CHOLESTEROL ESTIMATION BY A PIC16F877A MICROCONTROLLER BASED SYSTEM

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ABSTRACT

An approach towards estimation of cholesterol was done by setting up a simple redox reaction. The cholesterol oxidase enzyme[8] breaks down the substrate into an intermediate cholest-5-en-3-one, which is then converted to cholest-4-en-3-one[15]. In the present study this mechanism was exploited. The current produced by the release of electrons from the above reaction was determined by the Op-Amp and the pre-programmed micro-controller was used to determine the cholesterol concentration. And the cholesterol concentration was displayed in 16x2 alphanumeric display.

Keywords: Cholesterol oxidase, Op-Amp, Microcontroller, Liquid Crystal Display (LCD Display)

Introduction:

Source of chemicals: Cholesterol extra pure was obtained from Nice Chemicals and Triton X 100 from Himedia. Cholesterol oxidase extrapure from Streptomyces sp. was obtained from SRL Mumbai. Op-amp IC (Op-07), microcontroller, 20MHz crystal oscillator, wires, probe and alphanumeric 16x2LCD display was procured from the local market. All other chemicals used were of analytical grade.

Assay of free cholesterol oxidase: Reaction mixture consisting of 1.0 ml sodium phosphate buffer (0.05 M, pH 7.0) and 0.5 ml cholesterol oxidase was pre- incubated at 37^{O}C for 2 min. The reaction was initiated by adding 0.5 ml cholesterol solution (500 mg/dl). After incubating at 37^{O}C for 10 min, 1.0 ml color reagent was added and kept at room temperature for 15 min to develop the color and absorbance was read at 520 nm (A520) against control in a spectrophotometer and the content of H_2O_2 generated in reaction was calculated from standard curve between H_2O_2 concentration and (A520)[13]. The soluble protein in dissolved enzyme was determined by Lowry method[10]

Preparation of substrate solution: Cholesterol (50 mg) was dissolved in 1.0 ml of triton X 100 by slowly heating and stirring until solution was clear. Sodium phosphate buffer (0.05 M, pH-7.0) was added to it to get final concentration of 500 mg/dl. Solutions of different concentrations ranging from 50 to 500 mg/ dl were prepared and stored at $4^{O}C$.

Response measurement of the biosensor: The Op-07 ICwas used for its accuracy to measure, amplify and convert the current produced into its proportional voltage. The probes were immersed into 5ml sodium phosphate buffer (0.02 M, pH 7.0) in a beaker. Silver wires are used as the probe which are connected to the op-amp. 1ml of dissolved cholesterol solution was added to the beaker and resistance of the resultant solution was checked at 2000k with the help of the Op-Amp.

Materials and Methods:

The resultant output of the Op-amp was fed to the microcontroller for further calculation. The microcontroller used in this particular approach was a40pin PDIP PIC16F877A[12] made by microchip. The electrical signal (analog) which was sensed by the Op-Amp was connected to the ADC (analog to digital convertor) module of the microcontroller. The analog signal was converted to digital form with the analog to digital converter module which was built in the microcontroller. The 20 MHz crystal oscillator was used as the clock.

The microcontroller performs the calculation with a program encoded in it. A 16x2 alphanumericLCD display was interfaced with the microcontroller. The result was displayed in the 16x2LCD display in mg/dl.

Two 9V voltage sources are connected with the 4^{th} and 7^{th} pin of the Op-Amp IC as the –Vcc and +Vcc respectively. 6^{th} pin gives the output of the Op-Amp which was connected with the microcontroller as its input. The 2nd and 3rdpins are used for the input. In this case the 2nd pin was grounded and the 3rd pin attached with the probe was immersed in the solution.

The operating frequency range of the microcontroller was 0-24Mhz and the operating voltage was 2.7-6V. The flash memory, where the main program of the microcontroller was stored was 8k. The microcontroller also consists of 10 bit analog to digital converter module built in. The 2nd pin i.e. RA0/AN0 of the microcontroller was used for the input. The 6th pin of the op-amp, which was the output of the op-amp was connected to the 2nd pin of the microcontroller. The analog signal was converted to digital form with the built in analog to digital converter module of the microcontroller. After the conversion of the analog signal to its digital form the microcontroller performs the calculation part.The programming for the calculation of the cholesterol concentration was done in C language. The code was written and build in the MPLAB IDE software[17]. The hex code of the program which was required for the microcontroller to execute the program wasalso generatedwith MPLAB IDEsoftware[4]. The generated hex code was burnt in the microcontroller with the help of burner kit (VP812). The microcontroller was interfaced with the 16x2 LCD display which shows the cholesterol concentration. The interfacing was done by generating a separate hex code with the help of MPLAB IDE software and this particular hex code was also burnt in the microcontroller with the burner kit.

Cholesterol estimation from biological samples: Blood samples were procured from Centre for Genetic Studies (West Bengal University of Technology) and 1 ml of serum was collected by centrifuging it at 1500xg for 10 minutes at 4^{O}C.This study protocol was approved by the institute's ethics committee. The serum was added to 1.0 ml of 0.02 M sodium phosphate buffer at pH 7.0.The free cholesterol was estimated by performing the enzyme–substrate reaction and the current (μA) was measured and concentration of cholesterol was extrapolated from the standard curve between cholesterol solution (mg/dl) Vs electrical response in μA. No sample calculation was done.

Results :

Amperometric Detection: Cholesterol solutions ranging from 50 to 500 mg/dl were made and amperometric measurements were made after 0.5ml of cholesterol oxidase was added. The probes were immersed into the solutions and the displayed ampere was noted against the concentration of the solution. The same procedure was repeated with biological sample (serum).

Calculation of Vmax: From the lineweaver-burk plot the Y intercept (c) was found to be $4*10^{-5}$. Imax was thus calculated as 0.02A and Km was found to be 87.5mM.These values were used for plotting the MichaelisMenten graph for the enzyme catalyzed reaction.

Discussions: Current approach describes a simple redox mechanism utilized to determine serum cholesterol from blood samples with no loss of enzyme efficiency. The biosensor showed optimum activity within 60 seconds at a pH of 6. Showed linearity with cholesterol concentration between 2.78mM to 12.22mM and good accuracy. The resistance of the probes used were negligible as the probes used are good conductor and so did not cause a significant alteration of values. Though for better accuracy electrode system can be used.

Linearity: There was a linear relationship between current (μA) and cholesterol concentration ranging from 2.78mM(50mg/dl) to 12.22mM(220mg/dl) in the reaction mixture, which is comparable to earlier amperometric biosensors, using cholesterol oxidase, co-immobilized with peroxidase on polyaniline film (1-12.9 mM)[14], and was found higher than those immobilized on carbon paste electrode modified with hydroxymethylferrocene and hydrogen peroxide (0.15 mM)[16], pencil graphite rod (1.29to 10.3 mM)[6], epoxy resin medium (1 to 8 mM)[13], electro polymerized pyrrole (8 μM)[18].

Accuracy: In order to determine the accuracy of the biosensor, the values of cholesterol in serum were determined by commercial enzymatic colorimetric method and compared with those obtained by present methods. The values obtained by these two methods matched with each other and showed a good correlation (r= 0.96) with the following regression equation: Y(0.0035x + 4E-05). The values obtained by both the methods were also in agreement with the earlier study with epoxy resin bicomposite membrane (r=0.99)[1], cellulose acetate membrane (0.990)[7] and chitosan- modified glassy carbon electrode (r=0.99)[5].

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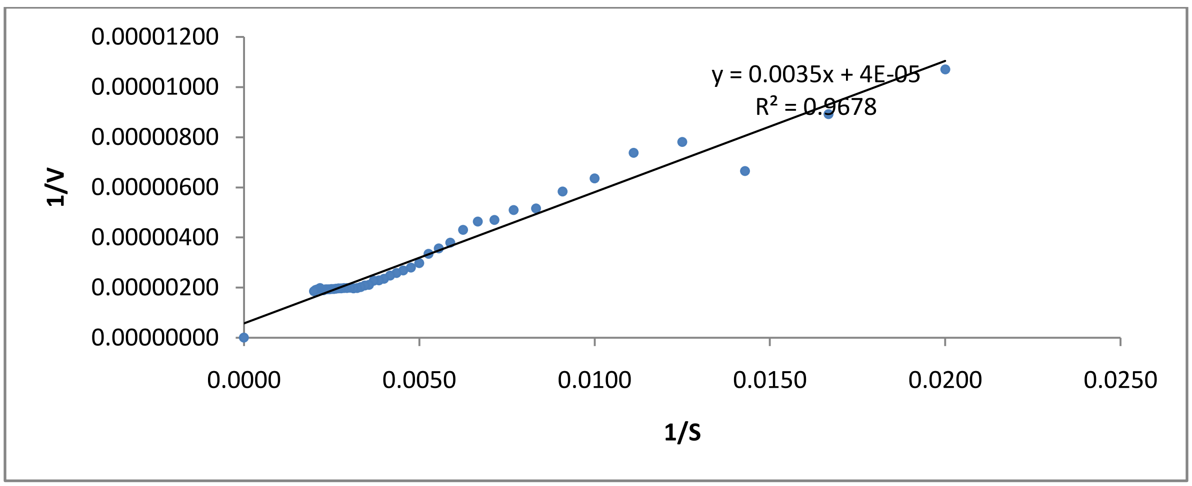
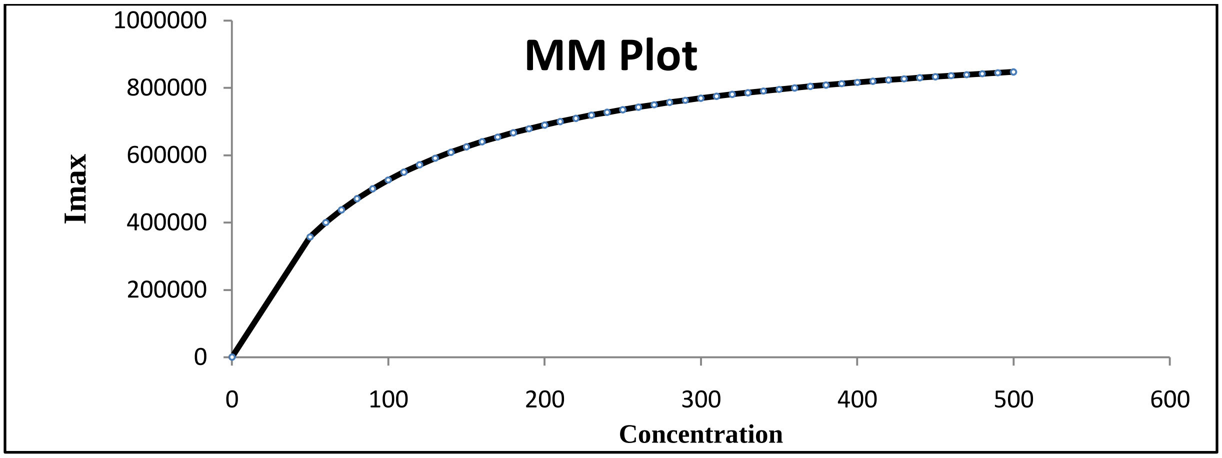
GRAPHS



Graph 1: Lineweaver - Burk Plot



Graph 2: Michealis – Menten Plot

Figures

Figure 1: PIC16F877A microcontroller chip Aritra Chatterjee

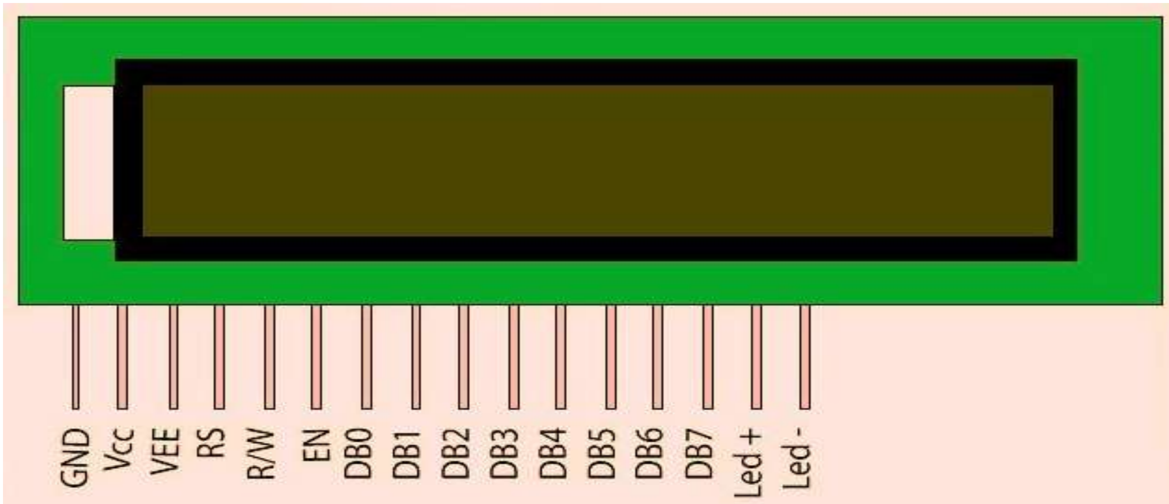


Figure 2: LCD 16x2 Display Aritra Chatterjee

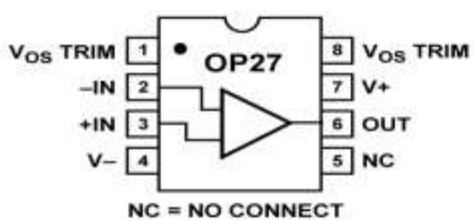


Figure 3: OP-27 Pin Configuration Aritra Chatterjee

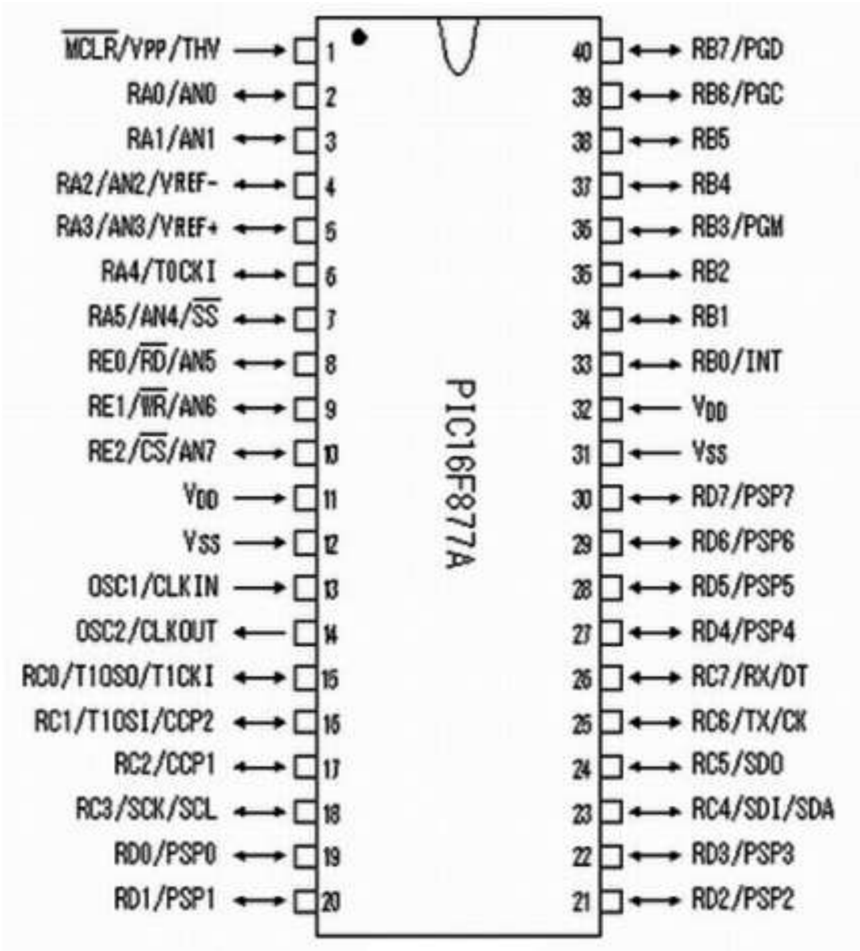


Figure 4: **PIC16F877A Microcontroller Pin Configuration Aritra Chatterjee**

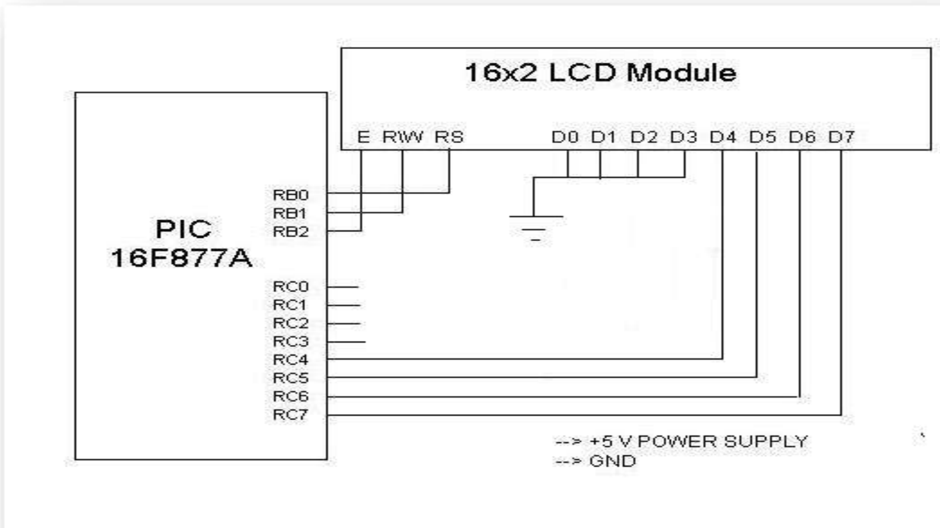


Figure 5: Circuit Diagram **Aritra Chatterjee**

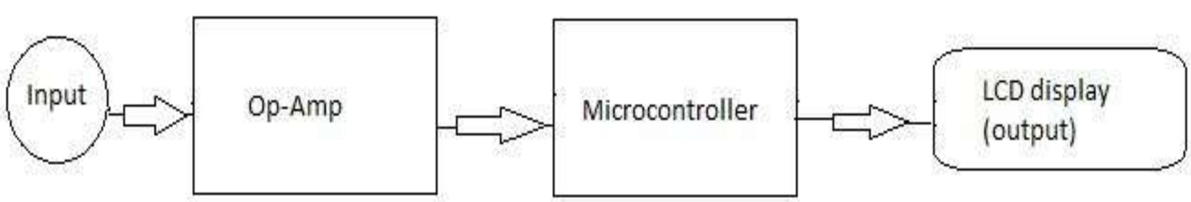


Figure 6: Block Diagram **Aritra Chatterjee**