ESTIMATION OF CHOLESTEROL BY IMPEDANCE MEASUREMENT BASED ON DSP TECHNIQUE

THESIS SUBMITTED TO GOA UNIVERSITY

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IN

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BY

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UNDER THE SUPERVISION OF

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CERTIFICATE

This is to certify that the thesis entitled, **"ESTIMATION OF CHOLESTEROL BY IMPEDANCE MEASUREMENT BASED ON DSP TECHNIQUE"** submitted by **Ms. INGRID ANNE P. NAZARETH** for the award of degree for Doctor of Philosophy in Electronics, is based on her original and independent research work carried out by her during the period of study, under my supervision. The thesis or any part thereof has not been previously submitted for any other degree or diploma of this or any other University or Institute.

(Prof. G. M. Naik)
Research Supervisor

(Ph.D. External Examiner)

Place: Goa University
Date: 26th October 2015

STATEMENT

I state that the present thesis entitled "**ESTIMATION OF CHOLESTEROL BY IMPEDANCE MEASUREMENT BASED ON DSP TECHNIQUE"**, is my original contribution and the same has not been submitted on any occasion for any other degree or diploma of this or any other University or Institute to the best of my knowledge the present study is the 1st comprehensive work of its kind in the area mentioned. The literature related to the problem investigated has been cited. Due acknowledgements have been made whenever facilities and suggestions have been availed of.

(Ingrid Anne P. Nazareth)
Candidate

Place: Goa University
Date: 26th October 2015

DEDICATED

TO MY BELOVED

MAMA AND PAPA

BRENDA & PHILIP NAZARETH

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Ingrid Anne P. Nazareth
26th October 2015

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ABBREVIATIONS

ABI:	Ankle Brachial Index
AHA:	American Heart Association
ALU:	Arithmetic Logic Unit
ANA:	Automated Network Analyzer
ANN:	Artificial Neural Network
ASIC:	Application Specific Integrated Circuit
BFC:	Bottom Fraction Cholesterol
BI:	Bioelectrical Impedance
BIA:	Bioelectrical Impedance Analysis
BMI:	Body Mass Index
BP:	Blood Pressure
BQ:	Beta Quantification
CHD:	Coronary Heart Disease
CHOL:	Cholesterol
CPU:	Central Processing Units
CVD:	Cardiovascular Diseases
DCM:	Designated Comparison Method
DDS:	Direct Digital Synthesizer
DSP:	Digital Signal Processing
DUT:	Device Under Test
EDTA:	Ethylene Diamine Tetra Acetic
EMI:	Electromagnetic Induction
FEM:	Finite Element Method
FPGA:	Field Programmable Gate Array
GC:	Gas Chromatography
GLC:	Gas Liquid Chromatography
GUI:	Graphical User Interface

HDL-C:	High Density Lipoprotein Cholesterol
HPLC:	High Pressure Liquid Chromatography
IDE:	Integrated Development Environment
IDL:	Intermediate Density Lipoprotein
IMD:	Implantable Medical Device
IP:	Intellectual Property
iPLS:	Interval Partial Least Square
ISA:	Instruction Set Architecture
LC:	Liquid Chromatography
LCD:	Liquid Crystal Display
LDL-C:	Low Density Lipoprotein Cholesterol
LE:	Logic Elements
Lp[a]:	Lipoprotein[a]
LPL:	Lipoprotein Lipase
MANOVA:	Multivariate ANalysis Of VAriance
μC:	Microcontroller
MF:	Multi Frequency
MIPS:	Millions of Instructions Per Second
MLR:	Multiple Linear Regression
NADH:	Nicotinamide Adenine Dinucleotide Hydride
NCD:	Non Communicable Diseases
NCEP:	National Cholesterol Education Program
NF:	Noise Figure
NIPALS:	Non linear Iterative PArtial Least Square
NIRS:	Near Infrared Spectroscopy
NMF:	Natural Moisturizing Factor
OGTT:	Oral Glucose Tolerance Test
PCA:	Principal Component Analysis
PCR:	Principal Component Regression

PLS:	Partial Least Square
PLSR:	Partial Least Square Regression
RBW:	Resolution Bandwidth
RF:	Radio Frequency
RISC:	Reduced Instruction Set Computer
RMSECV:	Root Mean Square Error of Cross-Validation
RMSEP:	Root Mean Squared Error of Prediction
SAM:	Self Assembled Monolayer
SE:	Standard Error
SEC:	Standard Error of Calibration
SHARC:	Super Harvard Architecture
SIMPLS:	SIMple Partial Least Square
SMA:	Sub Miniature version A
SNA:	Scalar Network Analyzer
SoC:	System-on-a-Chip
SoPC:	System-on-a-Programmable-Chip
TC:	Total Cholesterol
TRG:	Triglyceride
VAP:	Vertical Auto Profile
VAP-IIfs:	Vertical Auto Profile-II (fingerstick)
VBW:	Video Bandwidth
VCCS:	Voltage Controlled Current Source
VLDL:	Very Low Density Lipoprotein
VNA:	Vector Network Analyzer
WG:	Waveform Generator
WHO:	World Health Organization

PREFACE

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 other constituents in the human blood. It is important to develop an instrument wherein cholesterol percentage 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 cholesterol constituent. The set-up comprises of scalar network analyzer and a dielectric loss cell with which study on samples prepared in the laboratory in accordance with the various constituents present in whole blood is carried out, in the RF range of 10MHz-4000MHz. The data is later fed to a regression analysis matrix which is programmed in VLSI chip EP2C35F672C6N from Altera FPGA in order to calculate the constituent concentration.

Chapter 1 includes the introduction to the thesis along with 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.

Chapter 2 describes the various methods that the researchers have worked on over the past years, some of which include Electrophoresis Method, Centrifugation Method, Chromatographic Method, Deuterium Incorporation Technique, Impedance Method, Spectroscopic Techniques: NIRS & RF, Raman Spectroscopy, Precipitation Method, Body Mass Index, Fluorimetric, Direct Assay, Friedewald Equation & Enzymatic Method.

Chapter 3 gives elaborate details on the preparation of samples, designing of cell, experimental setup and the instruments used. Chapter 4 describes FPGA for Non-Invasive Cholesterol Measurement, the different soft-core Processors, NIOS II Processor, DE2 Board and the SoPC development board.

Chapter 5 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, is used for calculating unknown constituents.

Chapter 6 includes the results and conclusions and the future direction of the research. The RF response of the various laboratory prepared samples were modelled through curve-fitting and multivariate statistical applications to extend parameters to predict body constituents like Cholesterol, Glucose, Salt, Urea, Alanine & Lactate.

Ingrid Anne P. Nazareth
26th October 2015

Introduction

Good health is not merely the absence of disease or infirmity it also includes being physically hale and hearty, mentally fit and emotionally stable[1-2]. Health can be divided into 3 interdependent fields as Biomedical, Environmental and Lifestyle[3]. The advancement of health is accomplished through social, mental and physical well-being commonly known as the "Health Triangle"[4-5]. Concentrating more on lifestyle issues and their dealings with functional health, studies proposed that individuals can enhance their health by exercising, maintaining body weight, restricting liquor use, and abstaining from smoking.

Genetics plays an essential part in determining the wellbeing status of people. This can include the predisposition to specific diseases and the behaviours and habits which individuals develop through the way of life of their families. It might likewise play a role in the way in which individuals deal with stress, physically, emotionally or mentally. It is essential to maintain a healthy diet and weight in order to enjoy good health, thus bringing down the risk of coronary illnesses. Physical activity which includes exercise can have health benefits, thus reducing the risk of Cardiovascular Diseases (CVD), diabetes and cancer.

Good health is often marred by diseases and illnesses which are sometimes incurable[6-7]. The most feared diseases include strokes and CVD due to elevated cholesterol. Keeping good health is of utmost importance to every human being and to stay healthy, individuals need to check their blood parameters on a regular basis. Studies have demonstrated that high levels of anxiety can have adverse effects on health[8].

1.1 Strokes and Cardiovascular Diseases

CVDs consists of various disorders of the heart including heart vessels which consists of cerebrovascular disease, coronary heart disease, renal artery stenosis, peripheral arterial disease, cardiomyopathy, aortic aneurysm, heart failure, hypertensive heart illness, cardiac dysrhythmias, pulmonary heart disease, valvular heart ailment, inflammatory heart diseases, congenital heart disease, rheumatic heart disease, pulmonary embolism and deep vein thrombosis.

Strokes and heart attacks are typically intense events and are generally caused by a blockage that prevents blood flow to the brain or heart. It is mainly due to the build-up of plaque on the inner walls of the arteries[9-10]. Strokes can be brought about by blood clots or bleeding from blood vessels in the brain. The reason for strokes and heart attacks are typically the risk factors such as: unhealthy diet, harmful use of tobacco and alcohol, physical inactivity, obesity, diabetes and hypertension.

1.1.1 Risk Factors of CVD

The most essential risk factors for strokes and heart diseases are the harmful use of alcohol and tobacco, unhealthy diet and physical inactivity[11]. The effects of risk factors may appear in people, as increase in blood sugar and cholesterol, increase in blood pressure and obesity. These factors can be measured in primary health centres and demonstrate an increased risk of developing strokes, heart attacks and various complications.

Complete stoppage of tobacco use, consuming less salt, avoiding alcohol, increase in physical activity, increase intake of vegetables and fruits reduces the risk of CVD. In addition treatment with drugs, of diabetes, high cholesterol and hypertension may be important to reduce CVD and prevent strokes and heart attacks. In order to motivate people to sustain and adopt healthy behaviour, healthy choices are made affordable and available by health policies.

1.1.2 Common Symptoms of CVD

i) Strokes

Warnings of a stroke include sudden weakness and numbness of the leg, arm or face, particularly on one side of the body, intense headache, difficulty in speaking and comprehending speech, confusion, trouble seeing with one or both eyes, unsteadiness, loss of balance and coordination, severe headache and dizziness, unconsciousness and fainting with apparently no known cause.

ii) Heart Attacks

In actual fact there are no symptoms of the diseases of the blood vessels. A stroke or heart attack may be the first cautioning of the hidden disease. Discomfort or pain in the mid section of the chest and in the arms, elbows, left shoulder, back or jaw, shortness of breath, trouble in breathing, nausea or vomiting, feeling faint or light headed, becoming pale and breaking into a cold sweat are the symptoms of a heart attack.

1.1.3 CVD – A Development Issue in Middle and Low Income Countries

- Around 75% of the world's deaths occur in middle and low income countries from CVDs.
- People from these countries do not have the facilities of primary health care for revealing and treatment of disease.
- The poorest countries are the most affected. They experience the ill effects of CVDs and other non-transmittable diseases, which are noticed in the latter part of the disease and finally succumb to the same.

1.2 Strokes and CVD in the World

- CVDs are leading cause of death globally wherein more individuals die annually, than from some other cause.
- It was estimated that 17.5 million individual died from CVDs in 2012, which was around 31% of deaths worldwide, which is expected to increase to more than 23.6 million by 2030. Of these total deaths, around 7.4 million were because of coronary illness and 6.7 million were due to stroke.
- Over 75% of CVD deaths occur in middle and low income nations.

1.2.1 CVD Reduction Methods

World Health Organisation (WHO) has identified expensive interventions that can be used practically to prevent and control CVD. Some of the interventions, population wide, that can be executed to reduce CVDs include:

- Total tobacco and alcohol control strategies.
- Taxation to decrease the foods high in salt, sugar and fat that are consumed.
- In order to increase physical activity, cycle and walking paths to be constructed.
- Supplying nourishing meals to school children.

To prevent strokes and heart attacks, individual health care need to be focussed on patients having high CVD, which are more costly than a single risk factor, for example, hypercholesterolemia and hypertension.

If the above interventions are not followed, then the following treatment is necessary for prevention of CVD:

- Beta-blockers
- Statins
- Angiotensin-converting enzyme inhibitors
- Aspirin

In addition, surgical operations which are often costly are required to treat CVDs which include:

- Balloon angioplasty
- Heart transplantation
- Coronary artery bypass
- Valve repair and replacement
- Artificial heart operations

Medical devices such as prosthetic valves and pacemakers are required, to treat some CVDs.

1.2.2 WHO Response

194 Member State nations belonging to the WHO, agreed to diminish the avoidable Non Communicable Diseases (NCD) burden in 2013,on worldwide systems, taking into consideration preventing and controlling NCDs through a global action plan (2013-20). This aims to lessen unexpected deaths by 25% by NCDs, through 9 worldwide targets by 2025, which includes the following: since high B.P. is one of the main risk factors of CVD, 25% decrease is called for in the 6^{th} target. According to the 8^{th} target, 50% of adequate individuals should be entitled to counselling and treatment with drugs, to prevent strokes and heart attacks.

1.2.3 The 10 Leading Causes of Death in the World

The main leading causes that kill most individuals in the world are strokes and heart diseases, among others, during the last 10 years[12]. (Fig. 1.1 - 1.4)

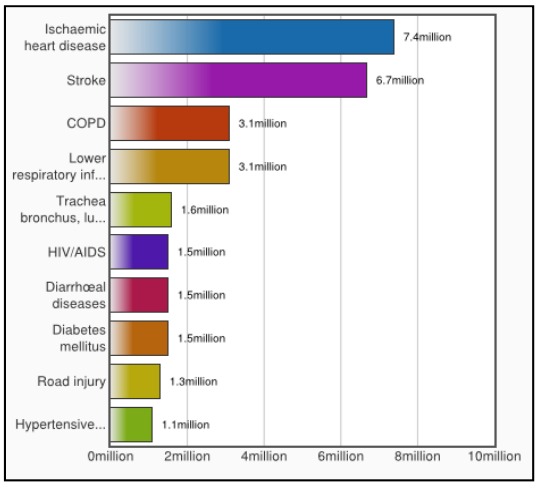


Fig.1.1:10 Leading Causes of Death Worldwide

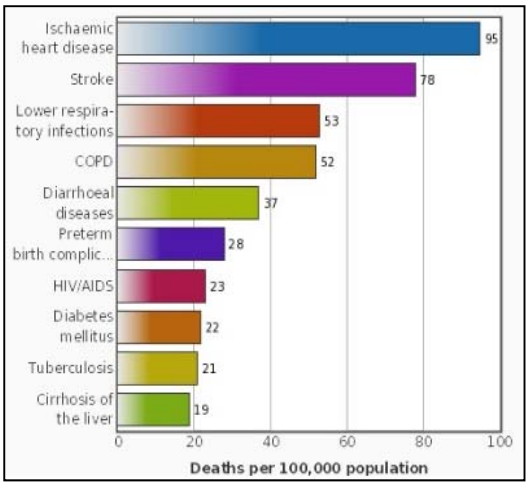


Fig.1.2: Causes of Death-Low Income Countries

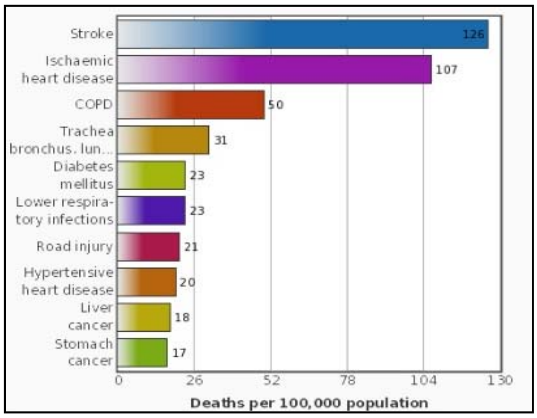


Fig.1.3: Causes of Death-Middle Income Countries

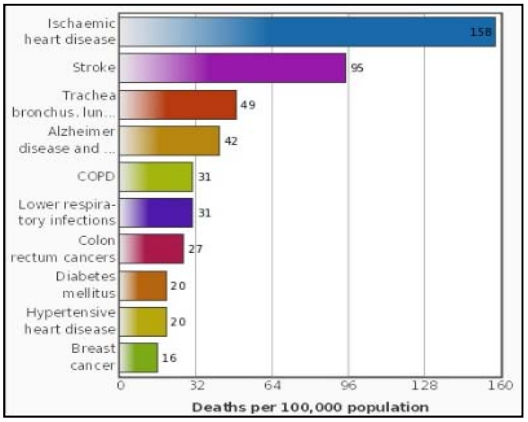


Fig.1.4: Causes of Death-High Income Countries

1.2.4 Stroke and Heart Disease Statistics

Mentioned here below are a few key statistics regarding stroke, heart disease and other CVDs and risk factors[13].

i) Heart Disease

- Heart disease is the No. 1 reason in the US, killing around 375,000 individuals a year.
- It is also the No. 1 killer of females claiming more lives, than all types of cancer together.
- In 2011, around 39,000 Afro-Americans succumbed to heart disease.

- Cardiovascular procedures and operations, expanded to around 28% from 2000 up to 2010, totaling around 7.4 million.

ii) Stroke

- In 2010, the occurrence of stroke was 33 million, with 50% individuals having a first stroke. Stroke was globally the second-leading cause of death, next to heart disease, representing 11.13% of the worldwide total deaths.
- In US stroke is No. 5, killing almost 129,000 individuals a year.
- Afro-Americans have double the risk for a first-ever stroke, as well as deaths, than the Americans.

iii) Other CVDs

- In 2008, cardiovascular deaths corresponded to 30% of global deaths, with 80% from low- and middle-income countries.
- Around 787,000 people in the U.S. died from CVD in 2011.
- CVD is the leading cause of death than all types of cancers combined.
- 85.6 million Americans and 50% Afro-Americans, have some type of CVD.
- The total expenses of stroke and CVD amount to more than $320.1 billion which include lost productivity and health expenditures.

iv) Sudden Cardiac Arrest

- Around 326,200 people experienced cardiac arrests out-of-hospital, in 2011, of which 10.6 % survived after being treated by emergency medical services.
- Every year, around 209,000 individuals have cardiac arrest while in the hospital.

1.2.5 Heart Disease, Stroke and Cardiovascular Disease Risk Factors

There are 7 important health behaviours and factors known as Life's Simple 7. The risks involved are: physical activity, not smoking, body weight, healthy diet, control of blood sugar, blood pressure and cholesterol. The goal of 2020 according to AHA is to reduce deaths in U.S. by 20% due to strokes and CVDs, as well as to improve the health by 20%.

i) Physical Activity

- Around 31% adults in the U.S. do not take part in physical activities at all.
- 36.6% boys and 17.7% girls are physically active for 1 hour per day per week.

ii) Smoking

- In 2010, tobacco smoking worldwide was one of the leading risk factors, which contributed to around 6.2 million deaths.
- 20% men, 16% women and 16% students were reported to be heavy smokers.
- In 2012 there were roughly 6,300 new individual smokers daily.

iii) Overweight / Obesity

- 69% U.S. adults and around 32% children are overweight or obese.
- Worldwide, around 1.46 billion adults in 2008 were overweight or obese.

iv) Healthy Diet

- Under 1% of U.S. adults and basically no children have a healthy diet according to AHA.
- Eating habits have changed drastically of late. Research over 34years (1971- 2004) demonstrates, that females consumed around 22% more calories, while men consumed 10% more.

v) Blood Sugar / Diabetes

- In 2010, adults affected by diabetes were estimated to be around 6.4% (285 million) worldwide and is anticipated to rise to 7.7% (439 million) by 2030.
- In U.S. around 9% (21 million) adults have contracted diabetes and 35% are diagnosed with pre-diabetes.

vi) High Blood Pressure

- Around 33% of U.S. adults have high B.P.
- Around 69% of individuals who experience a first heart attack have B.P. more than 140/90 mm Hg of which 46% adults do not have it under control.
- Hypertension is anticipated to increase around 8% from 2013 to 2030.

- Hypertension among Afro-Americans is the highest of any population worldwide.
- Around 972 million adults in the world had high B.P. in 2000.

vii) Cholesterol

- In U.S. around 43% adults have total cholesterol of more than 200 mg/dL.
- Around 33% adults have high levels of LDL-C &20% have low levels of HDL-C.

1.2.6 Indian and South Asian Scenario

i) Heart Disease and Stroke among Indians & South Asians

- Heart disease is the leading cause of deaths in India which represents approx. 60% of the heart disease worldwide, in spite of having <20% of the world's total population[14]. About 7,400,000 persons die of heart disorders annually in the world, of which 2,400,000 are Indians.
- Most Indians are affected at an early age with heart disease, without any warning. 50% Indian men experience heart attacks below the age of 50, and so do Indian women[15].
- This is due to an underlying hereditary predisposition to cardiomyopathy and metabolic deregulation and also a modern shift of risk factors, in the increase of consuming red meats, junk foods, saturated and trans fats.
- WHO has predicted that 20 million people will succumb to heart disorders, by the end of 2015 especially in India.
- Strokes are the next principal source of death at around 6,700,000 of which 1,600,000 are Indians. Estimated results prove that 40% of deaths due to strokes occur mostly in South Asia.
- WHO has also approximated that 1.6 million strokes per year will occur by 2015, subsequently resulting in 33% disabilities in India.

ii) Cholesterol among Indians & South Asians

- Cholesterol related problems are very frequent among Indians & South Asians due to hereditary factors, dietary habits and lack of exercise. In India, the heart disease rate due to high cholesterol is twice that of worldwide averages, according to demographic information acquired.

- People are inclined to have 3 times more lower HDL-C than LDL-C, which increases risk of untimely coronary diseases.

1.3 Cholesterol

Cholesterol appears to be a yellow fat-like material which is waxy in nature[16]. It is transported in the blood by lipoproteins. It is important for normal body functioning by building a membrane around the cells, which acts like a protective barrier. It is present all through the body, especially in the skin, brain, adrenal glands and nervous tissue which is essential for life. The chemical formula is $C_{27}H_{46}O$ (Fig. 1.5).

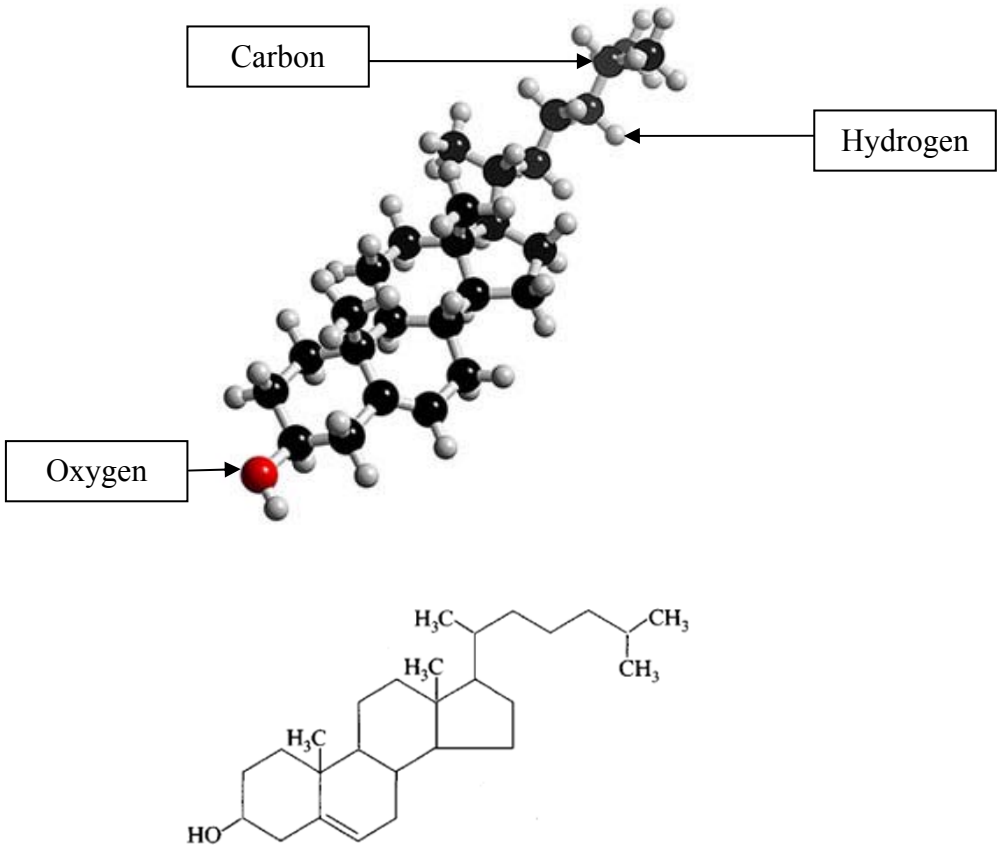


Fig. 1.5: 3D Molecule and Structure of Cholesterol

1.3.1 Cholesterol Bonds

i) Cholesterol is a Molecule

Cholesterol is a lipid molecule, wherein every atom is covalently bonded to each other, having no ionic bonds[17]. It consists of an extraordinary covalent bond, known as polar covalent bond.

ii) Cholesterol's Hydroxyl Polar Group

Hydroxyl group made up of the 2 atoms, out of the 74 atoms, exist in the cholesterol molecule. It is the only water soluble polar part of cholesterol. The hydroxyl group makes cholesterol an alcohol. It comprises of a hydrogen atom bound to an oxygen atom, which are individually neutral having equal no. of electrons and protons. Fig. 1.6 shows hydrogen and oxygen atoms bonded together.

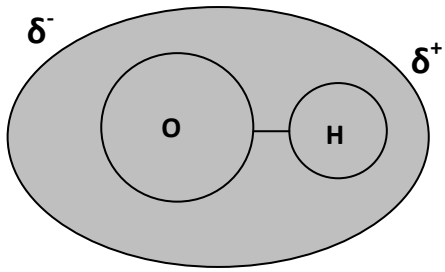


Fig. 1.6: Hydrogen and Oxygen Atoms Bonded Together

Since the electrons have a tendency to hang out much more on one side than the other, so their negativity is focused more on that side. Hence the other side becomes positive. The entire hydroxyl group is surrounded by the electron cloud (grey colour), more on the oxygen side as shown in Fig. 1.6. Partial charges exist due to the uneven distribution of charges, even though it is neutral leading to the hydrogen being partially positive and the oxygen being partially negative. Since positive and negative charges exist on either side of the hydroxyl group, it is known as polar.

iii) Cholesterol is Amphipathic

Cholesterol consists of a small water soluble hydroxyl group and a hydrocarbon tail along with the steroid ring, being water insoluble. This shows that cholesterol is amphipathic in nature, which is partly water insoluble, water soluble but not enough to dissolve in blood. Hence it is transported in lipoproteins.

This property is shown in Fig. 1.7. Water gets attracted to the hydroxyl part of cholesterol. The hydrogens shown in red are slightly positive, whereas the oxygens shown in blue are slightly negative. The hydrogen of cholesterol gets attracted to the oxygens of water and vice versa, thereby breaking the water molecules apart and mixing them as well.

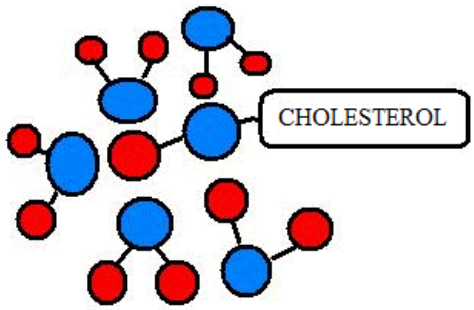


Fig. 1.7: Amphipathic Property of Cholesterol

It may be noted that, major portion of the cholesterol molecule are not attracted to water. If cholesterol is dissolved in water, it would float on top, but the polar hydroxyl groups would point towards the water.

iv) Hydrocarbon Rings in Cholesterol

Cholesterol contains an area with 4 hydrocarbon rings, in which carbon atoms are joined to one another. There are normally 6 carbons yet it can differ. The corner of each ring has a carbon atom bonded to 2 hydrogen atoms. Cholesterol contains 4 hydrocarbon rings, of which 3 are 6 carbon rings and 1 of them is a 5 carbon ring.

There is a double bond in one ring, a hydroxyl group protruding off one ring, hydrocarbon tail and two protruding CH_3 groups in the middle. Since hydrocarbon rings have a bond between hydrogen and carbon, it is non polar and therefore fat soluble.

v) The Hydrocarbon Tail is Non-Polar

The hydrocarbon tail of cholesterol is made up of carbon and hydrogen. The electronegativity of carbon and hydrogen are similar, therefore the electron cloud is equally distributed over the 2 atoms. Hydrogen-Carbon bonds are non-polar, as they do not have negative or positive poles within themselves.

1.3.2 Production and Function

Cholesterol has 3 main functions in the body[18]. It aids in the production of Vitamin D and steroid hormones, acts as a structural component of cell membranes and is used to produce bile acids which helps the digestion and retention of fats in the diet. Cholesterol is produced endogenously i.e. within the body and exogenously i.e. from diet. Saturated fat influences the cholesterol, that is produced endogenously and exogenously thereby increasing cholesterol in the blood. It also helps to absorbs fat molecules in the intestines as well as vitamins A, E, D &K. Cholesterol may also act as an antioxidant.

1.3.3 Transport and Removal

Since cholesterol is produced in the liver, it should be transported to the required tissue which needs it. Cholesterol is expelled from the body as bile salts or simply cholesterol. The enterohepatic circulation involves the process of reabsorbing 98% of bile salts excreted from the gall bladder, by the large intestines and re-expelled as bile, the remainder is discharged through faeces. About 1 gm of cholesterol is disposed from the body daily in this way.

1.3.4 Structural

The most important function of cholesterol is the structural element of cell membranes[19]. The average membrane consists of a mixed double layer of phospholipid and cholesterol molecules, perpendicular to the plane of the membrane, such that the hydroxyl groups face outwards from either surface. The cholesterol can exist only in unesterified or free form in the tissues of the membranes. Cholesterol is also a component of plasma lipoproteins and coupled with phospholipids and protein, to form a structure covering the lipoprotein surface and hence enabling the non-polar lipid, to be carried in soluble form.

1.3.5 Total Cholesterol (TC)

TC is made up of 3 main lipoproteins namely HDL, LDL and VLDL[20].

$$[TC] = [HDL\text{-}C] + [LDL\text{-}C] + [VLDL\text{-}C]$$

Using the enzymatic method, cholesterol is measured in plasma or serum in coupled reactions. H_2O_2 is a by-product of one of the reactions wherein colour is produced. The intensity of the colour is directly proportional to the concentration of cholesterol. The series of reactions are as follows:

$$\text{Cholesteryl ester} + H_2O \xrightarrow{\text{cholesteryl ester hydrolase}} \text{cholesterol} + \text{fatty acid}$$

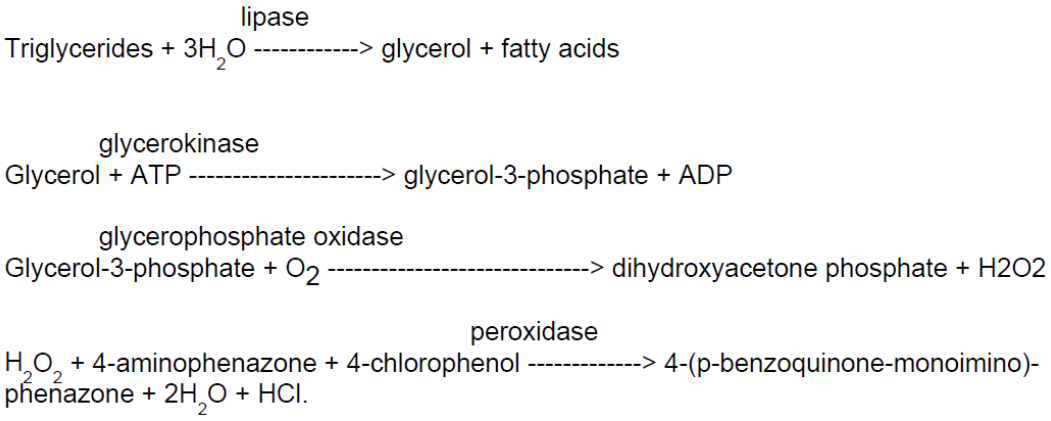
$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{4-(p-benzoquinone-monoimino)-phenazone} + 4\ H_2O$$

Normal Cholesterol levels should be under 250mg/dL in adults and under 170mg/dL in children.

1.3.6 Triglycerides (TRG)

TRG being an important lipid is also found in foods, but the body also tends to produce its own. Individual having high TRG also have high TC, low HDL-C and high LDL-C which is a danger for the increase of plaque build-up and coronary diseases. High TRG attack people who are obese and diabetic. Using the enzymatic method, TRGs are measured in plasma or serum, in coupled reactions. The series of reactions are as follows:



Normal TRG should be under 200mg/dL. The risky TRGs are as follows: borderline TRG ranges between 200-400mg/dL and high TRG above 400mg/dL. Pancreatitis can result if TRGs are extremely high and treatment is of utmost importance.

1.3.7 HDL Cholesterol

HDL-C is known as the “good cholesterol” as it ensures against stroke and heart attack. The liver produces these particles. They are comprised of 50% phospholipids and cholesterol and 50% protein. HDL-C particles carry the cholesterol away from the arteries to the liver, where it can be excreted by the body. By doing this, there is less cholesterol at the site of the artery to form plaques. Hence if the HDL-C is high, there would be less cholesterol to form plaques in the arteries. HDL-C is measured by the direct method in serum. The series of reactions are as follows:

ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO_4 ---> soluble non-reactive complexes with apoB-containing lipoproteins

HDL-cholesteryl esters $\xrightarrow{\text{PEG-cholesteryl esterase}}$ HDL-unesterified cholesterol + fatty acid

Unesterified chol + O_2 $\xrightarrow{\text{PEG-cholesterol oxidase}}$ cholestenone + H_2O_2

H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'_succinyl ethylene diamine + H2O + H^+ peroxidase > qunoneimine dye + H_2O

Normal HDL-C should be above 60mg/dL and low HDL-C which is risky is under 35mg/dL.

1.3.8 LDL Cholesterol

LDL-C is known as the bad cholesterol as it carries cholesterol to the tissues of the body which forms plaque on the inner walls of the arteries. This leads to atherosclerosis leading to a constricted blood flow. If the arteries in the heart get completely blocked, it causes a heart attack. Similarly if the arteries that carry blood to the brain get blocked, it can lead to a stroke. LDL-C should be low in order to reduce the risk of strokes and heart attacks. 70% of the TC exists in the form of LDL-C. It is computed using the following equation

[LDL-C] = [TC] - [HDL-C] - [VLDL-C]

[LDL-C] = [TC] - [HDL-C] - [TRG]/5

wherein [TRG]/5 is approximation of VLDL-C. All values are calculated in mg/dL.

Normal LDL-C levels should be under 130mg/dL in adults and under 110mg/dL in children.

1.3.9 Chylomicrons

Chylomicrons are the less intense and biggest of lipoproteins that are formed in the walls of the intestines from cholesterol and fats. They are used to transport triglycerides from the intestines to the cells which require energy. Lipoprotein Lipase (LPL) removes TRGs from chylomicrons. When chylomicrons exist in huge quantities, plasma appears to be milky.

1.3.10 Very Low Density Lipoproteins (VLDL)

VLDLs are produced in the liver. It distributes TRGs to skeletal muscles and adipose tissues used for energy and storage. The way in which TRGs are expelled from the circulation is similar to chylomicrons. After TRGs and proteins are removed, VLDLs convert to LDL. High VLDL plasma is found in people with diabetes mellitus, hypertriglyceridaemia, individuals with high consumption of alcohol and others having underactive thyroid.

1.3.11 Intermediate-Density Lipoproteins (IDL)

IDL produced from the degradation of VLDL, allows cholesterol and fats to travel in the blood[21]. They contain a series of cholesterol esters and triacylglycerols. They are passed from plasma to the liver or degraded to produce LDL particles. IDL transports various cholesterol, triglyceride fats and promotes the growth of atheroma. IDLs are intermediate between VLDL and LDL. The overview of lipoprotein function is given in Table 1.1 below.

1.3.12 Overview of Lipoprotein Function

Table 1.1: Characteristics of the Major Classes of Lipoproteins in Human Plasma

Lipoprotein Class	Major Apoproteins	Origin of Apoproteins	Transport Function	Mechanism of Lipid Delivery	Density ($g.cm^{-3}$)	Particle diameter (A)	Physiologic function
Chylomicrons	A-I, A-II, A-4, B-48, C-I, C-II, C-III, E	Small intestine	Dietary triglyceride	Hydrolysis by lipoprotein lipase	<0.95	750-12000	Absorption of dietary fat
VLDL	B-100, C-I, C-II, C-III, E	Liver and small intestine	Endogenous triglyceride	Hydrolysis by lipoprotein lipase	<1.006	300-800	Transport of triglyceride from liver to other tissues
IDL	B-100, C-I, C-II, C-III, E	VLDL	Endogenous cholesterol	Receptor-mediated endocytosis in liver	1.006-1.019	250-350	Initial product formed in VLDL catabolism
LDL	B-100	IDL	Endogenous cholesterol	Receptor-mediated endocytosis in liver or extrahepatic tissues	1.019-1.063	180-250	Cholesteryl ester transport
HDL	A-I, A-II, C-I, C-II, C-III, D, E	Liver and small intestine	Facilitates removal of cholesterol from extrahepatic tissues	Cholesteryl ester transfer to IDL cholesterol and LDL	1.063-1.210	50-120	Removal of excess cholesterol from tissue and lipoproteins

In order to generate and transport lipids in the body, 3 major pathways are responsible which include the endogenous, the exogenous and the reverse cholesterol transport pathway as shown in Fig. 1.8. The exogenous pathway involves the dietary fats and bile acids absorbed by the intestines. Here it forms chylomicrons which are processed by LPL in the capillaries to release fatty acids which are used by the adipose tissues and muscles. The remnants of chylomicrons are then absorbed by the remnant receptor in the liver. The endogenous pathway includes the synthesizing of lipoproteins in the liver. Cholesterol and TRG are produced by the liver, packed into VLDLs and discharged in the circulation[23-24]. It is then processed by LPL in the capillaries to release glycerol and free fatty acids. The fatty acids are used by the adipose tissues for storage and the muscles for energy. The remnants of VLDL which become IDL (smaller and denser than VLDL) are then passed onto the liver via the LDL receptors and some IDLs form LDL (smaller and denser than IDL) is absorbed by the extrahepatic tissue. Here HDL is formed and is the main lipoprotein in the reverse transport of cholesterol[25]. It is the smallest and the densest of lipoproteins. The cholesterol in the extrahepatic tissue which is in excess is taken back to the liver for use there or excreted though bile.

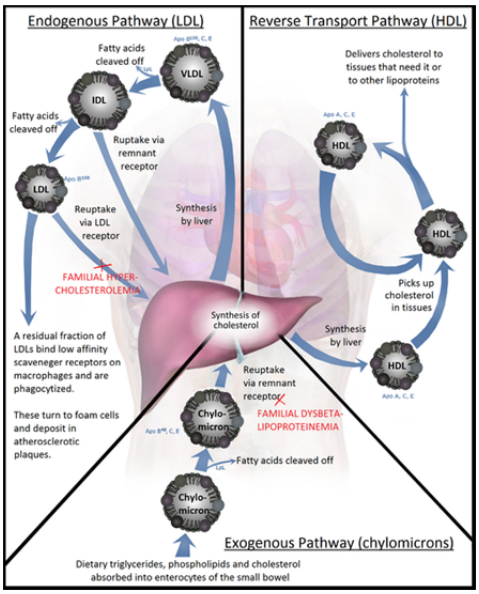


Fig. 1.8: Exogenous, Endogenous & Reverse Transport Pathways in Cholesterol

1.4 Testing of Blood Cholesterol Invasively

1.4.1 Finger Stick Method

The following are the steps of the Finger Stick Method[26]

1. The index or middle finger and the site are selected.
2. The finger is then cleaned with alcohol and air dried.
3. A little prick is made in the finger using a lancet.
4. Initial drop of blood wiped away.
5. 4 blood drops are removed utilizing a capillary tube.
6. A bandage is then placed on the finger.
7. The blood is collected in a cassette.
8. The cassette is then put into the Cholestech LDX instrument.
9. This instrument takes under 6 minutes to process the sample and the results of cholesterol are displayed onto a digital screen.

It takes under 90 seconds to draw blood using the Finger Stick method and the achievement rate in acquiring the 4 blood drops is 99.9%.

However this method has some drawbacks.

- A pain is often experienced when a lancet is pricked into the finger, but other than experiencing this minimal pain, the discomfort of the finger is negligible.
- In fewer than 10% of the patients, there is often a bruise caused by bleeding under the skin.
- The pricked area may be noticeable for a brief period of time after the blood is collected.
- The patient may experience a dizzy feeling.
- The danger of infection is under 0.1%.

1.4.2 Venous Blood Draw Procedure

The following are the steps of the venous blood draw method

1. Either arm is chosen and a tourniquet is placed above the draw site of the arm.
2. The median cubital vein is often selected.
3. Using a sterile alcohol prep pad, the site is cleaned.
4. A needle is pierced into the vein and blood is drawn, until the collection tube is full.
5. Within a minute the tourniquet is removed.
6. Once the tubes have been collected, the needle is removed.
7. 8.5mL blood is required per tube which may vary up to 3 tubes.
8. A cotton pad and band-aid are put on the pricked area.
9. The blood tube is labeled with the patient's details.
10. The tube is then kept aside in order to clot for around 15 to 30 minutes and immediately centrifuged.
11. The tubes are shipped overnight to a specific laboratory.
12. The laboratory tests the samples in less than 24 hours of taking delivery of the shipment.
13. Finally the results are mailed to the patient after a few days.

It takes around 5 minutes to draw blood using the venous blood draw method and the achievement rate in acquiring the required blood in about 2 attempts is 98.2%.

However it has more drawbacks than the Finger Stick method

- Excess bleeding can occur in patients using blood thinners or having problems with clotting after the venous blood draw.
- Blood clots under the skin can occur due to the prick of the needle, size of the vein chosen, insufficient pressure after the blood draw and immediate removal of the Band-Aid.
- The patient may feel light headed or faint.

- The danger of infection is under 0.1%.

- Nerve injury, arterial nicks and inflammation of the vein may occur resulting in hematoma.

1.4.3 Specimen Handling

1. Blood is collected in a glass collection tube.

2. It is then allowed to clot for 45 min. in a sealed tube at room temperature. If not kept for the required time secondary clots may occur.

3. Centrifuge the tubes at 1,500 x g for half an hour at 4°C and place it immediately into an ice bath and maintain it at 2-4° C onwards.

4. Samples ought to be kept frozen in a non-self defrosting freezer at -20°C, till sent to the laboratory.

1.4.4 Reagent Preparation

Cholesterol, HDL-C and TRG are analyzed on a Hitachi 704 analyzer. Cholesterol and TRGs are measured enzymatically using the cholesterol high performance reagent. Direct HDL-C reagent is analyzed along with TRGs and cholesterol. Should analysis be delayed, the specimens can be kept frozen at -80° C & can be stored for up to 1 year until analyzed.

i) To Operate Instrument, the Following Reagents are Required

1. Hitergent, a solution containing 5% ethanolamine, an antibacterial agent and a non-ionic detergent.

2. Cell Clean 90, a solution of NaOH used to keep reaction cells free of protein deposits.

3. 2% Hitergent Working solution. To deionized water add 20 mL Hitergent and bring to 1L.

ii) Test Specific Reagents

Cholesterol Reagent: The constituents of cholesterol high performance system pack reagents include: $\geq$0.15 U/mL cholesterol oxidase, 75 mmol/L PIPES buffer,

cholesterol reagent, ≥0.5U/mL cholesterol esterase, ≥4.2mmol/L Phenol, ≥0.25U/mL Peroxidase, 0.15mmol/L 4-Aminophenazone, 0.2mmol/L Sodium cholate, 10mmol/L Mg^{2+}, 1% Fatty alcohol-polyglycol ether, buffer, unspecified preservative, unspecified stabilizers. The reagent is supplied as a ready to use solution. After opening, it is stable for around a month at 2-12 °C or up to a week at room temperature. It should be stored away from light.

1.4.5 Meaning of Results

The results of the cholesterol tests can be either Desirable, High or Very High.

1. Desirable: (<250mg/dL): This is the normal cholesterol.

2. High: (250-300mg/dL): This is the range of medium risk. Control the cholesterol level by exercising regularly, maintaining a healthy diet and check again within 8 weeks.

3. Very High: (>300mg/dL): This is the area of high risk. A doctor's advice is extremely necessary and medication is a must.

The fundamental aim of treating hyperlipidaemic patients is to reduce the risk of untimely heart attacks and to lessen the occurrence of heart attacks in patients, who suffer from coronary disorders. Certain patients listed below should control their cholesterol level, which ought to be no higher than 4mmol/l and the LDL-C should be less than 2mmol/l:

- Patients having coronary and atherosclerotic disorders.
- Patients with dyslipidaemias, high B.P., family history of premature CHD, diabetes, thereby having a high risk of developing coronary and atherosclerotic disorders.

1.5 Computing Blood Cholesterol

There are quite a few numbers of ways to compute blood cholesterol in humans. They can be categorized into chemical tests and physical tests, as well as invasive and non-invasive. The significant ones are based on Photo Acoustic Spectroscopy, Stimulated Emission

Spectroscopy, Thermal Emission Spectroscopy, Optical Absorption Spectroscopy, Liquid Chromatography method, Chemical method, Reference method, Enzymatic Colorimetric test, Ultracentrifugation, Electrophoresis, classification of TC level using Body Mass Index (BMI), PreVu Non-Invasive cholesterol test and Impedance measurement. A few of the above important techniques, with their working principles and the merits & demerits are discussed below.

1.5.1 Near Infrared Spectroscopy (NIRS)

The principle of NIRS is that, constituents absorb IR light at their characteristic wavelength. Light is emitted into a sample using an NIR spectrometer over the optical wavelength range. The signal returned is plotted as a graph of wavelength against absorbance and is known as a spectrum. Absorbance is caused when NIR light interacts with molecular bonds (CH, OH, NH, etc), wherein the frequency of the incoming light is equal to the vibrational frequency of the bonds. The absorption level is comparative to the constituents present and hence, the contents present can be predicted. It uses a physical rather than a chemical technique. 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 fibre, for guiding the light to the tissue[27].

1.5.2 Chemical Method

In order to determine plasma cholesterol, the chemical procedure of Abell-Kendall is used which comprises of the Liebermann-Burchard response after hydrolysis and eradication of cholesterol. Plasma cholesterol & TRG content determinations are usually examined by computerized techniques at clinical research facilities. Default values for plasma TC are achieved using auto analyzer frameworks to which either the Liebermann-Burchard test or the ferric chloride–sulfuric acid technique could be applied. A fluorometric investigation is

utilized to decide the TRG reference values. Basic plasma estimations of TRGs and TC can be relied on for the analysis of the diverse lipoprotein tissues. It is an invasive method and there is wastage of chemicals in testing[28].

1.5.3 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[29].

1.5.4 Electrophoresis

Using various buffers along with electrophoretic media (agarose gel, paper, polyacrylamide gel and cellulose acetate membrane) lipoproteins can be separated. Under the control of a spatially uniform electric field, the motion of particles dispersed with respect to a fluid is called electrophoresis. Laboratories use the electrophoresis method to separate macromolecules according to sizes. The method applies a negative charge so that proteins are attracted towards the positive charge. The quantity of every lipoprotein is measured in an electrical field based on its movement. One of the electrophoresis methods requires nearly 24 hours storage of the gels prior to quantitation.

1.5.5 Ultracentrifugation

The ultracentrifugation is a method wherein a rotor spins at a very high speed as much as 2,000,000g. The analytical and the preparative are 2 types of ultracentrifuges. The lipoprotein fractions (HDL, LDL, IDL, and VLDL) can be segregated by ultracentrifugation of serum or plasma. Quantification and isolation of the lipoprotein fractions are performed, along with the estimation of TRGs, TC or different analytes to give a complete evaluation of risk of coronary illnesses. The lipoproteins can be separated based on particle density using the ultracentrifuge method. The most common technique is the flotation of VLDL at non-protein solvent plasma density (d = 1.006), with HDL, LDL and IDL existing in the bottom fraction or infranate. The next common technique involves the flotation of IDL and VLDL at a density of 1.019, attained by adding KBr to plasma or serum before ultracentrifugation[30].

1.5.6 Body Mass Index

The BMI is a value obtained from the height and weight of a person. It is defined as the weight divided by the square of the height, and is stated in units of kg/m^2, height calculated in metres and weight calculated in kilograms. A chart or table is also available to determine BMI as a function of height and weight using colours or contour lines for different categories. The BMI is an effort to measure the amount of tissue mass which include bone, fat and muscle in a person and then classify that person based on that value as obese, overweight, normal weight or underweight. The various ranges of BMI are obese: >30, overweight: 25 to 30, normal weight: 18.5 to 25 and underweight: <18.5. When BMI is high, people are at a greater risk of developing strokes, heart diseases and other serious diseases.

1.5.7 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[31]. The impedance is correlated to the amplitude of the signal. Impedance 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[32-33].

1.5.8 Enzymatic Method

An enzymatic method is used to determine cholesterol by using the enzymes horseradish peroxidase, cholesterol oxidase and cholesterol esterase along with Tninder reagent. Samples including cholesterol reagent are converted from cholesterol esters to cholesterol by cholesterol esterase. Cholesterol is oxidised by cholesterol oxidase to give out hydrogen peroxide. This reaction along with 4-aminoantipynine/phenol/peroxidise system emits a colour at 520nm. The procedure is reproducible and the results correlate well with those obtained by automated Liebermann-Burchard method.

1.5.9 Radio Frequency (RF)

The RF measurements consist of gain, spurious, harmonics, 1dB compression point, noise figure, image rejection, frequency stability and many more RF device measurements. Gain Measurement is the fraction of o/p to i/p power in dB. Spurious frequencies are undesirable frequencies at the o/p of the Device Under Test (DUT) normally with mixer devices. Harmonic frequencies are undesirable frequencies at the o/p of the DUT with integer multiples of the i/p frequency and is used for measuring harmonics. 1dB compression point is also known as gain compression point. It is the o/p power until DUT

functions well such that it does not reach saturation. Noise Figure measurement is the amount of noise given out by DUT and it has a relationship to noise temperature given as: NF = 10 log (T / 290 + 1) where NF is in dB and T is in Kelvin. The difference between 2 power levels when individual frequencies are fed at the i/p and is known as image frequency rejection specified in dBc. It exists in the receiver section of the RF transceiver. The DUT frequency which changes due to temperature and aging, is known as frequency stability measurement. It consists of 2 types: long and short term, measuring as parts per million/year and parts per million/day.

The chapter describes the various methods that the researchers have worked on over the past few years, some of which include Electrophoresis Method, Centrifugation Method, Chromatographic Method, Deuterium Incorporation Technique, Impedance Method, Spectroscopic Techniques (NIRS, RF & Raman Spectroscopy), Precipitation Method, BMI, Fluorimetric, Direct Assay, Friedewald Equation and Enzymatic Method.

2.1 Electrophoresis Method

The separation of lipoproteins is carried out using the electrophoretic techniques, in a quick and single operation according to G. R. Warnick *et al*[34]. Depending on the lipid content present, the bands can be distinguished from other constituents by means of lipophilic dyes. W. Neubeck *et al* devised a technique with phosphotungstic acid using precipitation of bands, after agarose gel electrophoresis was found to permit more consistent quantification of lipoprotein cholesterol, but gives inconsistent results in a typical lipoprotein[35]. D. R. Conlon *et al* used particular enzyme reagents, like cholesterol oxidase and esterase along with a peroxidase indicator, so as to stain electrophoretic plates, to improve the cholesterol quantification[36]. Although the method is reliable and quick, it does not give precise and accurate results for HDL-C quantitation. J. Contois *et al* used cholesterol dehydrogenase with an stable and insoluble dye, nitroblue tetrazolium chloride, which gives accurate quantification of HDL-C[37]. A colour system also gives accurate quantification of lipoproteins and HDL-C, according to P. Benlian *et al*[38]. M. Nauck *et al* specified that the separation was acceptable, when the content of apolipoprotein characterized the fractions implying no cross-contamination[39]. L. M. Baudhuin *et al* measured plasma Lp(a) cholesterol with and without ultracentrifugation, using the electrophoretic method and results were compared to Lp(a) mass and to each other[40]. Whole plasma and ultracentrifuged Lp(a) cholesterol levels showed high correlation (R = 0.964). Lp(a) mass >30 mg/dl did not have significant Lp(a) cholesterol. Whereas D. L.

Sparks *et al* measured the surface charge of lipoprotein by agarose gel electrophoresis, wherein a Bio-Rad model 702 power supply was used to apply a voltage of 100±2 V, across a distance of 5.5 cm[41]. There is a very high correlation *(r*= 0.99) between the electrophoretic mobility regions: pre-beta, beta, alpha and serum albumin region.

2.2 Centrifugation Method

A method determining cholesterol by ultracentrifugation and agarose gel electrophoresis was developed by M. Nauck *et al*, A. Sawle *et al* and D. L. Sparks *et al*. Nauck used enzymatic staining for cholesterol, which is highly accurate and reproducible. Due to the ultracentrifugation step, the method is lengthy and less sensitive than most methods, its distinctive features is used to cross refer Lp(a), according to Marz *et al*[42]. Sawle made use of iodixanol to separate and examine plasma lipoproteins, which offers an accurate, reproducible and quick method that is simple[43]. J. Jabbar *et al*, G. R. Warnick *et al*, K. R. Kulkarni *et al* worked on the estimation of cholesterol, using the centrifugation method. According to Jabbar, a positive correlation existed between HDL-C, false high TC and lipemia[44]. They suggested that the elimination of turbidity by airfuge/ultracentrifugation is less time consuming, costly and gives reliable and accurate results of HDL-C and TC. Warnick proposed that separation using ultracentrifugation is time-consuming and tedious but is highly useful in research, as the lipoproteins can be altered by means of centrifugal forces used and high salt concentrations. A microvolume VAP method having a high resolution, was developed by Kulkarni for the estimation of cholesterol[45]. However, VAP-I method needs a large volume of 1.3 ml plasma[46]. The VAP-IIfs is highly sensitive, accurate, reliable, reproducible and requires only 18 μl of plasma.

2.3 Chromatographic Method

Serum HDL-C and LDL-C were determined by J. Dong *et al,* by HPLC and ultracentrifugation without Lp[a] interference[47]. $BFC_{1.063ME(-)}$ was greater than $BFC_{1.063ME(+)}$, and hence the difference was not correlated with HDL (r = -0.093), but with Lp[a] (r = 0.848). The results of HPLC or ultracentrifugation correlate accurately with the DCM results. The method requires a less amount of sample and is simple to operate. L. Amigo *et al* separated and quantitated cholesterol carriers in bile, by ultracentrifugation, confirmed by gel filtration chromatography[48]. The method having an advantage of removal of the dilutional effect of buffers, is required for gel filtration chromatography. However N. Ayyad *et al* worked on a better ultracentrifugation method for the separation of micelles and vesicles[49]. The procedure described is quick, simple and agrees well with the gel filtration chromatography method. N. S. Ling *et al* characterized globulin which is cholesterol-bound by chromatographic and electrophoresis methods[50]. The analytical procedure is reproducible with a good standard deviation of ±5%. J. Siedel *et al* describe a method which is sensitive for quantifying cholesterol ester cleavage (≥ 99.5%) by using HPLC method[51].

2.4 Deuterium Incorporation Technique

Cholesterol is separated from lipids using thin layer chromatography. Cholesterol is removed from silica using hexane chloroform diethyl ether, in the ratio 5:2:1. It is then transferred to a combustion tube containing silver wire and copper oxide. The tube was then sealed and combustion of cholesterol to H_2O and CO_2 took place. Water was separated from CO_2 by distilling the water into the tubes containing zinc in vacuum. Under vacuum the tubes are sealed and water reduces at 520ºC for half an hour to obtain HD or H_2O gas. The deuterium incorporation method is used to measure the synthesis rate of cholesterol.

Apparently there was no specific difference between sterol balance and absorption of deuterium into cholesterol[52]. The method is simple, reliable with a correlation r=0.745 according to P. J. H. Jones *et al*. Biosynthesis of absolute and fractional cholesterol synthesis rate was measured using mass isotopomer distribution analysis and deuterium incorporation method. Correlation of synthesis rate of absolute cholesterol was r=0.79 and fractional cholesterol was r=0.84[53].

2.5 Impedance Method

A method was recommended by M. S. Mohktar *et al* to estimate the cholesterol level in blood utilizing neural network & bioimpedance techniques non-invasively[54]. Bioelectrical Impedance Analysis (BIA) estimation is executed utilizing the bio impedance analyzer. A current signal < 1mA at 50 kHz is applied to 2 black sensor cables whereas 2 red sensor cables are used to identify the signal. The measurement procedure takes approximately 3 minutes to get the results. Artificial Neural Network (ANN) methods are used and compared for calculating the high TC level in the blood. The specificity, sensitivity & accuracy were calculated by comparing the Multiple Linear 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. M.V. Malahov *et al* recommended to recognize hematological & biochemical blood parameters that can be precisely estimated by means of BIA technique[55]. Samples of blood from 46 people were poured into four test tubes. Blood (2.5ml) was collected in test tubes with Ethylene Diamine Tetra Acetic (EDTA) 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 ABC-01 "Medass" was utilized to perform BI spectroscopy of blood (1.5ml) from 5–500 kHz. Results show that the principle extracellular plasma particles: Na+ & Cl- concentrations are not related to extracellular fluid resistance of the blood. 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[56]. 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%.

E. Aristovich *et al* recommended a non-invasive impedance technique estimation of blood cholesterol by 3D finite field modelling[57]. This process supports the variation in 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. The paper prevails over the setback, by implementing a useful particle aggregation technique in FE modelling, exclusively influencing the accurateness of the field calculation. K. Cheng *et al* proposed to design a current source which includes a Voltage Controlled Current Source (VCCS), a microcontroller (μC) and a Waveform Generator (WG)[58]. The μC is used 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 kΩ. The phase difference output current varies from 0° to 19.6°, over the above mentioned frequency range. The proposed MF BI measuring system provides an inexpensive solution for BI applications. During system testing, the output current signal is constant. H. S. H. Matsumoto *et al* proposed a simple non-invasive technique to measure cholesterol by using a solvent to extract the skin component[59]. A Self-Assembled Monolayer (SAM) sensor and a HPLC are utilized to analyze the extracted solution. The strong attraction towards hydrophobic cholesterol gives the SAM electrode high responsiveness & sensitivity. Higher cholesterol is shown by the person with high cholesterol of the skin. The coefficient of correlation of non-invasive & invasive method is 0.9408, hence this method could be used practically. Table 2.1 gives a list of instruments based on bio-electrical impedance.

Table 2.1: List of Instruments Based on Bio Electrical Impedance

Sr. No.	Name	Measured at	Description	Used to find
1	Matron BF906 Body Composition Analyzer - Body Fat Analyzer	This portable, battery operated, tetrapolar method of 4 electrodes which are applied to the right side of the body on the hand, wrist, foot and ankle.	Bio-electrical, Impedance Analyzer Frequency: 50Khz, Resolution: Measures body fat in increments of 0.1%, Impedance Range: 200-1000 Ohms.	Body Fat %, Body Fat Weight, Target Fat %, BMI, BI, BMR, Target Weight (min / max), Lean Weight, Lean %, Water Litres, Water %, Target Water.
2	Maltron Body Fat Analyzer 900	Enter personal details into the unit and place electrode pads on one hand and one foot for instant results.	BI Analyzer, Frequency: 50Khz, Resolution: Measures body fat in increments of 0.1% , Impedance Range: 200-1000 Ohms	Body Fat% , Target Fat% , BMR, Body Impedance
3	Alkaline's VLA Bioimpedance Analysis	A low voltage electrical current	Alkaline VLA ™ is a completely non-invasive tool for measuring your bioimpedance, or your body composition.	Fat and muscle, ideal fat to muscle ratio and weight for the frame, Hydration levels, quality of muscle tissue, cellular age, level of risk for metabolic disease
4	Omron HBF-306 Body Fat Analyzer	50 kHz and 500 µA are used.	Body fat % (4.0 to 50.0%) BI Method, BMI (7.0 to 90.0), BMI classification.	Body fat volume and percentage by weight.

5	ImpediMed SFB7 Body Composition Analyzer	The ImpediMed SFB7 is a single channel, tetra polar bioimpedance spectroscopy (BIS) device that scans 256 frequencies between 4 kHz and 1000 kHz.	Highly Accurate Body Composition Analysis, Readings in Less than a Second, Advanced Options - User Definable Hydration Coefficient, Full Access and Disclosure of all Raw Data.	Fat-Free Mass, Fat-Free Mass %, Fat Mass, Fat Mass %, Total Body Water, Total Body %, ECF, ECF %, ICF, ICF %.
6	Tanita BC1000 with D1000 Wireless Display	The BC1000 Body Composition Scale offers consumers the ability to wirelessly link data to remote displays, such as a PC.	Ant+ Wireless Connectivity, Healthy Edge Software, Garmin Compatible, Forerunner & Edge 800, Compatible with Tanita D1000 Wireless Display, 200kg Weight Capacity.	Total Weight, Body Fat %, Muscle Mass, Total Body Water, Bone Mass, Physique Rating, BMR, Visceral Fat, Metabolic Age.
7	Tanita BC545 Body Composition Scale	The measured resistance to the flow (BIA) is fed into researched equations to provide accurate and personalized body composition readings.	8 Electrodes Segmental BIA Technology, Advanced Dual Frequency (ADF), 5 Segmental Fat Readings, Athlete and Guest Mode, Adult and Child Healthy Range Indicator, 150kg Weight Capacity.	Total Weight, Body Fat %, Total Body water %, Muscle Mass, Bone Mineral Mass, Daily Calorie Intake, Physique Rating, Metabolic Age up to 80 years, Visceral Fat, Visceral Fat Indicator.

2.6 Spectroscopic Technique

2.6.1 Near Infrared Spectroscopy

An SEC of 0.36mmol/L and a correlation of r=0.92 are obtained according to J. W. Hall *et al* by using a linear equation with wavelength for cholesterol at 1674nm and TRGs at 2330nm[60]. A model of 8 factors consisting of the spectrum segments1635-1800 nm and 2035-2375 nm produces an SEC of 0.18mmol/L and a correlation r=0.99 for the PLS calibration. The description of atherosclerotic plaques by using the NIRS techniques was started by L. S. Cassis *et al*[61]. During surgery, NIRS was used to image the content of lipid in plaques according to R. J. Dempsey *et al*[62]. NIR scan was performed ex vivo on the plaque after surgery and spectra predicted the composition of lipoprotein, also cross verified by gel electrophoresis. NIRS was also used to determine content of cholesterol also determined by HPLC method wherein a correlation of r=0.96 was observed by W. Jaross *et al*[63]. NIRS was also used to accurately measure the cholesterol to collagen ratio reported by V. Neumeister *et al*[64]. If a fibre optic probe is fitted on a NIR spectrometer, the correlation increases in comparison to ex vivo measurements, in a recent study by J.

Wang *et al*[65]. In order to identify chemical composition of plaques, J. D. Caplan *et al* used NIRS which had 88% sensitivity and 79% specificity[66].

TC was measured by S. Shimawaki *et al* using a spectrophotometer and a cholesterol kit[67]. Low cholesterol and high cholesterol groups showed a significant change in brightness. The correlation of low and high cholesterol levels is r=0.449. K. Z. Liu *et al* determined HDL-C and LDL-C concentrations using IR spectroscopy[68]. The SE between clinical laboratory assays and IR-predicted values was 0.15 mmol/L for HDL-C with r=0.91 and 0.22 mmol/L for LDL-C with r=0.98. The drawback for routine use is the cost factor as barium fluoride windows are used. Cholesterol was estimated by H. Z. Gao *et al* in serum without any reagent using iPLS and NIRS[69]. A PLS model having high r=0.975 a low RMSEP is selected in the cholesterol region of 1688-1760nm. J. Jiang *et al* studied the outcome of cholesterol on the measurement of glucose through NIRS and OGTT[70]. The coefficient of Pearson's correlation for the diabetic group −0.8440 and Sig. is 0.0722 suggesting that the correlation is good. Hence in conclusion, a negative correlation between cholesterol and glucose concentration exists. E. Peuchant *et al* determined cholesterol using NIRS which is a reproducible and accurate method without the use of reagents[71]. The results have a correlation of r=0.963 using the reference method and r=0.985 using the enzymatic method.

2.6.2 Radio Frequency Method

A measuring system which includes an IMD with an RF signal source and a transmitting antenna to generate and transmit RF signals, in a subject body, surrounded by tissue was developed by H. Abrahamson *et al*[72]. In order to monitor change in blood fats and cholesterol an IMD can be used through RF measurements. It has many advantages over the optical and enzymatic which are used by sensors that are implantable. Even if tissue

grows around the RF antennas and IMD, it is still effective over a large period of time. It can acquire tissue constituents and blood measurement without extracting blood from individuals and does not require an uninterrupted connection between the tissue and antennas. It works in the range from about 1 MHz - 1 THz. I. A. P. Nazareth *et al* developed an algorithm based on regression technique to estimate blood cholesterol[73]. As the frequencies increases, the attenuation also increases at 575MHz, 995MHz, 1145MHz, 1285MHz and 2185MHz. The blood constituents have unique spectra and in order to find out unknown value of blood constituents like cholesterol, urea, glucose, etc., the data is fed to a PLSR model.

2.7 Raman Spectroscopic Method

Cholesterol determined by Raman spectroscopy was found to be consistent with excellent repeatability and good method to get a non-destructive analysis according to P. Weinmann *et al*[74]. D. Qi *et al* reported that the TC had an improvement, with a lower RMSECV, accurate prediction of samples and a good correlation between reference and predicted concentrations[75].Blood was also analyzed using PLS prediction and it took <1second to predict the concentration of an unknown sample[76] J. Bood *et al* reported IR absorption and Raman spectroscopy diagnostics of tissue[77]. The Raman spectra of NADH and purified cholesterol were recorded as 150 seconds integration time, 1200 g/mm grating and 792.5 nm excitation. The cholesterol spectrum exhibits an intense absorption band at 7225 cm^{-1}[78]. A skin composition analyzer was used to measure content present in 10 μm depth in the skin. Around 17,000 Raman spectra were used to reveal alterations in the amounts of skin constituents such as ceramides, cholesterol, NMF, lactate and urea of which NMF decreases with age and the rest increases. Raman spectroscopy was used to analyze blood cholesterol which has a correlation between the reference and the predicted as r=0.66 according to A. M. K. Enejder *et al*[79].

2.8 Precipitation Method

The precipitation methods were able to achieve precise and reliable results with the targets set by NCEP, as shown by their ability in laboratories specializing in lipids, but not in routine laboratories, because of manipulations which includes pipetting[34]. J. Aufenanger *et al* worked on the precipitation of the lipoproteins. The disadvantages include, 3 hours for precipitation time and 80 minutes for electrophoresis time. P. N. M. Demacker *et al*, assessed 6 precipitation methods for determining HDL-C: Tung-B, Tung-L, Hep, PEG/Dex, Dex and PEG method[80]. The PEG/Dex and Tung-B precipitation methods have less precipitation/sample ratio < 0.4. Similar values were given out by the PEG/Dex, PEG, Dex and Tung-B methods. A total error of 10.6% was shown by the Tung-B method in comparison with the HDL-C method. It is a cost-effective and reliable method for routine analysis. The turbidity was hardly seen with Mg^{2+} precipitation than with the Heparin-Mn^{2+} precipitation method according to M. F. L. Virella *et al*[81]. Mg^{2+} precipitation is reproducible, very stable, simple and does not require expensive equipment. The correlation between the 2 precipitation methods is r = 0.95. To analyze LDL-C & HDL-C, J. Jabbar *et al* compared the fully automated enzymatic and precipitation manual method, which provided accurate, precise and reliable results[82]. The fully automated method needs less labour and provides a high throughput.

2.9 Body Mass Index

A relationship between ABI, LDL, adult cholesterol and childhood BMI was studied by M. E. Sundaram *et al*[83]. The 2008-09 BMI z-score associated positively with an increase in serum LDL and ABI score. BMI of 1982 & 2008-09 had a correlation of r = 0.56 in males and r = 0.58 in females. M. Faheem *et al* also made a study whether BMI would influence cholesterol[84]. Cholesterol gave a positive correlation r = 0.205 with BMI when bivariate

correlation analysis was done. Western studies confirmed the results[85-88]. Studies performed in Asia also gave a positive correlation[89-90]. However some studies gave mixed results[91]. The correlation between BMI and lipid profile was studied by H. S. Sandhu *et al*[92]. A significant positive correlation in males and significant negative correlation in females was found for TRGs. A negative correlation was found in males for HDL-C whereas a negative correlation was found in females in LDL-C. BMI increased with decrease in HDL-C and increase in TRG according to K. C. Maki *et al*[93]. A good relationship was noticed between LDL-C, BMI and cardio respiratory fitness[94]. I. Janssen *et al* was of the opinion that waist circumference and BMI contribute independently to the estimation of visceral and subcutaneous fat[95]. Cholesterol levels increased with increase in BMI in males as well as females. As levels of BMI increased, mean levels of HDL-C decreased and low HDL-C increased as studied by C. D. Brown *et al*[96].

2.10 Automated Fluorimetric Method

In order to determine serum lipoprotein and serum cholesterol, G. Robertson *et al* proposed an automated fluorimetric method to estimate cholesterol[97]. The method was initially originated by R. W. Albers *et al*[98], it was then modified by K. J. Carpenter *et al*[99] and D. B. McDougall *et al*[100]. The method suggested by Carpenter was further automated by A. Antonis *et al*[101]. The method is highly sensitive and fairly specific, necessary for accurate estimation of low cholesterol concentrations. High emission when stimulated with 365 mμ light gave blue fluorescent light, activated at 546mμ and measured red-orange fluorescent light at 590mμ gives high specific cholesterol. A back reservoir is not needed and there is a marked gain in the sampling rate of 60/hour instead of 30/hour with the automated system and using a sampler II. The method is reproducible, precise and has a good degree of correlation compared to the manual methods used in the laboratory.

2.11 Direct Assay Method

W. G. Miller *et al* estimated LDL-C and HDL-C, by comparing 7 different direct assay methods with ultracentrifugation[102]. Due to the need for specificity towards unusual lipoproteins, all the methods failed. W. J. Korzun *et al* measured LDL-C and HDL-C by 8 direct assay methods[103]. 25% of the frozen pools were commutable for nearly all the LDL-C methods, for diseased as well as non diseased patients. On the other hand, all the frozen pools were generally commutable for each and every HDL-C methods, for diseased as well as non diseased patients. S. Sahu *et al* used Friedewald's equation along with a direct homogenous assay kit, to measure HDL - C and LDL-C[104]. The Standard Deviation and mean of LDL-C, showed a major difference at TRG, in the lower ranges. The correlation was r=0.88, between direct assay estimation and Friedewald's equation. 5 LDL-C methods, correlated well with precipitation / ultracentrifugation, with the correlation r ranging from 0.93 – 0.95 as studied by E. Bairaktari *et al*[105]. The direct assay and Friedewald equation were hardly affected by high LDL-C levels, and thereby they showed a higher negative predictive value and higher sensitivity. LDL-C and HDL-C were estimated using direct methods and LDL-C was estimated using Friedewald's equation, a study made by C. M. M. Cordova *et al*[106]. The 2 methods had a good correlation with each other. The Friedewald formula was more positively biased to the direct method and more asserted with > 201 mg/dL TC levels. The Friedewald formula was negatively biased with TRG levels from 301 – 400 mg/dL. Plasma LDL-C was estimated by W. T. Friedewald *et al*[107]. The large % errors in Type IV patients are due to the fault of the laboratory errors in the ultracentrifugation procedure and not the inaccuracy of the direct assay method. H. Fei *et al*[108] compared a detergent assay LDL-C and a polyethylene/cyclodextrin assay. The concentrations of LDL-C attained with detergent assay were higher than the other.

Y. Iwasaki *et al* examined the performance of a New-Daiichi assay method for LDL-C and evaluated the results with those obtained by Denka-Seiken assay[109]. The New-Daiichi assay is reproducible, stable and linear. In some patients, cholesterol was detected by both methods, but the New-Daiichi assay method is more specific and less reactive for LDL-C. S. Usui *et al* studied the differential reactivity of VLDL and LDL subfractions to LDLd (Daiichi Pure Chemical) and LDLk (Kyowa Medex)[110]. The LDLk (x) and LDLd (y) methods highly correlate for entire serum samples (r = 0.966). The LDLk method has lower reactivity (88.4-92.0%) than the LDLd method (95.6-98.7%), but poor recovery is shown by both methods. P. Bayer *et al* evaluated 4 direct LDL-C assays namely Wako, Kyowa, Denka Seiken and Daiichi[111]. When compared with a BQ assay, a very good correlation was found for the 4 methods. Wako, Kyowa and Daiichi methods showed a positive correlation. However the reliability of the Denka Seiken method was the best amongst the rest. Roche and Wako are 2 direct methods to estimate LDL-C according to M. E. Salán *et al*[112]. Both the methods are precise but showed error of 7.72% and 4.46% respectively. The Wako method had a high correlation of r=0.966 whereas the Roche method had a lower correlation of r=0.929.A study was conducted by N. Rifai *et al* comparing 3 laboratories and using various analyzers, showed a correlation r = 0.99[113]. The direct HDL-C assay also agreed well with the precipitation / ultracentrifugation method (r = 0.98). M. Nauck *et al* assessed a liquid assay for the direct estimation of HDL-C[114-115].The correlation between the precipitation method and the liquid HDL-C assay was r = 0.992. The method is robust, comparable and precise. The analytical performance of precipitation Spinpro, N-geneous of HDL-C assays was reported by N. Harris *et al*[116].All results were highly correlated with the reference procedure ($r \geq 0.96$). M. L. A. Peña *et al* reported the performance of 4 HDL-C assays: Kyowa, Daiichi, precipitation and ultracentrifugation procedures[117]. The homogeneous HDL-C assays correlated well with

ultracentrifugation and precipitation methods. The accuracy, precision and specificity of the Kyowa method were better than the Daiichi method. J. M. Simó *et al* evaluated the homogeneous assay for estimating HDL-C and compared it with a precipitation method[118-119]. The performance of the method is adequate with respect to detection limit, linearity, accuracy and precision.

2.12 Using Friedewald Equation

The Friedewald equation is not applicable to samples wherein TRGs are greater than 400mg/dL, non fasting patients or patients with Type III chylomicronemia or hyperlipoproteinemia. When TRG increases from 200 - 400 mg/dL, the equation becomes inaccurate[120-121]. The Friedewald formula miscalculated LDL-C by around 10% and agreed within 10% of BQ[122]. A poor correlation was seen between ultracentrifugation and the Friedewald equation[123]. LDL-C was underestimated using the Friedewald formula according to H. Scharnagl *et al*[124]. The more the negative bias, the LDL-C concentration decreased when compared with BQ. A study by L. Baruch *et al* described disagreement between results measured with a direct LDL-C assay and Friedewald equation, but >25% of the results varied by around 30mg/dL, in spite of a good correlation[125].The performance goals were reviewed by J. H. Contois *et al* for lipoprotein and lipid testing and established that the bias %, precision % & total error % for HDL-C was ≤ 5, ≤ 4 & ≤ 13 for LDL-C was ≤ 4, ≤ 4 & ≤ 12 and TC was ≤ 3, ≤ 3 & ≤ 9[126].

2.13 Enzymatic Method

In order to measure esterified and cellular free cholesterol, P. Robinet *et al* determined a simple, high-throughput, precise and sensitive enzymatic method[127]. By pretreating the solution with catalase, the sensitivity increased in this method. Using cholesterol reagents, P. Srisawasdi *et al* created user-defined methods to estimate the analytic performance of a

kinetic or an end point method, for the determination of cholesterol enzymatically[128]. Lower reagent cost and good precision are the benefits of the end point method. The kinetic method requires lesser analysis time and shows reduced interferences. J. D. Belcher *et al* proposed a micro-enzymatic method to estimate triglycerides and cholesterol, separated by ultracentrifugation in lipoprotein subfractions[129]. It requires 200 µl of serum, but the equipments are costly. The method has a sensitivity and detection limit < 2 mg/dl and imprecision and accuracy <3%.

J. Aufenanger *et al* and I. R. Kupke *et al* estimated cholesterol enzymatically which was separated electrophoretically. Aufenanger found that disadvantages of the method are, 3 hours precipitation time and 80 mins electrophoresis time[130]. The advantages are lack of interference of proteins, independence of actual components of lipoproteins and high specificity. Kupke discovered that the recovery was 98-100% and the precision of estimating cholesterol was excellent with a correlation of r=0.943[131]. C. C. Allain *et al* described an enzymatic method by means of a single reagent to estimate cholesterol[132]. The method has better specificity, is reproducible, has very good precision and correlates well. L. Lillienberg *et al* tested the effectiveness of enzymatic or GLC method to determine cholesterol. The enzymatic method gave 2% lesser value than the GLC method which in turn gave 12% lesser value than the colorimetric method[133]. The summary of cholesterol measurements methods are given in Table 2.2.

Table 2.2: Summary of Cholesterol Measurements Methods

	Method	Principle	Advantages	Disadvantages
Invasive	Reference method	Chain of chemical reactions followed by colour intensity measurement	Very accurate	Time-consuming, not suitable for automation
	Ultracentrifugation	Ultraviolet light absorption test after high speed centrifugation	Accurate	Low capacity, time-consuming
	Enzymatic colorimetric test	Chain of chemical reactions followed by colour intensity measurement	Accurate	Is a low priority test performed in hospitals
	Electrophoresis	Electrophoretic separation followed by product staining and colour intensity analysis	Reliable, accurate	Time-consuming, not suitable for automation
	HPLC	Lipoproteins are separated in accordance with their mass, then Ultraviolet absorption measured	Reliable	Needs ultracentrifugation prior to chromatography
	Accutrend Plus	Chemical reaction producing colour on a test strip	Portable system, quick results	Indicates cholesterol level as "high" or "low"
Non invasive	Classification BIA	Rule based system	Non-invasive	Low accuracy
	PreVu	Chemical reaction results are measured with portable spectrometer	Non-invasive	Measured cholesterol levels do not correlate with serum cholesterol

2.14 Cholesterol Kits

Since cholesterol kits help to measure total cholesterol invasively, it is advantageous to own one, so as to check the level at least once in a way. Table 2.3 shows the list of cholesterol kits available.

Table 2.3: List of Cholesterol Kits

Sr. No.	Name	Tests for
1	Complete Blood Cholesterol Levels at-Home Test	Detailed analysis of good and bad cholesterol, measures the total blood cholesterol levels
2	Lipid Profile (LP)	Measures cholesterol levels, no doctor's referral necessary.
3	Statin & Cholesterol Check Complete Package - Laboratory Blood Test	Checks for possible liver damage caused by cholesterol drugs
4	Cholesterol Full Panel Test Kit - CheckUp America Home Access FDA Cleared Home Cholesterol Testing	Tests for Complete Panel: Total Cholesterol, HDL, LDL and Triglycerides,
5	CholesTrak - Cholesterol Home Testing Kit	The test gives an estimate of the amount of cholesterol in the blood.

6	Boots Cholesterol Home Test Kit	The test gives an estimate of the amount of cholesterol in the blood.
7	Cholesterol & HDL Cholesterol Test Kit	Total & HDL Cholesterol. No interference from Bilirubin up to 20 mg/dl, Haemoglobin in up to 20 gm/dl, Triglycerides up to 1000 mg/dl.
8	Sterol Skin Cholesterol Analysis PREVU* Test	Measures cholesterol levels
9	CHEMCARD® TEST KIT	Measures cholesterol levels
10	Cholesterol Home Scan Testmedica	Measures cholesterol levels
11	CHOLESTECH LDX LIPID CONTROLS	Cholesterol (TC), Triglyceride (TRG), HDL, Glucose, Alanine Transaminase and Aspartate Transaminase
12	CHOLESTECH LDX MULTI-ANALYTE CONTROLS (TRIPLE PACK)	TC, HDL, TRG, glucose, alanine aminotransferase and aspartate aminotransferase test procedures
13	CHOLESTECH LDX/HEMOSENSE INRATIO2 KITS	Measures cholesterol levels
14	Accutrend Plus Cholesterol Meter	For the determination of total cholesterol and glucose in capillary whole blood.
15	Q. Steps Biometer Cholesterol Biometer Glucose Monitoring System	Measures cholesterol and glucose levels
16	CholesTrak HDL & Total Cholesterol Testing Kit	TC ,HDL cholesterol
17	ADA CheckUp America American Diabetes Association Cholesterol Panel	TC, HDL-cholesterol, Triglycerides, LDL-cholesterol.
18	CardioChek PA Lipid Panel	Measures total cholesterol, HDL and triglycerides. Calculates LDL cholesterol and TC/HDL ratio.
19	CardioChek PA Glucose-Cholesterol Test Strips	Measures total cholesterol and glucose
20	CardioChek PA Combo Cholesterol-HDL Test Strips	Measures total cholesterol and HDL cholesterol, calculates TC/HDL ratio
21	CardioChek PA Cholesterol, HDL, Glucose Test Strips	Measures total cholesterol, HDL, glucose and calculates TC/HDL ratio
22	CardioChek Cholesterol Starter Kit	Measures cholesterol levels
23	CardioChek Home Cholesterol Analyzer Promo	Self-monitoring cholesterol, triglycerides, HDL cholesterol, glucose or ketones.
24	PRIMA Cholesterol and Triglycerides 2 in 1 Home Test/Meter Kit Monitoring System FDA Approved.	Prima home test meter is designed to self-test cholesterol and triglycerides in the comfort of the home and its Very Accurate quality made Meter.
25	EasySure Cholesterol Test Strip	Measures Blood Glucose, Cholesterol & Haemoglobin levels
26	SELF Check Cholesterol Level Test	Measures cholesterol levels
27	Easy Touch Cholesterol Test Strip	Measures cholesterol levels
28	Prima Home Test Cholesterol Test Kit - Pack of 2 Test	Measures cholesterol levels
29	CHEMCARD™ CHOLESTEROL	Measures cholesterol levels
30	Easymate 3 in 1 Glucose, Cholesterol and Uric Acid Test Kit	Cholesterol, glucose, uric acid

2.15 Objectives

Since strokes and heart attacks are the major cause of deaths in the world it is of utmost importance to develop an instrumentation using advanced microelectronics circuits, which is programmable and having interpretation mechanism to enable a common man to know the level of cholesterol. Due to increase in fear and hence negligence on the part of the patients, it is necessary to develop a non invasive instrument, to determine the level of cholesterol. Hence this research study has been undertaken, with the main objective in mind, of developing a non invasive instrument to measure cholesterol, which would be inexpensive, measure quickly with increased accuracy and user friendly.

Thereby the objectives of this research are as mentioned below:

1. To design a dielectric cell to test the major constituents found in human blood.
2. To use multivariate system approach to enhance cholesterol signature in DSP domain.
3. To develop an easy method to measure the level of cholesterol that would be portable, less time consuming and inexpensive.
4. To make the system user friendly by displaying it on a LCD.

Various techniques such as ultracentrifugation, electrophoresis, spectroscopy, impedance, etc. were discussed in the previous chapters, for the measurement of cholesterol in blood. The current work is based on RF spectroscopy and various designs based on 4 probe method and by using a dielectric cell, were setup which are discussed in the following sections.

3.1 Design Using 4 Probe Method

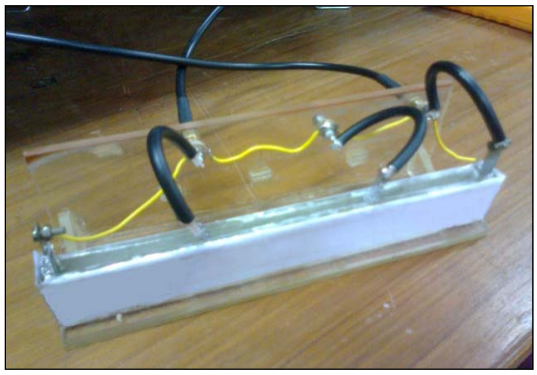


Fig. 3.1: Actual 4 Probe Measurement Cell

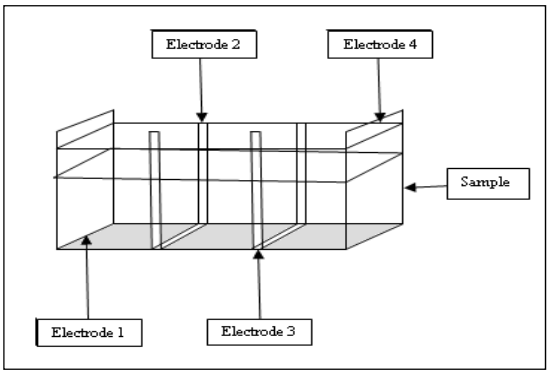


Fig. 3.2: Schematic of 4 Probe Measurement Cell

The measurement cell shown in Fig. 3.1 is designed based on the 4 probe method having dimensions, 9" x 1.5" x 1.5". At electrode 1, the signal is injected and the transmitted signal through the liquid column is seen at electrodes 2 & 3 as shown in the schematic in Fig. 3.2. The difference of the signal at 2 & 3 gives the absorption in the region of 2 & 3 liquid column. The 2 inner electrodes (electrode 2 and electrode 3) are made of Stainless Steel (SS) plates which are kept at a distance of 3 inches. SS sheet is used to avoid the localized pitting corrosion leading to the creation of small holes on the electrode surface. Initially the experiments were performed and there was a loading effect. This drawback was overcome by reducing the cross section area, by introducing a number of plates, thereby increasing the impedance accordingly. RF cables along with SMA connectors conforming to RG-58/U were used to connect the cell to a plastic container, in order to reduce the mechanical disturbances and reflection losses. The entire cell was placed in a

heavy metal box with an iron lid, in order to reduce the electrical disturbances. The metal box and the lid were individually grounded. The experimental setup is seen in Fig. 3.3.

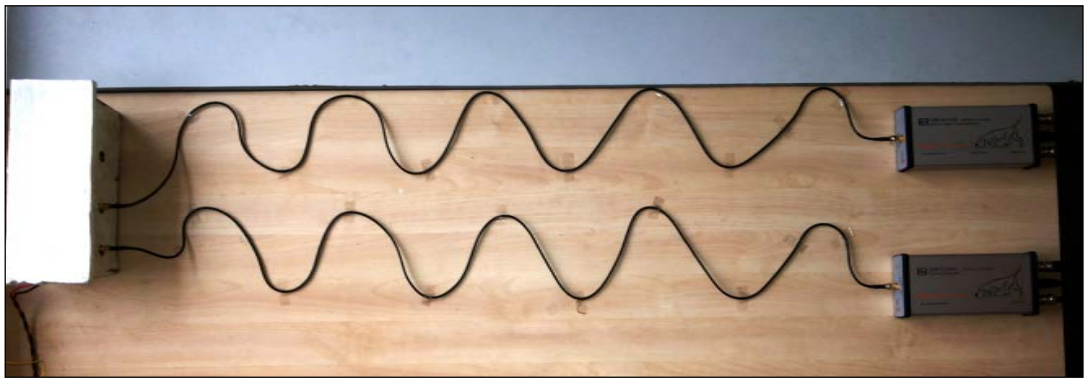


Fig. 3.3: Experimental Setup Using 4 Probe Method

3.2 Design of Dielectric Cell

The frequency response for the test sample using the 4 probe method was unstable. The attenuation of the signal is due to the presence of a large number of signals present in the environment or due to the cable length. The wavelength at high frequencies becomes comparable to the length of the cables being used in designing the cell. Due to its radiation into free space, a loss is experienced, thus giving an unstable response. Taking all of the above into consideration, a new cell was constructed based on the dielectric principle. The dielectric property of a material establishes its performance, under the influence of electric field. The speed of propagation of an EM wave depends on the EM characteristics of a medium and is controlled by 2 factors: the electrical permittivity and the magnetic permeability. The dielectric property of a biological tissue, results from the interaction of EM radiation, with its components at the molecular and cellular level.

A rectangular shaped cell is required to determine the RF responses of different blood constituents. In order to design the cell, two acrylic sheets also known as plexiglass are required, the dimensions being 12.5cms x 5cms and 2cms x 2cms. The first acrylic sheet is cut into three parts: two sheets of 12.5cms x 2cms and one sheet of 12.5cms x 1cm. A

scribing knife is used to score the sheet. The sheet is held firmly in place and a scriber is drawn along a straight line, several times. The sheet is then clamped at the end of a table. A sharp downward pressure is applied to break the sheet. A sand paper is used in a circular motion to smoothen any sharp edges. The sheet is then polished by moving it back and forth, across the buffing wheel, without applying too much pressure in order to prevent heat buildup. The other acrylic sheet is then cut into 2 halves of 2cms x 1cm each, in a similar manner. The acrylic sheets are placed together to make an open box (cell). A sheet of 12.5cms x 1cm is placed at the base, 12.5cms x 2cms are fixed on the sides of the box & the smaller sheets of 2cms x 1cm as the other sides, in order to make a cell of 12.5cms length, 2cms height and 1cm breadth as shown in Fig. 3.4. The cell is fixed together using carbon tetrachloride and is tested for leakage by filling the entire cell with water and leaving it overnight. Next, two holes of 0.5cm diameter are drilled in the side supporting plates, for connector 1 & connector 2, in order to fix the 2 SMA female connectors. These connectors are next soldered to a gold wire, placed in the centre of the cell and are stuck individually to supporting plates for connector 1 & connector 2 using araldite. Subsequently, two more holes of 1mm wide are drilled in the plates 1 & 2, to pass the central wire through them. The central wire is also fixed between plates 1 & 2, using araldite. Once again the cell is filled with water and tested for leakage overnight. The cell can hold 15mL of liquid.

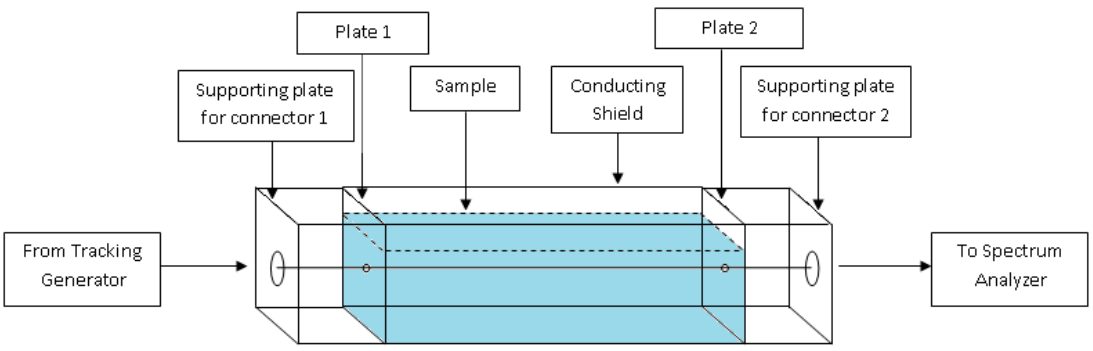


Fig. 3.4: Cell Design

3.3 EMI Isolation Box

Next this cell needs to be shielded from the EM radiations. It is covered entirely with a thin copper foil on the outside and a thin gold foil on the inner side, using superglue and the copper & gold foils are then individually grounded. An EMI isolation box is constructed having dimensions 20cms x 6cms x 6cms. The box is constructed using 4 plates of 20cms x 6cms, 2 plates of 6cms x 6cms and a hinge for the lid. The entire box is welded and the cell is then placed in this EMI isolation box and covered with the lid. The EMI isolation box is then grounded. This forms the dielectric loss cell.

The cell is then connected via RF cables to the tracking generator and signal analyzer. The RF cables are 10cms in length and have connectors at both the ends which are "MX SMA male to male". It is a coaxial cable, having screw type device with 50Ω impedance. The cable has good electrical performance between DC and 18GHz. The connectors have a high-performance, excellent durability and are also compact.

The characteristics of the connectors are as follows:

- The connectors cannot de-couple in applications having intensive vibrations, since the cable has a threaded interface along with a coupling nut.
- A strong signal is obtained since the cord has a clean connection.
- As cable trimming is expensive and laborious and considering the delays involved phase adjusted connectors ensure effortless screw adjustments.
- In order to prevent corrosion the connectors are plated with 24K gold.

The EMI isolation box along with the cell, the tracking generator and the signal analyzer, are placed on the wooden plank separately and screwed on securely, in order to prevent any mechanical movements as shown in Fig. 3.5. The tracking generator and the signal analyzer are connected to the PC via USB 2.0 cable in order to power them on. Next one

end of the BNC cable is connected to the ‘TG Sync’ of the tracking generator and ‘Self Test/Sync Out’ of the spectrum analyzer, for the fast sweep. It is also used for spans greater than 100 kHz. Another BNC cable is used for the slow sweep and is connected to the ‘10 MHz Ref In/Out’ of the tracking generator and the other end to the ‘10MHz Ref In’ of the spectrum analyzer. Initially the spectrum analyzer and tracking generator are connected nose-to-nose, using the SMA barrel (SMA male to male connector) and the spike software is launched.

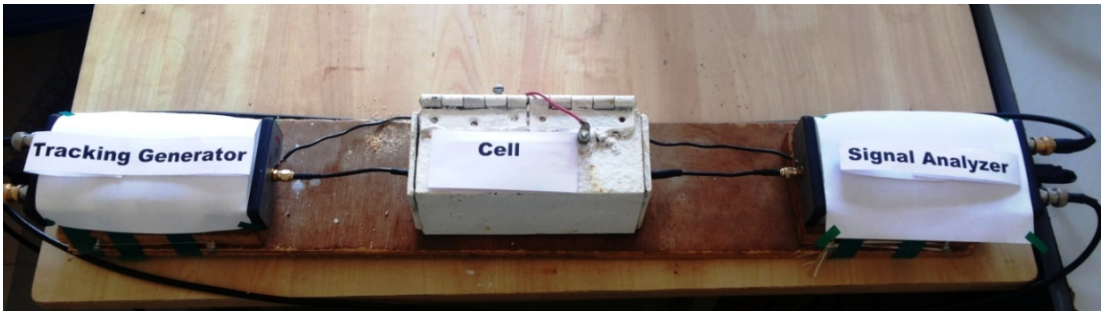


Fig. 3.5: Experimental Setup

The start, stop, center and span frequencies are set to the necessary sweep range and reference level is set to +10dBm. The 0 insertion loss line will appear one section from the peak point of the graticule. Then the sweep size is selected in order to establish the step size. While the tracking generator is connected to the spectrum analyzer, nose-to-nose, await the total sweep to terminate. Then the ‘Store Thru’ button is selected which will rectify the amplitude offsets linking spectrum analyzer to tracking generator which produces a steady line at 0dB.

Now insert the cell to test and take measurements. Tracking generator injects a signal at connector 1, which then passes through a copper wire in the liquid column and observed at connector 2. Experiments are carried out in slow and fast sweeps at a certain time. The results are then cross verified by repeating the experiment after an hour and a 2 hour gap, in order to nullify the environmental effect and are found to show good repeatability. The

measurement is performed wherein a Signal Hound USB-SA44B spectrum analyzer and a USB-TG44A tracking generator are used. The MF BI spectrum are modeled through curve-fitting and multivariate statistical applications, to predict the main blood constituents like cholesterol, urea, glucose, lactate and alanine.

3.4 Preparation of Samples

Human blood consists of over 100 constituents. The major components of blood are cholesterol, glucose, urea, alanine and lactate. The cholesterol in the normal human blood is 150-250mg/dL, urea ranges from 10-20mg/dL, glucose ranges from 70-110mg/dL, lactate ranges from 10-15mg/dL and alanine ranges from 10-20mg/dL. The blood constituents have to be scaled down to 15mL since the cell can hold only 15mL. Hence the experiment is performed with average concentrations of urea 2.25mg/15mL, of glucose 13.5mg/15mL, of lactate 1.9mg/15mL, of alanine 2.25mg/15mL and of cholesterol 34mg/15mL. Constituents are measured, using a weighing machine having an error accuracy of 0.01mg, as shown in Table 3.1.

Table 3.1: Constituents of Different Concentrations Used in the Experiment

Conc. Of Samples	Cholesterol mg/15mL	Glucose mg/15mL	Urea mg/15mL	Alanine mg/15mL	Lactate mg/15mL
0.5	17	6.8	1.13	1.13	0.95
0.75	25.5	10.15	1.69	1.69	1.43
1	34	13.5	2.25	2.25	1.9
1.25	42.5	16.85	2.81	2.81	2.38
1.5	51	20.3	3.38	3.38	2.86
1.75	59.5	23.65	3.94	3.94	3.33
2	68	27	4.5	4.5	3.8
2.25	76.5	30.35	5.06	5.06	4.27
2.5	85	33.7	5.62	5.62	4.74
3	102	40.5	6.75	6.75	5.7

Experiments are conducted with the above constituents. Samples are prepared using 14mL distilled water, 1mL alcohol and the above constituents in varied concentrations. Alcohol is used since cholesterol does not dissolve in water. The average concentration of a

constituent is denoted as ‘1’, half the average is denoted as ‘0.5’ and ‘2’ for twice the concentration and so on. The experiments are conducted with various concentrations as well, which are over the standard range & for extreme cases. All the constituents are measured carefully and combined.

Measure 14mL of water using a measuring cylinder. Add 1mL alcohol to it and pour this mixture into the cell. Close the lid of the EMI isolation box and start the software as discussed in section 3.7. Next open the lid of the box and extract the liquid using a syringe and wash the cell using distilled water. Next, a combination of the major constituents in a certain percentage, are taken in a beaker and dissolved in 1mL of alcohol added to 14mL of water and stirred for some time. This mixture is poured into the cell and the lid is closed. Start taking the readings and save the results. Then open the box and wash the cell thoroughly with water. Continue doing so until there are no traces of any residue. Repeat the procedure with 14mL water with 1mL alcohol to compare it with the initial reading. Once it is constant proceed to the next combination. Repeat the above steps till the last combination is completed. Scalar network analyzer is used to obtain attenuation data of these solutions.

3.5 Network Analyzers

Slotted line in microwave measurements have a disadvantage of performing measurement of phase and amplitude limited to one frequency. More time is spent to perform broadband measurement of frequencies over a wide range. With the designing of network analyzers which measures phase and amplitude over a short time, the problem is solved. An accurate reference signal at the initial setup requires to be generated. With this reference signal, phase and amplitude of the emitted signal of the DUT is assessed.

A general network analyzer consists of 4 parts as shown in Fig. 3.6[134]. They are as follows:

i) **Signal Source:** The signal is produced by the signal source by which DUT is stimulated. DUT reacts by transmitting a fraction of the signal and reflecting the remaining. The response frequency of the DUT can be verified by sweeping the source frequency. There are 2 major kinds of sources i.e. synthesized signal generator and sweep oscillator.

ii) The transmitted, reflected and incident signal are separated by the **separation device**. The measurement of the phase and amplitude signal can be performed and differences can be determined. This can be done with the use of high impedance probes, bridges, power splitters or directional couplers.

iii) In order to simplify the process microwave frequencies are converted by the receiver to permit exact measurement. There are 3 major **receiver techniques** i.e. diode, harmonic mixing and fundamental mixing. Diode being a broadband detector, changes an RF signal into a relative DC voltage. This is the most common technique in scalar network analyzer. Harmonic and fundamental mixing techniques are broadband based, which changes an RF signal into an IF signal. At IF frequencies both the techniques have BPF to reject the unwanted frequencies.

iv) The user obtains results on a CRT screen which is used to project the information in the **Display** section. The formats of displaying information can be either Cartesian, polar, impedance or table format.

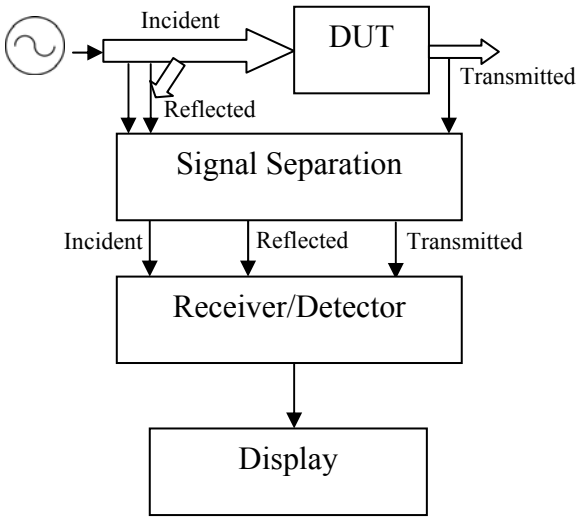


Fig. 3.6: Network Analyzer Block Diagram

3.5.1 Types of Network Analyzers

i) Scalar Network Analyzer (SNA)

SNA is a very simple type of RF network analyzer which measures the properties of amplitude of the DUT[135]. The working of an SNA is like a tracking generator together with a spectrum analyzer. When both are used together, the operation is closely linked electrically. The tracking generator generates a frequency signal that is similar to the signal received by the spectrum analyzer. If the output and input of the tracking generator and spectrum analyzer respectively are connected directly, a steady line is seen on the analyzer screen specifying the output amplitude. Should a device be connected between the 2 instruments, subsequently the spectrum analyzer will display any change in amplitude. Therefore SNA can be used to measure the amplitude response of different components.

ii) Vector Network Analyzer (VNA)

VNAs are also known as Automated Network Analyzer (ANA). Originally network analyzers were corrected by hand manually. Return loss estimations could not decide >20dB in several cases. Phase, insertion and gain loss were computed from a through connection followed by a DUT. Then the 1st automatic network analyzer was launched. A minicomputer which was launched after manipulating the vector data resulted in accurate phase and magnitude of the 4 S-Parameters and automatic error correction. Nowadays the VNAs are automatic, with a built in error correction. These VNAs comprises of a sweep oscillator, RF cables, an information display, a control panel and a test set including more than 2 ports. Frequently each and every port of the test set comprises of an intricate ratio measuring gadget and a twofold directional coupler. Other choices include bias current or voltage injection and a PC to manipulate and then to store the data. The procedure of describing the systematic errors by evaluating calibration standards in the network analyzer is called vector error correction. The impacts of the systematic errors are removed

mathematically from raw estimations. Hence the measurements on devices are provided by the network analyzer with a higher accuracy.

3.5.2 Spectrum Analyzer

RF signals can be viewed in the frequency domain using the spectrum analyzer. It changes signals in the time domain into frequency domain. The accuracy of the power levels displayed by the spectrum analyzer is around ±0.5dB. However it is not accurate for frequency measurement and hence frequency counters are the best choice.

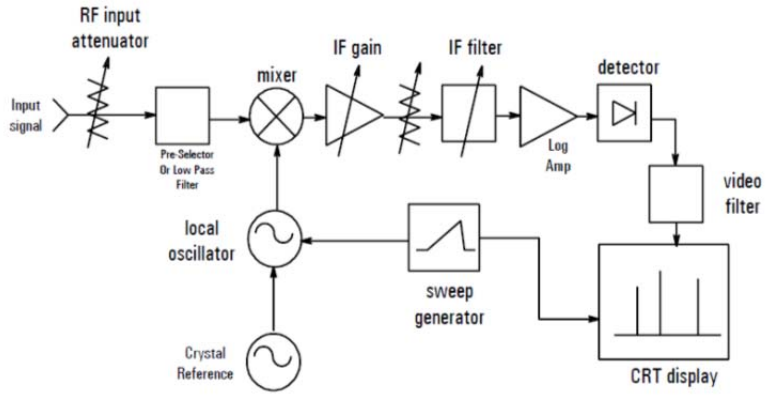


Fig. 3.7: Spectrum Analyzer Block Diagram

The major components which exist in a spectrum analyzer are RF input attenuator, local oscillator, mixer, sweep generator, IF filter, video filter, detector, and CRT display. After the mixer, the IF gain amplifier is also important although not included in Fig.3.7. IF frequency is generated from the mixer which is filtered using an IF Band Pass Filter. The CRT displays the signal as power vs. frequency. The signal which requires to be determined, is connected to the RF i/p port of the spectrum analyzer and the following needs to be setup: Video Bandwidth (VBW), Resolution Bandwidth (RBW), Sweep Time, Span or Frequency Range and RF Centre Frequency.

It is difficult to recognize the 2 signals that appear as 1 which is created by the RBW. VBW smoothens out the signal with the help of a LPF. Even if noise prevails, it is feasible

to perform averaging of video signals on a spectrum analyzer. The time needed to sweep the frequency of the LO over the frequency span is known as 'Sweep Time'.

Sweep Time = 1 / √ (RBW)

Spectrum analyzer is needed for harmonics, spurious, phase noise and frequency measurements.

3.5.3 Difference between Network Analyzer and Spectrum Analyzer

Tuned receivers are used in both the network and spectrum analyzer which operates on a frequency range, very similar to each other. The differences between them are as mentioned below in Table 3.2:

Table 3.2: Differences between Network and Spectrum Analyzer

Sr. No.	Network analyzer	Spectrum analyzer
1	Is used to measure S parameters , return loss, insertion loss, transmission coefficients, reflection coefficients and much more	Is used to measure phase noise, spurious, sidebands, harmonics, carrier power level and many more.
2	It is designed to take care of measurements related to group delay	It is not possible to take care of measurements related to group delay
3	It helps measure known signals.	It helps discover unwanted signals.
4	It comprises of multiple and source receivers and evaluates broadband frequency signal using techniques such as frequency and power sweep.	It is just a receiver which displays the signal fed to its RF input port from any RF transmitting device through cable or with antenna
5	It provides higher measurement accuracy due to vector error correction feature.	It has lower measurement accuracy.
6	Estimations are difficult while placing a marker on the display, but understanding the results is very simple.	Estimations are difficult while placing a marker on the display, but understanding the results is difficult.

3.6 Instrumentation

3.6.1 USB-TG44ATracking Generator

The tracking generator used is a "**Signal Hound USB-TG44A"** ranging from10 Hz – 4400 MHz which is used to measure amplifiers, attenuators, filters, etc along with USB-SA44B spectrum analyzer (Fig. 3.8). It is simple to use, compact and trouble free[136-137].

Fig. 3.8: Tracking Generator - USB-TG44A

Some features of the tracking generator include the following

- It measures 8" in length and its weight is 10 ounces.
- Ranges: Frequency (10Hz – 4400MHz) and amplitude (-30dBm to -10dBm).
- External power supply not needed as it draws power from the USB.
- Cost is one for both hardware and software.
- Works on any Windows OS.
- Step size ranges from 10Hz - 10MHz.
- Frequency accuracy is ±1ppm.

3.6.2 USB-SA44B Spectrum Analyzer

The spectrum analyzer used is **Signal Hound USB-SA44B** ranging from 1Hz – 4400MHz (Fig. 3.9). It is simple to use, compact and trouble free. It has a dynamic range, sensitivity and accuracy at a very low cost. In addition there is a thermometer permitting accurate estimation of amplitude measurement. It also has a preamplifier for the reduction of LO leakage and improving sensitivity. The features of the spectrum analyzer are similar to the tracking generator[138-139].

Fig. 3.9: Signal Analyzer – USB-SA44B

3.6.3 Theory of Operation

The tracking generator consists of a 32-bit Direct Digital Synthesizer (DDS) in which the firmware is exactly set to 10Hz steps. The DDS gives out frequencies 10Hz - 28MHz. This signal needs to be multiplied by a factor ranging between 5 and 200 in order to create frequencies >28MHz. For frequencies <28MHz, the output harmonic content produced at the output is lower. Whereas >28MHz, harmonics which are generated, remain not filtered. The harmonics present has negligible impact, as they are beyond the input bandwidth. Phase noise of USB-TG44A is greater than the USB-SA44B.

3.6.4 The Signal Hound Front & Rear Panels

The front panel has a SMA RF i/p of 50Ω which is DC-coupled. Damage will be caused when DC is applied and signals are greater than +20dBm. Every time a command processed from the PC, the Busy or Ready LED flashes orange. The rear panel has 3 connectors: a) TG Sync - to be connected to the spectrum analyzer's 'Sync Out' when fast sweeps are required, b) USB type B connector – to be connected to PC using USB cable and c)10MHz Ref o/p – to be connected spectrum analyzer's '10MHz i/p' when slow sweeps are required.

3.6.5 Setup of the Tracking Generator

The Slow / High dynamic range is selected when the dynamic range is greater than 70dB. Sweep "thru" immediately is selected to create the 1st sweep as the "thru" and the later sweeps plotted are comparative to it. An offset frequency can be added to the tracking generator via the "Use Mixer Offset". The Absolute Amplitude (-30dBm to -10dBm) or Relative Amplitude (0dBm to 30dBm) control mode is used depending on the type of application (Fig. 3.10).

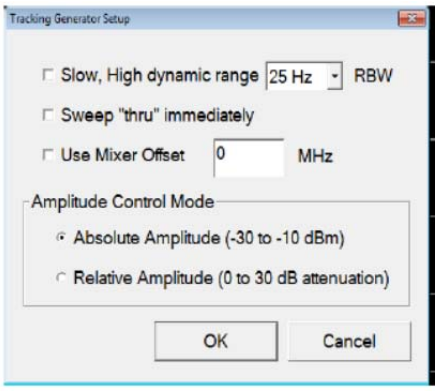


Fig. 3.10: Tracking Generator Setup

3.6.6 The Graphical User Interface

Fig 3.11 shows the Graphical User Interface (GUI), which is designed in order that the SA44B can run on its own or together with the TG44A. The GUI has a control panel in order to send commands to the TG44A and SA44B through the USB. It processes and receives the signal data through the USB to the PC, onto the screen.

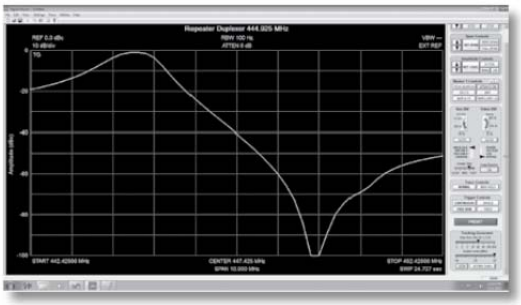


Fig. 3.11: Graphical User Interface

3.7 The Software

3.7.1 Frequency Controls

The middle frequency of the sweep can be changed by using the centre mode. The span reduces if the centre frequency is changed and the stop or the start frequencies falls outside the scope. In order to modify the stop or the start frequency, the start or stop mode is used. If a frequency is chosen outside the range of the instrument, then it is terminated to the instrument's range. If the step size needs to be changed then the step mode is used.

3.7.2 Amplitude Controls

The 'Ref Level' can be changed to set the level of power of the highest point in the graticule line. A matrix of squares is used as a reference to display sweeps, when measurements are made is known as 'graticule'. The selected units will change the units that are shown through the whole system. When automatic and attenuation gain is set, measurements upto the reference level can be anticipated. Using arrows the reference level by the dB/div amount can be altered. dB/div sets the scale to a positive value for y-axis. The value chosen symbolizes the vertical height of a single square on the graticule. The dB/div control is not used in linear mode. Electronic attenuator is set in the Atten to automatic so that the gadget can improve for compression and dynamic range when measurements are made.

3.7.3 Span Controls

The set span is used to control the start and stop frequency difference. If a frequency is chosen outside the range of the instrument, then it is terminated to the instrument's range, thereby resulting in a smaller span. The span can be altered by using the arrow buttons to vary between the sequence 1, 2, 5 or 10. The full span selects the largest spans available by

changing centre, span, start and stop frequencies. The zero span mode is obtained by using the centre frequency, as the start centre frequency, in order to capture zero span.

3.7.4 Bandwidth Controls

A range of RBW can be used for every span (Fig. 3.12). The signal processing and FFT size are controlled by the RBW. Native RBWs are available from <1 Hz - 10.1MHz, in powers of 2. Arrow buttons are used to select a particular RBW. Non-native RBWs are available from 10Hz - 10MHz, when using the arrow buttons, in a 1-3-10 sequence mode when using the arrow keys. Only native bandwidth values in Real-Time mode are allowed, ranging from 2.4kHz - 631kHz in powers of two. When the signal passes through an RBW filter, it gets converted into amplitude, which is then passed through the VBW filter. All RBW choices are available as VBW, with the limit that VBW must be ≤ RBW. VBW is not selectable in Real-Time mode. Having auto RBW selected will choose fast and reasonable RBWs relative to the span. Having auto VBW selected will force VBW to match RBW all the time. This is for performance and convenience reasons.

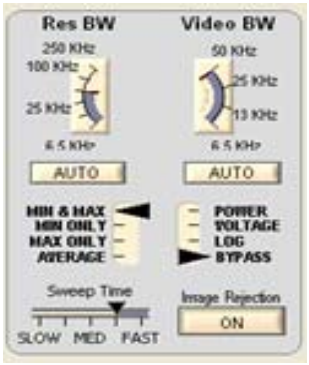


Fig. 3.12: Bandwidth Controls

Fig. 3.13: Tracking Generator Controls

On the Virtual display of the GUI, the tracking generator under the PRESET button exists as shown in Fig. 3.13. The "RF out" of the tracking generator is connected to the "RF in" of the spectrum analyzer, via a SMA adapter along with the BNC cables, connected to the Sync or Trigger of both network and spectrum analyzer. Then the Start button is selected.

The default options are selected and a vertical line around - 10dBm appears which represents the level of the o/p of the tracking generator into the spectrum analyzer. If the RF Out of the tracking generator is disconnected, a low level signal appears. The bandwidth, amplitude and frequency range can be controlled by adjusting the sweep settings.

3.7.5 The Detail Steps in Acquiring the Signal

1. The tracking generator and spectrum analyzer are connected to the PC via USB cables.

2. The “RF out” of the tracking generator is connector to plate 1 of the cell and the “RF in” of the signal analyzer is connected to plate 4 of the cell using 2 RF cables.

3. For fast sweeps the TG Sync of the tracking generator is connected to the Sync Out of the spectrum analyzer.

4. For slow sweeps, the 10MHz Reference o/p of the tracking generator is connected to the 10MHz i/p of the spectrum analyzer.

5. The Signal Hound software is launched.

6. The span / center / start / stop frequencies are selected in the required range.

7. The Linear button changes the units from mV to dB/div and vice versa.

8. Adjust the Sweep Time to mid fast for fast sweeps and mid slow for slow sweeps accordingly.

9. Select Single under Trigger Control for single sweeps at a time.

10. Next the Start button under Tracking Generator is selected.

11. The “sweep thru immediately” is selected.

12. After the sweep is over, markers are placed and readings are taken.

Embedded system means the processor is embedded into that application, where in various devices can be programmed. The hardware platform comprises of an i/p device, an o/p display, a microcontroller (μC) / microprocessor (μP), 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 time used on debugging & development. μPs whose behaviour & architecture are completely described utilizing a subset of a Hardware Description Language are called soft-core processors. They can be synthesized on any Field Programmable Gate Array (FPGA) or Application Specific Integrated Circuit (ASIC) technology. Hence they provide 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 core processor 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.

4.1 Signal Processing

The number of additions and multiplications limit the computation time, required by Digital Signal Processing (DSP) algorithms[140]. The most familiar DSP technique, is the multiply & accumulate and FIR digital filter implementation. Usually the i/p signal is convolved with a transfer function having coefficient as a_i. There could be a few or many coefficients, in the said function response which consists of a_i where $0 \leq i \leq p$.

$$y[n] = a_0x[n] + a_1x[n-1] + \ldots + a_px\ [n-b]$$

Such filters have output y[n] which is used to perform the computation by multiplying input signals x[n] and adding products as shown in Fig. 4.1.

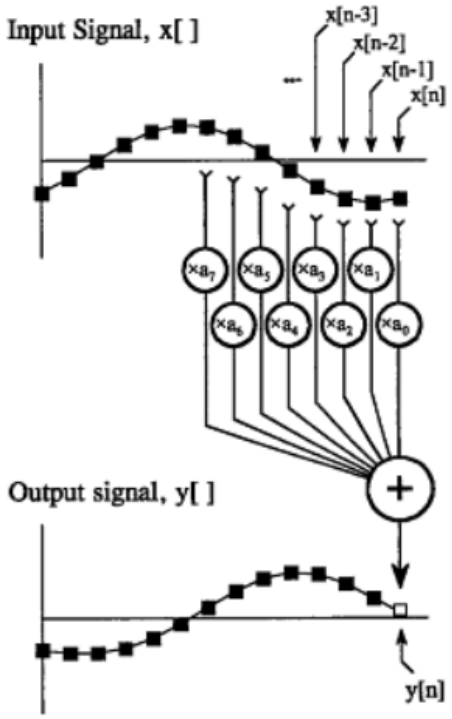


Fig. 4.1: FIR Digital Filter

A fast DSP is in demand but not necessary, as there are some drawbacks such as design difficulty, cost, power consumption, etc.

4.1.1 DSP Algorithms

FIR filters and similar computation are carried out by DSP. Algorithms are needed to comprehend the hardware. Real time and offline processing need to be initially differentiated. The complete i/p remains in the computer in offline processing and then analyzed later, whereas as soon as the i/p signal is received, the o/p signal is generated in real time processing or even after a short delay. Real time DSP can be implemented using

circular buffer. A memory location having 4 pointer parameters, form a circular buffer. They include the start, the end, the next sample and the step size.

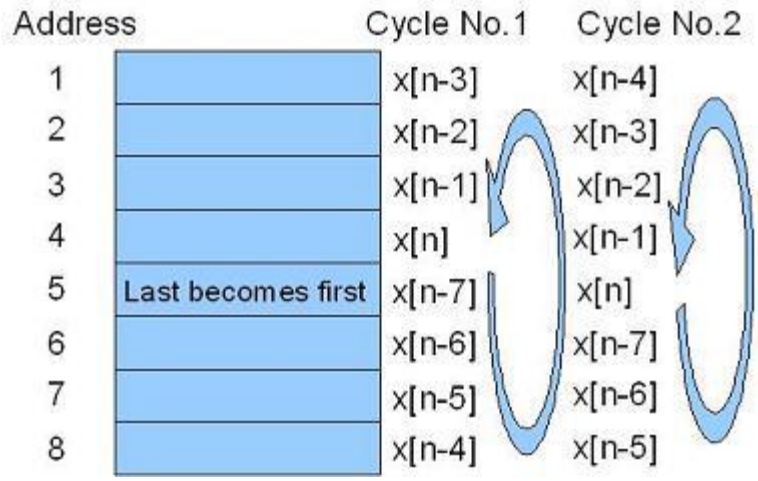


Fig. 4.2: Circular Buffer for Real Time DSP

Fig. 4.2 shows a circular buffer having 8 memory locations 1 to 8. Cycle No.1 shows how data is engaged at a specific time and Cycle No. 2 shows how the data is updated at next clock cycle.

DSP system can also be implemented as general purpose or dedicated DSP based processor. There are 2 kinds of DSP Algorithms: Signal Analysis and Filtering Algorithm. The basis of these algorithms can be Discrete Fourier Transform or difference equation. Software, Firmware and Hardware are used to implement these algorithms.

- Software: The algorithm is carried out as a program running on a minicomputer, workstation, programmable DSP chip or a PC.
- Firmware: The algorithm is carried on a Read Only Memory chip.
- Hardware: The algorithm is carried out using digital circuitry.

4.1.2 DSP Architecture

In order to transfer data to memory, to and fro, a major hurdle in the execution of DSP algorithm is experienced. This involves programming instructions as well as data. For example, if 2 numbers are multiplied from memory, then 3 binary values are needed, i.e. program instructions as well as the numbers which are to be multiplied. Fig. 4.3 shows the Von Neumann Architecture wherein the task is performed using a single memory.

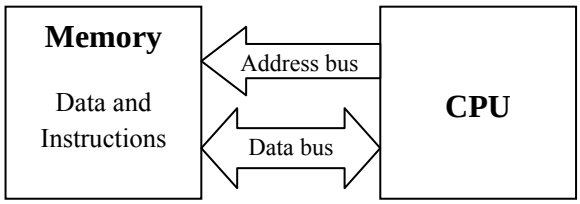


Fig. 4.3: The Von Neumann Architecture

Fig. 4.4 shows the Harvard Architecture where fast processing is needed which is expensive and complex. Since it has a dual memory, instruction and data can be received simultaneously which improves the speed. Hence this architecture is used nowadays.

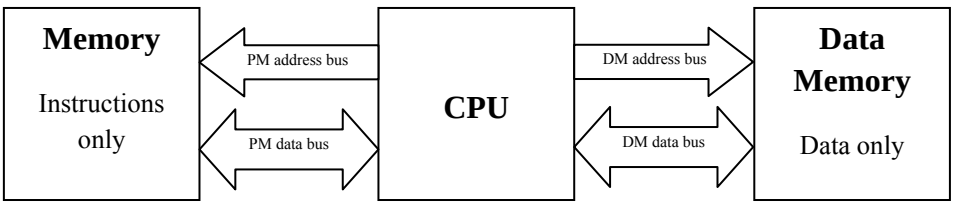


Fig. 4.4: The Harvard Architecture

Fig. 4.5 depicts the Super Harvard Architecture (SHARC). In order to improve the throughput, special features such as i/o controller and instruction cache are added to Harvard Architecture. The drawback of the Harvard design basically is that the program memory bus is not as busy as the data memory bus. For example if 2 numbers are multiplied, then 2 numbers need to be passed over the data bus whereas one instruction needs to be passed over the program bus. To betterment this situation, data memory is relocated to program memory. Filter coefficients and instructions need to be placed in the

program memory. Only the i/p signal remains in the data memory. The situation still remains the same with the program memory bus becoming busier. But the execution of DSP algorithms is normally in loops. Hence the SHARC includes an instruction cache. The Arithmetic Logic Unit (ALU) and multiplier in a SHARC are parallel accessed. In one clock cycle, a set of data can be fed to the ALU from the registers and data can be fed to the multipliers from the registers simultaneously. The results can be received either in either of the registers.

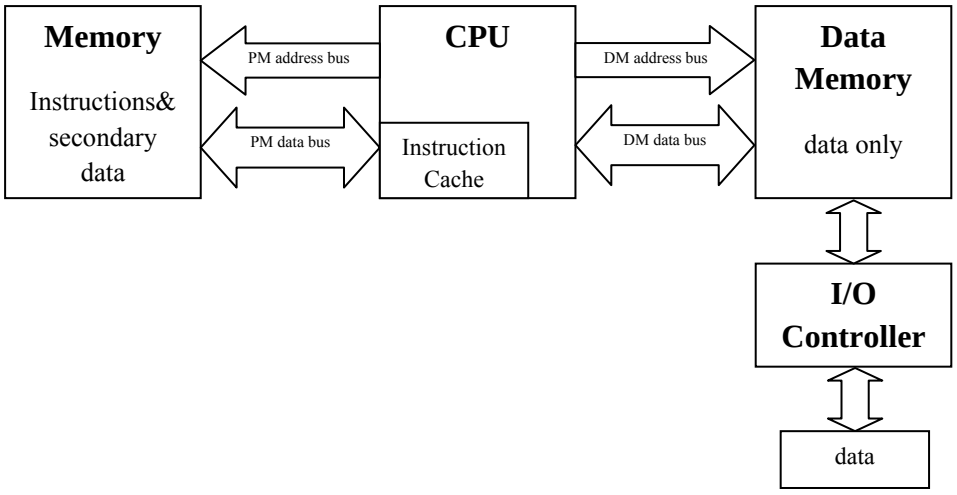


Fig. 4.5: The Super Harvard Architecture

Floating vs. Fixed point: DSP can also be separated into floating and fixed points. Floating point uses 32-bits to save data and hence the bit patterns are more. Fixed point uses only 16-bits and sometimes 24-bits to store data. There are 4 ways to represent a number using fixed point, i.e. Signed Integer (-32768 to 32767), Unsigned Integer (0 to 65535), Signed Fraction (-1 to 1) and Unsigned Fraction (0 to 1).

4.1.3 Multiprocessor Configuration of DSP

Many DSPs combine together in a single system, can handle highly powered tasks known as parallel processing or multiprocessing. In SHARC DSPs external hardware logic are not required to link the buses together, since arbitration logic is present. The DSP is arranged

in different configurations as shown in Fig. 4.6 and Fig. 4.7, i.e. Data Flow and Cluster Type. In data flow type, every processor performs each step in assembly line. In cluster type, the processor interacts with bulk memory over a parallel bus.

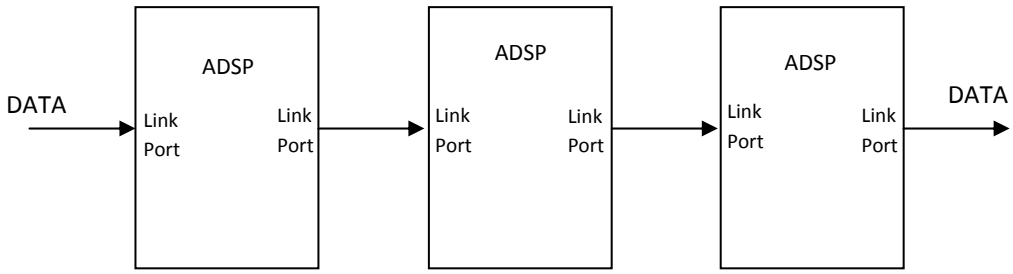


Fig. 4.6:Data Flow Multiprocessing

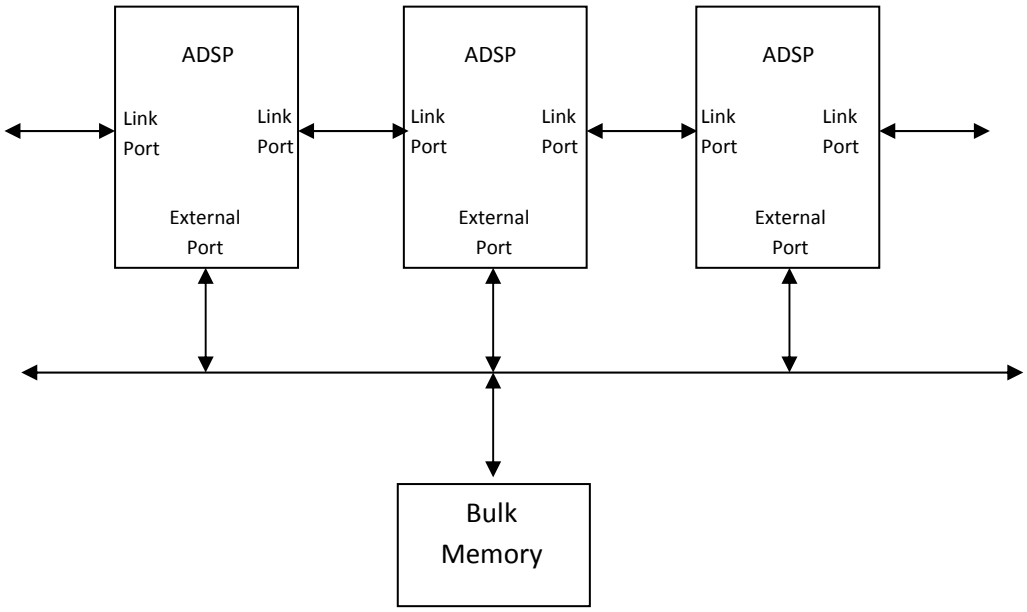


Fig. 4.7: Cluster Type Multiprocessing

4.2 Field Programmable Gate Array

A semiconductor device where functions can be characterized, subsequent to fabricating is called an FPGA. An FPGA can be adapted to new standards, to reconfigure hardware for definite applications and to program product functions and features, even after being established in the field; hence it is termed as Field Programmable. Gate Arrays are 2-D

arrays of logic gates[141]. An FPGA is flexible in designing and can change functionality without being costly and risky. FPGAs consists of interconnect resources and logic blocks (grid of free circuit elements) but the function is achieved through programming. A structure of the FPGA is shown in Fig. 4.8. FPGAs have a high logic capacity and are accountable for the designing of digital circuits.

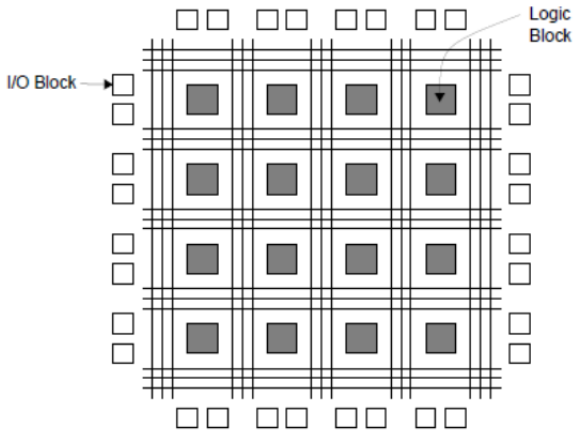


Fig. 4.8: Structure of an FPGA

FPGAs nowadays comprises of configurable Flash or SRAM, high-speed i/p or o/p pins (I/Os), routing and logic blocks. FPGA consists of Logic Elements (LEs) and interconnects which are reconfigurable, in order to connect LEs physically to one another. LEs can be configured to perform simple or complex functions. The FPGA core is an array of wires and logic gates etched in an IC, so that it can be reconfigured. Rich functionality, low power and low cost are provided by hard Intellectual Property (IP) which is built in an FPGA fabric. Central Processing Units (CPUs), protocol controllers, transceivers calculating circuits and memory blocks are examples of hard IP. Only the hard IP is not customizable in comparison to the rest of the FPGA. FPGAs have multiple hard core processors, some million bits of internal memory and quite a few million gates of programmable hardware logic. In recent times FPGAs are huge enough, that a mixture of hard and soft cores can be placed in one FPGA. Using the programmable logic, they can

also execute fast integer add circuits, making the FPGAs a practical design platform in DSP applications.

4.3 Difference between an FPGA and an ASIC

In order to choose between an FPGA and an ASIC, the value propositions have to be evaluated carefully. FPGAs are used for any type of design since they have unique features of high-speed serial, clocking, DSP blocks and embedded processors at low prices. FPGAs are flexible and cost-effective compared to ASICs since they can be programmed by a customer according to ones needs, instead of hiring a contractor. Any logical function can be implemented using FPGAs and ASICs, but the FPGAs have an advantage of changing the functions of the chip after manufacture and can be used in many applications. Although ASIC can run faster than a most advanced FPGA, it would be extremely expensive, very difficult and risky. FPGA reduces design errors, shortens design time, has greater flexibility, reduces power consumption and offers a low cost than an ASIC and hence is the best choice in the world of technology. Small parts of FPGAs can be customized for use without affecting the rest of the design and hence, FPGA can be chosen as it offers great configurability and less risk to the development schedule.

4.4 Soft Core Processor

Two kinds of processors are available on an FPGA chip: Soft core and Hard core processors[142]. Soft core processors cost less due to the mass manufacturing methods. Depending upon the size of an FPGA, designers are offered higher flexibility to implement number of cores. Nevertheless, soft core processors have some drawbacks like higher power consumption, reduced processor performance and larger size. FPGA sellers have introduced soft core processors particularly for FPGA implementation. The processors have features such as ALU, Instruction Sets and Register, precisely manufactured for

efficient use. The performance of soft core processors has improved tremendously as compared to hard core processors. Application development tools such as Integrated Development Environment (IDE) are available on a FPGA soft core processor. It enables setting of parameters, cache instantiation, etc. Fig. 4.9 shows the soft core processor development platform.

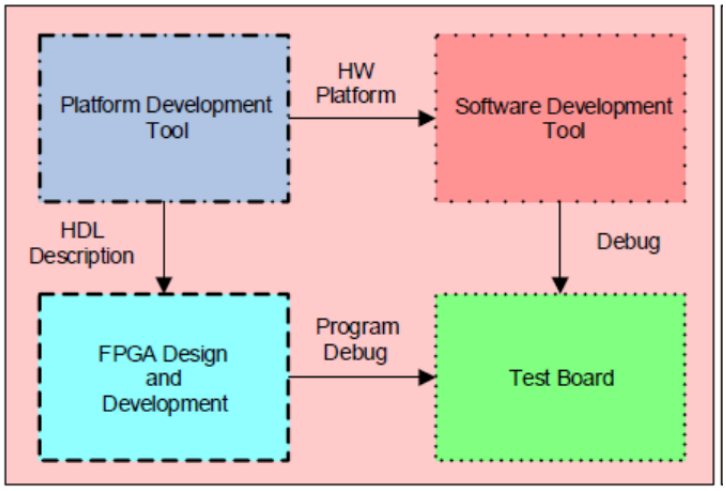


Fig. 4.9: Soft Core Processor Development Platform

The criteria for selecting a soft core processor are:

i) Performance and Power: The factors for a system design are power requirement and operational performance. Product designers have to exchange the two parameters to execute the desired system functionality.

ii) IDE Tools: Analysis and effective tool estimation is important. The following factors can have important consequences on design cycle efficiency: verification and debug capabilities, design tool flow, ease of use and feature set, quality of tool tutorials and available training.

iii) Operating System (OS) Considerations: An abstract interface level provides embedded designs, in order to lessen the design time by including an OS. It requires low level software in order to connect it to the hardware.

Soft core processors, in addition to development and supporting tools, are commercially emerging nowadays. Some of the main products which are available are Xilinx MicroBlaze, OpenRISC 1200, LEON3 and Altera NIOS /NIOS II. They offer logic

elements and memory with many IP peripherals for the quick advancement of System on-Programmable-Chip (SoPC).

4.4.1 MicroBlaze Soft Core Processor

Xilinx's MicroBlaze is one of the most common soft core processor, which can be modified with several memory and peripheral configurations[143]. It is a 32-bit RISC processor with 3-stage pipeline varying from one to three cycles. Xilinx Platform Studio is used to achieve the design and produce a MicroBlaze based system, configuring and instantiating cores from the default libraries. MicroBlaze uses a Harvard memory architecture wherein 2 Local Memory Buses are used to link the data and instruction memories. The user defines the number of peripherals used, as well as the size of the memory used in a specific design. In addition, the on-chip-peripheral bus, relieves system performances and is designed to support low-speed/performance peripherals, such as external bus controllers, USB, GPIO and UART. The MicroBlaze operates within a Virtex-4 component at up to 200MHz. Depending on the configuration of the processor, the required range of resources to execute a MicroBlaze soft core processor, ranges from 900 to 2,600 Xilinx Look-Up Tables.

4.4.2 Altera NIOS II Processor

Altera Organization is a top supplier of PLDs and FPGAs. Stratix, Stratix II and Cyclone FPGA groups are well known products, widely used in the design of embedded systems and in DSP applications. NIOS II Processor, which shows Harvard memory architecture, is a Reduced Instruction Set Computer (RISC) processor. The different features of the processor are 32 general purpose registers, 32-bit Instruction Set Architecture (ISA), instructions for 64-bit & 128-bit multiplication and single-instruction 32x32 multiply & divide operations. It is feasible to build, debug & run software of a number of platforms

utilizing the NIOS II IDE. SoPC builder from Altera is used for the evaluation and creation of embedded systems. Reusable custom components are recognized in an easy way, by reducing the time required to set up an SoC, design and construct from weeks to hours.

4.4.3 OpenRISC 1200

The OpenRISC 1200 (OR1200) is a synthesizable open source implementation of the OpenRISC 1000 RISC architecture. Verilog HDL is used to implement the IP core of the OR1200. The design is freely available and can be downloaded, as well as modified by any individual. The implementation details memory management hardware, Central Processing Unit (CPU), Programmable Interrupt Controller (PIC), tick timer, debug unit and power management unit. The OR1200 CPU is a functioning of the 32-bit ISA. The ISA has 2 addressing modes: PC-relative and register indirect with displacement and 5 instruction formats. Most instructions have a 5-stage pipeline implementation and are able to execute a single cycle. A MAC unit exists in the CPU, in order to support DSP applications.

4.4.4 LEON3

Based on the SPARC V8 architecture, the 32-bit LEON3 processor supports multiprocessing configurations. The processor has around 16 CPU cores and is completely synthesizable, which can be executed in synchronous or asymmetric multiprocessing configurations. A configuration consisting of 4 processors can deliver a performance of around 1400 Dhrystone MIPS. For educational, research and evaluation purposes, the LEON3 multiprocessor core can be obtained, under the GNU GPL license, in full source code. For the purpose of commercial applications, a low cost license is accessible. At lower frequencies, the multiprocessor solution provides higher performances as compared to the single processor solutions as well as lowers costs, power savings, and gives full

compatibility with EDA tools. The multiprocessor system designs are simplified hence reduces design costs and time-to-market.

The Comparison of NIOS II, Microblaze, OpenRISC1200 and Leon3 are shown in Table 4.1. Different features are used to compare between the soft core processors. It can be seen that the NIOS II processor can provide up to 256 custom instructions and this feature is not applicable to Microblaze and LEON3. NIOS II has 6 stages which are not readily available in LEON3 due to high resources required.

Table 4.1 Comparison of Soft-Cores

	Nios II (Fast Core)	MicroBlaze	OpenRISC 1200	LEON3
Speed MHz (ASIC / FPGA)	200 MHz (FPGA)	200 MHz (FPGA)	300MHz (ASIC)	125MHz/400MHz (FPGA/ASIC)
FPGA/ASIC Tech.	Stratix/ Stratix II	Virtex-4	0.18 micron	0.13 micron
Reported DMIPS	150 DMIPs	166 DMIPs	300DMIPS	85DMIPs
ISA	32-bit RISC	32-Bit RISC	32 or 64-bit RISC	32-bit RISC
Cache Memory (I/D)	Up to 64KB	Up to 64KB	Up to 64KB	Up to 256KB
Floating Point Unit (optional)	IEEE 754	IEEE 754	as peripheral	IEEE 754
Pipeline	6 Stages	3 Stages	5 Stages	7 Stages
Custom Instructions	Up to 256 Instructions	None	Unspecified limit	None
Register File Size	32	32	32	2-32
Implementation	FPGA	FPGA	FPGA, ASIC	FPGA/ASIC
Area	700-1800 LEs	1269 LUTs	N/A	N/A

(1) – Using 1,2 or 4-way set associative configuration

4.5 NIOS II Processor

The NIOS II soft core processor has a 32-bit pipelined RISC architecture. It also has 16-bit instructions some of which requires 1 clock. It has a large internal register file. The performance of Altera FPGA is 30 to 80 MIPS. The data path can be configured to either 16-bit having 1100 LEs or 32-bit having 1700 LEs. Load and Store instructions are used to move data between the general purpose registers and memory. The NIOS II processor wordlength is 32-bits. At configuration time, the user can select either big-endian or little-

endian assignment style in 32-bit word byte addresses. The NIOS II soft core processor is shown in Fig. 4.10[144].

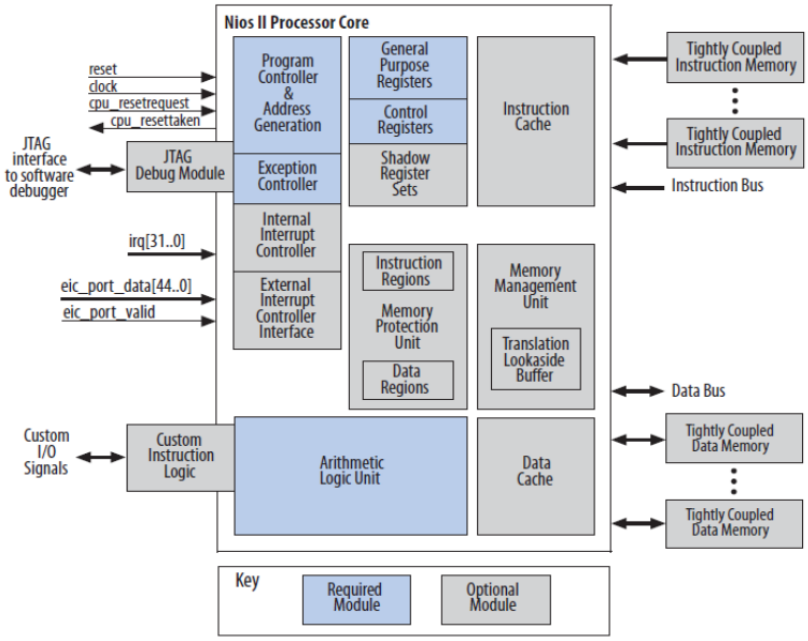


Fig. 4.10: NIOS II Soft Core Processor

The NIOS II processor operates in the following modes:

- Debug mode – In order to implement features such as watchpoints and breakpoints, software debugging tools are used.

- User mode –The purpose of the mode is to avoid implementation of certain instructions meant for system purposes.

- Supervisor mode – If the processor is reset, then it permits it to perform available functions and instructions.

The foundation of the NIOS II instruction set forms the functional units of the architecture. But none of the units are implemented in hardware. An implementation on NIOS II is a set of design selections embodied by a certain NIOS II soft core processor. Every implementation has objectives, such as higher performance or smaller core size. The flexibility permits the NIOS II architecture to adjust to various target applications. Implementation variables are of the following features: software emulation or hardware

implementation of a feature; exclusion or inclusion of a feature; and more or less of a feature. The NIOS II processor system is shown in Fig. 4.11[145].

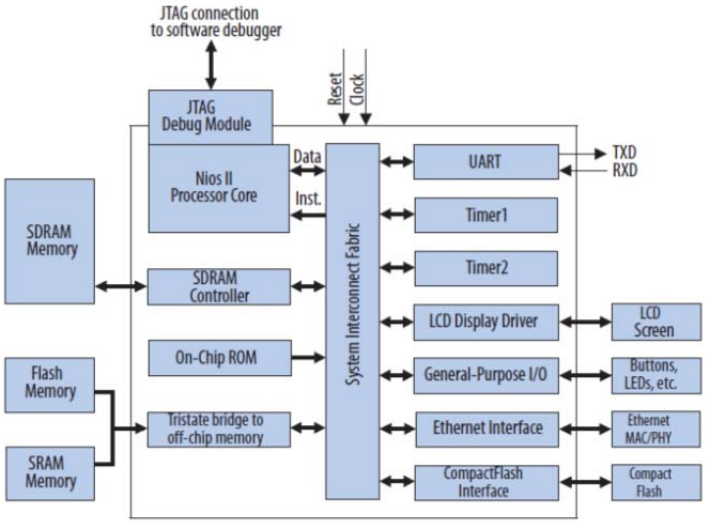


Fig. 4.11: NIOS II Processor System

It is feasible to build, debug & run software of a number of platforms utilizing the NIOS II IDE. SoPC builder is provided which helps in rapid evaluation and development (validation / verification) of the embedded system. The comparison between NIOS II Economy, Standard and Fast Processors is shown in Table 4.2.

Table 4.2: Comparison of NIOS II Variants

Features	Nios II Economy	Nios II Standard	Nios II Fast
Objective	Optimized for size	Balance between size and Speed	Optimized for High Performance
Caches (Instruction/Data)	None	Up to 64KB / None	64KB / 64KB
Pipeline Stages	1	5	6
Hardware Multiply	Software Emulated	3 Cycles per MUL	1 Cycle per MUL
Logic Elements used	600-700	1200-1400	1400-1800
Custom Instructions	256 Custom Instructions		

LEs used in NIOS II Standard are well balanced as compared to economy and fast processors. The Hardware Multiply uses 3 Cycles/MUL unlike the fast variant having 1 Cycle/MUL. The balance between size and speed is better than the others. The NIOS II standard provides real-time, high performance and deterministic results.

4.6 Altera DE2 Board

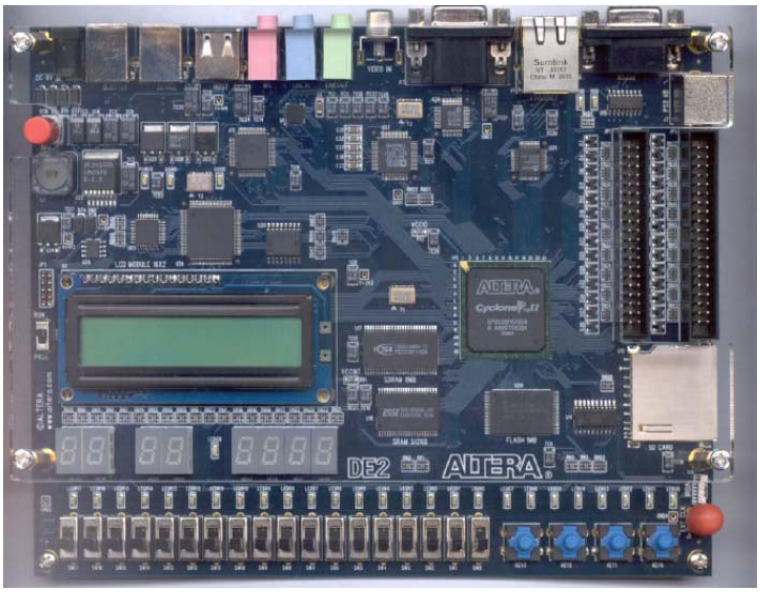


Fig. 4.12: DE2 Board

The Altera DE2 Board in Fig. 4.12 is used for storage, advanced design prototyping and networking in the multimedia. It uses the technology in both CAD and hardware tools to give the designers a range of topics. The board has an affluent set of features that make it befitting for use in various laboratories, for a variety of design projects and for the development of classy digital systems.

Some of the DE2 board resources are listed as follows[146]:

- FPGA: Cyclone II EP2C35
- Flash memory: 4-Mbyte
- Static memory: 512-Kbyte
- Dynamic memory: 8-Mbyte
- LCD, LED, key, switch
- 27-MHz, 50-MHz crystals, oscillators
- 4 pushbutton switches &18 toggle switches
- USB Blaster for programming

4.7 Block Diagram of the DE2 Board

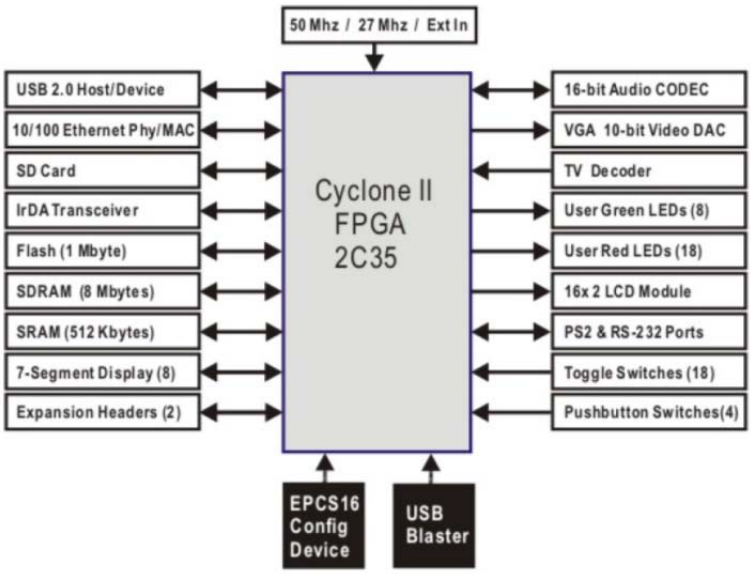


Fig. 4.13: Block Diagram of the DE2 Board

The block diagram of DE2 board is depicted above in Fig. 4.13[147]. Connections are supplied through the FPGA, to give the user maximum flexibility so that any system design can be implemented. The processor and the interfaces which are to be connected to various chips on the DE2 board can be executed in the FPGA device. The Avalon Switch Fabric interconnects components. On-chip memory is provided by the memory blocks in the FPGA device for the NIOS II processor. Appropriate interfaces are used to access the memory chips like SDRAM and SRAM on the DE2 board. I/p and o/p interfaces are connected to the I/O devices which are used in the system. A JTAG UART interface connects the host PC to the DE2 board via a USB link provided by a circuitry along with software known as USB-Blaster. The NIOS II processor is controlled by the host PC which is provided by the JTAG Debug Module. Thereby, it performs various operations such as collecting trace data, setting program breakpoints, stopping and starting execution and downloading programs into memory. SoPC Builder tool is used to execute a system by selecting the necessary components and parameters that would fit on the system.

4.8 Embedded Development Software

In order to develop an FPGA design in embedded system, software and hardware logic, Quartus II, SoPC Builder, the NIOS II IDE etc. were used. Quartus II integrates simulation, verification, layout, threading, combination, programmable and system logic components design. A user can create a complete system by defining their own commands or peripherals, or by using the available components that are incorporated in SoPC Builder. A system can be built with reasonable resources. Moreover, SoPC Builder generates automatically in-chip bus structures, arbitrations, interrupts logic for every hardware component, headers that are compliant with system features for designing of consecutive software (these headers describe data structure , interrupt priority and memory mapping of every peripheral register space). SoPC Builder updates the headers automatically when the hardware system is changed and offers software designers with a configuration interface, which shows flexibility that cannot be achieved by ordinary hard core processors. The NIOS II IDE is used to edit, compile, debug and download programs as well as software development tasks can be completed, hence it is a basic tool for NIOS II processor. Data can be written into the NIOS II processor by using a computer, a JTAG-capable download cable and an Altera FPGA.

4.9 SoPC Development Boards

SoPC development boards are used to make it possible for designers to study the complicated tool flow and also to provide for starting software/hardware co-design projects. The boards enable a large FPGA with many megabytes of external memory, as well as various built-in i/o features that are capable of supporting a soft core processor. FPGAs also include hard core processors. As a PCB is designed for a SoPC-based product, work can still be carried out in parallel on the software development tasks and the

hardware configuration using the development board. The advantages of SoPC design are the short development cycle, flexible nature and it is reconfigurable. Nonetheless, the drawbacks include relatively high power consumption, higher unit costs in production and lower maximum processor performance. The resources required for the implementation on the Cyclone II FPGA are selected as shown in Fig. 4.14.

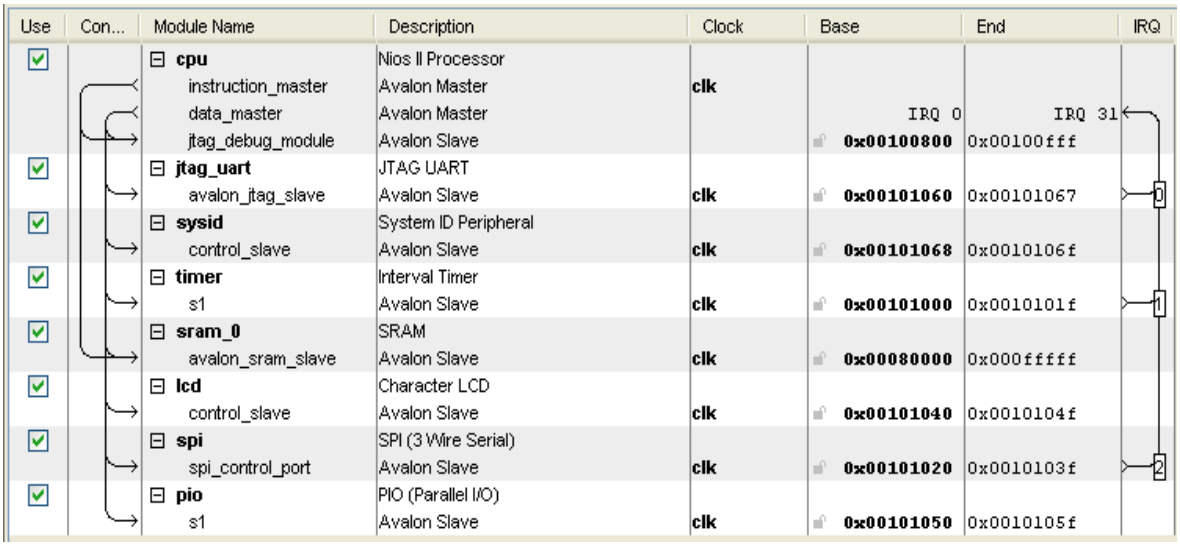


Fig. 4.14: Altera SoPC Builder

The GUI used for configuring Processor Core is a drag and drop style menu used for set processor options and to add I/O hardware. The selected modules are the NIOS II Standard Processor, JTAG UART, timer, SPI, SRAM, System ID, Parallel I/Os and LCD display. Once the modules are selected, the core is generated. Pin assignments are included at this stage. The FPGA core developed to find unknown cholesterol is shown in Fig. 4.15.

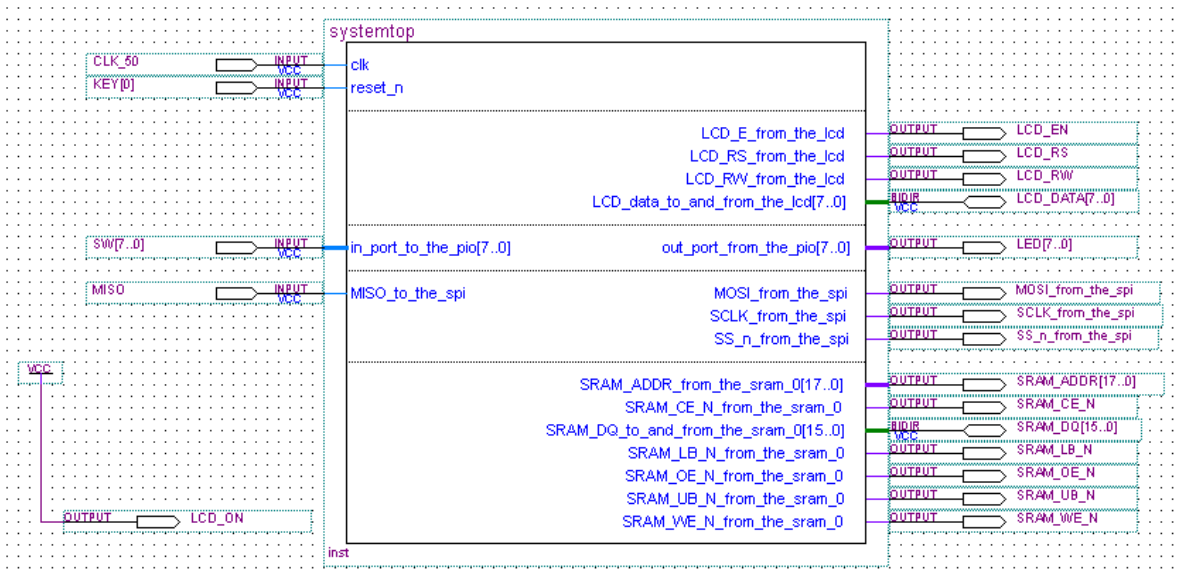


Fig. 4.15: FPGA Core

Next the design is compiled and downloaded in the FPGA. The Compilation Report is given in Fig. 4.16. The NIOS II IDE is used to write a program in C for the PLSR algorithm which is programmed into the Cyclone II FPGA and given in Annexure I.

Flow Status	Successful - Sat Oct 24 12:30:59 2015
Quartus II Version	7.2 Build 207 03/18/2008 SP 3 SJ Web Edition
Revision Name	test
Top-level Entity Name	test
Family	Cyclone II
Device	EP2C35F672C6
Timing Models	Final
Met timing requirements	Yes
Total logic elements	2,608 / 33,216 (8 %)
Total combinational functions	2,335 / 33,216 (7 %)
Dedicated logic registers	1,505 / 33,216 (5 %)
Total registers	1505
Total pins	77 / 475 (16 %)
Total virtual pins	0
Total memory bits	46,208 / 483,840 (10 %)
Embedded Multiplier 9-bit elements	0 / 70 (0 %)
Total PLLs	0 / 4 (0 %)

Fig. 4.16: Compilation Report

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, is 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 and allows an analyst to observe the inner or unknown structure, of big data sets and to visually recognize the factors, which influence the outcome.

5.1 Multivariate Analysis

Multivariate analysis contains a group of techniques with more than one variable, which are dedicated to the testing of data sets. From early 1900's, the analysis has been a branch of statistics with the development of microcomputers, mainframes and software which has made calculations fast and simple. Multivariate analysis can be performed in a non experimental design or in a classic experimental design. A data matrix, wherein, variables are represented by columns and subjects are represented by rows is analyzed. The matrix can be cross-product /sum-of-squares matrix, covariance/variance matrix or correlation matrix. A matrix is solved using linear algebra and gives linear combination of variables, which is based on the solution. The variance is maximized by composite scores and is associated between the set of independent variables (X set) and the dependent variables (Y set). The significance of a variable is decided by the ratio of weight, to the set of variables and is specified by a numerical coefficient. The division of a trait in a population is represented by variance. When 2 variables correlate or associate together, then a factor or

trait is common between them and they tend to vary equally on the scores present in the data set. The fundamental trait is important for them to co-vary along with each other. Scores vary in a similar manner and is measured as variance. 2 sets of variables which correlate with each other have a good variance. Multivariate analysis can be used for individual units or multiple variables.

5.1.1 Simple Linear Regression

Based upon the weight of X, Y is estimated using a regression equation, where X is the predictor and Y is the criterion. The slope of regression line is the predicted Y for every subject. It is also known as weight which is the coefficient of X and for every change in X, Y changes per unit time. The line is formed which implies that the variance is maximized and the error of the sum of squares is minimized. The idea of the ordinary least squares is similar to the sum of squares (X-MeanX) as the variation of scores from the mean is less.

5.1.2 Multiple Linear Regression (MLR) Analysis

A linear regression where a set of independent variables X, describes a dependent variable Y is known as MLR. In this case the data matrix is simultaneously solved, such that the linear composite of X variables gives a correlation with Y, also known as multiple R. A plane is constructed instead of a line, for the estimation which functions in the similar manner, so that SS regression is maximized and SS error is minimized. Many Independent Variables (IV) are used in MLR to estimate at least one dependent variable. If the IV's are perpendicular to each other, the difficulty decreases to a group of univariate regressions and when they are correlated, their value is calculated from partial coefficients. When an IV is predicted from any variables, a major difficulty arises as the calculations needed by MLR, cannot be performed and this is known as multicollinearity. Some probable solutions

to the problem of regression having many dependent variables with numerous predictors are given below[148-149].

i) Partial Least Square (PLS) Regression (PLSR)

The above problem of multicollinearity is solved by calculating latent vectors and is used to estimate many dependent variables. PLSR is a bilinear form of the 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 focused on the minimum probable number of constituents.

ii) Principal Component Regression (PCR)

A statistical process that converts a group of correlated variables into a group of uncorrelated variables using an orthogonal transformation is called Principal Component Analysis (PCA). The number of variables (known as Principal Components) is equal to or less than the total number of variables. The transformation is described in the best possible way, so that, the 1st Principal Component has a large variance and every subsequent component has the maximum variance, under the limitation that it is perpendicular to the previous components. The remaining variables are uncorrelated. The principal components are eigenvectors of a symmetric covariance matrix and hence are orthogonal. PCA is susceptible to comparative scaling of unique variables. The independent variables are fed to a PCA and the predictors used in MLR, are scores of units. Factor variation is explained through X-scores. This gives much information in factor space, however may not be involved with the estimated surface.

iii) Ridge Regression (RR)

By adding a small ridge (constant) to the diagonal of the matrix (correlation), the problem of multicollinearity is accommodated by RR. The calculation of the predicted values is made possible for MLR.

iv) Reduced Rank Regression (RRR) or Redundancy Analysis

Dependent Variable are initially submitted to PCA in RRR and the scores are used as Dependent Variables where the original IVs are then used as estimators as Dependent Variables in series of MLRs.

5.1.3 Multivariate ANalysis Of VAriance (MANOVA)

ANOVA has one dependent variable, whereas MANOVA has many dependent variables. Hypothesis could be tested by means of MANOVA. Multivariate F values could be obtained instead of univariate F value, based on the contrast of the error covariance/ variance matrix and the effect of the same. Covariance is needed, since cognitive psychology and statistics maybe correlated and it is necessary for performing important tests. By producing dependent variables, the multiple dependent variables can be tested to accomplish maximum group difference. The variables produced are linear combination of the calculated dependent variables.

5.1.4 Logistic Regression

Logistic Regression is one of the extensions of multiple regressions with the exception that the output Y variable is a definite variable and not a continuous one. The reason of this analysis is to classify subject, into 1 of 2 categories using predictor variables, such that the accuracy is high. Since the output is discrete, the correlation between X and Y is non-linear, hence the aim is to estimate the possibility, that an individual belongs to either of the groups. A probability of 0 implies that the individual is not in the main group and a

probability of 1 implies that the individual is in the main group. The analysis of the logistic regression does not involve ordinary least squares, but uses a composite procedure of maximum likelihood estimation, in weighting X variables.

5.1.5 Canonical Correlation

Canonical correlation is also an expansion of multiple regressions, 2 or more X predictor variables are used to predict 2 or more Y variables. The aim is to describe the relationship between X and Y variables. The analysis is a two-way process, Ys predicting Xs and Xs predicting Ys, but generally predictor X normally determines the criterion Ys. Shared variance has two or more geometric axes or dimensions, as canonical correlation consists of multiple Xs and Ys. The smaller of the 2 sets contain the no. of axes or dimensions, which is equal to the no. of variables. Two linear composites represent every dimension, 1 for the X set and 1 for the Y set of variables. The correlation between the 2 linear composites is known as Canonical correlation, RC. It is similar to the multiple R in MLR and eigenvalue, RC_2, specifies that the quantity of variance is shared between the composites.

5.1.6 Advantages and Disadvantages of Multivariate Analysis

The advantages of using a Multivariate Analysis are that each method varies in type or amount of Dependent and Independent Variables; multiple levels of analyses are provided and richer realistic design. The disadvantages of using a Multivariate Analysis are robustness of assumptions (less known), extremely difficult to interpret and large Ns are required[150].

5.2 Partial Least Squares (PLS)

Herman Wold, a Swedish statistician introduced the econometric technique, PLS in 1960s who later developed it with Svante Wold, his son. PLS regression (PLSR) is used mostly in bioinformatics, chemometrics, anthropology, neuroscience, sensometrics, electrochemical, chromatographic, UV and NIR data. On the contrary, PLS modelling is also used in econometrics, social sciences, strategic management and marketing. PLS is used when the factors are highly collinear and numerous for the construction of predictive models. Consecutive pairs of X and Y scores are selected such that the relationship between them is strong. This structural equation modelling method is component based and not covariance based. PLS is a statistical method related to PCR. By projecting observable and predicted variables onto a new space, PLS finds a regression model. PLS methods are called bilinear factor models since data X and Y are projected to new spaces. PLS is useful in finding the basic relations between matrices X and Y.

A PLS model is used to locate the multidimensional direction of X in order to locate the maximum direction in Y space. PLSR is appropriate when a multicollinearity exist among X values and when the predictor matrix has extra variables compared to observations. Standard regression is impossible in these cases. Principal Components of dependent data Y and Independent data X are the basis of PLS models. The main idea is to compute the scores of Principal Components of data matrices X and Y and to establish a regression model between them. The main aim when developing a PLS model is to decide the number of Principal Components in a PLS model. By cross validation, the number of components can be determined empirically using a large number of components. A low PRESS (PRedictive Error Sum of Squares value) value indicates a good prediction model.

5.2.1 Partial Least Squares Regression: Basic Ideas

PLSR is another extension of MLR model[151]. A linear model indicates the link between predictor variable, X and dependent variables, Y, such that

$Y = c_0 + c_1X_1 + c_2X_2 + ... + c_qX_q$

In this equation, c_0 is the regression coefficient (i=0) and the c_i values are the regression coefficients (i=1 to q) calculated from the data. The following two significant properties in the multivariate methods are common. The prediction functions have to be less than the least of the X and Y variables and factors have to be extracted from matrices X'X and Y'Y and not from matrices of cross product. PLSR is also an extension of MLR without imposing a limit used by canonical correlation, PCR and discriminant analysis. In PLSR, factors extracted from matrix Y'XX'Y, represent the prediction functions, which exceeds the highest of the number of X and Y variables.

PLSR is the least restricted extension of MLR, which permits it to be employed where there are more predictors than observation variables whereby, there is a limitation of multivariate methods. PLSR is used to choose an appropriate predictor variable and to recognize outliers prior to classical linear regression. PLSR has been used in different fields such as pharmaceutical science, psychology, medicine, economics and chemistry wherein predictive linear modelling is essential. Especially in chemometrics, PLSR is a standard tool for forming linear relations involving multivariate measurements.

5.2.2 Computational Approach: Basic Model

The main aim of PLSR is to construct a linear model **Y=XB+E,** where Y is a response matrix of b x c, X is a predictor matrix of b x d, B is a regression coefficient matrix d x c, and E is a noise term with dimensions, b x c. The factor scores produced by the PCR and PLSR have no correlation between the factor score variables. Matrix ‘X’ consisting of a

huge number of predictors correlate with Matrix 'Y', which is the response variable and forms a data set. Factor scores **T=XW** are first computed by the regression for a suitable weight matrix W and then the model **Y=TQ+E** is calculated where Q is the loading and E is the error term. Next the loadings Q are calculated using **B=WQ** and hence **Y=XB+E** is determined.PCR and PLSR have different methods used for removing factor scores. The weight matrix W reflects the covariance structure amid the predictor variables, which is produced by PCR whereas the weight matrix W reflects the covariance structure amid the response and predictor variables which is produced by PLSR.

A weight matrix W of p x c is produced by PLSR for creating a model for X, such that **T=XW**, i.e., weight vectors form the columns of W for the X columns, generating the equivalent factor score matrix T of n x c. The weights are calculated such that each of these maximizes covariance between corresponding factor scores and responses. Ordinary least squares methods for the regression of Y on T produces weights for Y and Q, hence **Y=TQ+F**. Finally the prediction model completed, once Q is calculated and **B=WQ**, hence **Y=XB+F**. Another important matrix i.e. factor loading matrix, P of p x c is used in the factor model **X=TP+E,** where E is the error term.

5.3 Algorithms for Computing PLS

5.3.1 NIPALS Algorithm

H. Wold developed the NIPALS Algorithm, for PCA and subsequently for PLS. It is used for computing the principal components and for extracting eigenvectors. The results are accurate but the calculations are slow. NIPALS is the model algorithm for calculating PLSR factors. These come in many variants which normalize or not, particular factors. One of most competent NIPALS algorithms is as follows:

For every i=1,…,c, where $A_o=X^TY$, $M_o=X^TX$, $C_o=I$, and c given,

1. compute q_i, the dominant eigenvector of $A_i^TA_i$
2. $w_i=C_iA_iq_i$, $w_i=w_i/||w_i||$, → store w_i into column i of W
3. $p_i=M_iw_i$, $c_i=w_i^TM_iw_i$, $p_i=p_i/c_i$, → store p_i into column i of P
4. $q_i=A_i^Tw_i/c_i$, → store q_i into column i of Q
5. $A_{i+1}=A_i - c_ip_iq_i^T$ and $M_{i+1}=M_i - c_ip_ip_i^T$
6. $C_{i+1}=C_i - w_ip_i^T$

end loop

W is the weight matrix for X, T is the PLS factor scores matrix such that **T=XW** & PLSR coefficients B of Y on X are calculated as **B=WQ**.

5.3.2 SIMPLS Algorithm

An alternative method for computing PLSR is the SIMPLS algorithm, which is as follows and is depicted in a flowchart in Fig. 5.1.

For every i=1,…,c, where $A_o=X^TY$, $M_o=X^TX$, $C_o=I$, and c given,

1. compute q_i, the dominant eigenvector of $A_i^TA_i$
2. $w_i=A_iq_i$, $c_i=w_i^TM_iw_i$, $w_i=w_i/sqrt(c_i)$ → store w_i into column i of W
3. $p_i=M_iw_i$ → store p_i into column i of P
4. $q_i=A_i^Tw_i$ → store q_i into column i of Q
5. $v_i=C_ip_i$ then normalize $v_i=v_i/||v_i||$
6. $C_{i+1}=C_i - v_iv_i^T$ and $M_{i+1}=M_i - p_ip_i^T$
7. $A_{i+1}=C_iA_i$

end loop

Compute **T=XW** and $\mathbf{B=WQ^T}$.

Q is the Factor loadings matrix for Y such that $\mathbf{Y=TQ^T+F}$,

P is the Factor loadings matrix for X such that $\mathbf{X=TP^T+E}$ and

B is the PLS regression coefficients of Y on X

This algorithm is very fast, accurate, gives optimal results and more efficient.

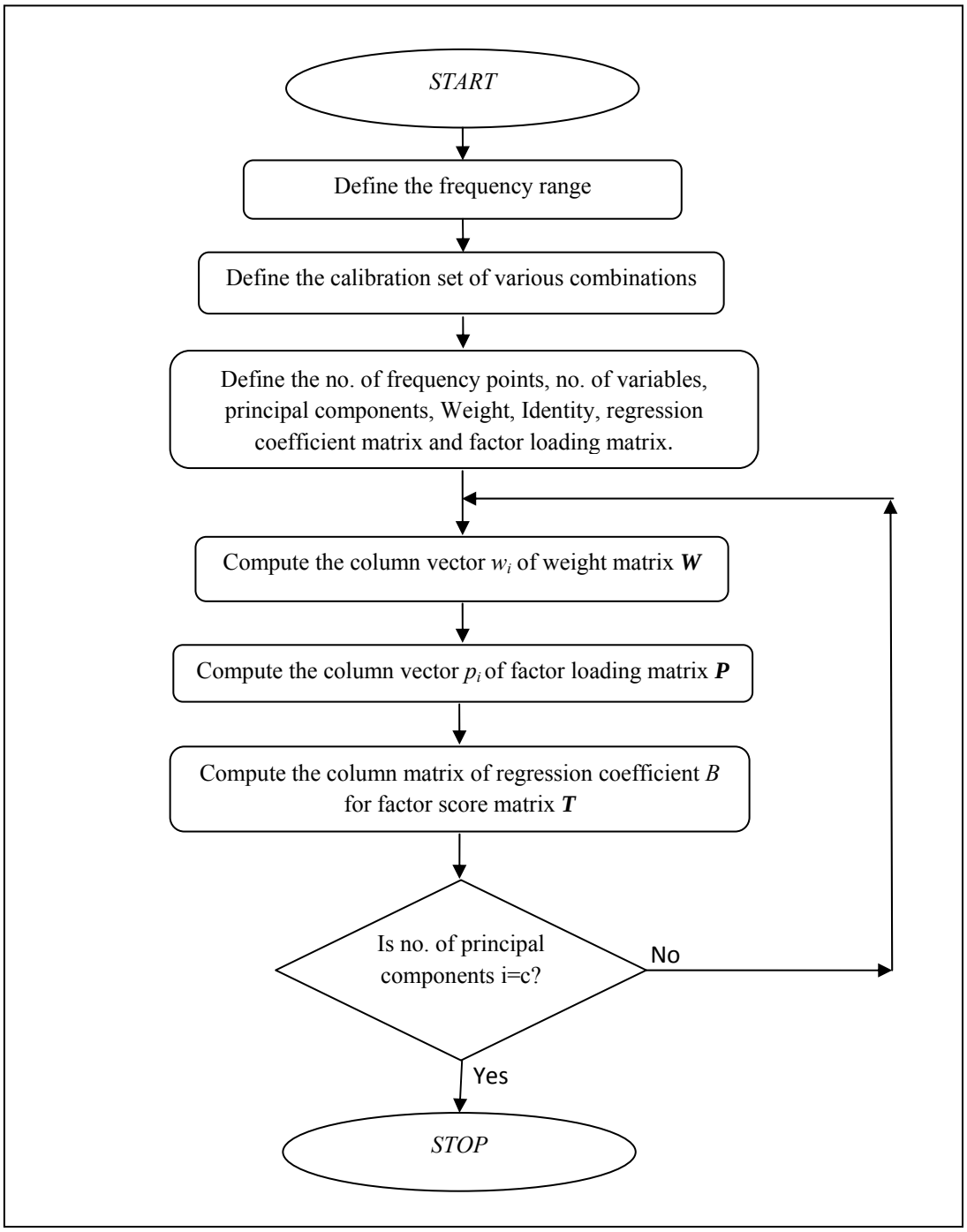


Fig. 5.1: Flowchart of the SIMPLS Algorithm

5.4 ParLeS Software

Besides being used in other types of multivariate data, ParLeS is a shareware that is developed for research and teaching in spectroscopy and chemometrics[152]. ParLeS is used to pretreat, pre-process and transform spectra using different algorithms. It is also used to implement PLSR with cross validation, PCA and bagging-PLSR (bootstrap aggregation-PLSR). ParLeS aids in the implementation of a huge number of pre-processing techniques, whereby the accuracy and robustness of PLSR improves by bagging-PLSR. In addition the unique features comprise of user-friendly functionality as well as the provision of several graphical output and assessment statistics.

The software structure is shown in Fig. 5.2. Initially the data is loaded into ParLes, thereafter the user can pretreat, pre-process and transform the spectra before modelling using PLSR and PCA. When PLSR is carried out, the cross validation step is overlooked and PLSR modelling can be directly performed. The prediction file can be loaded into ParLeS to predict unknowns or to confirm the PLSR models. Results can be saved in ASCII format for additional analysis and external plotting.

The options used to save the o/p of the analysis is depicted as trapeziums, assessment statistics which are provided by ParLes are shown as rounded rectangles, the chemometrics capabilities are displayed by circles and double lines symbolizes data import operations respectively.

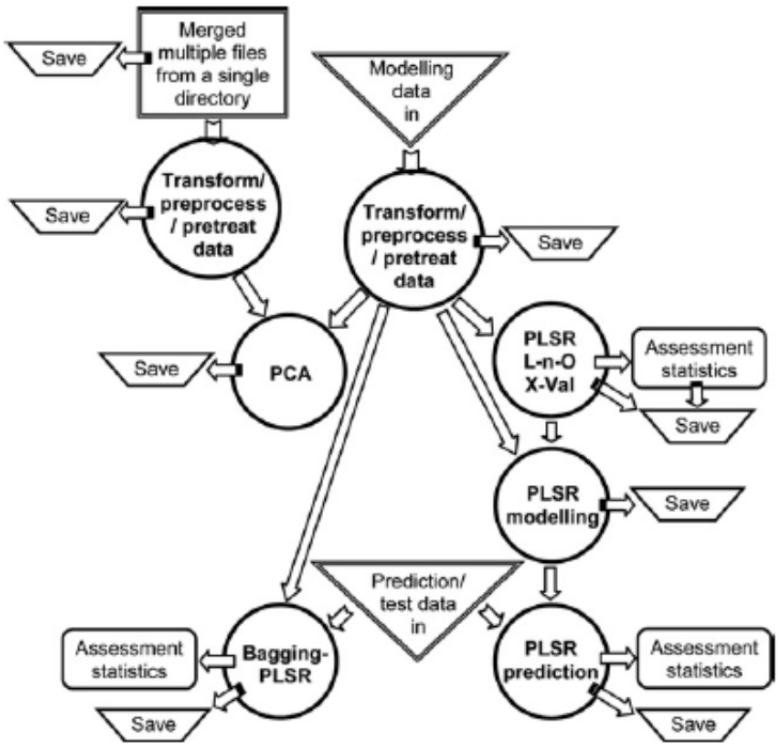


Fig. 5.2: Software Structure of ParLes

ParLeS has a unique feature of being user-friendly through its GUI and other features mentioned earlier as shown in Fig. 5.3.

Fig 5.3: The ParLeS Software GUI

The "Data In" tab is selected and the calibration file in the "Data For Modelling" column is chosen. The "Total Number of y variables" is selected as '5' since the total no. of

constituents present in the experiment are '5'. The "Select y variable for modelling" is selected as '5' in order to estimate cholesterol through this multivariate system. Next the unknown data file which is to be predicted is selected in the "Data For Prediction" column. The "Total Number of test y variables" is selected as 5 as well as the "Select test y variable for prediction" as 5 (Fig. 5.4).

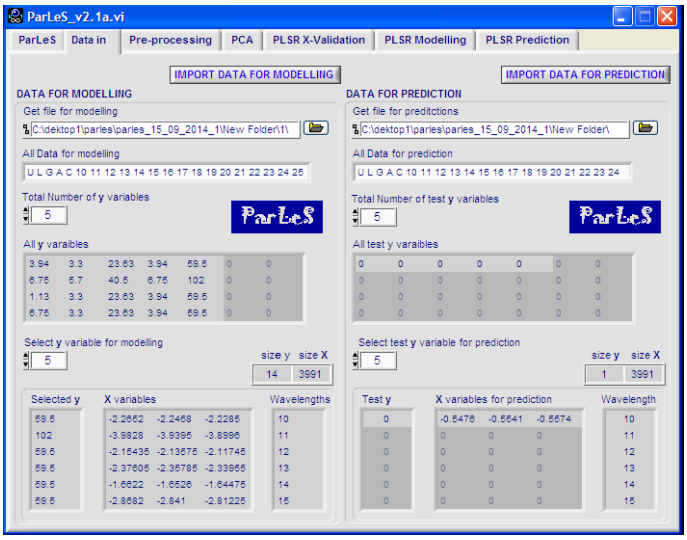


Fig. 5.4: Input Data for Prediction

In order to do the Pre-Processing of data the "Run Selection" button is selected. Pre-Processing is done to filter out noise signals. (Fig. 5.5)

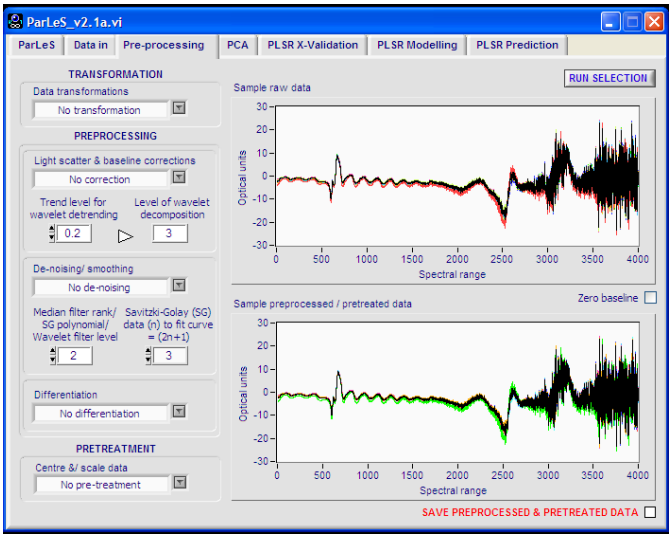


Fig. 5.5: Pre-Processing of Data

The PCA tab is selected and the No. of principal components is selected as twice the no. of input variables, in this case 10 (Fig.5.6).

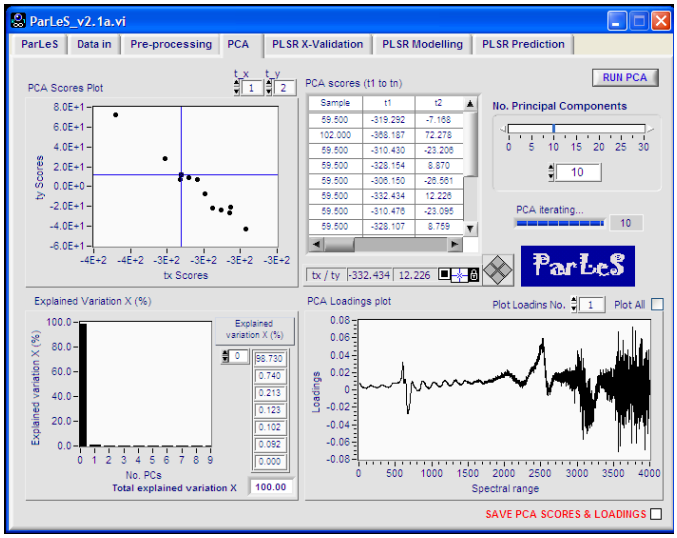


Fig. 5.6: Principal Component Analysis

As shown in Fig. 5.7, the no. of factors for X-Validation in the PLSR X-Validation tab is 10 and the Run X-Val is selected.

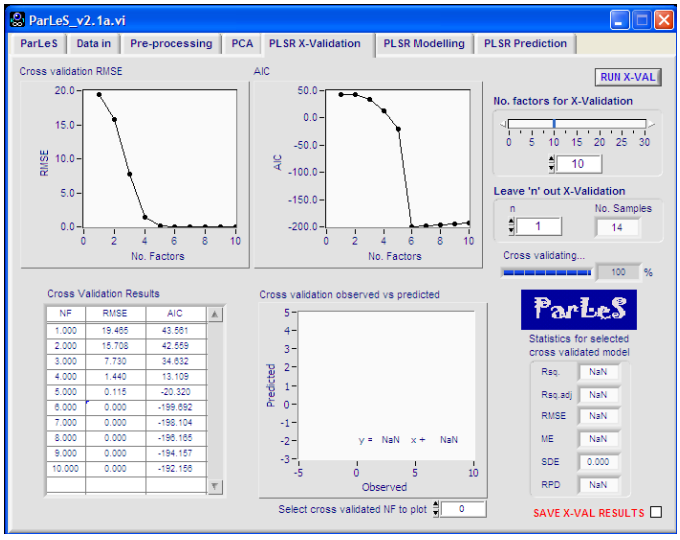


Fig. 5.7: Partial Least Square Regression X-Validation

As shown in Fig. 5.8, the no. of factors for PLS in the PLSR Modelling tab is 10 and the Run PLSR Modelling is selected where delete-n-cross validation is performed.

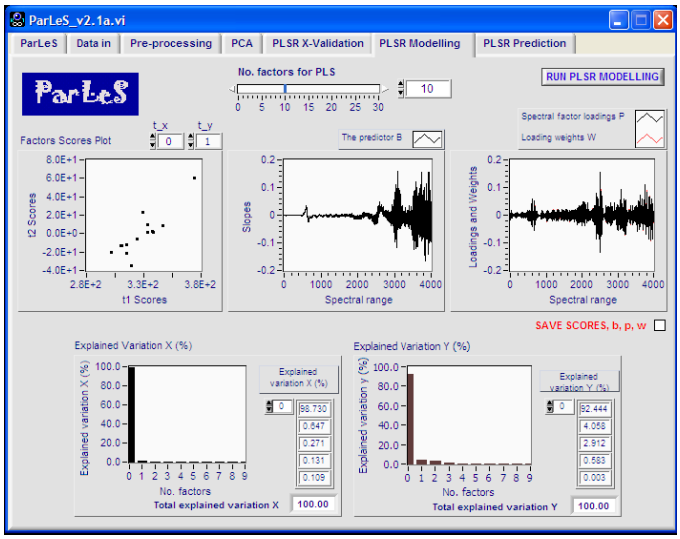


Fig. 5.8: Partial Least Square Regression Modelling

The last stage of the ParLes Software includes the prediction of cholesterol wherein the RMSE value is displayed along with the graph of PLSR Predicted vs. Observed, etc.(Fig.5.9).

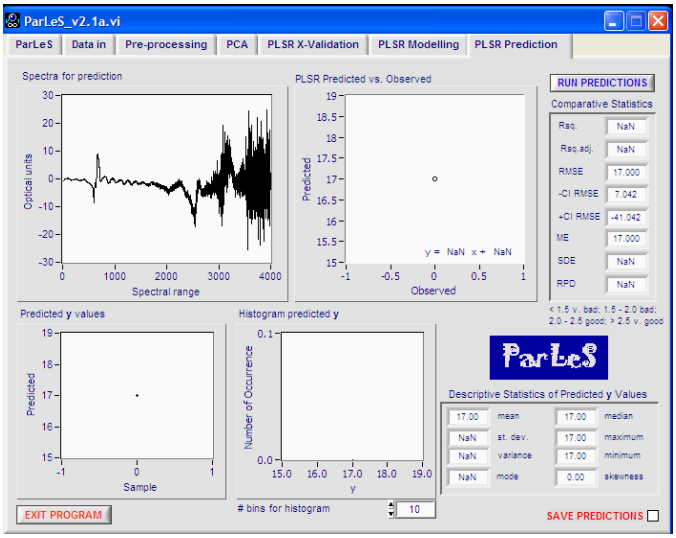


Fig. 5.9: Partial Least Square Regression Prediction

This chapter includes the results and conclusions and the future direction of the research. The RF response of the various laboratory prepared samples were modelled through curve-fitting and multivariate statistical applications, to extend parameters to predict body constituents like cholesterol, glucose, urea, alanine & lactate.

The various components with known concentrations were mixed in different ways and were used in the calibration file. While a few additional samples were prepared to test the efficacy of the algorithm. The predicted concentration was then compared with the actual concentration to find out the percentage errors.

6.1 Variations in Cholesterol

A typical spectrum of cholesterol in different concentrations of 0.5, 1, 2 & 3 in the RF range of 10MHz to 2200MHz is shown in Fig. 6.1.

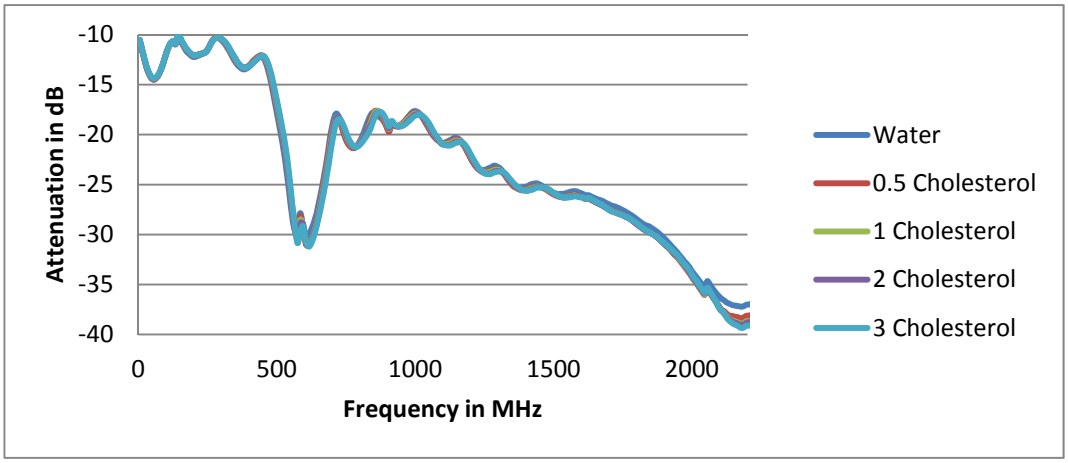


Fig. 6.1: Spectra of Various Concentrations of Cholesterol from 10MHz to 2200MHz

The cholesterol shows a good variation only in certain regions at specific frequencies (575MHz, 995MHz, 1145MHz, 1285MHz & 2185MHz) and is shown in an expanded form in Fig. 6.2-6.6.

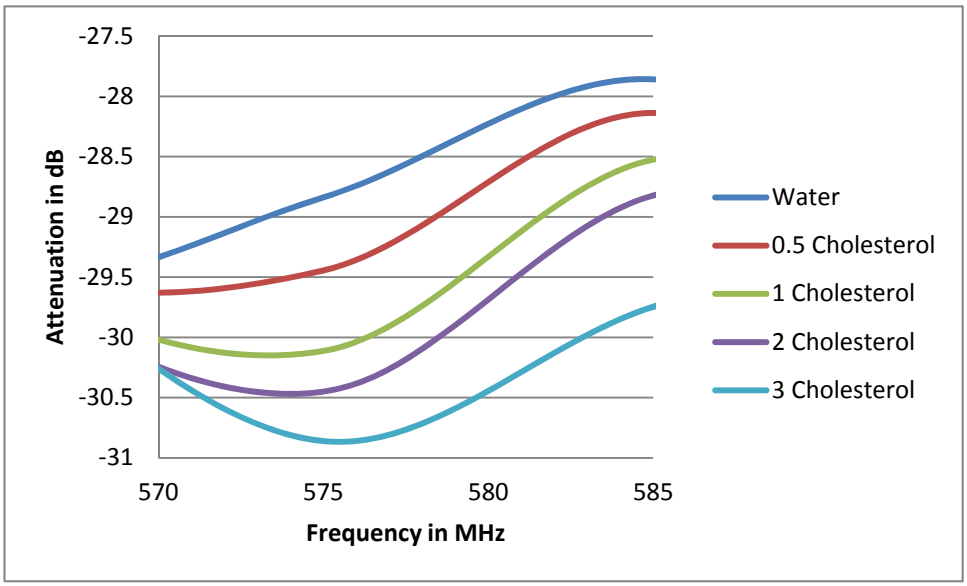


Fig. 6.2: Graph of 570MHz to 585MHz

At 575MHz there is no trough seen in water, but as the concentration of cholesterol increases from 0.5 to 3 the trough is more visible. (Fig. 6.2)

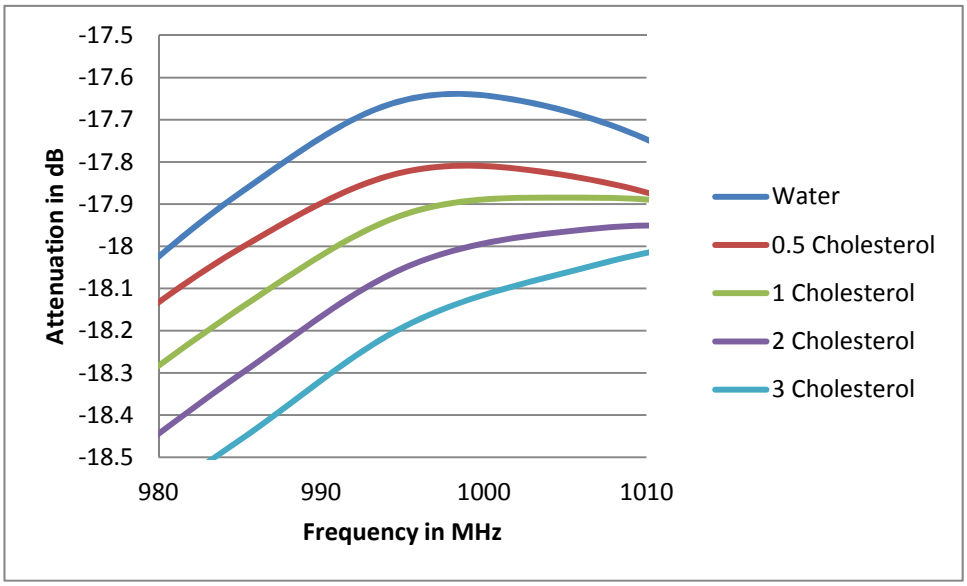


Fig. 6.3: Graph of 980MHz to 1010MHz

At 995MHz, a peak is seen in water which disappears when cholesterol is added. The attenuation of solution increases as the concentration of cholesterol increases. (Fig. 6.3)

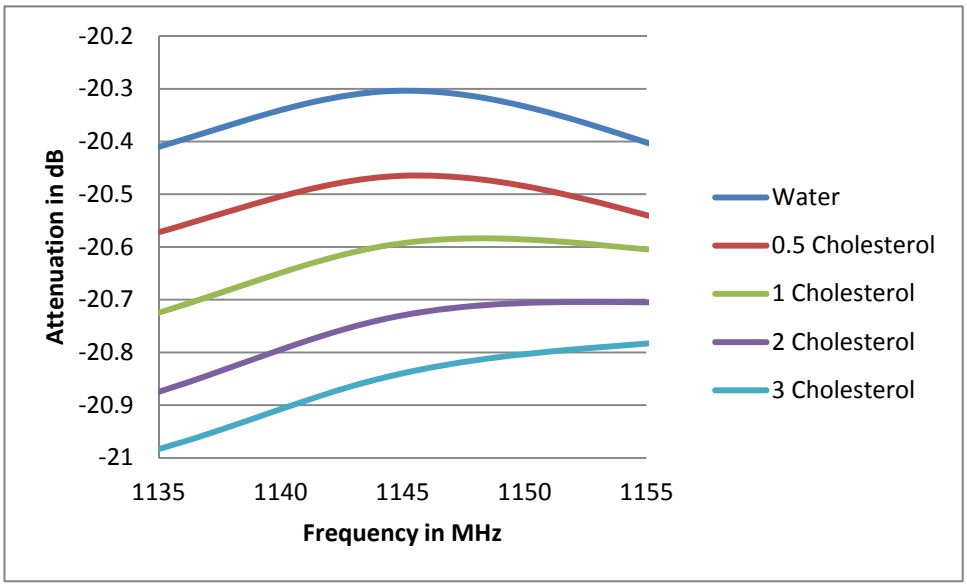


Fig. 6.4: Graph of 1135MHz to 1155MHz

A peak is also seen in water at 1145MHz which disappears when cholesterol is added. As the concentration of cholesterol increases, the attenuation level of solution increases. (Fig. 6.4)

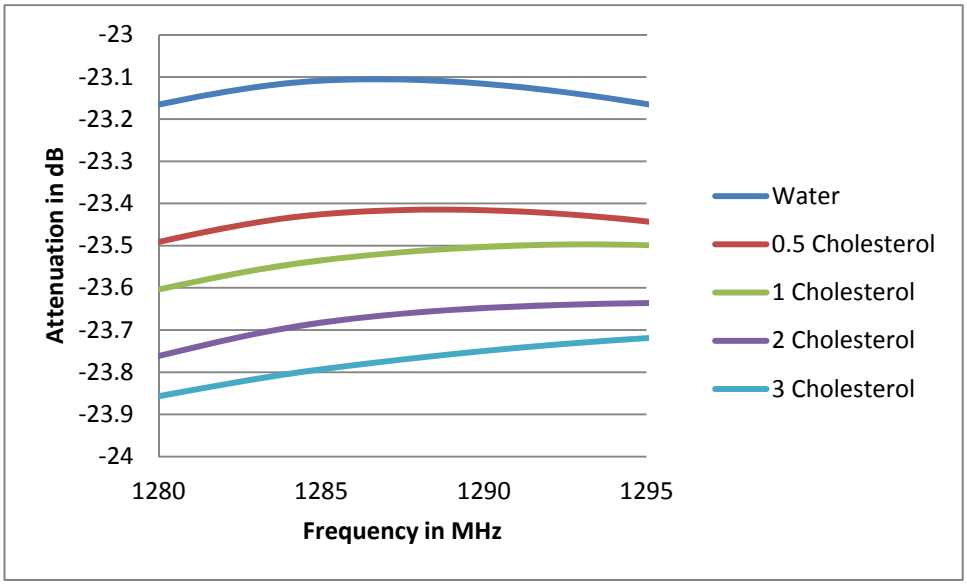


Fig. 6.5: Graph of 1280MHz to 1295MHz

A peak is seen in water at 1285MHz which disappears when cholesterol is added. As the concentration of cholesterol increases, the attenuation level of solution increases. (Fig. 6.5)

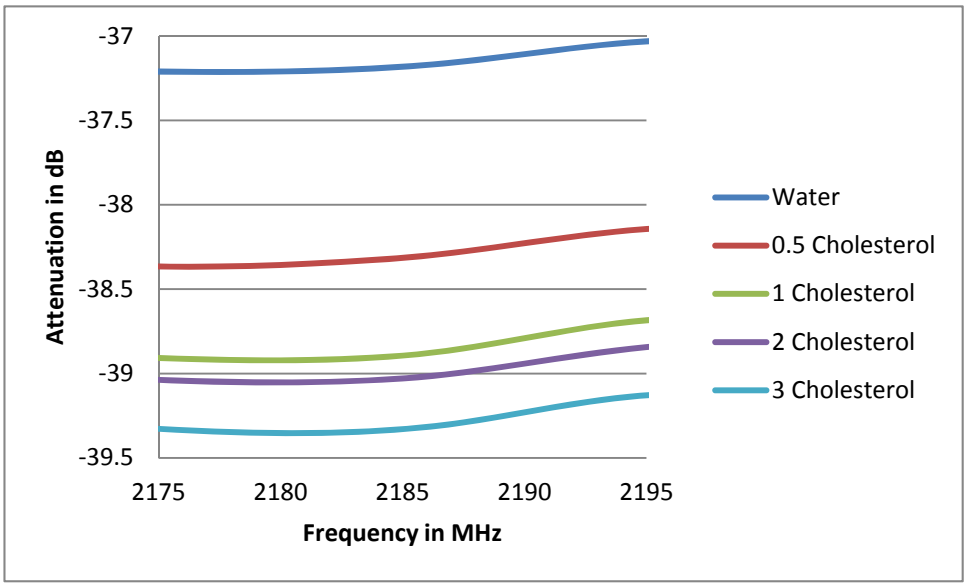


Fig. 6.6: Graph of 2175MHz to 2195MHz

A trough is also seen in water at 2185MHz which disappears when cholesterol is added. As the concentration of cholesterol increases, the attenuation level of solution increases. (Fig. 6.6)

Table 6.1: Variation of Attenuation Level of Cholesterol Solution in dB

Freq in MHz	Water	Concentration			
		0.5	1	2	3
575	-28.84	-29.44	-30.11	-30.45	-30.86
995	-17.65	-17.83	-17.93	-18.05	-18.19
1145	-20.30	-20.47	-20.59	-20.73	-20.84
1285	-23.11	-23.43	-23.53	-23.68	-23.79
2185	-37.18	-38.31	-38.89	-39.03	-39.33

The figures in Table 6.1 show the attenuation of cholesterol solution in dB. The attenuation increases at frequencies 575MHz, 995MHz, 1145MHz, 1285MHz and 2185MHz as the concentration of cholesterol increases. Therefore, cholesterol gives a unique RF output in the frequency range of 10MHz-2200MHz which is used for building a multivariate system.

6.2 Variations in Glucose

The attenuation graphs for glucose are shown in Fig. 6.7 in the 10MHz-1200MHz frequency range. The concentration of glucose is varied from 0.5 to 3 and the RF spectrum for each concentration is obtained. Glucose has a peak at 445MHz wherein the concentration of glucose increases, the attenuation level decreases. It also has a peak at 585MHz wherein the concentration of glucose increases, the attenuation level increases. It has a trough at 625MHz wherein the concentration of glucose increases, the attenuation level increases. It has a peak at 1005MHz wherein the concentration of glucose increases, the attenuation level decreases. Glucose has a trough at 1095MHz wherein the concentration of glucose increases, the attenuation level decreases. It has a peak at 1155MHz wherein the concentration of glucose increases, the attenuation level decreases.

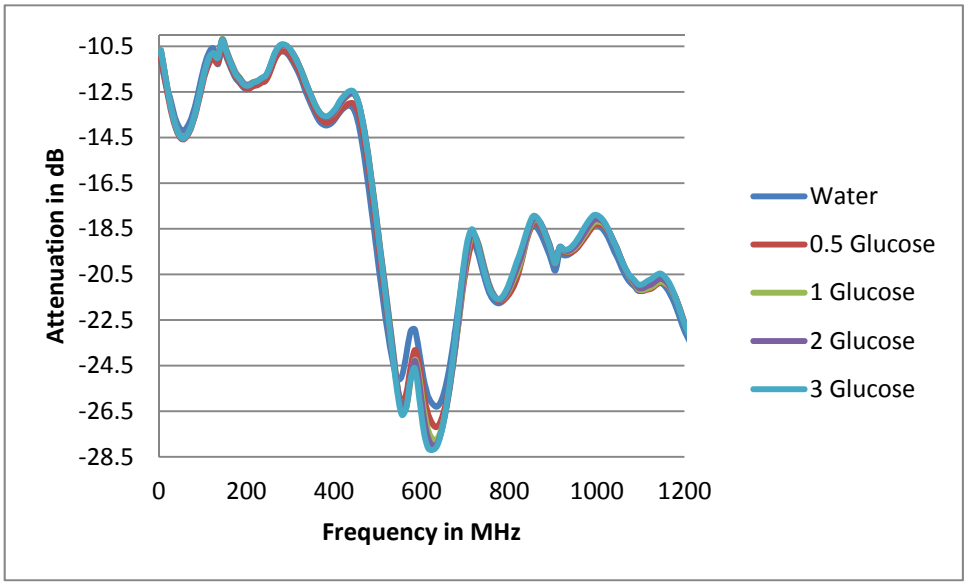


Fig. 6.7: Spectra of Various Concentrations of Glucose from 10MHz to 1200MHz

The Table 6.2 shows the attenuation level of glucose solution in dB. The attenuation decreases at frequencies 445MHz, 1005MHz, 1095MHz and 1155MHz, as the concentration of solution increases and as the concentration of solution increases the attenuation increases at frequencies 585MHz and 625MHz.

Table 6.2 : Variation of Attenuation Level of Glucose Solution in dB

Freq in MHz	Water	Concentration			
		0.5	1	2	3
445	-13.26	-13.02	-12.59	-12.57	-12.49
585	-22.93	-23.82	-24.24	-24.30	-24.62
625	-26.17	-27.08	-27.71	-28.01	-28.20
1005	-18.37	-18.30	-18.19	-18.10	-17.95
1095	-21.23	-21.19	-21.15	-21.09	-20.95
1155	-21.01	-20.92	-20.89	-20.78	-20.61

6.3 Variations in Urea

A typical spectrum of urea in different concentrations of 0.5, 1, 2 & 3 in the RF range of 10MHz to 500MHz is shown in Fig. 6.8.

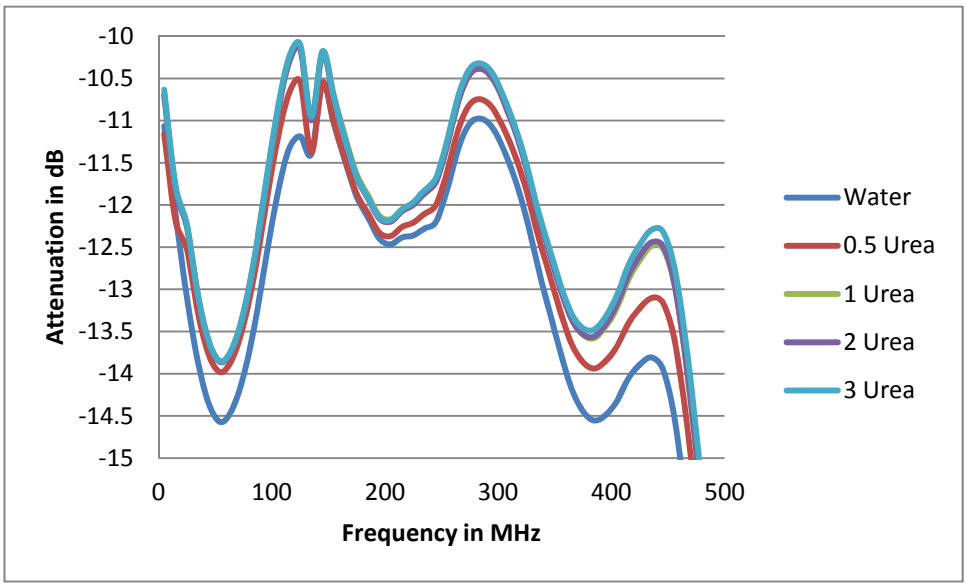


Fig. 6.8: Spectra of Various Concentrations of Urea from 10MHz to 500MHz

The graph shown in Fig. 6.8 shows that as the concentration of solution increases at 85MHz, the attenuation decreases. Further at 115MHz, the attenuation decreases as per the concentration of solution increases. A trough is observed at 245MHz wherein the attenuation decreases as the concentration of solution increases. The graph also shows that as the concentration of solution increases at 385MHz, the attenuation decreases. The attenuation decreases at 425MHz as the concentration of solution increases.

Table 6.3: Variation of Attenuation Level of Urea Solution in dB

Freq in MHz	Water	Concentration			
		0.5	1	2	3
85	-13.416	-12.797	-12.628	-12.626	-12.603
115	-11.342	-10.687	-10.434	-10.316	-10.259
245	-12.202	-11.999	-11.721	-11.725	-11.677
385	-14.555	-13.938	-13.577	-13.557	-13.476
425	-13.894	-13.226	-12.657	-12.604	-12.473

The figures in Table 6.3 show the absorption level of urea in dB. Here we notice that as the concentration of urea increases the absorption decreases at frequencies 85MHz, 115MHz, 245MHz, 385MHz and 425MHz.

6.4 Determination of Cholesterol using ParLes Software

From the tables shown above, the spectra of every major blood constituents are unique and hence the data of spectra along with combination of major constituents of blood, are fed to a PLSR model. The dielectric cell can hold only 15mL of liquid. The concentration of constituents needs to be scaled down to 15mL from 100mL, as the blood constituents in a human are measured in mg/dL. Table 6.4 gives the constituents of different concentrations used, measured in mg/15mL, which are used in the experiment.

Table 6.4: Constituents of Different Concentrations Used in the Experiment

Conc. Of Samples	Cholesterol mg/15mL	Glucose mg/15mL	Urea mg/15mL	Alanine mg/15mL	Lactate mg/15mL
0.5	17	6.8	1.13	1.13	0.95
0.75	25.5	10.15	1.69	1.69	1.43
1	34	13.5	2.25	2.25	1.9
1.25	42.5	16.85	2.81	2.81	2.38
1.5	51	20.3	3.38	3.38	2.86
1.75	59.5	23.65	3.94	3.94	3.33
2	68	27	4.5	4.5	3.8
2.25	76.5	30.35	5.06	5.06	4.27
2.5	85	33.7	5.62	5.62	4.74
3	102	40.5	6.75	6.75	5.7

6.4.1 Case 1: Calibration Set having Samples at Average Levels

The data obtained from the graph are then used in a calibration set, to predict constituents present in the blood. When the calibration set consists of combination of constituents only at the average levels of normal concentrations, then the error is minimal at the average of normal concentration of '1' up to '2' and increases outside the range as shown in Figures 6.9-6.13 and as seen in Tables 6.5-6.9.

Table 6.5: Cholesterol in Combination with Other Constituents at Normal Concentration

Sr. No.	Cholesterol (actual)	Cholesterol (predicted)	Error %	Cholesterol	Urea	Lactate	Glucose	Alanine
1	25.5	37.54	47.216	0.75	1	1	1	1
2	34	34	0	1	1	1	1	1
3	42.5	42.5	0	1.25	1	1	1	1
4	51	51	0	1.5	1	1	1	1
5	59.5	59.5	0	1.75	1	1	1	1
6	68	68	0	2	1	1	1	1
7	76.5	66.49	13.085	2.25	1	1	1	1

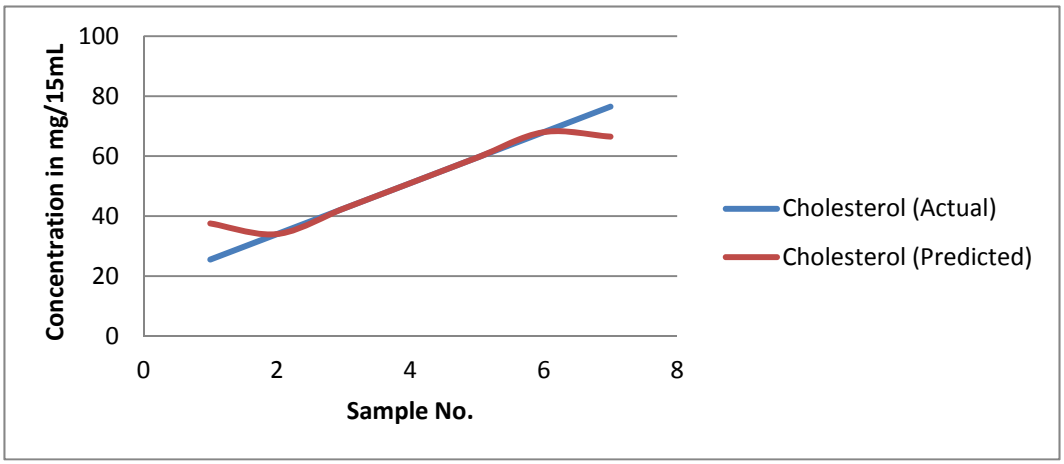


Fig. 6.9: Cholesterol in Combination with Other Constituents at Normal Concentration

Table 6.5 shows the variation of cholesterol changing from 0.75 to 2.25 times the normal blood cholesterol, keeping the other constituents at the average concentration found in a normal healthy body. As seen in the line graph in Fig. 6.9, the actual and predicted cholesterol has minimum or no error when concentration of cholesterol is changed between normal and double the normal. Table 6.5 shows that when the concentration is changed from normal to 0.75 times the normal, the error increases from 0 % to 47.216 %. It is also

seen that when the concentration of cholesterol is changed from double the normal to 2.25 times the normal, the error increases from 0 % to 13.085 %.

Table 6.6: Effect of Urea on Cholesterol Estimation

Sr. No.	Cholesterol (actual)	Cholesterol (predicted)	Error %	Cholesterol	Urea	Lactate	Glucose	Alanine
1	34	29.29	13.853	1	0.75	1	1	1
2	34	34	0	1	1	1	1	1
3	34	34	0	1	1.25	1	1	1
4	34	34	0	1	1.5	1	1	1
5	34	34	0	1	1.75	1	1	1
6	34	34	0	1	2	1	1	1
7	34	32.36	4.824	1	2.25	1	1	1

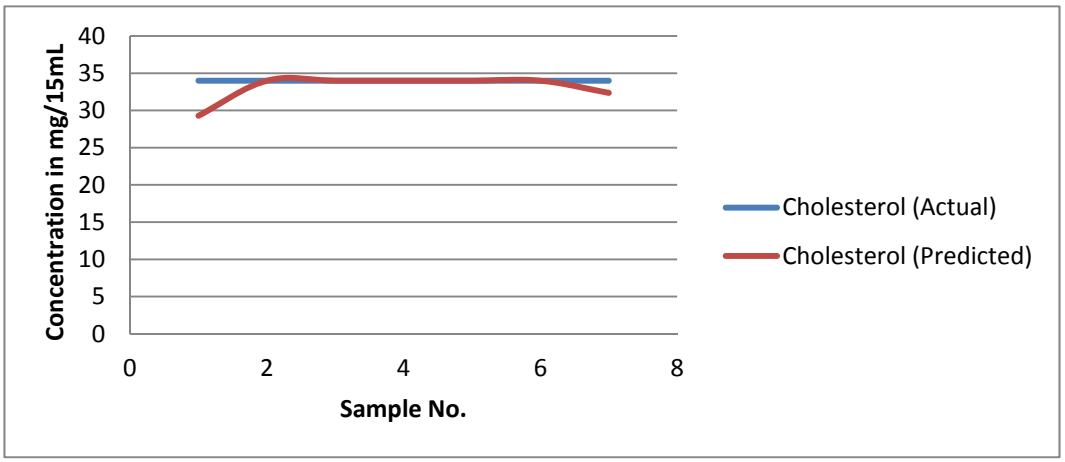


Fig. 6.10: Effect of Urea on Cholesterol Estimation

Table 6.6 shows the variation of urea changing from 0.75 to 2.25 times the normal blood urea, however the other constituents are at the normal concentration and the effect of urea can be seen on cholesterol. As seen in the line graph in Fig. 6.10, the actual and predicted cholesterol show minimum or no error when concentration of urea is changed between normal and 2 times the normal. Table 6.6 shows that the error increases from 0 % to 13.853 % when the concentration is below the normal value. It is also seen that when the concentration of urea is changed from two times the normal to 2.25 times the normal, the error increases from 0 % to 4.824 %. The error is more when it is changed to below the normal concentration.

Table 6.7: Effect of Lactate on Cholesterol Estimation

Sr. No.	Cholesterol (actual)	Cholesterol (predicted)	Error %	Cholesterol	Urea	Lactate	Glucose	Alanine
1	34	36.16	6.353	1	1	0.75	1	1
2	34	34	0	1	1	1	1	1
3	34	34	0	1	1	1.25	1	1
4	34	34	0	1	1	1.5	1	1
5	34	34	0	1	1	1.75	1	1
6	34	34	0	1	1	2	1	1
7	34	40	17.647	1	1	2.25	1	1

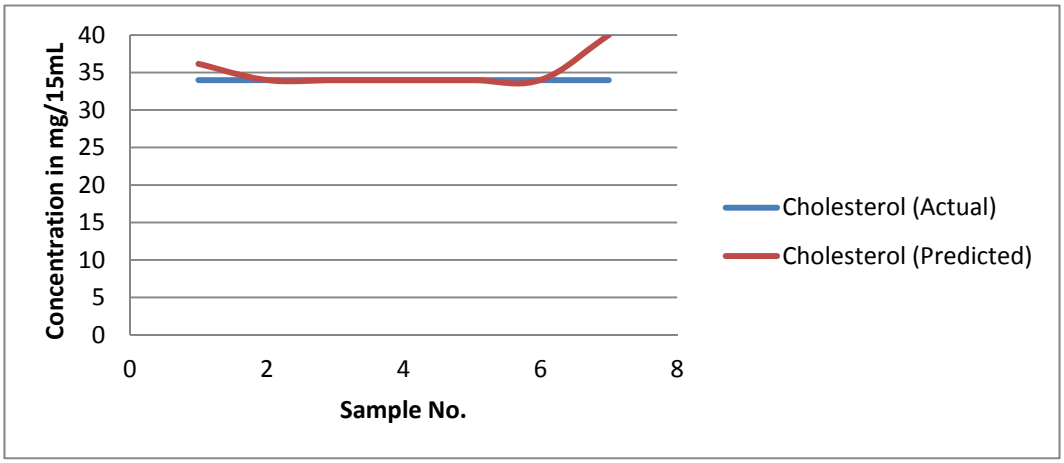


Fig. 6.11: Effect of Lactate on Cholesterol Estimation

Table 6.7 shows the variation of lactate changing from 0.75 to 2.25 times the normal blood lactate, keeping the other constituents normal and the effect of lactate can be observed on cholesterol. As seen in the line graph in Fig. 6.11, the actual and predicted cholesterol shows minimal or no error when concentration of lactate is changed between normal and double the normal. Table 6.7 shows that when the concentration is changed from normal to 0.75 times the normal, the error increases from 0 % to 6.353 %. It is also seen that when the concentration of lactate is changed to beyond twice the normal range, the error increases from 0 % to 17.647 %. This implies that there is more error when it is changed to 2.25 times the normal concentration.

Table 6.8: Effect of Glucose on Cholesterol Estimation

Sr. No.	Cholesterol (actual)	Cholesterol (predicted)	Error %	Cholesterol	Urea	Lactate	Glucose	Alanine
1	34	18.84	44.588	1	1	1	0.75	1
2	34	34	0	1	1	1	1	1
3	34	34	0	1	1	1	1.25	1
4	34	34	0	1	1	1	1.5	1
5	34	34	0	1	1	1	1.75	1
6	34	34	0	1	1	1	2	1
7	34	32.9	3.235	1	1	1	2.25	1

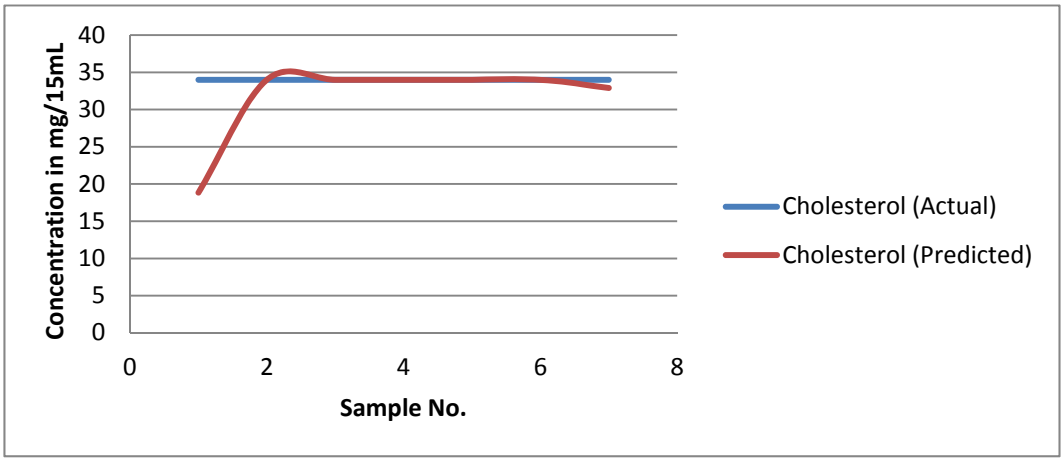


Fig. 6.12: Effect of Glucose on Cholesterol Estimation

From Table 6.8, it can be observed that the variation of glucose changes from 0.75 to 2.25 times the normal blood glucose, keeping the other constituents normal and the effect of glucose can be seen on cholesterol. As seen in the line graph in Fig. 6.12, the actual and predicted cholesterol shows minimum or no error when concentration of glucose is changed between normal and twice the normal. Table 6.8 shows that when the concentration is changed from normal to 0.75 times the normal, the error increases from 0 % to 44.588 %. It is also noticed that when the concentration of glucose is changed from twice the normal to 2.25 times the normal, the error increases from 0 % to 3.235 %. Here there is a huge error of 44.588% when it is changed to below the normal concentration which shows that glucose has a greater effect on the estimation of cholesterol.

Table 6.9: Effect of Alanine on Cholesterol Estimation

Sr. No.	Cholesterol (actual)	Cholesterol (predicted)	Error %	Cholesterol	Urea	Lactate	Glucose	Alanine
1	34	35.13	3.324	1	1	1	1	0.75
2	34	34	0	1	1	1	1	1
3	34	34	0	1	1	1	1	1.25
4	34	34	0	1	1	1	1	1.5
5	34	34	0	1	1	1	1	1.75
6	34	34	0	1	1	1	1	2
7	34	34.48	1.412	1	1	1	1	2.25

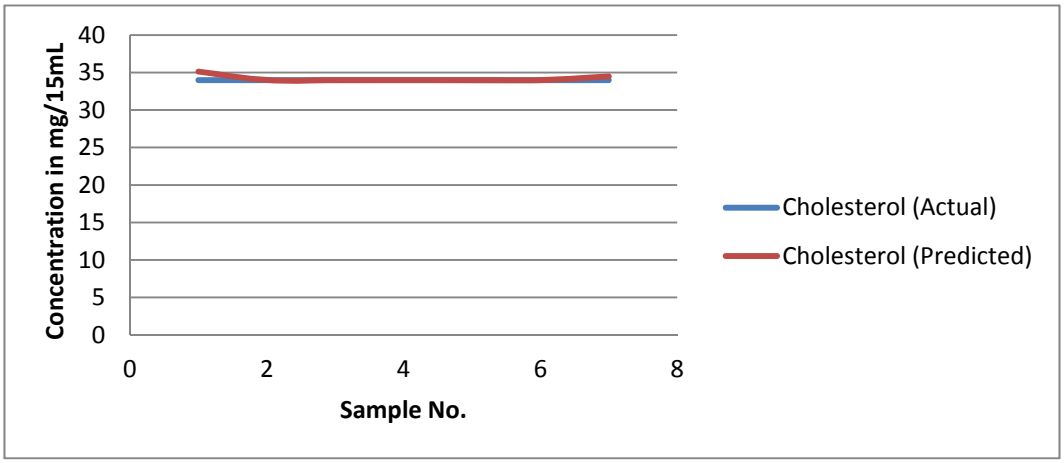


Fig. 6.13: Effect of Alanine on Cholesterol Estimation

It can be observed from Table 6.9 that the variation of alanine changes from 0.75 to 2.25 times the normal value, i.e. 1, keeping the other constituents normal and the effect of alanine can be seen on cholesterol. As illustrated in Fig. 6.13, the actual and predicted cholesterol shows minimum or no error when concentration of alanine is changed between normal and double the normal. Table 6.9 shows that when the concentration is changed from normal to 0.75 times the normal, the error increases from 0 % to 3.324 %. It is also noticed that when the concentration of alanine is changed from double the normal to 2.25 times the normal, the error increases from 0 % to 1.412 %. A minimal error is noticed outside the normal range.

6.4.2 Case 2: Calibration Set having 16 Samples.

As seen in Case 1, the errors increase greatly outside the normal range. In Case 2, the calibration set includes 16 different combinations of the major components of blood in the entire range.

Table 6.10: Predicted Cholesterol having 16 samples in Calibration Set

Sr. No.	Cholesterol (actual)	Cholesterol (predicted)	Error%	Cholesterol	Urea	Lactate	Glucose	Alanine
1	51	50.81	0.373	1.5	0.5	1.25	0.75	1
2	51	50.88	0.235	1.5	0.75	1.25	0.5	1
3	51	50.91	0.177	1.5	0.75	1.25	1	0.5
4	51	50.72	0.549	1.5	0.75	0.5	1	1.25
5	17	16.72	1.647	0.5	0.75	1.5	1	1.25
6	25.5	25.34	0.627	0.75	0.5	0.75	0.5	0.75
7	25.5	25.35	0.588	0.75	0.5	0.75	0.5	0.5
8	42.5	42.69	0.447	1.25	1.5	1.25	1.5	1.25
9	42.5	42.46	0.094	1.25	1.5	1.25	1.5	1.5
10	17	16.63	2.176	0.5	1	0.5	1.5	1.5
11	51	50.996	0.008	1.5	1	1.5	0.5	0.5
12	34	33.9	0.294	1	0.5	1.25	1	0.5

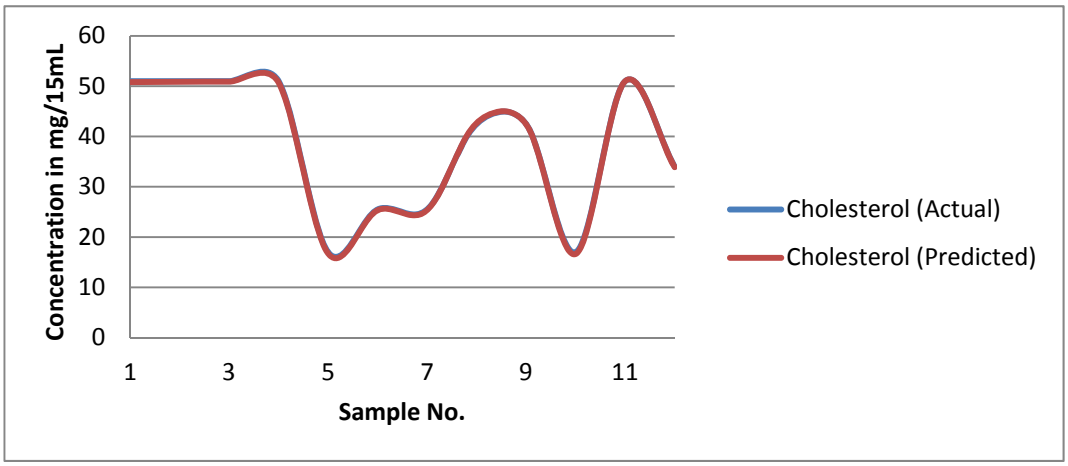


Fig. 6.14: Predicted Cholesterol having 16 Samples in Calibration Set

It is observed from Table 6.10 that the error increases from 0.008 % to 2.176 %. Values below normal concentration of blood constituents of 0.75 or even 0.5 times the normal concentrations show hardly any error. Fig. 6.14 shows the different samples used in the experiment and the predicted cholesterol matches the actual cholesterol with minimal error. In order to make the system portable, a smaller calibration set is necessary whereby Case 3 has a calibration set with 12 samples.

6.4.3 Case 3: Calibration Set having 12 Samples.

When the calibration set has more than 15 samples, it shows that it has less error but requires high resources. In Case 3, the calibration set has 12 samples in and out of the range of normal constituents.

Table 6.11: Predicted Cholesterol having 12 Samples in Calibration Set

Sr. No.	Cholesterol (actual)	Cholesterol (predicted)	Error %	Cholesterol	Urea	Lactate	Glucose	Alanine
1	25.5	25.763	1.031	0.75	0.5	0.5	0.5	0.5
2	17	16.96	0.235	0.5	0.5	0.5	0.75	0.5
3	17	17.389	2.288	0.5	0.75	0.5	0.5	0.5
4	17	16.613	2.276	0.5	0.75	0.75	1	0.5
5	34	33.8858	0.336	1	0.75	0.75	0.75	0.75
6	34	33.9638	0.107	1	1.25	1.25	1.25	1
7	42.5	43.7521	2.946	1.25	1.5	1.5	1.25	1.25
8	42.5	42.7085	0.491	1.25	1.5	1.5	1.5	1.25
9	51	48.7510	4.41	1.5	0.75	1	1.25	1.5

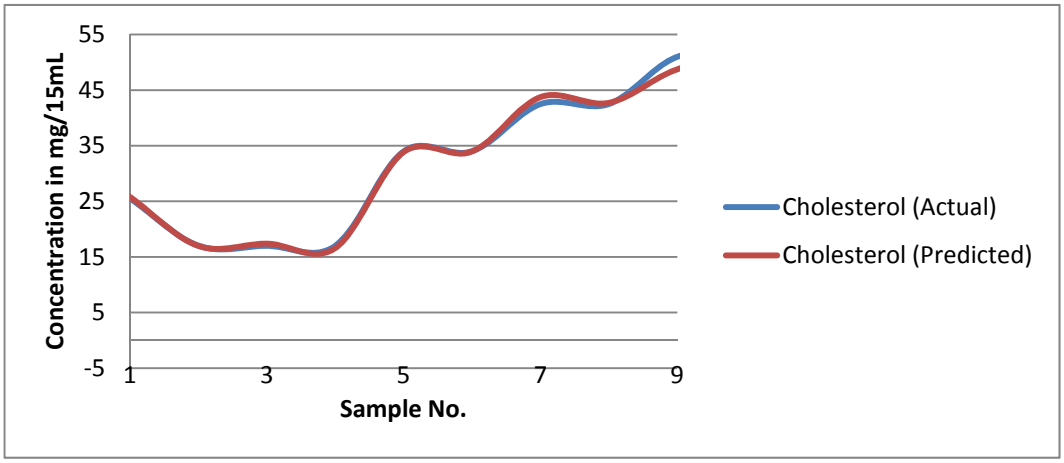


Fig. 6.15: Predicted Cholesterol having 12 Samples in Calibration Set

It is observed from Table 6.11 that the error increases from 0.107 % to 4.41 %. Fig. 6.15 shows the different samples used in the experiment and the predicted cholesterol matches the actual cholesterol with a maximum error of 4.41%.

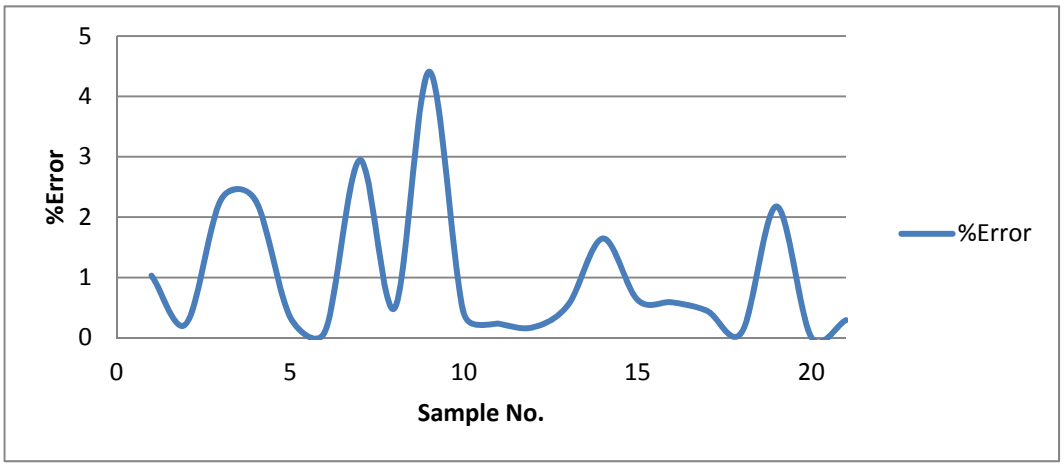


Fig. 6.16: %Error with 12 Sample Calibration Set

The results attained are within ±5% of the actual content in the sample seen in Fig. 6.16 & are within the limits of the percentage error, defined by WHO.

6.5 User Friendly Interface for Monitoring Cholesterol

As observed in Section 6.4 the various constituents of blood play an effective role on cholesterol. The calibration set was made to include out of range variables to reduce the large errors of constituents on cholesterol. The errors % is incomprehensible by a common man. Hence this section explains how the error % is converted and displayed on an LCD and hence the common man will be able to know if the cholesterol levels present is normal, high or whether a doctor needs to be consulted.

The flowchart of user friendly interface for monitoring cholesterol is depicted in Fig. 6.17. Initially the input value of predicted cholesterol is compared with the different ranges and accordingly the cholesterol levels are displayed on the LCD of the DE2 board. As people get older their cholesterol levels are naturally on the higher side, therefore if the value is ≥0.75 and ≤1.25 i.e. cholesterol is within satisfactory range (i.e. 150 mg/dL - 250 mg/dL), wherein the display shows “CHOLESTEROL IS NORMAL”. If not, the value of cholesterol is then compared with the range of 1.25 – 1.5, (i.e. 250mg/dL - 300mg/dL),

then the display shows “CHOLESTEROL IS HIGH” and the patient needs to control the level of cholesterol through diet and exercise. If it does not match any of the above ranges then the cholesterol levels can be either over 300mg/dL or below 150mg/dL wherein a doctor needs to be consulted and “CONSULT A DOCTOR IMMEDIATELY” is displayed on the LCD.

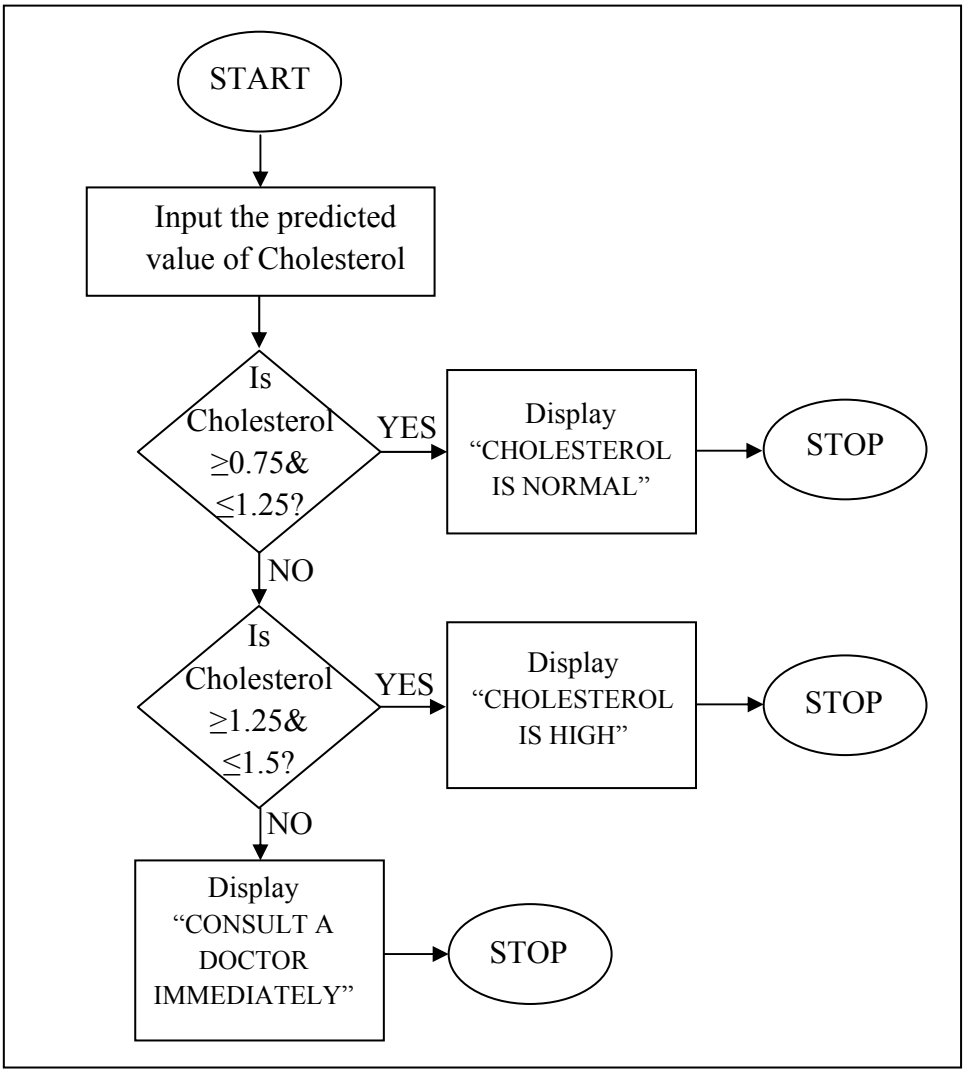


Fig. 6.17: Flowchart of User Friendly Interface for Monitoring Cholesterol

Figure 6.18 illustrates the photographs of DE2 board LCD display for 3 different conditions when cholesterol is normal, cholesterol is high and cholesterol is very high or low. The NIOS II IDE is used to write a program in C for the user friendly interface for

monitoring cholesterol which is programmed into the Cyclone II FPGA and given in Annexure II.

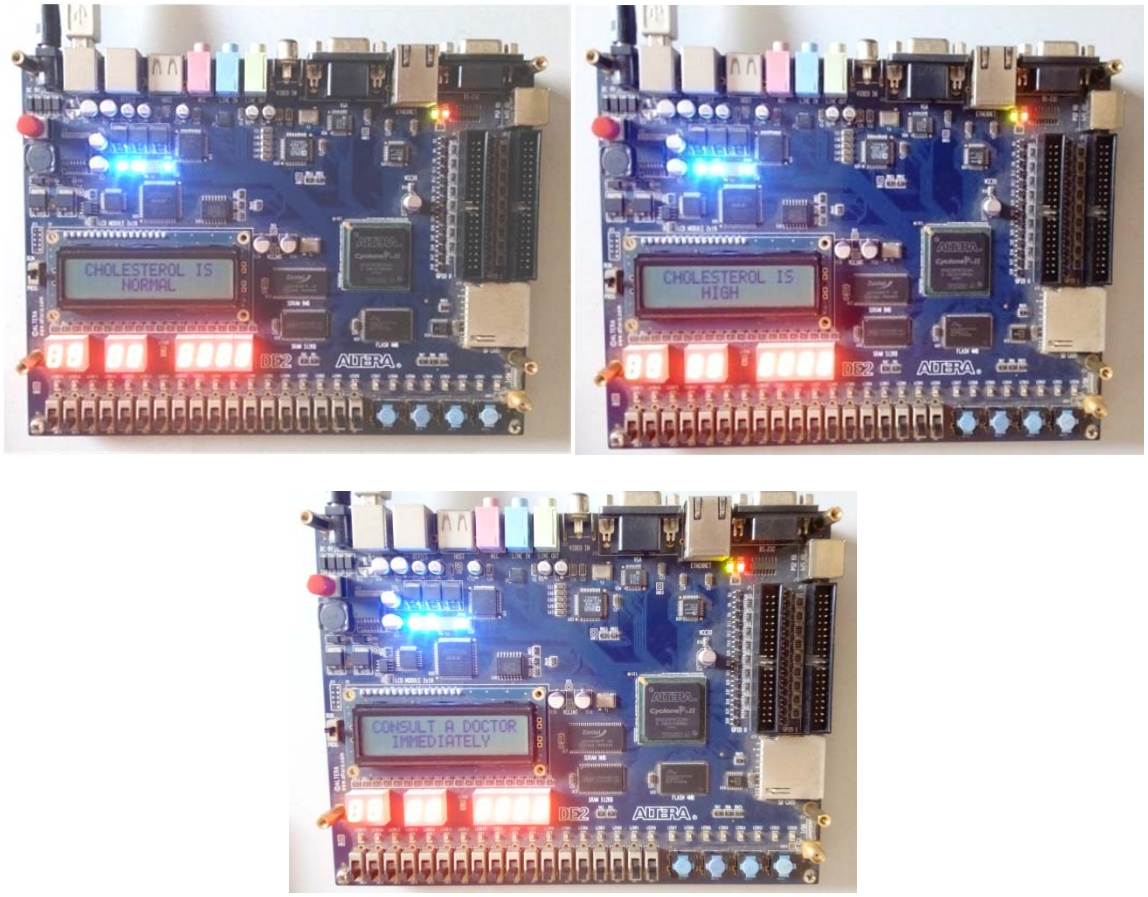


Fig. 6.18: Photographs of User Friendly LCD Display

6.6 Conclusion

The thesis describes a novel method to estimate blood cholesterol, using dielectric loss at RF frequency range. The method proposed uses RF signals, which range from 10MHz to 2200 MHz and analyses blood constituents. The spectra received from the RF spectroscopic sensor are analysed by embedded technology. The data of the spectra is later fed to a regression analysis matrix which is programmed in VLSI chip EP2C35F672C6N from Altera FPGA in order to calculate the constituent concentration. C language is used in order to develop PLSR model using SIMPLS Algorithm which is embedded on the NIOS II platform in order to estimate cholesterol level and hence make the instrument portable.

The sensor had a good repeatability after being tested for accuracy many times over and results were attained within ±5% of the actual content in the sample. A user friendly interface for monitoring cholesterol by a common man was developed to display content of cholesterol as “CHOLESTEROL IS NORMAL”, “CHOLESTEROL IS HIGH”, and “CONSULT A DOCTOR IMMEDIATELY” for various ranges of cholesterol levels.

6.7 Future Scope

The research can be further extended for the prediction of other blood constituents in a sample. The device can be used to test various blood samples. The analysis of the spectra was limited up to 2200MHz even though the instrument had a higher range of 10MHz-4.4GHz. More errors were incurred at higher frequencies limiting the research to this range. In future, studies could be made by exploring the higher frequencies and by adding noise filters. The present setup can be made portable by designing oscillators which generate particular frequencies, where the variations of cholesterol are noted, and embedding them in an FPGA.

Annexure I

Code for PLSR Algorithm

```
#include <stdio.h>
#include <float.h>
#include <math.h>
#define RW_1 4
#define RW_2 4
#define CL_1 4
#define CL_2 4
#define Q 4
double plus(double**, int, int);
double** min(double** ,double**, int );
double** dvsn(double**, int, int, double);
double** mlt(double**,double**, int, int, int);
double** pqr(double**, int, int);
double** idn(int);
double** col(double**, int, int);
double** intls(double**, int, int);
double** trns(double**, int, int);
void gtt(double**, int, int);
double** stt(double**, int, int);
void Jac_Cylc_Mtd(double eigenvalues[CL_1],
        double *eigenvectors[CL_1][CL_1],double *P, int n);

void main()
{
int i,j; //row1=0,col1=1,row2=0,col2=0;
double**matrx1,**matrx2,**AO,**MO,**trns1,**CO,**AO_trns,**g,M[CL1][CL1];
double eigenvalues[CL_1],**qh,**Wh,**Wh_mat,**Ch,**W,**ph,**p,**q,**vh;
double eigenvectors[CL_1][CL_1],Ch_sq,m=2.0,**X_pre;
double **v_trns,**C1,**p_trns,**M1,**A1,**q_trns,**B,**T,av_vh;
clrscr();
```

```
matrx1=intls(matrx1,RW_1,CL_1);
matrx2=intls(matrx2,CL_2,CL_2);
stt(matrx1,RW_1,CL_1);
stt(matrx2,RW_2,CL_2);
clrscr();
trns1=trns(matrx1,CL_1,RW_1);
AO=mlt(trns1,matrx2,CL_1,CL_2,RW_1);
MO=mlt(trns,matrx1,CL_1,CL_1,RW_1);
CO=idn(CL_1);
AO_trns=trns (AO,CL_2,CL_1);
g=mlt(AO_trns,AO,CL_2,CL_2,CL_1);
        for(i=0;i<CL_1;i++)
        {
                for(j=0;j<CL_1;j++)
                {
                        M[i][j]=*(*(g+i)+j);
                }
        }
        Jac_Cylc_Mtd (eigenvalues,*eigenvectors,*M,CL_1);
                qh=intls(qh,CL_1,CL_1);
                        for(i=0;i<CL_1;i++) {
                                for(j=0;j<CL_1;j++) {
                                        if(i==j)
                                                qh[i][j]=eigenvalues[i];
                                        else
                                                qh[i][j]=0.0;
                                }
                        }
                Wh=mlt(AO,qh,CL_1,CL_1,CL_2);
                Wh_mat=col(Wh,CL_1,CL_1);
                Ch=trns(Wh_mat,1,CL_1);
                Ch=mlt(Ch,MO,1,CL_1,CL_1);
                Ch=mlt(Ch,Wh_mat,1,1,CL_1);
                Ch_sq=sqrt(**Ch);
```

```
                Wh_mat=dvsn(Wh_mat,CL_1,1,Ch_sq);
                W=pqr(Wh_mat,CL_1,1);
                Wh_mat=col(W,CL_1,1);
                ph=mlt(MO,Wh_mat,CL_1,1,CL_1);
                p=pqr(ph,CL_1,1);
                Wh_mat=col(W,CL_1,1);
                qh=mlt(AO_trns,Wh_mat,CL_2,1,CL_1);
                q=pqr(qh,CL_2,1);
                ph=col(p,CL_1,1);
                vh=mlt(CO,ph,CL_1,1,CL_1);
                av_vh=plus(vh,CL_1,1);
                av_vh=av_vh/m;
                vh=dvsn(vh,CL_1,1,av_vh);
                v_trns=trns(vh,1,CL_1);
                C1=mlt(vh,v_trns,CL_1,CL_1,1);
                C1=min(CO,C1,CL_1);
                p_trns=trns(ph,1,CL_1);
                M1=mlt(ph,p_trns,CL_1,CL_1,1);
                M1=min(MO,M1,CL_1);
                A1=mlt(CO,AO,CL_1,CL_2,CL_1);
                q_trns=trns(q,1,CL_1);
                B=mlt(W,q_trns,CL_1,CL_1,1);
                T=mlt(matrx1,W,RW_1,1,CL_1);
                gtt(T,RW_1,1);
                ph=trns(p,1,CL_1);
                X_pre=mlt(T,ph,RW_1,CL_1,1);
                matrx1=trns(matrx1,CL_1,RW_1);
                X_pre=trns(X_pre,CL_1,RW_1);
                gtt(X_pre,CL_1,RW_1);

getch();
free(matrx1);
free(matrx2);
} /* end of main */
```

```
double plus(double** matrx, int row,int col)
{
	int i,j=col-1;
	double plus=0.0;
	for(i=0;i<row;i++)
		plus+= *(*(matrx +i)+j);
	return plus;
}

double** min(double** matrx1,double** matrx2,int col)
{
	int i,j;
	double **diffc;

	minus=intls(diffc, col, col);
	for(i=0;i<col; i++)
	{
		for(j=0;j<col;j++)
			*(*(diffc +i)+j)=( *(*(matrx1+i)+j) - *(*(matrx2+i)+j) );
	}
	return diffc;
}

double** dvsn(double** matrx, int row,int col, double Ch_sq)
{
	int i,j,k=0;
	double **dvde;
	dvde=intls(column, row, col);
	for(i=0,j=(col-1);i<row;i++)
	{
		*(*(dvde +i)+k)=*(*(matrx +i)+j) / Ch_sq;
	}
	return dvde;
}
```

```
double** mlt(double** ari1,double** ari2,int row,int col,int col1)
{
	double **rslt;
	int i=0,j=0,k=0;
	rslt=intls(rslt, row, col);
	for(i=0; i<row; i++)
	{
		for(j=0; j<col; j++)
		{
			for(k=0; k<col1; k++)
			{
				*(*(rslt + i)+j)+=(*(*(ari1+i)+k))*(*(*(ari2+k)+j));
				if (k!=(col1-1))
				printf ("+");
			}
			printf("\t");
		}
		printf("\n");
	}
	return (rslt);
}

double** pqr(double** matrx, int row,int col)
{
	int i,j,k=0;
	double **pqr;
	pqr=intls(pqr, row, col);
	for(i=0;i<row;i++)
	{
		*(*(pqr+i)+k)= *(*(matrx +i)+k);
	}
	return pqr;
}
```

```
double** idn(int dim)
{
	double **CUO;
	int i,j;
	CUO=intls(CUO, dim, dim);
	for(i=0;i< dim; i++)
	{
		for(j=0;j< dim; j++)
		{
			if(i==j)
			{
				CUO[i][j]=1.0;
			}
			else
			{
				CUO[i][j]=0.0;
			}
		}
	}
	return CUO;
}

double** col(double** matrx,int row,int col)
{
	int i,j,k=0;
	double **colmn;
	colmn=intls(colmn,row,col);
	for(i=0,j=(col-1);i<row;i++)
	{
		*(*(colmn+i)+k)=*(*(matrx+i)+j);
	}
	return colmn;
}
```

```
double** intls(double** ari,int row, int col)
{       int i=0,j=0;
        ari=(double**)malloc(sizeof(double)*row*col);
        for(i=0;i<row;i++) {
                for(j=0; j<col; j++) {
                        *((ari+i)+j)=(double*)malloc(sizeof(double));
                        *(*(ari+i)+j)=0.0;
                } }
        return ari;
}

double** trns(double** ari, int row1,int col1)
{
        double **trns1;
        int i,j;
        trns1=intls(trns,row1,col1);
        for(i=0;i<col1;i++) {
                for(j=0;j<row1;j++)
                *(*(trns1+j)+i)=*(*(ari+i)+j);
        }
        return trns1;
}

void gtt(double** ari, int row,int col)
{
        int i=0,j=0;
        for(i=0; i<row; i++) {
                for(j=0; j<col; j++)
                {
                        printf("%lf\t",*(*(ari+i)+j));
                }
                printf("\n");
        }
}
```

```
double** stt(double** ari, int row,int col)
{
	int i=0,j=0;
	double val=0.0;
	for(i=0; i<row; i++)
	{
		for(j=0; j<col; j++)
		{
			printf("Enter value for row %d col %d :",(i+1),(j+1));
			scanf("%lf", &val);
			*(*(ari+i)+j)=val;
		}
	}
	return ari;
}
//https://www.codechef.com/viewplaintext/509513[153]
void Jac_Cylc_Mtd(double eigenvalues[Q], double *eigenvectors[Q][Q],double *A, int n)
{
	int row, i, k, j, m;
	double *pAb, *pAp , *p_e ,*p_r;
	double threshold;
	double threshold_norm;
	double tn_phi, sn_phi, cs_phi, tn2_phi, sn2_phi, cs2_phi;
	double sn_2phi, cs_2phi, ct_2phi;
	double dm1;
	double dm2;
	double dm3;
	double max;
	double r;
	if ( n < 1)
		return;
	if ( n == 1)
	{
```

```
        eigenvalues[0] = *A;
        *eigenvectors[0][0] = 1.0;
        return;
}
for (p_e = eigenvectors, i = 0; i < n; i++)
        for (j = 0; j < n; p_e++, j++)
                if (i == j)
                        *p_e = 1.0; else *p_e = 0.0;
for (threshold = 0.0; pAp = A; i = 0; i < ( n - 1 ); pAp += n; i++)
        for (j = i + 1; j < n; j++)
                threshold += *(pAp + j) * *(pAp + j);
                threshold = sqrt(threshold + threshold);
                threshold_norm = threshold * DBL_EPSILON;
                max = threshold + 1.0;
                while (threshold > threshold_norm) {
                        threshold = 10.0;
                        if (max < threshold) continue;
                                max = 0.0;
        for (pAp = A, k = 0; k < (n-1); pAp += n, k++) {
                for (pAb = pAp + n, m = k + 1; m < n; pAb += n, m++) {
                        if ( fabs(*(pAp + m)) < threshold ) continue;

ct_2phi = 0.5 * ( *(pAp + k) - *(pAb + m) ) / *(pAp + m);
dm1 = sqrt( ct_2phi * ct_2phi + 1.0);
if (ct_2phi < 0.0) dm1 = -dm1;
        tn_phi = -ct_2phi + dm1;
        tn2_phi = tn_phi * tn_phi;
        sn2_phi = tn2_phi / (1.0 + tn2_phi);
        cs2_phi = 1.0 - sn2_phi;
        sn_phi = sqrt(sn2_phi);
        if (tn_phi < 0.0) sn_phi = - sn_phi;
           cs_phi = sqrt(cs2_phi);
           sn_2phi = 2.0 * sn_phi * cs_phi;
           cs_2phi = cs2_phi - sn2_phi;
```

```
                p_r = A;
                dm1 = *(pAp + k);
                dm2 = *(pAb + m);
                dm3 = *(pAp + m);
                *(pAp + k) = dm1 * cs2_phi + dm2 * sn2_phi + dm3 * sn_2phi;
                *(pAb + m) = dm1 * sn2_phi + dm2 * cs2_phi - dm3 * sn_2phi;
                *(pAp + m) = 0.0;
                *(pAb + k) = 0.0;
        for (i = 0; i < n; p_r += n; i++)
        {
        if ( (i == k) || (i == m) ) continue;
                if ( i < k ) dm1 = *(p_r + k); else dm1 = *(pAp + i);
                        if ( i < m ) dm2 = *(p_r + m); else dm2 = *(pAb + i);
                                dm3 = dm1 * cs_phi + dm2 * sn_phi;
                        if ( i < k ) *(p_r + k) = dm3; else *(pAp + i) = dm3;
                                dm3 = - dm1 * sn_phi + dm2 * cs_phi;
                        if ( i < m ) *(p_r + m) = dm3; else *(pAb + i) = dm3;
        }
        for (p_e = eigenvectors, i = 0; i < n; p_e += n, i++)
        {
                dm1 = *(p_e + k);
                dm2 = *(p_e + m);
                *(p_e + k) = dm1 * cs_phi + dm2 * sn_phi;
                *(p_e + m) = - dm1 * sn_phi + dm2 * cs_phi;
        }
}
        for (i = 0; i < n; i++)
                if ( i == k ) continue;
                else if ( max < fabs(*(pAp + i))) max = fabs(*(pAp + i));
        }
}
        for (pAp = A, k = 0; k < n; pAp += n, k++)
                eigenvalues[k] = *(pAp + k);
}
```

Annexure II

User Friendly Interface for Monitoring Cholesterol

```
#include <unistd.h>
#include <stdio.h>
#include <string.h>
#include <io.h>
#include "system.h"
#include "altera_avalon_timer_regs.h"
#include "alt_types.h"
#include "altera_avalon_lcd_16207_regs.h"
#include "altera_avalon_pio_regs.h"
#define LCD_RD_DATA_REG 3
#define LCD_WR_DATA_REG 2
#define LCD_RD_STATUS_REG 1
#define LCD_WR_COMMAND_REG 0

void LCD_Init(void)
{
usleep(15000); /* Wait for 15 ms */
/* 8-bit, 2 line, 5x7 mode */
IOWR(LCD_BASE, LCD_WR_COMMAND_REG, 0x38);
usleep(4100); /* Wait for 4.1 ms */
IOWR(LCD_BASE, LCD_WR_COMMAND_REG, 0x38);
usleep(100); /* Wait for 100 us */
IOWR(LCD_BASE, LCD_WR_COMMAND_REG, 0x38);
usleep(5000); /* Wait for 5ms */
IOWR(LCD_BASE, LCD_WR_COMMAND_REG, 0x38);
usleep(100); /* Wait for 100 us */

/* Set Display to OFF*/
IOWR(LCD_BASE, LCD_WR_COMMAND_REG, 0x08);
usleep(100);
```

```
/* Set Display to ON */
IOWR(LCD_BASE, LCD_WR_COMMAND_REG, 0x0C);
usleep(100);

/* Set Entry Mode -- Cursor increment, display doesn't shift */
IOWR(LCD_BASE, LCD_WR_COMMAND_REG, 0x06);
usleep (100);

/* Set the Cursor to the home position */
IOWR(LCD_BASE, LCD_WR_COMMAND_REG, 0x02);
usleep (2000);

/* Display clear */
IOWR(LCD_BASE, LCD_WR_COMMAND_REG, 0x01);
usleep(2000);
}

int main()
{
        LCD_Init();
        FILE *fp;
        float prompt=0.0;
        char Txt1 [] = "CHOLESTEROL IS NORMAL";
        char Txt2 [] = "CHOLESTEROL IS HIGH";
        char Txt3 [] = "CONSULT A DOCTOR IMMEDIATELY";
        fp=fopen("Estim_Cho.txt","r");
        if (fp == NULL) {
                printf("Error in opening of file \n"); }
        else
                fscanf (fp, "%f", &prompt);
                lcd_write_cmd(LCD_BASE,0x01);
                usleep(5000) ;
                printf("%f",prompt);
```

```
if ((prompt>150) && (prompt< 250))
{
        int i;
        for(i=0;i<strlen(Txt1);i++) {
                lcd_write_data(LCD_BASE,Txt1[i]);
                usleep(2000); }
}

else if ((prompt>250) && (prompt< 300))
{
        int i;
        for(i=0;i<strlen(Txt2);i++){
                lcd_write_data(LCD_BASE,Txt2[i]);
                usleep(2000); }
}

else
{
        int i;
        for(i=0;i<strlen(Txt3);i++) {
                lcd_write_data(LCD_BASE,Txt3[i]);
                usleep(2000); }
}

fclose(fp);
return 0;
}
```

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