# 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. Epidemiological studies have shown a strong correlation between coronary heart disease and blood cholesterol level. There is need for a method that is sufficiently flexible to yield good results in clinical laboratory. Numbers of cholesterol biosensors have been developed over the past 30 years. Fibre-optic fluorescence, Fibre-optic luminescence, Potentiometric, Spectrophotometric and Fluorometric biosensors, which determine cholesterol enzymatically. Some of these methods suffer from interference from other substances found in the blood such as ascorbic acid and uric acid. Therefore amperometric biosensor was designed based on titanium oxide nano particle with Advanced RISC (Reduced Instruction Set Computing) Machine processor to determine the cholesterol level in human blood.

*Index Terms*— Spectrophotometric, Flurometric, Advanced RISC Machine.

## I. 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 cds nanoparticles modified electrode [1], cl-plasma treated Ag/Agcl reference electrode [2], immobilization of glucose oxidase in Chitosan on a glassy carbon electrode with gold platinum alloy multiwall carbon nano tubes [3], bioelectrocatalytical glucose oxidation with phenoxazine modified glucose oxidase [4], nonenzymatic glucose sensor in alkaline media with carbon nano tube on glassy carbon electrode [5].

The platinum nanoparticles supported on multiwall Carbon nanotubes for the detection of glucose concentration range from 1 to 26.5 mM [6]. 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 -0.3V [7]. Needle enzyme electrode was used to measure lactate invivo [8]. $H_2O_2$ biosensor [9], Insulin sensor [10], NADH sensor [11] were designed to estimate $H_2O_2$, 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 oxidase [12].

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 $SiCO_2 + Si_3N_4$ dielectric gate of the said electrode, poly (pyrrole –co- N- methyl pyrrole)-sensor [14], surface plasmon resonance based biosensor [15], membrane permeability based sensor [16] .

A high cholesterol level in human blood is related to arteriosclerosis, hypertension, myocardial infarction and many heart disorders [17]. 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 biomolecules [18]. The elcrtochemical 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 substrate [19]. The photolithography technique is used to fabricate the nanogap based on the CMOS technology [20]. Cholesterol oxidase catalysis the aerobic oxidation of cholesterol to Δ4 –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 $H_2O_2$ at electrode surface [21].

$$H_2O_2 \xrightarrow{\quad ELECTRODE \quad} 2H^+ + O_2 + 2e^-$$
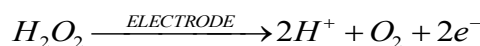
## II. EXPERIMENTAL PROCEDURE

### REAGENTS

Cholesterol Powder (E.C.Number – 200-353-2) - 3β-Hydroxy-5-Cholestene, $C_{27}H_{46}O$. Molecular weight is 386.65 g/mol and Cholesterol Oxidase (CHOX) is a

monomeric flavor protein containing FAD (E.C. Number – 1.1.3.6). Molecular mass is 55 kDa, $K_M = 3.5$x $10^{-4}$ M (Cholesterol). One unit will convert 1.0 μMole of cholesterol to 4-cholesten-3-one per minute at pH 7.5 at $25^{o}$C purchased from Sigma-Aldrich.

$$Cholesterol + O_2 - \rightarrow cholest - 4 - en - 3 - one + H_2O_2$$
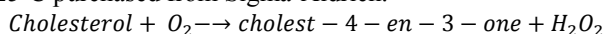
### PREPARATION OF POTASSIUM BUFFER SOLUTION

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 $k_2HPO_4$ was mixed with 1 M of 38.5 ml of $KH_2PO_4$ to get 0.1 M potassium Phosphate buffer solution. This Potassium Phosphate 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

$TiO_2$ nano powders was prepared by dissolving 8 ml of titanium tetraisopropoxide [Ti{OCH(CH$_3$)$_2$}$_4$] 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. Obtained white precipitate was filtered and washed with distilled water to remove impurities. Finally the powder was dried at $100^{o}$C.

### PREPARATION OF TITANIUM OXIDE SOLUTION

The Titanium oxide nano powder solution was prepared by dissolving 100 mg of $TiO_2$ Nano powder in diluted sulphuric acid, heated to $185^{o}$C and the dissolved titanium oxide solution color turns to brownish yellow.

### PREPARATION OF CHOLESTEROL SOLUTION

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

### 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.

### III. CHOLESTEROL MEASUREMENT SYSTEM BASED ON ARM PROCESSOR

The amperometric cholesterol biosensor System designed consists of a number of hardware modules, which include: Enzyme coated platinum electrode, ARM processor LPC 2148 and LCD display. The current from the working electrode for the particular 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.1 shows the block diagram of the system.
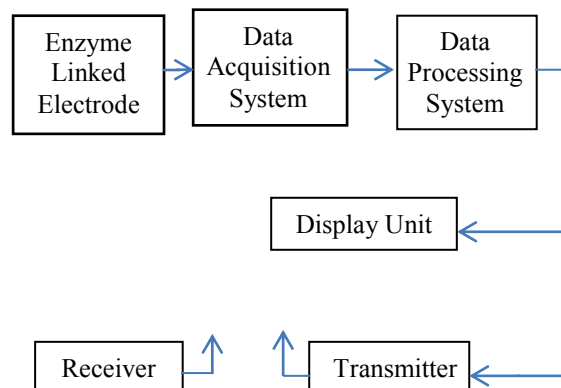


Fig.1. Block diagram of the biosensor system

### IV. METHODOLOGY

The biosensor system consisting of ARM processor continuously reads the data from the electrode and displays the value on the LCD screen. The observed values were stored in a register for further analysis. The processing and display software was written in C using Keil μVision3 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.
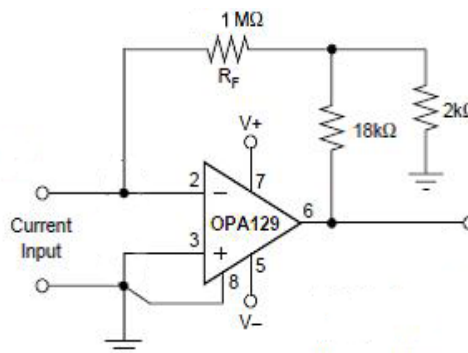
Fig.2. Current Amplifier circuit

Fig. 2 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.

## V. 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. Then the electrode current was amplified by the operational amplifier using OPA 128 and displayed in the microcontroller unit.

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.3 and Fig.4.
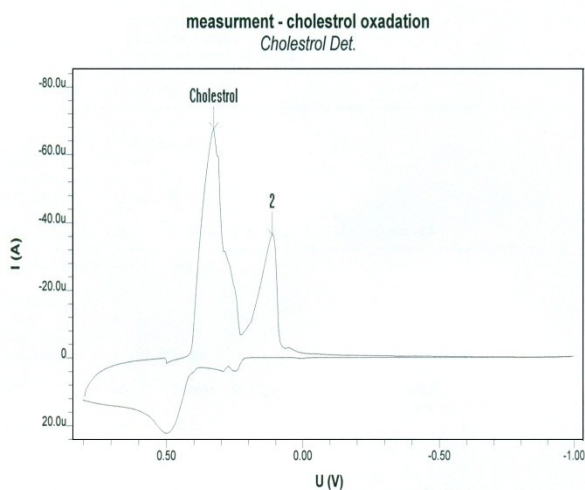
Fig.3. Cyclic voltammograms of enzyme coated Platinum electrode in the Electrolyte with the application of 0.05 ml cholesterol powder solution.
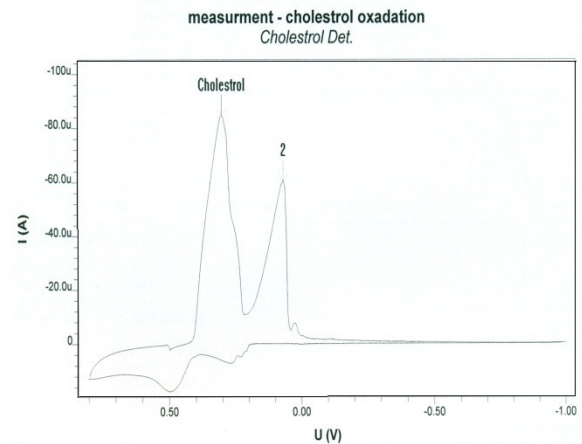
Fig.4. Cyclic voltammograms of enzyme coated platinum electrode with the application of 0.1 ml cholesterol powder solution.

## VI. 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.

The developed sensor is under trials. After taking reading from many samples the sensor was calibrated to know the cholesterol concentration in the blood. This project will pave a new way for specific, additional electrochemical based, cost effective method for the determination of cholesterol in blood.

## VII. FUTURE SCOPE

A further study can be done to effectively measure other parameters in the blood using amperometric biosensors. The concept of the developed algorithm can be used to measure the thyroxine (T4),triiodothyronine (T3) and Thyroid stimulating hormone (TSH) level in the blood. The electrode current changes based on the enzyme (Thyroglobulin) coated on the electrode with different nanoparticles can be studied for better results. The data from the enzyme electrode can be further processed and displayed in the display unit.

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