Original Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

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

RAID SALEEM AL BARADIE*[1] AND ANANDH SAM CHANDRA BOSE[2]

[1]Associate Professor, Department of Medical Lab, Majmaah University, Kingdom of Saudi Arabia
[2]Lecturer, Department of Medical Equipment, Majmaah University, Kingdom of Saudi Arabia

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 α = 0.05. Fabricated amperometric biosensor was found to be sensitive in determining the cholesterol level in the human blood.

KEY WORDS: Spectrophotometric, Flurometric, luminesence, Amperometric

RAID SALEEM AL BARADIE*

Associate Professor, Department of Medical Lab, Majmaah University, Kingdom of Saudi Arabia.

*Corresponding author

Received on: 23-09-2016
Revised and Accepted on : 21-03-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.b409-414

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 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 nanotubes[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 glucose concentration ranging from 1 to 26.5 mM was detected using platinum nanoparticles[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] and 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 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 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 the electrode surface [21].

$$H_2O_2 \xrightarrow{ELECTRODE} 2H^+ + O_2 + 2e^- \text{ ----- (1)}$$

MATERIALS AND METHODS

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 = 3.5x 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°C purchased from Sigma-Aldrich.

$$\text{Cholesterol} + O_2 \longrightarrow \text{cholest-4-en-3-one} + H_2O_2 \text{ -------(2)}$$

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 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. 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 TiO_2 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.

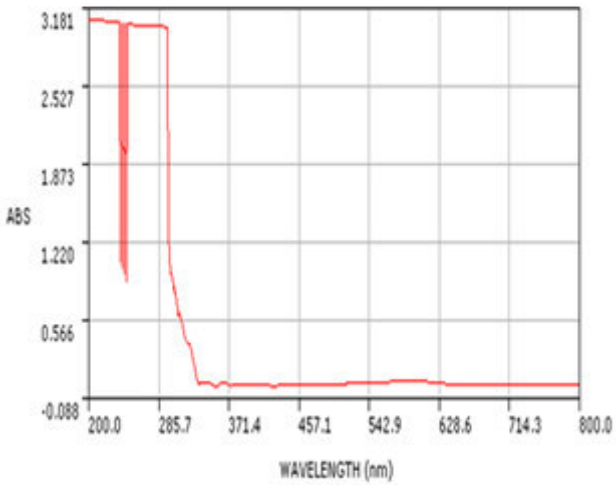

Figure 1
UV Spectrum of TiO_2

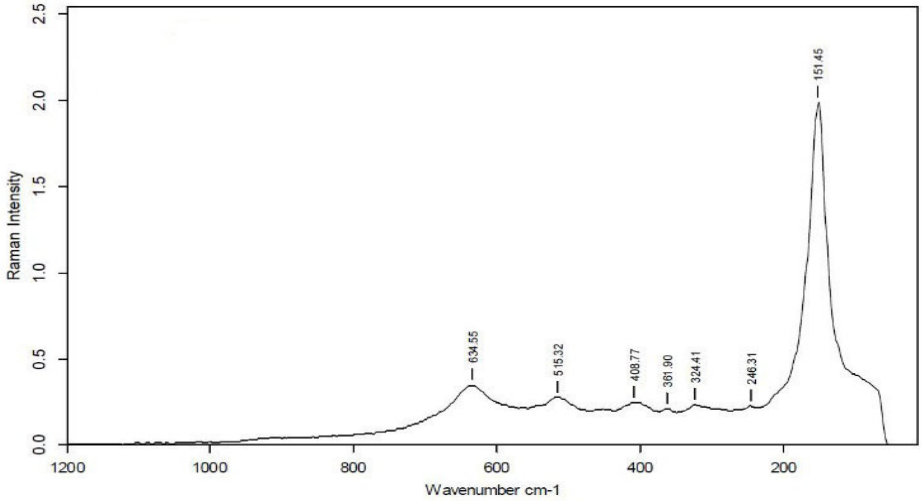

Figure 2
Raman Spectrum of TiO_2

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.

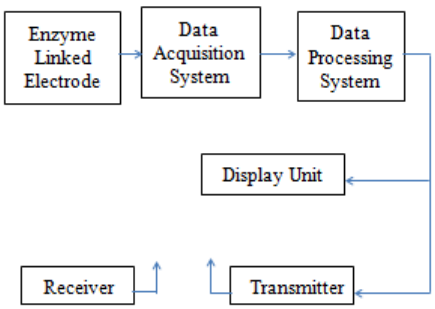

FIGURE 3
Block diagram of the biosensor system

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 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.

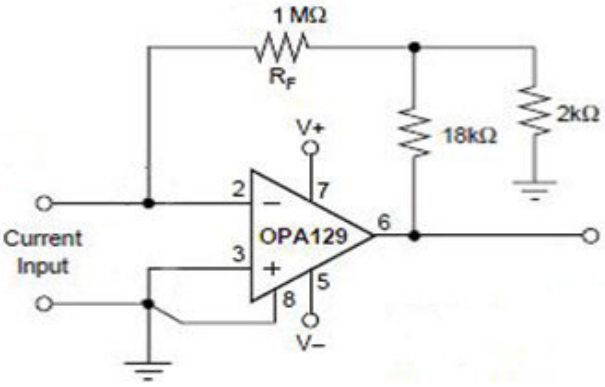

Figure 4
Current Amplifier circuit

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.

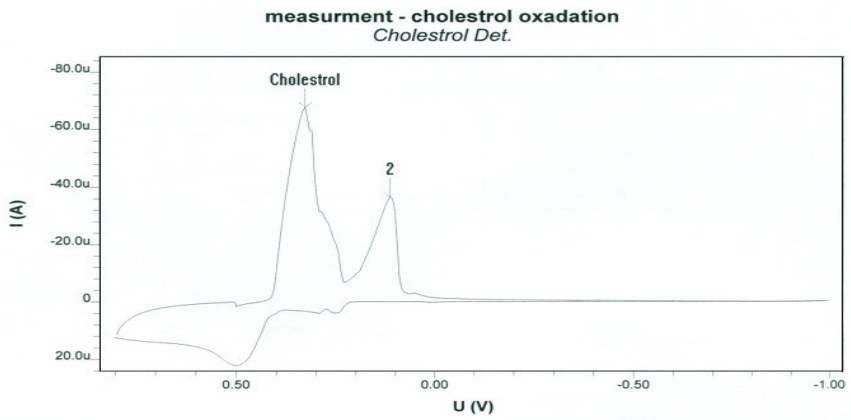

Figure 5
Cyclic voltammograms of enzyme coated Platinum electrode in the Electrolyte with the application of 0.05 ml cholesterol powder solution.

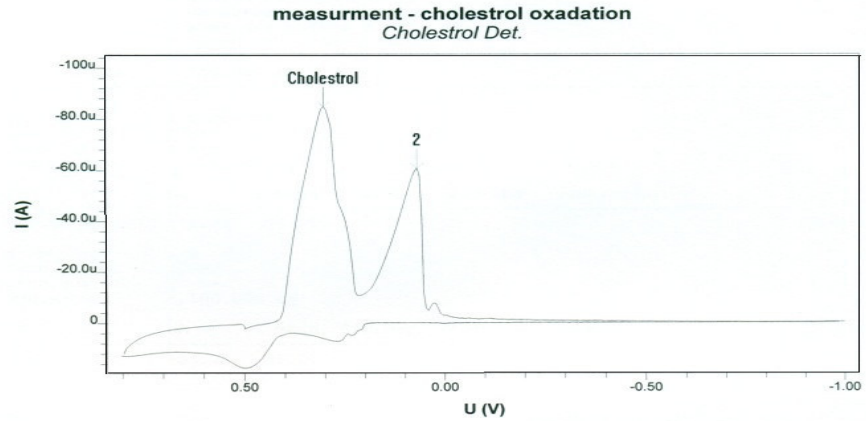

Figure 6

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 α = 0.05. The statistical value U_{stat} is 47 which is greater than the critical value U_{crit} is 23.

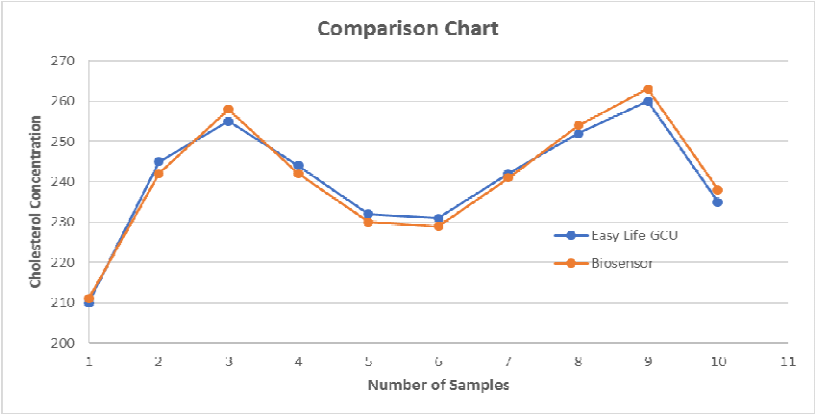

Figure 7

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.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to Dr. Khalid Bin Saad Megrin, Rector of Majmaah University for his support and the students who participated in this study. This research has been financed by Stroke Research Chair, Majmaah University, Kingdom of Saudi Arabia.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Huang Y, Zhang W, Xiao H, Li G. An electrochemical investigation of glucose oxidase at a CdS nanoparticles modified electrode. Biosens Bioelectron. 2005 Nov 15;21(5):817-21.
2. Park SI, Jun SB, Park S, Kim HC, Kim SJ. Application of a new Cl-plasma-treated Ag/AgCl reference electrode to micromachined glucose sensor. IEEE Sens J. 2003 Jun;3(3):267-73.
3. Kang X, Mai Z, Zou X, Cai P, Mo J. A novel glucose biosensor based on immobilization of glucose oxidase in chitosan on a glassy carbon electrode modified with gold–platinum alloy nanoparticles/multiwall carbon nanotubes. Anal Biochem. 2007 Oct 1;369(1):71-9.
4. Krikstopaitis K, Kulys J, Tetianec L. Bioelectrocatalytical glucose oxidation with phenoxazine modified glucose oxidase. Electrochem commun. 2004 Apr 30;6(4):331-6.
5. Kang X, Mai Z, Zou X, Cai P, Mo J. A sensitive nonenzymatic glucose sensor in alkaline media with a copper nanocluster/multiwall carbon nanotube-modified glassy carbon electrode. Anal Biochem. 2007 Apr 1;363(1):143-50.
6. Rong LQ, Yang C, Qian QY, Xia XH. Study of the nonenzymatic glucose sensor based on highly dispersed Pt nanoparticles supported on carbon nanotubes. Talanta. 2007 Apr 30;72(2):819-24.
7. Bian C, Xia S, Xu Y, Chen S, Cui Z. A micro amperometric immunosensor based on two electrochemical layers for immobilizing antibody. InSensors, 2005 IEEE 2005 Oct 30 (pp. 4-pp). IEEE.
8. Rong Z, Leitao E, Popplewell J, Alp B, Vadgama P. Needle enzyme electrode for lactate measurement in vivo. IEEE Sens J. 2008 Jan;8(1):113-20.
9. Chen S, Yuan R, Chai Y, Zhang L, Wang N, Li X. Amperometric third-generation hydrogen peroxide biosensor based on the immobilization of hemoglobin on multiwall carbon nanotubes and gold colloidal nanoparticles. Biosens Bioelectron. 2007 Feb 15;22(7):1268-74.
10. Wang J, Tangkuaram T, Loyprasert S, Vazquez-Alvarez T, Veerasai W, Kanatharana P, Thavarungkul P. Electrocatalytic detection of insulin at RuOx/carbon nanotube-modified carbon electrodes. Anal Chim Acta. 2007 Jan 2;581(1):1-6.
11. Nassef HM, Radi AE, O'Sullivan CK. Electrocatalytic sensing of NADH on a glassy carbon electrode modified with electrografted o-aminophenol film. Electrochem commun. 2006 Nov 30;8(11):1719-25.
12. Shi QC, Peng TZ, Chen JY. A novel cholesterol oxidase biosensor based on Pt-nanoparticle/carbon nanotube modified electrode. Chem Res Chin Univ. 2005 Aug 1;16(8):1081.
13. Solanki PR, Kaushik A, Ansari AA, Malhotra BD. Nanostructured zinc oxide platform for cholesterol sensor. Appl Phys Lett. 2009 Apr 6;94(14):143901.
14. Singh K, Basu T, Solanki PR, Malhotra BD. Poly (pyrrole-co-N-methyl pyrrole) for application to cholesterol sensor. J Mater Sci. 2009 Feb 1;44(4):954-61.
15. Gehlot R, Sharma K, Mathew M, Kumbhat S. Surface plasmon resonance based biosensor for label free detection of cholesterol. Indian J Chem. 2008; 47A: 1804-08.
16. Srivastava RC, Sahney R, Upadhyay S, Gupta RL. Membrane permeability based cholesterol sensor—A new possibility. J Memb Sci. 2000 Jan 4;164(1):45-9.
17. Aravamudhan S, Kumar A, Mohapatra S, Bhansali S. Sensitive estimation of total cholesterol in blood using Au nanowires based micro-fluidic platform. Biosens Bioelectron. 2007 Apr 15;22(9):2289-94.
18. Neetika G, Shalini S, Irfan AM. Advances in sensors based on conducting polymers. J. Sci. Ind. Res. 2006; 65: 549-57.
19. Khan R, Solanki PR, Kaushik A, Singh SP, Ahmad S, Malhotra BD. Cholesterol biosensor based on electrochemically prepared polyaniline conducting polymer film in presence of a nonionic surfactant. Journal of polymer research. 2009 Jul 1;16(4):363-73.
20. Yogeswaran U, Chen SM. A review on the electrochemical sensors and biosensors composed of nanowires as sensing material. Sensors. 2008 Jan 21;8(1):290-313.
21. Wisitsoraat A, Karuwan C, Wong-ek K, Phokharatkul D, Sritongkham P, Tuantranont A. High sensitivity electrochemical cholesterol sensor utilizing a vertically aligned carbon nanotube electrode with electropolymerized enzyme immobilization. Sensors. 2009 Oct 29;9(11):8658-68.

Reviewers of this article

Dr. J. Shekaina, Ph.D.

Assistant Professor, Electronics and Communication Department, C.S.I. Institute of Technology, Thovalai - 629302, Kanyakumari Dt, Tamilnadu, India

Asst.Prof.Dr. Sujata Bhattacharya

Assistant Professor, School of Biological and Environmental Sciences, Shoolini University, Solan (HP)-173212, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript