To Design A Biosensor For The Determination Of Cholesterol In Human Blood

Ashwini Basavaraju, Ankith Rajashekar Mullangi, Sanjay Menon, Lakshmishree S S

Abstract— The field of nanotechnology is truly fascinating. The use of nanomaterials in the development of biosensors has paved the way for the emergence of many signal transduction technologies. Biosensors play a significant role in detection and monitoring wide range of medical applications. Cholesterol monitoring in human blood is one of the most important routine analysis performed in medical screening. Epidemiological studies have shown a strong correlation between coronary heart disease and blood cholesterol level. There is need for an effective method that is sufficiently flexible to yield good results in clinical diagnostics. Over the past 30 years large number of cholesterol biosensors have been developed. Various methods such as Fibre-optic fluorescence, Fibre-optic luminescence, Potentiometric, Spectrophotometric and Fluorometric biosensors, have been proposed to determine cholesterol enzymatically. But 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—Nanotechnology,Nanomaterials,Biosensors,Cholesterol,Flurometric,Fibre-optic flourescence,Spectrophotometric.

1 INTRODUCTION

The development of a cholesterol biosensor is important due to the prevalence of cardiovascular diseases as a major health threat around the world. Cardiovascular diseases and cardiac arrest are the number one cause of death globally. One of the most important reason is hypercholesterolemia that is increased concentration of cholesterol level in blood. An estimated 17.3 million people died from cardiovascular diseases in 2008, representing 30 percent of global deaths. The number of people who die from cardiovascular diseases, mainly from heart disease and stroke, will increase to reach approximately 24 million by 2030.Cardiovascular diseases are projected to remain the single leading cause of death. Estimation of cholesterol level in blood hence is the most important and challenging task for medical industry. Development and improvement of existing cholesterol biosensor has got a worldwide attention. Nanotechnology plays a significant role for the development of cholesterol biosensors. The emergence of nanotechnology offers great opportunities to improve the sensitivity, stability, anti-interference ability and the performance of the biosensing systems.

The concentration of cholesterol in blood is an important parameter for the diagnosis and prevention of disease. Ideally, the total cholesterol concentration in a healthy person's blood should be less than 200 mg/dL (<5.17 mM). The borderline value is defined as 200-239 mg/dL (5.17-6.18 mM), and the high value is defined as above 240 mg/dL (g6.21 mM).2 The measurement of blood cholesterol concentration is a routine practice in medical screening or diagnosis. Therefore, a simple, portable and practical sensitive cholesterol biosensor is desirable and can be useful in the prevention and the management of cardiovascular diseases.

Blood cholesterol level chart	Desirable mg/dL	Borderline(high) mg/dL	High risk mg/dL
Total cholesterol	<200	200-240	>240
Triglycerides	<150	150-500	>500
Low density cholesterol	>130	130-160	>240
High density cholesterol	>50	50-35	<35

Table.1:Cholesterol Level Chart

2 RESEARCH GAP

The progress of reliable, high sensitive and robust technique for the active and fast detection of cholesterol is an interesting topic recently. It is also desirable to build up a reliable and a portable cholesterol biosensor, which allows instantaneous detection of cholesterol level in blood. Different methods have been carried out for the recognition of cholesterol such as, biochemical investigation using radioactive labels, HPLC analysis, and electrochemical detection. The key drawbacks of these methods are their pitiable sequential, spatial resolutions, high power requirement, shorter life time and difficulty of the supplementary technical arrangements. With various methodology, the cholesterol biosensors were achieved a substantial interest owing to their linearity, sensitivity, reusability, selectivity, shelf-life, fast response time, repeatability, and stability. The mediator free electrochemical biosensors are based on suitable immobilization of selective enzyme on proper matrixes offers a portable, economical, disposable, and fast technique for the detection of different bio-molecules. In recent times,

- *Ashwini Basavaraju is currently pursuing bachelors degree program in Instrumentation Technology in R.V.College of Engineering,India,560065 E-mail:ashwini.b1711@gmail.com.*
- *Ankith Rajashekar Mullangi is currently pursuing bachelors degree program in Instrumentation Technology in R.V.College of Engineering,India,560065. E-mail:ankith.r.m@gmail.com.*
- *Sanjay A Menon is currently pursuing bachelors degree program in Instrumentation Technology in R.V.College of Engineering,India,560065. E-mail:sanjay12menon@gmail.com.*
- *Lakshmishree S S is currently pursuing bachelors degree program in Instrumentation Technology in R.V.College of Engineering,India,560065. E-mail:lakshmishreess@gmail.com*

researchers are investigated with bio-compatible composite materials as appropriate matrixes for the enzyme immobilization for the efficient recognition of different biological molecules. Among various immobilization techniques, the Au/TGA/ChOx fabricated bio-chips are one of the most promising matrixes which can be used for the immobilization of enzymes due to their numerous interesting properties such as non-toxicity, mediator-less detection, high-surface area, requiring low-sample volume, fast-response time, chemical stability, highly-sensitive, ease of handling, selective, and ease of enzymatic fabrication.

3 OBJECTIVES

Cholesterol biosensors used in clinical analysis are more accurate than home kits. Total cholesterol monitoring in human blood 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 an effective method that is sufficiently flexible to yield good results in clinical laboratory. 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.

3.1 Features On Which The Success Of A Cholesterol Biosensor Depends:

- Be stable under normal stage conditions and show good stability over large number of assays.
- The reaction should be as independent of physical parameters as pH and temperature.
- The response should be accurate, precise, reproducible and linear over the useful analytical range. It should also be free from electrical noise.
- It should be cheap, small and portable.
- Capable of being used by unskilled person.
- Variation of enzymes and electrode materials had improved performance in sensitivity storage and shelf life.

4 PRINCIPLE OF BIOSENSOR

- Analyte- Blood Sample
- Bioreceptor- Enzyme- Cholesterol oxidase
- Matrix- Platinum Electrodes
- Immobilisation of enzyme- Adsorption technique
- Transducer- Convert biosignals into electrical signals.
- Data processing- Arm- 7 TDMI-S reads the data from the electrode and displays on a LCD screen.

5 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 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.

Fig.1:Process diagram of the system

6 BLOCK DIAGRAM -

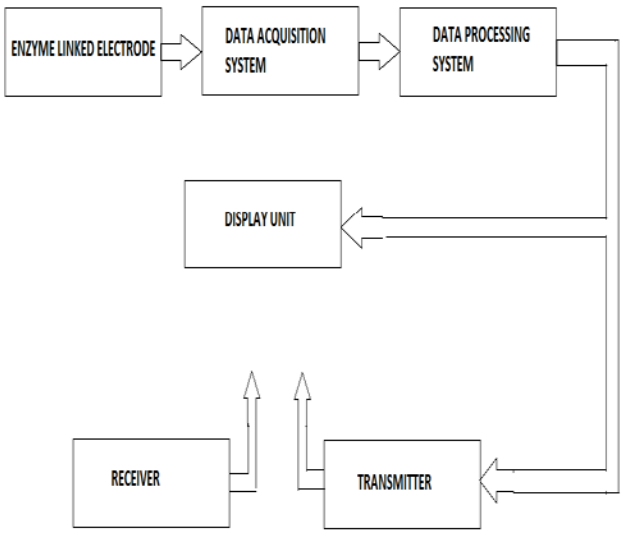

Fig.2: Functional block diagram of the system

7 EQUIPMENT/ COMPONENTS REQUIRED:

- Enzyme coated platinum electrode-
  Function: 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.
- Arm processor- The ARM7TDMI-S (LPC 2148) is a general purpose 32-bit microcontroller, which offers high performance and very low power consumption. It costs INR-1200/-
- LCD display- A display unit. Data related to the cholesterol concentration is dispayed. It costs INR-300/-.

8 BUDGET:

The development of new biosensors and devices based on nanotechnology is a highly capital intensive exercise. Although miniaturization allows for economies of scale to be achieved in the actual manufacturing of biosensors, huge capital investment is required for research and development.
According to the new market report published by transparency market research "Biosensors Market- Global industry analysis, size, share, growth, trends and forecast, 2012-2018" in 2011, the global biosensors market was valued at USD 9.9 billion and it is expected to grow at a CAGR of 9.6 percent from 2012-2018 to reach a market of USD 18.9 billion by 2018.

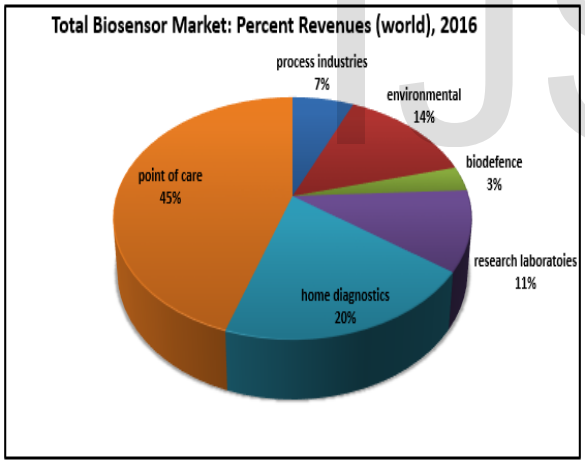

Fig.3: Biosensors market revenues for 2015

9 APPLICATION:

Development of cholesterol biosensors in therapeutic diagnostics has gained much attention in health care and biomedical fields. With the different experimental parameters, detection of cholesterol in blood sample has considered incredibly significant since its enhancement is related with diabetes, heart diseases, nephrosis, and obstructive jaundice. It is also useful in wireless monitoring for people in hazardous operations.
GENERAL APPLICATION OF BIOSENSOR:
Clinical and Diagnostic Applications-One well known example of a clinically applied biosensor is the glucose monitor, which is used on a routine basis by diabetic individuals to check their blood sugar level. These devices detect the amount of blood glucose in undiluted blood samples allowing for the easy self-testing and monitoring that has revolutionized diabetes management.
Applications in industry-Biosensors are used in the food industry to measure carbohydrates, alcohols and acids, for example, during quality control processes. The devices may also be used to check fermentation during the production of beer, yoghurt and soft drinks. Another important application is their use in detecting pathogens in fresh meat, poultry or fish.
Environmental applications-Biosensors are used to check the quality of air and water. The devices can be used to pick up traces of organophosphates from pesticides or to check the toxicity levels of wastewater, for example.

9.1 Other Applications:

- Food analysis
- Study of biomolecules and their interaction
- Drug development
- Crime detection
- Medical diagnosis
- Environmental field monitoring
- Quality control
- Industrial process control
- Manufacturing of pharmaceuticals and replacement organs.

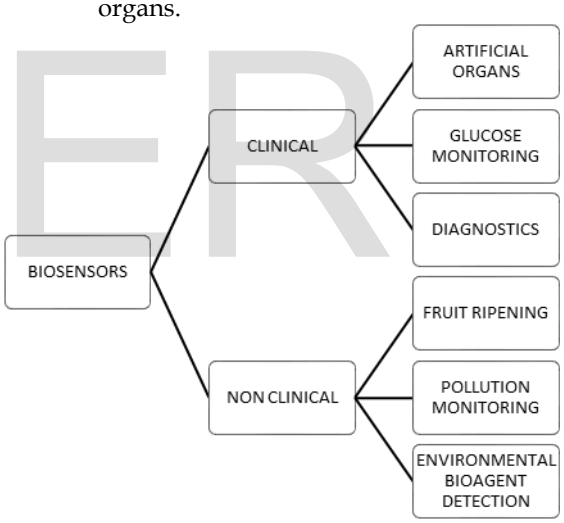

Fig.4: Potential applications of biosensors

10 CONCLUSION

Biosensor based on a nanoparticle is implemented to estimate the total cholesterol level in blood. The basic parameters such as linearity, sensitivity, response time, reusability, shelf life and detection limit to check the efficiency of cholesterol biosensors were determined. Thus biosensor based on nanoparticle is advantageous over the existing method for the detection of cholesterol which involves blood test.

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