

# Calculating blood components of cholesterol research design



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Good health is absolutely important to a human being and to remain healthy people need to check their blood level parameters. Cholesterol is a very important constituent of over 100 constituents in human blood. It is important to develop an instrument wherein blood parameters can be calculated which will be non-invasive, user friendly, portable and reliable. The thesis explains the designing and making of an instrumentation setup to calculate the blood constituents. It comprises of the study of samples made in the laboratory according to the various constituents present in whole blood in the RF range of 10MHz-4000MHz. The data is later fed to a regression analysis matrix which can be programmed in VLSI chips such as Altera FPGA in order to calculate the constituent concentration.

This thesis is proposed to contain 6 chapters with proposed chapters as given below

#### Chapter I (Introduction)

This chapter includes the introduction to the thesis, health and diseases, overview of cholesterol, types of cholesterol, role of cholesterol in humans, the various diseases due to high cholesterol, the worldwide scenario, the testing of cholesterol & blood test range of different constituents.

Total mental, physical & social wellness is a condition of health as well as the presence of infirmities or diseases. <sup>[1][2][3]</sup> Good health is often marred by diseases and illnesses which are sometimes incurable. <sup>[4][5]</sup> The most dreaded diseases include Cardio Vascular Diseases (CVD) and Strokes due to high Cholesterol. About 7, 000, 000 persons die of heart disorders annually in

the world, of which 2, 400, 000 are Indians. Strokes are the next principal source of death at 6, 200, 000 of which 1, 600, 000 are Indians. Cholesterol is important for normal body functioning, which appears to be a fat-like material which is waxy in nature.

It is used in making of hormones and for cellular functions. The Total Cholesterol (TC) in the blood consists of High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) & triglycerides. The cholesterol obstructs the arteries when it amasses in the body resulting in the limitation of blood flow. It could be tested invasively by visiting a doctor & extracting the blood using a needle & syringe technique. Since this procedure is painful, it develops a fear among the patients & could also be infection prone. Non-invasive methods are easier to use in one's home. Instant report could be attained & therefore non-invasive technique is gaining a lot of importance as the electronics industry now offers many smart sensors. Blood has many constituents and it depends on aspects such as age, diet, state of health and other particulars. [6][7][8]

The chief blood components are Cholesterol, NaCl, Glucose, Urea, Lactate & Alanine.

There are quite a few number ways to compute blood cholesterol in humans, invasive & non-invasive. They can be categorised into chemical tests and physical tests. The significant ones are based on Photo Acoustic Spectroscopy (PAS), Stimulated Emission spectroscopy, Thermal Emission Spectroscopy (TES), Optical absorption spectroscopy, Liquid Chromatography method, Chemical Method, Ultracentrifugation, Electrophoresis and

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Impedance measurement. The important techniques together with their working principles and the merits & demerits are discussed below.

Near Infrared Spectroscopy (NIRS): The principle of NIRS is that constituents absorb Infrared light at their characteristic wavelength. The absorption level is comparative to the constituents present. Hence the contents present can be predicted. It uses a physical rather than a chemical technique.

1. It is rather sensitive to calibration errors, but probes for non-invasive measurement are not available. However, new spectroscopic methods are now available with IR optical fiber for guiding the light to the tissue.
2. Chemical Method: In order to determine plasma cholesterol, the chemical procedure of Abell-Kendall is done which comprises of the Liebermann-Burchardt response after hydrolysis and eradication of cholesterol. Plasma cholesterol & triglyceride content determinations are usually examined by computerized techniques at clinical research facilities. Default values for plasma TC are achieved using autoanalyzer frameworks to which either the Liebermann-Burchardt test or the ferric chloride-sulfuric acid technique could be applied. A fluorometric investigation is utilized to decide the triglyceride reference values. Basic plasma estimations of triglycerides and TC can be relied on for the analysis of the diverse lipoprotein issues. It is an invasive method and there is wastage of chemicals in testing.
3. Chromatography: Chromatography techniques can be sorted out into 2 categories, i. e. Gas chromatography (GC) and Liquid Chromatography(LC). GC is a typical kind of chromatography utilized

scientifically for dividing and analyzing constituents that can be vaporized without decay. GC is used to test the purity of a specific substance, or segregating the distinctive parts of a mixture. In High Performance Liquid Chromatography (HPLC), a mobile phase comprises of either polar or non polar solvents. The specimen is constrained by a fluid at a huge pressure through a section that is filled with a stationary phase for the most part made out of sporadically or roundly formed particles picked or derivatized to achieve specific sorts of separations. Chromatography has low uncertainty, high precision, high accuracy and good linearity but it is expensive and not portable.

4. Impedance Measurement: An Impedance Plethysmograph framework is made up of a V-I converter and a sine generator. Passing current into a body section is done with the assistance of two current electrodes. The current path which produces the voltage signal is sensed with the assistance of an alternate pair of voltage electrodes. <sup>[9][10]</sup> The impedance is correlated to the amplitude of the signal. Impedance qualities measured at a series of frequencies or at a few distinct frequencies may aid in clarifying the differences in body composition more accurately than impedance estimation at a specific frequency. <sup>[11][12]</sup>

## Chapter II (Objectives & Literature Review)

Mas S. Mohktar et al recommended a method to estimate the cholesterol level in blood utilizing neural network & bioimpedance techniques non-

invasively. Bioelectrical Impedance Analysis (BIA) estimation was executed utilizing the bio impedance analyzer, Biodynamic Model 450.

1. A current signal  $< 1\text{mA}$  at 50 kHz was applied to 2 black sensor cables whereas 2 red sensor cables were used to identify the signal. The measurement procedure took approximately 3 minutes to get the results. 4 Artificial Neural Network (ANN) methods were used and eventually compared for calculating the high TC level in the blood. The specificity, sensitivity & accuracy were calculated by comparing the Multiple Logistic Regression (MLR) model results with the reference default values from the dataset. The sensitivity of the MLR model is 34.5%, the specificity is 97.6% and therefore the total accuracy is 71.4%. The ANN & BIA techniques could be implemented on chronic disease patients and diabetic invalids. [13]

E. Aristovich et al recommended a non-invasive impedance technique estimation of blood cholesterol by 3D finite field modelling.

1. This process supports the variation of calculating impedance over a conducting medium since the concentration of particles is altered. To calculate impedance, the current is computed between 2 electrodes throughout the conducting media created by the electric field distribution. It is obtained by computing & modelling 3D electric fields for known voltages connected between the electrodes utilizing Finite Element Method (FEM). The intricacy of FE models is accredited to particle distribution, the material & geometrical parameters, and the

size & shape that can be of several orders of degrees lesser as when compared to the general problem domain under investigation.

2. The paper prevails over the setback by implementing a useful particle aggregation technique in FE modelling exclusively influencing the accurateness of the field calculation. [14]
3. J. Nyström et al proposed to study a set of 34 men with various degrees of diabetic levels, including Multi Frequency (MF) BIA and skin changes by NIR. A fiber-optic probe to measure skin reflectance spectra was used on 4 sites. A joint multivariate analysis was carried out on the spectral range of 400-2500nm, using a lead sulphide detector (1100nm-2500nm) and a silicon detector (400nm-1100nm). NIR method can recognize skin conditions identified with diabetes. The 2 procedures combined together can offer a higher possibility for discrimination & classification of skin condition with exact classification rising from 63% to 85%. [15]
4. K. Cheng et al proposed to design a current source which includes a voltage controlled current source (VCCS), a microcontroller (uC) and a waveform generator (WG). The uC is made use of to program the WG to produce a sine voltage signal from 100 Hz - 100 kHz. The VCCS based Howland current pump converts the signal to current. The total harmonic distortions of the o/p current are 0.25% at 1 kHz & 0.40% at 100 kHz for the load resistance of 1 k $\Omega$ . The output current's phase difference varies from 0° to 19.6° over the above mentioned frequency range. The proposed multi-frequency BI measuring system

provides an inexpensive solution for BI applications. During system testing, the output current signal is constant.

Hiroshi Shiigi Hiroaki Matsumoto et al proposed a simple non-invasive technique to measure cholesterol by using a solvent to extract the skin component.

1. A self-assembled monolayer (SAM) sensor and a HPLC were utilized to analyze the extracted solution. The SAM electrode having an excellent responsiveness & sensitivity, attributed to its strong attraction towards hydrophobic cholesterol. Higher cholesterol was shown by the person with high cholesterol of the skin. The coefficient of correlation of non-invasive & invasive method was 0.9408, hence this method could be used practically. [17]

M. V. Malahov et al recommended to recognize hematological & biochemical blood parameters that can be precisely estimated by means of BI technique. Samples of blood from 46 people were poured into four test tubes.

2. Blood (2.5ml) was put in test tubes with Ethylenediaminetetraacetic acid for hematological investigation, next blood (3ml) was collected in tubes having heparin for BIA, later blood (2ml) was collected in tubes having sodium citrate for fibrinogen estimation and finally blood (4ml) was collected into unfilled tubes for biochemical serum examination. BIA analyzer DBC-01 "Medass" was utilized to perform BI spectroscopy of blood (1.5ml) from 5-500 kHz. Results show that the principle extracellular plasma particles:  $\text{Na}^+$  &  $\text{Cl}^-$  concentrations are not related to extracellular fluid resistance of the blood. [18]



## Objectives

The objective of the research is to design and develop an easy method to measure the level of cholesterol. The work envisages a development of an instrumentation using advanced microelectronics circuits, which is programmable and having interpretation mechanism to enable a common man to know his level of cholesterol.

It is proposed to use multivariate system approach to enhance cholesterol signature in DSP domain.

## Chapter III (Methodology and Instrumentation)

This chapter gives elaborate details on the preparation of samples, designing of cell, experimental setup and the instruments used. Human blood consists of many constituents; the major ones are Cholesterol (225mg/dL), Glucose (70-110mg/dL), Urea (10-20mg/dL), Lactate (10-15mg/dL) and Alanine (10-20mg/dL). Experiments are conducted with the above constituents. Samples are prepared using 14 mL distilled water, 1mL alcohol and the above constituents in varied concentrations. The average concentration is denoted as '1', half the average is denoted as '0.5' and approximately '0.75' to '1.25' is the actual range of blood components. The experiments are conducted with various concentrations as well, which are over the standard range & for extreme cases, & are denoted as 1.5, 1.75, 2, 2.25 & 3.

A cell was designed which was rectangular in shape having dimensions 12.5cms x 1cm x 2cms. The cell was used to measure RF response of various blood constituents. The cell was lined with a thin Cu foil and a copper wire

was connected to 2 connectors which were placed on extreme ends of the cell. The external radiations were reduced by placing the cell in an iron box which was earthed. This forms the dielectric loss cell. The cell was then connected via RF cables to the tracking generator and signal analyzer. The entire setup was secured firmly avoid mechanical movements. Experiments were carried out using the slow sweep and the fast sweep. The experiment was conducted after an hour and 24 hours to verify the accurateness of the results.

In comparison to the initial results, these were precise.

The tracking generator used is Signal Hound USB-TG44A which ranges from 10 Hz - 4400 MHz and the signal analyzer used is Signal Hound USB-SA44B which ranges from 1 Hz - 4400 MHz. A separate power supply is not essential as it is fed from the USB cable. The tracking generator and signal analyzer are approximately 8" long, light in weight and could be used practically anywhere.

#### Chapter IV(FPGA for Non-Invasive Cholesterol Measurement)

Software and hardware components operating together to perform a definite application is called Embedded Systems. The hardware platform comprises of an i/p device, an o/p display, a microcontroller (uC) / microprocessor (uP), application software and an onboard memory.

Designing embedded systems is getting more complicated nowadays due to the stiff restraints on power consumption, performance, size & area usage.

Hence, the software/hardware co-design procedure is utilized to plan embedded systems to decrease the measure of time used on debugging & development. uPs whose behaviour & architecture are completely described utilizing a subset of an Hardware Description Language (HDL) are called soft-core processors. They can be synthesized for any Field Programmable Gate Array (FPGA) or Application Specific Intergrated Circuit (ASIC) technology; hence they supply designers with much flexibility.

A platform for combining multiple design functions into a package or a group of packages is provided by an FPGA device. Incorporation of functionality results in reduced power & higher performances.

Design combination can be accomplished by integrating soft or hard processor cores in an FPGA to execute processing functionality and required control. The capability to incorporate design functionality and system-level components can reduce schedule, cost and risk.

#### Nios II - Altera Organization

Altera Organization is a top seller of FPGAs and Programmable Logic Devices (PLDs). They proffer the Cyclone, Stratix and Stratix II groups of FPGAs and are extensively utilized in DSP applications and design of embedded systems. Nios II Processor being a Reduced Instruction Set Computer (RISC) processor depicts Harvard memory architecture. The various features of this processor are single-instruction 32×32 divide and multiply operations, instructions for 128-bit & 64-bit multiplication, 32-bit Instruction Set Architecture (ISA) & 32 general purpose registers.

## Chapter V(Multivariate Data Analysis)

This chapter describes the multivariate data analysis, Partial Least Square Regression (PLSR), the different algorithms, i. e. Non-linear Iterative PARTial Least Square (NIPALS) and SIMple Partial Least Square (SIMPLS), the advantages and disadvantages of the algorithms, the ParLes software which is priority software developed for research applications, used for calculating unknown constituents.

Nowadays several factors add to numerous problems which are multivariate.

Multivariate analysis is a tool to obtain relationships and patterns amongst several variables concurrently. It can predict how an alteration in one variable affects other variables. It is very graphical which allows an analyst to observe the inner or unknown structure of big data sets and to visually recognize the factors which influence the outcome.

PLSR is a bilinear form of technique where information in x data is assigned onto a small amount of latent variables known as PLSR components.

The y data that are used in predicting the underlying variables to guarantee the first components are those that are most applicable for calculating the y variables. The relationship between x and y data is simplified as it is focussed on the minimum probable number of constituents.

## Chapter VI (Results & Conclusions)

This chapter includes the results and conclusions and the future direction of research.

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The multi-frequency BI spectrum was modelled through curve-fitting and multivariate statistical applications to extend parameters to predict body constituents like Cholesterol, Glucose, Salt, Urea, Alanine & Lactate.

The various components were mixed in different ways and some were used in the calibration file and the rest were treated as unknown. The spectra of cholesterol in different concentrations of 0.5, 1, 2 & 3 in the RF range of 10MHz to 4GHz was shown in Fig. 1.

The cholesterol shows a good variation only in certain regions at specific frequencies (575 MHz, 995 MHz, 1145 MHz, 1285 MHz & 2185 MHz) and one of them i. e. 575 MHz is shown in an expanded form in Fig. 2.

The data obtained from the graph is then used in a calibration set to determine the unknown constituents presents in the blood. When the calibration set has more than 20 samples, it shows that it has less error. Since the spectra of every blood constituent are unique, the data of the spectra is fed to a ParLes software to get out unknown values of blood constituents. Table I gives the actual concentration of blood constituents in the experiment.

Unknown concentration of cholesterol and known concentration of others were fed to a multivariate system. Table II shows the results of predicted values of cholesterol which are 43.75mg and 48.75mg whereas the actual values of cholesterol are 42.5mg and 51mg respectively. The results attained are within  $\pm 5\%$  of the actual content in the sample & are within the limits of the percentage error defined by WHO.