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Utilization of Polyimide Materials for Enhanced Electrochemical Detection of Cholesterol*

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ABSTRACT

The objective of this study was to utilize a range of polyimide materials for the determination of cholesterol. Cholesterol is a waxy, fat-like substance produced by the liver and is essential for constructing cells and producing vitamins and other hormones. Blood cholesterol levels are an important parameter the diagnosis and treatment of several clinical diseases, including arteriosclerosis, cardiovascular ailments, hypertension, and others. Cholesterol levels in the blood were measured using several chemical and enzymatic methods. Electrochemical tests were conducted using Cyclic Voltammetry (CV) and Time Base (TB) techniques. The surface morphologies of the modified electrode surfaces were examined in detail using scanning electron microscopy (SEM) techniques. In the enzymatic reaction, hydrogen peroxide was formed due to the enzymatic reaction between cholesterol and cholesterol oxidase, which was measured at 0.650–0.750 V against Ag/AgCl. In this study, poly[1,3,5-tri(aminophenyl)hegzahydro-1,3,5-pyromellithimide] and poly[2,4,6-triaminopirimidyn benzophenondiimide] were employed as polymeric materials to immobilize cholesterol oxidase. It was hypothesized that these two polyimides could be utilized as an appropriate medium for the immobilization of cholesterol oxidase.

Keywords: polyimide, cholesterol oxidase, polymer matrix, biosensor.

1. Introduction

Cholesterol is a fat (also referred to as a lipid) that is transported through the bloodstream by lipoproteins. The two main types of lipoproteins are low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Cholesterol is a sterol, a type of lipid, formed from the combination of alcohol and transported in the blood. It has a waxy and oily consistency. The discovery of cholesterol in gallstones in 1754 led to the naming of this substance, derived from the Greek words chole- (bile) and stereo (solid) and the -ol suffix in chemistry. Cholesterol is present in all organs, with the highest concentrations observed in the brain, nerves, heart, intestines, muscles, and liver.

The concentration of blood cholesterol is a clinically significant parameter for the diagnosis and treatment of various clinical conditions, including cardiovascular disease, hypertension, and atherosclerosis [1-4]. The concentration of cholesterol in human blood is typically quantified through the use of various chemical or enzymatic methods. The chemical reactions employed for the determination of cholesterol are associated with certain difficulties, including lack of specificity and selectivity due to interfering reactions and the use of unstable and corrosive reagents. Enzymatic methods utilizing cholesterol ester hydrolase (CEH) and cholesterol oxidase (COx) for the measurement of total cholesterol are more sensitive than chemical methods, but both techniques are time-consuming [5]. The enzymatic method exhibits high selectivity, a short reaction time, a small electrode surface, a low cost, and high reproducibility. In studies of amperometric biosensors, the immobilization of enzymes on the electrode surface is the most crucial step [6,7]. Various classes of polymers have been utilised as immobilization media.

The measurement of cholesterol was monitored by the following reactions, which utilised the enzyme cholesterol oxidase (COx).

$$\text{Cholesterol} + O_2 \xrightarrow{COx} \text{Cholest-4-en-3-one} + H_2O_2 \text{ [8,9]}$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \text{ [10,11]}$$

The amperometric detection of hydrogen peroxide is typically conducted anodically (e.g., oxidation at +0.7 V with a Pt working electrode). However, this method is significantly influenced by numerous readily oxidizable

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interfering substances in authentic samples. [12].

Polyimides are durable polymers that are chemically inert and resistant to heat and environmental conditions. Polyimide films have a diverse range of applications, including use as a sensor [13-15]. In particular, with regard to their mechanical, chemical, and especially selectivity properties, the permselectivity of these polymeric films should be considered as a promising approach for their use as enzyme immobilization media.

This study successfully employed polyimide polymeric materials as support materials for immobilizing cholesterol oxidase. Physical methods were employed to achieve immobilization, and utilizing these electron mediators, cholesterol was detected electrochemically. In order to ascertain the optimal conditions for the generation of H_2O_2 via an enzymatic reaction between cholesterol and cholesterol oxidase, cyclic voltammetry (CV) and time-base (TB) techniques were employed. In the time-base technique, an applied potential of +0.75 V facilitated the generation of H_2O_2 via enzymatic catalysis of a cholesterol-cholesterol oxidase reaction.

2. Material and Method

2.1. Material

Cholesterol oxidase (COx) (E.C.1.1.3.6. Pseudomonas sp. = E.coli) with a specific activity of 1.5 U/mg solids was obtained from MP Biomedicals. KCl, NaCl, Na_2HPO_4 used for PBS (phosphate buffer salts) were purchased from Merck (www.merck-chemicals.com). Hexane, KH_2PO_4 for PBS, and cholesterol as reagent were purchased from Riedel-De Haen (www.riedeldehaen.com), Carlo Erba (www.carloerbareagenti.com), and Sigma (www.sigmaaldrich.com), respectively.

2.2. Instrumentation

All aqueous solutions were prepared using deionized and double-distilled water. The nitrogen gas employed for purging and blanketing during electropolymerization was of high purity. Electrochemical tests were conducted using cyclic voltammetry (CV) and the Time base (TB). The CV and TB techniques were conducted using an electrochemical analyzer, the BAS 100W (Bioanalytical Systems, Inc., West Lafayette, IN, USA). The BAS 100W electrochemical analyzer employed the standard three-electrode system, comprising a platinum disk (BAS, MF-2013, 1.98 mm^2) as the working electrode, an Ag/AgCl reference electrode, and a platinum wire coil as the auxiliary electrode. The surface morphologies of the modified electrode surfaces were examined in detail using scanning electron microscopy (SEM) techniques. Scanning electron microscopy (SEM) analyses were conducted using the LEO EVO40 microscope. During the analyses, a 20-nanometre Au-Pd coating was applied using the BALTECK brand sputter coating technique. The pH was determined using a Jenway 3010 pH meter.

2.3. Preparation of biosensor

At the outset of the electrochemical studies, the Pt disc working electrode was prepared in accordance with the standard procedure outlined by Ekinci (1999), which involved cleaning and polishing to a 0.05 µL finish with aqueous alumina slurry [16]. Two distinct polyimide derivatives were employed for this purpose. The aforementioned polyimide derivatives were prepared and characterised by chemical methods in accordance with the literature sources [17,18]. The enzyme electrodes were formed by the deposition of COx onto the polyimide-modified electrodes. This was achieved by depositing the solution, prepared by dissolving 0.1 g of polyimide in 2 mL NMP, onto the electrode surface and allowing it to dry. The electrochemical measurements of the prepared enzyme electrodes were conducted in 0.1 M phosphate-buffered saline (PBS) at a neutral pH.

In the preparation of the biosensor, a variable quantity (1-10 µl) of cholesterol oxidase was added to the polymer electrode and allowed to react for a period of 2 hours at a temperature of +4°C, thus allowing for immobilization. For the amperometric experiments, the PBS solution was aerated for approximately 15 minutes and maintained at room temperature. A potential of +0.75 V was applied to the cell system due to the generation of H_2O_2 by the enzymatic reaction between cholesterol and cholesterol oxidase. The biosensor responses were registered as a current signal (nA-µA) by tracking the oxygen consumption at +0.75 V in relation to the Ag/AgCl electrode, due to the enzymatic activity. Once the constant background current value had been reached, the substrate was added to the reaction cell. Subsequently, the buffer solution was replenished following each measurement.

In the presence of surfactants, cholesterol is soluble in water. The literature contains numerous procedures for the preparation of standard solutions of cholesterol [19-24]. In the literature, a variety of solvents, including alcohol, hexane, and triton, were employed to prepare standard solutions of cholesterol. A bare platinum electrode was employed to examine the effects of the solvents. To achieve this, steady-state amperometric behavior was assessed by time-based measurements at +0.75 V in 0.1 M PBS. In order to pursue further research, hexane was selected as the solvent for the preparation of the cholesterol stocks. The results of this experiment are presented in Figure 1.

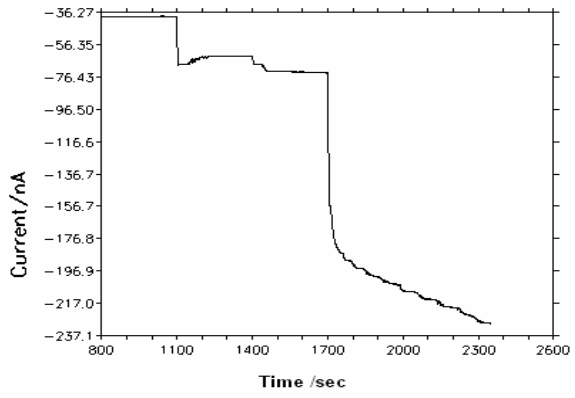

Figure 1. Injection of different solvents onto bare Pt electrode; 900 sec hexane, 1000 sec hexane + Cholesterol, 1100 sec 2-propanol, 1400 sec 1% Triton X-100 + 0.1 M PBS, 1700 sec Triton X-100

3. Results and Discussions

3.1. Poly[1,3,5-tri(aminophenyl) hexahydro-1,3,5-triazine pyromellithimide]–COx Electrode

A series of drops of Poly[1,3,5-tri(aminophenyl)hexahydro-1,3,5-triazine pyromellithimide] were applied to a bare Pt electrode, with 2, 5, 10 and 30 µL of the solution used. The amperometric current responses of poly[1,3,5-tri(aminophenyl)hexahydro-1,3,5-triazine pyromellithimide] and the adsorbed cholesterol oxidase-containing enzyme electrode (polyimide + COx) against cholesterol injections are presented in Figures 2-6.

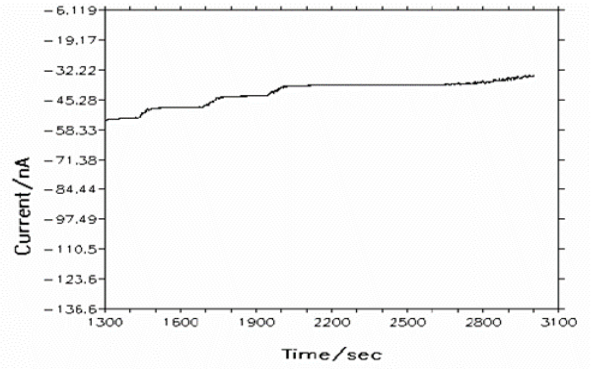

Figure 2: 5 µL of poly[1,3,5-tri(aminophenyl)hexahydro-1,3,5-triazinepiromellitimide] electrode response to cholesterol injections: at 1600, 1900, 2200, 2500, 2800 sec.

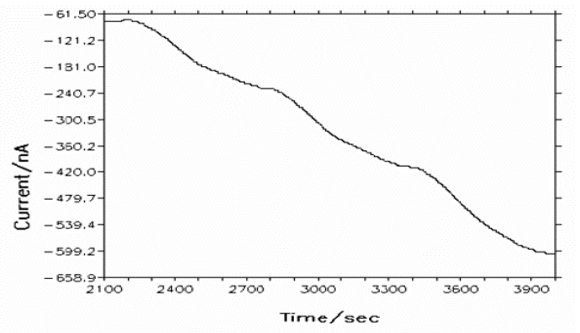

Figure 3: 5 µL of poly[1,3,5-tri(aminophenyl)hexahydro-1,3,5-triazinepiromellitimide] + 2 µL COx doped electrode response to cholesterol injections: at 2200, 2800, 3400 sec.

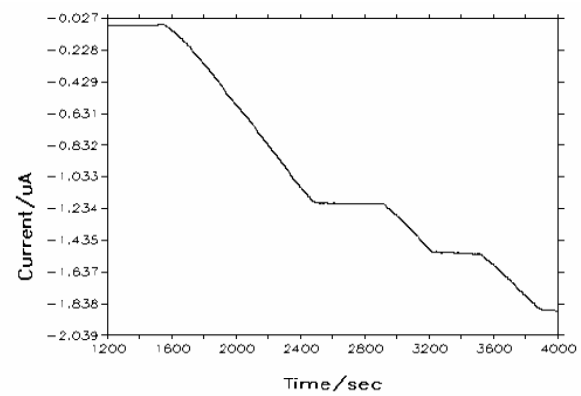

Figure 4: 5 µL of poly[1,3,5-tri(aminophenyl)hexahydro-1,3,5-triazinepiromellitimide] + 10 µL COx doped electrode response to cholesterol injections: at 1550, 1950, 2950, 3500 sec.

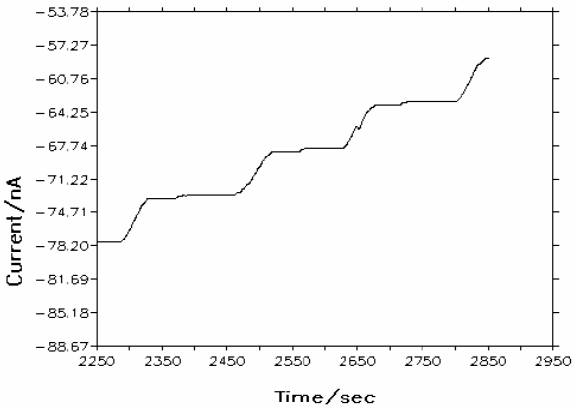

Figure 5: 30 µL poly[1,3,5-tri(aminophenyl)hexahydro-1,3,5-triazinepiromellitimide] electrode response to cholesterol injections: at 2550, 2650, 2750 sec.

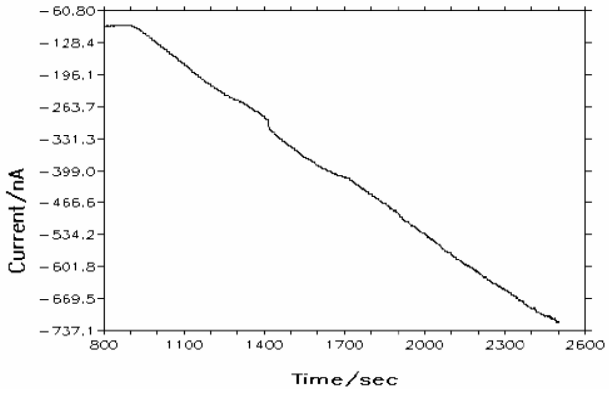

Figure 6: 30 µL of poly[1,3,5-tri(aminophenyl)hexahydro-1,3,5-triazinepiromellitimide] + 10 µL CO_x doped electrode response to cholesterol injections: at 900, 1300, 1700, 2100, 2500 sec.

Scanning electron microscopy (SEM) was employed to obtain structural information about the poly[1,3,5-tri(aminophenyl)hexahydro-1,3,5-triazinepyrrolomellitimide] electrode and the poly[1,3,5-tri(aminophenyl)hexahydro-1,3,5-triazinepyrrolomellitimide]-COx electrode. Figure 7 illustrates the structural differences between polyimide and polyimide-CO_x.

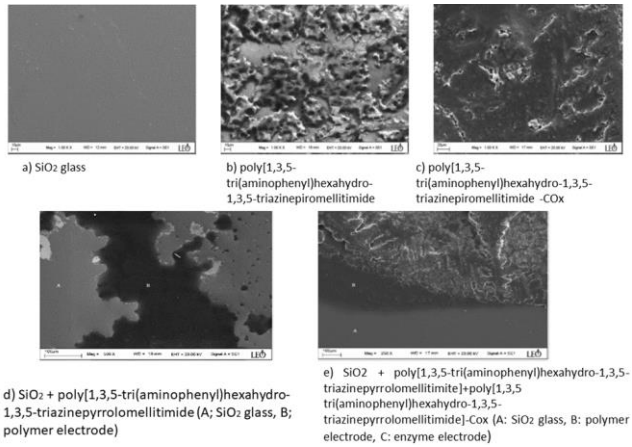

Figure 7: SEM images of bare SiO_2 surface, polymer electrode, and enzyme electrode

3.2. *Poly[2,4,6-triaminopyrimidine benzophenondiimide]- CO_x Electrode*

We obtained the CVs of poly[2,4,6-triaminopyrimidine benzophenondiimide] and enzyme-adsorbed polyimide electrode. We also investigated the attachment of the enzyme to the polymeric structure by comparing the CVs obtained. A comparison of Figures 8 and 9, which display the CVs of polyimide and enzyme-immobilized polyimide, suggests that immobilization may have occurred.

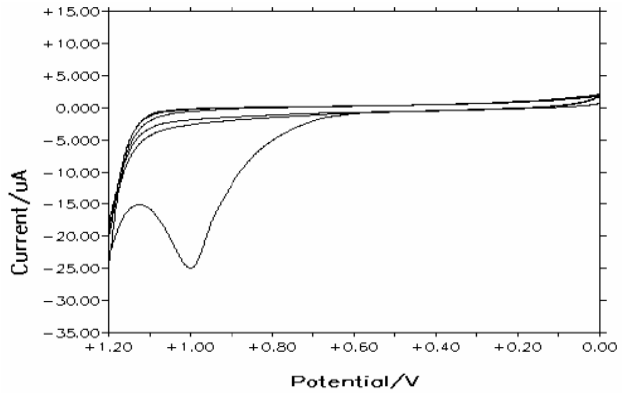

Figure 8: 2 µL of Poly[2,4,6-triaminopyrimidine benzophenondiimide] electrode 0-1200 mV and 8-cycle CV at 50 mV/s

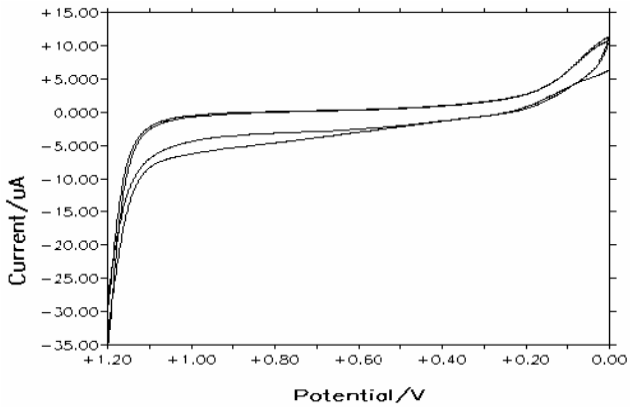

Figure 9: 2 µL of Poly[2,4,6-triaminopyrimidine benzophenondiimide]+ 2 µL of CO_x CV of the formed electrode at 0-1200 mV, 50 mV/s for 8 cycles.

The behavior of poly[2,4,6-triaminopyrimidine benzophenonediimide] and enzyme-adsorbed polyimide electrodes were examined in the presence of cholesterol injections with TB. The experiments conducted in 0.1 M PBS solution at 0.650 V demonstrate that the enzyme binds to the polymeric structure and responds to cholesterol injections. This is illustrated in Figures 10 and 11.

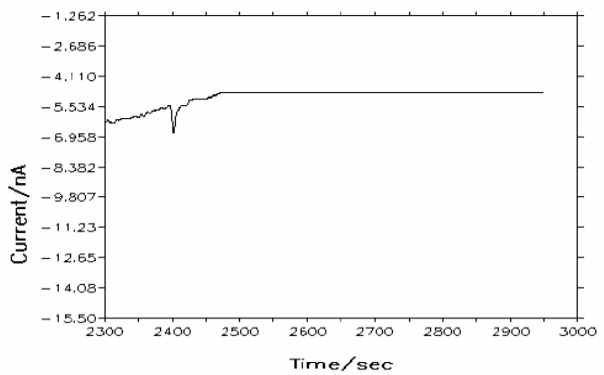

Figure 10: 1 µL poly[2,4,6-triaminopyrimidine benzophenondiimide] cholesterol response to injections: 2400, 2500, 2600, 2700, 2800, 2900 sec

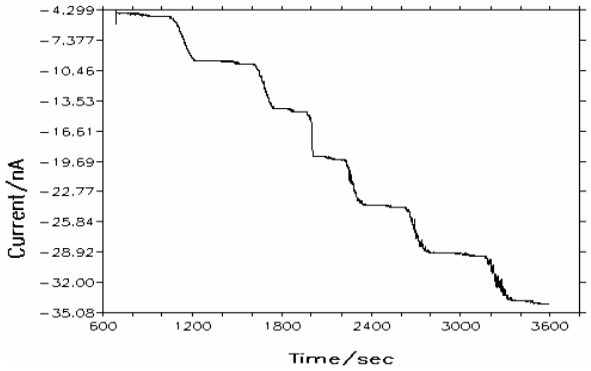

Figure 11: Response of the electrode composed of 1 µL Poly[2,4,6-triaminopyrimidine benzophenondiimide]+ 2 µL COx to cholesterol injections: 800, 1400, 2000, 2600, 3200 sec.

Figure 12 presents SEM images of the surface of SiO_2 glass, the surface of a polyimide electrode, and the surface of a polyimide-enzyme electrode. The images clearly demonstrate the differences in surface morphology.

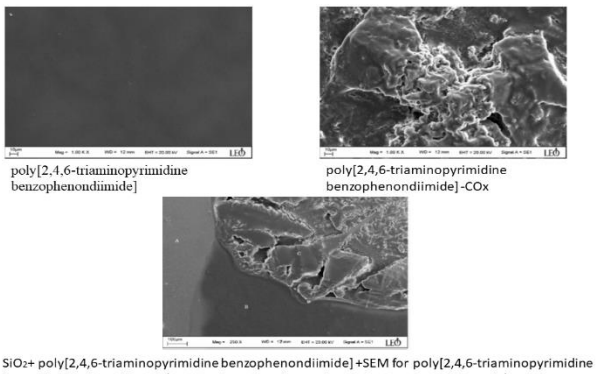

Figure 12. SEM images of bare SiO_2 surface, polymer electrode, and enzyme electrode

4. Conclusions

A comparison of the SEMs of SiO_2 glass, polyimide, and enzyme-immobilized polyimides reveals significant differences due to immobilization. The responses obtained for two polyimide derivatives indicate that the related polyimide films may be suitable as immobilization media for cholesterol oxidase.

It is postulated that there is a linear relationship between plasma cholesterol levels and the development of diseases such as cardiovascular disease, diabetes, high blood pressure, and gallstone formation. Approximately 70% of cholesterol in serum samples is in an ester structure, and two enzymes are used for the determination of total and free cholesterol. Among these enzymes, cholesterol esterase (CE) enables the conversion of cholesterol esters into cholesterol, while cholesterol oxidase (CO_x) converts the formed and/or free cholesterol into cholest-4-en-3-one and hydrogen peroxide, an electroactive species, in the presence of oxygen. In polymeric supports prepared by electrochemical and chemical methods, the current generated by the following reactions was measured in order to determine whether cholesterol oxidase was arrested.

Cholesterol $+O_2$ kolest -4-en-3-on $+ H_2O_2$

$$H_2O_2 \xrightarrow{COx} O_2 + 2H^+ + 2e^-$$

The current measured value is proportional to the substrate concentration (cholesterol).

A significant challenge was encountered during the preparation of the stock cholesterol solution. “Stock cholesterol solutions containing Triton X-100 are commonly referenced in the literature. Following numerous experiments, it was determined that Triton X-100 significantly influenced the response obtained]. Consequently, the use of Triton X-100 was discontinued, and hexane was identified as an appropriate solvent for the preparation of the stock cholesterol solution”[25].

In their study, Doukyu and Aono observed that the bioconversion of water-insoluble compounds was impeded due to their low solubility in aqueous media [26]. Furthermore, they observed that aqueous media-organic phase, or two-phase systems, are advantageous for the bioconversion of high concentrations of water-insoluble substrates. Nevertheless, the use of organic solvents can affect the stability and activity of enzymes. Enzymes exhibit high activity and stability under specific conditions. It has been postulated that these conditions can be highly beneficial for technological applications where organic solvents are employed.

Acknowledgements

This study is based on the author’s doctoral thesis.

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