrode

From the figure 2:(a), it was clear that the SEM image of bare glassy carbon electrode was a uniform layer of carbon, whereas the clogged globular structure from the figure 2:(b) confirms the immobilization of Hb together with AgNp beneath its layer.

3.3 Linearity for the detection of Acrylamide

The immobilized electrode was used for the amperometric detection of acrylamide, a correlation between varied acrylamide concentration and change in current was developed by plotting acrylamide concentration (mM) on X-axis and Change in current (µA) on Y-axis was shown in figure 3. The developed correlation follows a linear trend with a regression equation of $y = 0.0494x + 0.0504$ with an R^2 of 0.99. It is evident from the result the Change in current linearly increases with increase in acrylamide concentration. The obtained result shows fair agreement with Garabagiu *et al.*, [18].

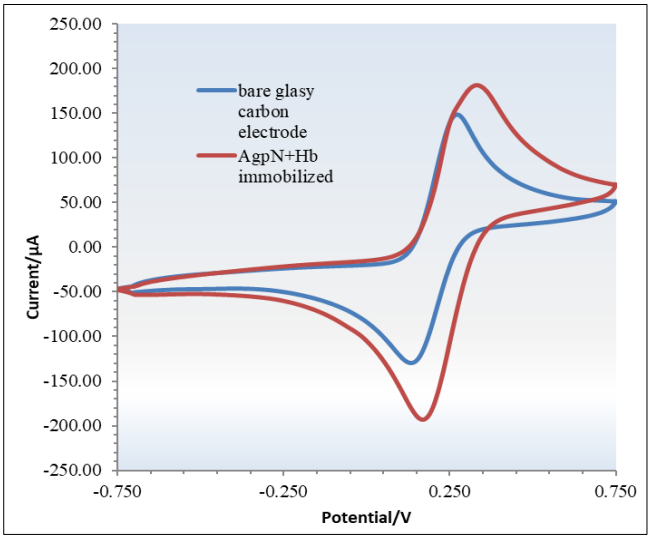

Fig 1: Cyclic voltammogram of Bare and immobilized galssy carbon electrode

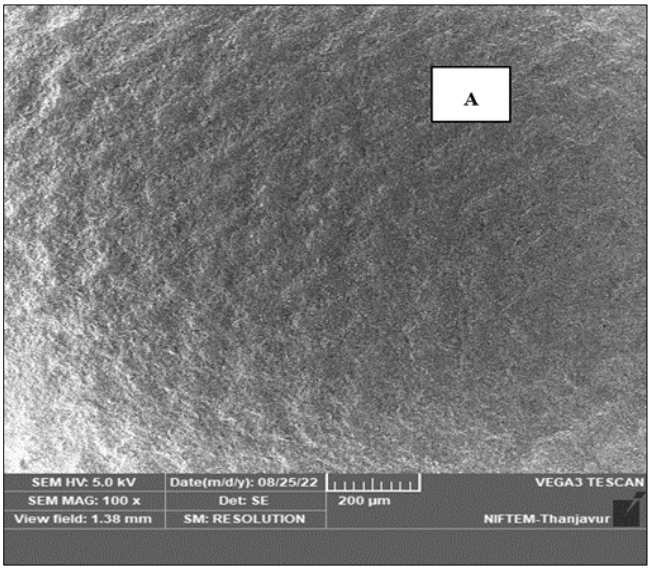

Fig 2(a): Morphology of bare electrode

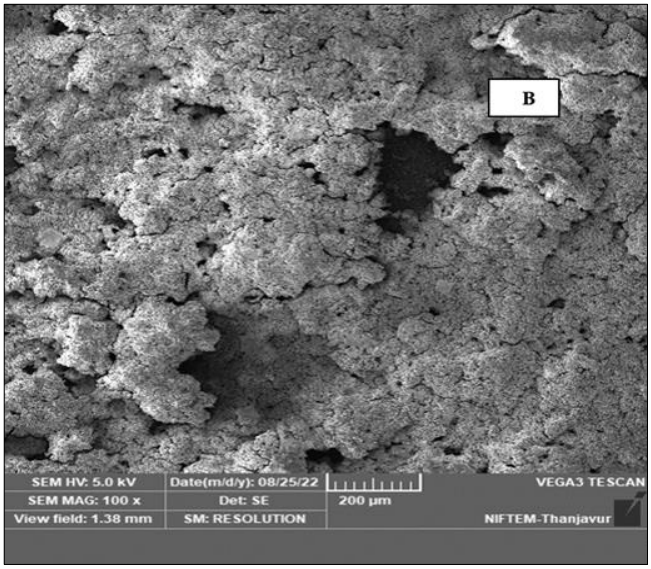

Fig 2(b): Morphology of immobilized electrode

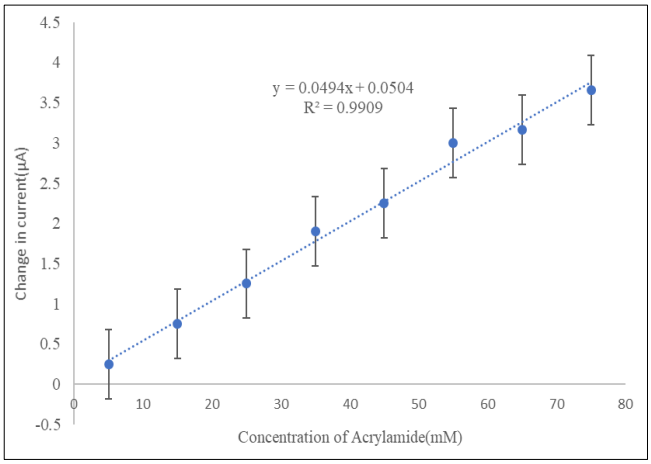

Fig 3: Linear plot between Current (µA) Vs Acrylamide (mM)

4. Conclusion

Immobilization and characterisation is a very important aspect in the design and fabrication of biosensors especially in case of electrochemical sensors. In this study, the focus was mainly on the modification of glassy carbon electrode using silver nanoparticles and haemoglobin. Characterisation of electrode before and after immobilization was analysed using cyclic voltammetry and scanning electron microscopy. Then a standard calibration plot was developed for the developed biosensor with a high linearity. Future studies can be focused on performance evaluation and stability of the developed biosensor.

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