



NANOPARTICLE BASED AMPEROMETRIC BIOSENSOR FOR THE QUANTITATIVE DETERMINATION OF CHOLESTEROL IN HUMAN BLOOD

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ABSTRACT

Total cholesterol monitoring in human blood serum is one of the most important routine analysis performed in clinical laboratory. There is a strong correlation between coronary heart disease and blood cholesterol level. Numbers of cholesterol biosensors have been developed over the past 30 years. Cholesterol is determined enzymatically by Fibre-optic fluorescence, Fibre-optic luminescence, Potentiometric, Spectrophotometric and Fluorometric biosensors. Some of these methods suffer from interference from other substances found in the blood such as ascorbic acid and uric acid. There is need for a method that is sufficiently flexible to yield good results in clinical laboratory. The attributes of the enzyme linked platinum electrode was studied with cholesterol powder solution using cyclic voltammeter 797 VA. The peak value of the current from the electrode was detected with the input potential changes from +0.5 V to -1 V. The Mann Whitney U Test was conducted for the sampled data, the results show that the data is significant with $\alpha = 0.05$. Fabricated amperometric biosensor was found to be sensitive in determining the cholesterol level in the human blood.

KEY WORDS: Spectrophotometric, Fluometric, luminescence, Amperometric



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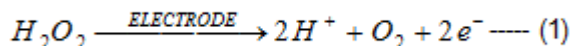
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INTRODUCTION

For many decades, scientists have recognized the power of incorporating biological principles and molecules into the design of artificial devices. Biosensors, an amalgamation of signal transducers and biocomponents play a prominent role in medicine. Many kinds of amperometric glucose sensors were fabricated based on Cadmium Sulfide (CdS) nanoparticles modified electrode¹, Cl-plasma treated Ag/AgCl reference electrode², immobilization of glucose oxidase in chitosan on a glassy carbon electrode with gold platinum alloy multiwall carbon nanotubes³, bioelectrocatalytic glucose oxidation with phenoxazine modified glucose oxidase⁴, nonenzymatic glucose sensor in alkaline media with carbon nano tube on glassy carbon electrode⁵. The glucose concentration ranging from 1 to 26.5 mM was detected using platinum nanoparticles⁶. The response sensitivity of the glucose slightly changes at more positive detection potentials. An immuno sensor to detect human immunoglobulin G based on two electrochemical layers for immobilizing antibody. The dose response was studied at working potential -0.3V⁷. Needle enzyme electrode was used to measure lactate *in vivo*⁸. H₂O₂ biosensor⁹, Insulin sensor¹⁰ and NADH sensor¹¹ were designed to estimate H₂O₂, Insulin and NADH respectively. Researchers recently have been made attempts to create sensitive, selective, reliable and low cost cholesterol sensors because of the clinical significance in the measurement of blood cholesterol level. Highly selective methods have been developed by utilizing the electrode modified with cholesterol oxidase¹². Nanostructured zinc oxide (nano-ZnO) film onto indium-tin-oxide (ITO) cholesterol sensor containing preferred (002) plane and 10 nm crystallite size using sol-gel technique for immobilization of cholesterol oxidase (CHOX)¹³. A novel potentiometric sensor based on the fabrication of ISFET (Ion Selective Field Effect Transistor) coated with molecular imprint of cholesterol on the SiCO₂ + Si₃N₄ dielectric gate of the said electrode, poly (pyrrole-co- N- methyl pyrrole)-sensor¹⁴, surface plasmon resonance based biosensor¹⁵, membrane permeability based sensor¹⁶. A high cholesterol level in human blood is related to arteriosclerosis, hypertension, myocardial infarction and many heart disorders¹⁷. There is considerable interest towards the application of silicon to biosensors. This has been attributed to their interesting properties such as biocompatibility, redox characteristics and the possibility direct electron transfer between electrode and active sites of biomolecules¹⁸. The electrochemical reaction was studied by covalently coupling cholesterol oxidase via glutaraldehyde onto electrochemically prepared polyaniline film in presence of TritonX-100 onto indium-tin-oxide (ITO) glass substrate¹⁹. The photolithography technique is used to fabricate the nanogap based on the CMOS technology²⁰. Cholesterol oxidase catalysis the aerobic oxidation of cholesterol to Δ^4 -cholestenone with stoichiometric production of hydrogen peroxide has

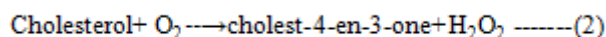
opened the way for the development of electrochemical sensors for the determination of cholesterol that are based on the amperometric determination of H₂O₂ at the electrode surface²¹.



MATERIALS AND METHODS

Reagents

Cholesterol Powder (E.C. Number – 200-353-2) - 3 β -Hydroxy-5-Cholestene, C₂₇H₄₆O. Molecular weight is 386.65 g/mol and Cholesterol Oxidase (CHOX) is a monomeric flavin protein containing FAD (E.C. Number – 1.1.3.6). Molecular mass is 55 kDa, K_M = 3.5x 10⁻⁴ M (Cholesterol). One unit will convert 1.0 μ Mole of cholesterol to 4-cholesten-3-one per minute at pH 7.5 at 25°C purchased from Sigma-Aldrich.



Preparation of Potassium Buffer Solution

1M of Potassium Phosphate buffer solution with pH 7.0 was prepared by dissolving 17.48 gm. of potassium phosphate dibasic in 100 ml distilled water and 13.609 gm. of potassium phosphate monobasic also in 100 ml distilled water. Then volume of 1M of 61.5 mL of K₂HPO₄ was mixed with 1 M of 38.5 ml of KH₂PO₄ to get 0.1 M potassium phosphate buffer solution. This buffer solution was converted to 50 mM by adding 50 ml of 0.1M potassium phosphate buffer with 50 ml of distilled water.

Preparation of Cholesterol oxidase solution

Cholesterol oxidase solution was prepared by dissolving the 100 UN of cholesterol oxidase in 50 mM of potassium phosphate buffer solution with pH 7.0

Synthesis of Titanium oxide

TiO₂ nano powders was prepared by dissolving 8 ml of titanium tetraisopropoxide [Ti(OCH(CH₃)₂)₄] in 50 ml ethanol under constant magnetic stirring. The solution obtained after 45 minutes was converted into a gel by adding 100 ml of deionized water. The white precipitate thus obtained was filtered and washed with distilled water to remove impurities. Finally, the powder was dried at 100°C.

Preparation of Cholesterol solution

The cholesterol solution was prepared by dissolving 100 mg of cholesterol powder was dissolved in ethanol.

Preparation of Titanium oxide solution

Titanium oxide nano powder solution was prepared by dissolving 100 mg of TiO₂ Nano powder in diluted sulphuric acid, heated to 185°C and the dissolved titanium oxide solution color turns into brownish yellow. The UV and Raman spectrum is shown in Fig.1 and Fig.2.

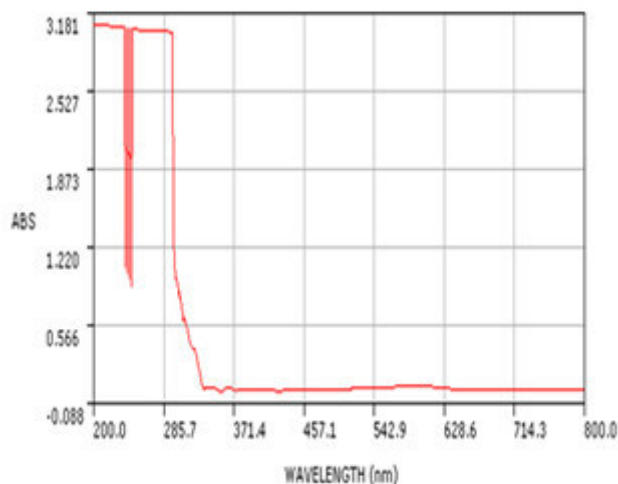


Figure 1
UV Spectrum of TiO₂

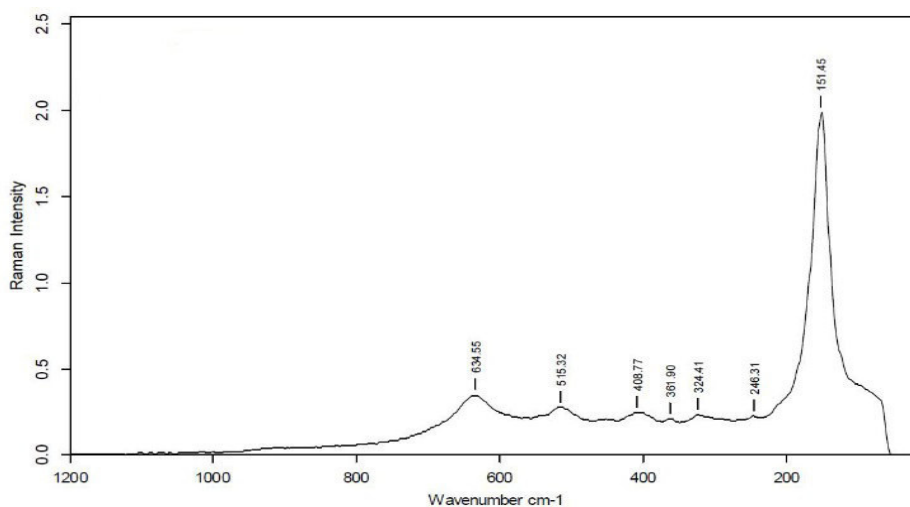


Figure 2
Raman Spectrum of TiO₂

Apparatus

All electrochemical experiments were carried out on a cyclic voltameter 797 VA Computrace (Metrohm, USA). A conventional three electrode system was used in this work. The enzyme coated platinum electrode was used as a working electrode with 2mm diameter. A platinum electrode was used as a counter electrode and an Ag/AgCl electrode was used as a reference electrode. Sodium Phosphate buffer solution (0.1M) was always employed as supporting electrolyte.

CHOLESTEROL MEASUREMENT SYSTEM BASED ON ARM PROCESSOR

The amperometric cholesterol biosensor system designed for the measurement consists of several hardware modules, which include: Enzyme coated platinum electrode, ARM processor LPC 2148 and LCD display. The current from the working electrode for the input voltage can be read by the processor with the application of the cholesterol powder solution in sodium phosphate buffer solution. Further the same data related to the cholesterol concentration can be transferred it to any android system using wireless technology. Fig.3 shows the block diagram of the system.

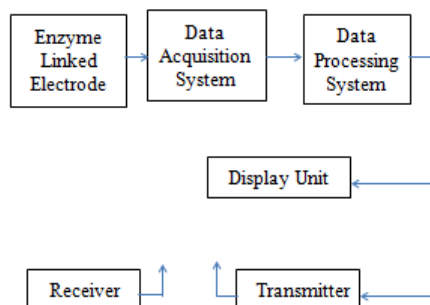


FIGURE 3
Block diagram of the biosensor system

METHODOLOGY

The biosensor system consisting of ARM processor continuously reads the data from the electrode and displays the value on the LCD screen. The data was validated with Easy life GCU system. The observed values were stored in a register for further analysis. The processing and display software was written in C using Keil μ Vision3 software and the Hex code was downloaded to the processor LPC 2148. Electrochemical behavior of the sensor was identified by using cyclic

voltammetry techniques. The stability of the nano particle mixed cholesterol oxidized biosensor has been analyzed for various temperature, pH, and cholesterol concentration. This fabricated biosensor has been characterized for cholesterol detection in the concentration range between 10mg/dl and 1gm/dl cholesterol by cyclic voltammetry measurement. The linear relationship between the analyte concentration and response current of the electrode was observed.

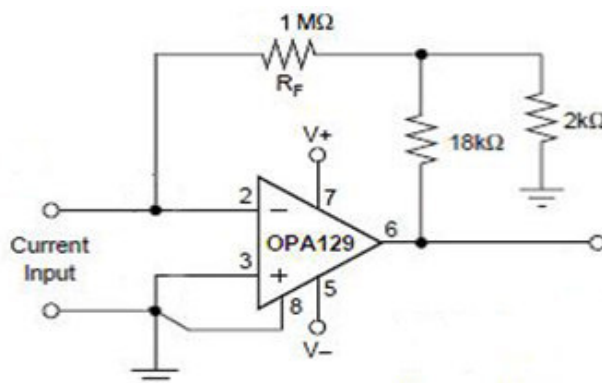


Figure 4
Current Amplifier circuit

Figure 4 shows an ultra-low bias current monolithic operational amplifier. The non-standard pin out of the operational amplifier was to achieve lowest possible input bias current. The negative power supply was connected to pin 5 to reduce the leakage current from the V- supply (Pin 4) to the op-amp input terminal. With this new pin out, sensitive inputs were separated from both power supply pins. The ARM7TDMI-S (LPC 2148) is a general purpose 32-bit microcontroller, which offers high performance and very low power consumption. The current developed from the working electrode based on the cholesterol present in the blood was read by the ARM processor and displayed in the display unit.

RESULTS

The characteristics of the enzyme linked Platinum electrode (Titanium oxide + cholesterol oxidase) were studied using cyclic voltammetry in various condition. The electrode is placed in the 0.1 M sodium phosphate

buffer solution and the current developed in the electrode was observed with the potential from +0.5 to -1V. The cholesterol powder solution was added and the output current from the cyclic voltammeter was studied. Initially the output was studied without the nano particles linked at the electrode. Then we changed the electrode (nanoparticle linked), the pH value of the buffer solution and the concentration of the cholesterol solution, the output characteristics were studied using cyclic voltammetry as shown below in the Fig.5 and Fig.6. By comparing the determined concentration of cholesterol with the Easy Life GCU system, it was found that there is a linear relation between the cholesterol concentration of blood and the calculated cholesterol concentration measured from the biosensor device. The percentage mean relative error of the sampled data calculated as 0.069. The comparison curve of the sampled data is shown in the figure 7.

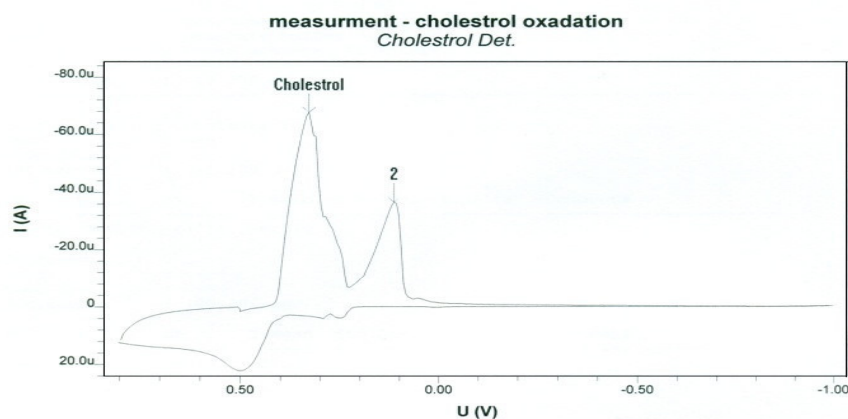


Figure 5
Cyclic voltammograms of enzyme coated Platinum electrode in the Electrolyte with the application of 0.05 ml cholesterol powder solution.

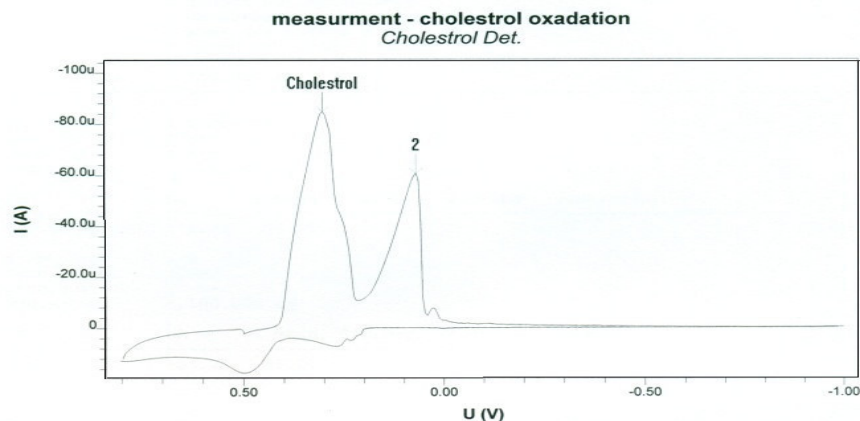


Figure 6
Cyclic voltammograms of enzyme coated platinum electrode with the application of 0.1 ml cholesterol powder solution. The Mann Whitney U Test was conducted, it was found that the results were significant with $\alpha = 0.05$. The statistical value U_{stat} is 47 which is greater than the critical value U_{crit} is 23.

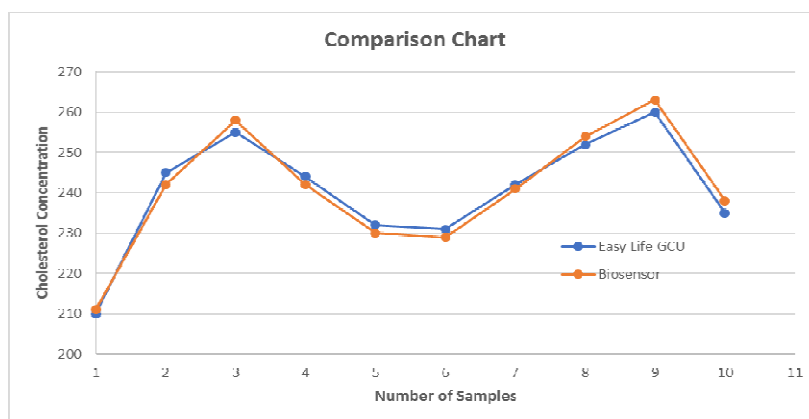


Figure 7
Comparison curve of cholesterol value (Biosensor system) and Easy Life GCU System.

DISCUSSION

A novel enzyme electrode was developed for the determination of cholesterol concentration in human blood using titanium oxide nanoparticle embedded in the platinum electrode. This study shows that the titanium oxide nanoparticle acts as a catalyst between the cholesterol solution and the enzyme electrode. The sweep voltage in the cyclic voltammetry 797 VA was changed to study the features of the enzyme electrode. At one voltage, the current in the nanoparticle coated electrode is maximum. This voltage acts as the reference voltage to measure the cholesterol concentration in the human blood.

CONCLUSION

A novel enzyme-electrode based cholesterol biosensor system has been developed that can measure the cholesterol level in the blood. The system is mainly built up with ARM processor. The 797 VA computrace instrument is used to measure electrochemical behavior of the enzyme coated platinum electrode. This study has

shown that the titanium oxide nanoparticle acts as effective mediator between the cholesterol oxidase and the platinum electrode. After taking reading from many samples the sensor was calibrated to know the cholesterol concentration in the blood. The results showed that the developed biosensor was sensitive, stable and cost effective method for the determination of cholesterol in blood.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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