1. ORGANIC NITRATES AND NITRITES AS CARDIAC DRUGS

1.1 Introduction:

Angina pectoris is a heart disease. It is characterized by a pressing pain in chest that often radiates to the neck area, arms and shoulders. Although its symptoms had been noted as early as 1632, it was first characterized as a clinical entity under the name of Pectoris Dolor by William Hebenden in 1768. For nearly a century following its first description, there was no treatment to relieve an angina patient of his agony. Brandy, ether, chloroform, ammonia and other stimulants or depressants have been attempted as remedy, but nothing seemed to bring any comfort to the patient. The breakthrough came in 1867, when T. Lauder Brunton a British physician reported his success with the use of amyl nitrite. Nitrates are the most important class of compounds used as antiangina agents. Amyl nitrite was the first among nitrates and nitrates to be introduced. He wrote in Lancet, "On pouring from five to ten drops of amyl nitrite on a cloth and giving it to patient to inhale, the pain completely disappeared and, generally did not return till its wanted time next night." In 1879, another British physician, Murrell reported his success with nitroglycerin. Unlike amyl nitrite, which has an offensive odour and had to be inhaled, nitroglycerin
can be administered inconspicuously in the form of a sublingual tablet. Nitroglycerin is the drug used most widely for the treatment of an angina attack.\textsuperscript{5} It is administered in tablet sublingually, because the compound is metabolized fast by the liver. The oral dose requirement is up to 20 times that of sublingual dose.\textsuperscript{6} Due to its ease of administration and potency, nitroglycerin soon became the drug of choice for the management of angina. Even today it is the most effective medicine for the treatment of acute anginal attack.

It is interesting to speculate about the events that led Brunton and Murrell to amyl nitrite and nitroglycerin as anti-anginal agents. Amyl nitrite was first prepared in 1844 by Balard from amyl alcohol and nitrous acid. Many of its pharmacological properties including its ability to cause "flushing of the face, throbbing of the carotids and acceleration of heart's action" were observed soon afterwards.\textsuperscript{7,8} Brunton had heard of amyl nitrite from a friend who had conducted some experiments with it and observed a marked decrease in arterial blood pressure among both humans and animals. In his attempts to relieve anginal pain, Brunton had observed earlier that withdrawal of blood of patients provided some comfort to them. He attributed this observation to the lowering of arterial pressure resulting from the removal of blood. So, on hearing about amyl nitrite he reasoned, "a substance which possesses the power of lessening it (arterial tension) in such an aminent
degree would probably produce the same effect (as the removal of blood, i.e. relief of pain) and want ahead with his historic trial.\textsuperscript{1}

Amyl nitrite is exceedingly volatile. It can be inhaled in order to obtain the therapeutic effects of the nitrite ion in the body rapidly. In actual practice, however, amyl nitrite is employed as a vasodilator in the treatment of attack of angina pectoris. Amyl nitrite is also used as drug in emergency treatment of cyanide poisoning, where nitrites are given to produce methemoglobin which temporarily inactivates the toxic cyanide ions by combining with it to form cyanomethemoglobin.

Nitroglycerin was synthesized two years after amyl nitrite by the Italian chemist, Sobrero who made the unstable oily liquid by slow addition of glycerol to a cooled mixture of nitric acid and sulfuric acid.\textsuperscript{9,10} The destructive power of the new compound was used to prepare many explosives. One person who fell victim to nitroglycerin was Emil Nobel who perished (1864) in an explosion in his brother's plant in Sweden, before the latter learned to soak the dangerous liquid into diatomaceous earth and make dynamite.\textsuperscript{11}

Sobrero reported that nitroglycerin caused headaches. Later, it was reported that workers in munition plants developed the same discomfort and also postural weakness and dizziness.
The similarity between these symptoms and the action of amyl nitrite caught the attention of Murrell. He tried nitroglycerin first on himself and then on his patients. In 1879, he reported in the Lancet "From a consideration of the physiological action of drug (nitroglycerin) and more especially from the similarity existing between its general action and that of nitrite of amyl, I concluded that it would probably prove of service in the treatment of angina pectoris and I am happy to say that this anticipation has been realized". Nitroglycerin is a general relaxant of smooth muscle. Its actions are directly on the smooth muscles and are independent of the types of innervation. Its actions can not be prevented by any known agents. It acts as a vasodilator on the finer blood vessels and thus, causes a fall in blood pressure. The hypotensive action is only occasionally sought clinically; however, its peripheral vasodilation finds extensive clinical application in the prophylaxis and relief of attack of angina pectoris. It does not have a direct action on the myocardium. Recent clinical evidence indicates that repetitive administration of nitroglycerin lowers pulmonary venous pressure and relieves pulmonary edema in congestive failure following myocardial infarction.

1.2 Manufacture and use of Nitrates:

To day, nitroglycerin is made in essentially the same way as Sobrero's method. Glycerol, sulfuric acid and nitric acid are mixed slowly.
True nitrocompounds in which the nitrogen is directly bonded to a carbon are not cardiovascular relaxants. However, it is clear that activity of the nitrate is not proportional to the number of \(-\text{O-NO}_2\) groups present in glycerol moiety.

Nitroglycerin tablets are made of methyl cellulose or lactose to which a small amount of nitroglycerin is absorbed. The tablet is administered sublingually. In contact with mucous tissue, it disintegrates readily and releases the active ingredient. The concentration of the drug in blood reaches peak level within five minutes and the relief from angina is observed within two minutes. Sublingual administration is essential for a rapid relief of pain, because it allows the drug to act without being first metabolized in the liver. The drugs administered orally are absorbed through the gastrointestinal tract into the portal vein and are first led to the liver. In the case of nitroglycerin this would mean an immediate breakdown to glyceryl dinitrate followed by a further slower denitration to glyceryl mononitrate. Since glyceryl dinitrate is only 10\% as effective as nitroglycerin and glyceryl mononitrate has no cardiovascular activity, the transportation of nitroglycerin to the heart before it is degraded by the liver, is imperative.
The shelf life of the tablets is about three months. It is not the instability of nitroglycerin molecule, but its volatility shortens its shelf life. Vapour pressure of nitroglycerin at 25°C is $5.5 \times 10^{-4}$ mm Hg. Its adsorption on an inert excipient lowers this value even further. Therefore, its tablets in an uncovered container lose quickly their potency. Another storage problem is the intertablet mobility of nitroglycerin. It is not uncommon even after a brief period of storage for some tablets to lose strength while others accumulate an extra dosage of the drug.

Amyl nitrite, although is affective as nitroglycerin in providing anginal relief, is now seldom used. Its foul smell and the conspicuous nature of administration have virtually put an end to its utilization for cardiovascular relief.

Over the years, a number of other organic nitrates have been shown to provide cardiovascular relaxation. Among them currently in use are isosorbide dinitrate (isordil) penta-erythritol tetranitrate (duotrate) and erythrityl tetranitrate (cardilate). Their structures and properties are given in the Table-I.
### Table - I: Properties of some organic nitrites

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Structure</th>
<th>Melting Point</th>
<th>Water solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sodium nitrite</td>
<td>NaONO</td>
<td>-</td>
<td>Soluble</td>
</tr>
<tr>
<td>2.</td>
<td>Ethyl nitrite</td>
<td>C₂H₅-O-NO</td>
<td>Liquid</td>
<td>Slightly Soluble</td>
</tr>
<tr>
<td>3.</td>
<td>Amyl nitrite</td>
<td>(CH₃)₂-CH-(CH₂)₂-O-NO</td>
<td>Liquid</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>4.</td>
<td>Glyceryl Trinitrate</td>
<td>CH₂-O-NO₂</td>
<td>Liquid</td>
<td>Soluble</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH-O-NO₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH₂-O-NO₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Isosorbide dinitrate U.S.P.</td>
<td>O₂N-O-CH-CH-CH₂-CH₂-O-NO₂</td>
<td>70°</td>
<td>1.1</td>
</tr>
<tr>
<td>6.</td>
<td>Pentaerythritol tetranitrate N.F.</td>
<td>C(CH₂-O-NO₂)₄</td>
<td>140°</td>
<td>0.01</td>
</tr>
<tr>
<td>7.</td>
<td>Erythritol tetranitrate N.F.</td>
<td>CH₂ONO₂</td>
<td>61°</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(CHONO₂)₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH₂ONO₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Triethanolamine trinitrate</td>
<td>N(CH₂CH₂ONO₂)₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Mannitol hexanitrate</td>
<td>O₂N-O-C-C-C-C-C-ONO₂</td>
<td>106°-108°</td>
<td>Insoluble</td>
</tr>
<tr>
<td>10.</td>
<td>2-Aminoethanol nitrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mono p.toluene sulphonate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Next to nitroglycerin, isosorbide dinitrate is the most widely used long acting organic nitrate drug. Chemically, it is a dinitrate ester of dianhydroisorbital. It can be administered sublingually, but generally, it is given orally. Its action begins within 30 minutes of its administration and last for 2-12 hours. Its oral dose must be much larger, as compared to sublingual route of administration, because the drug metabolizes in the liver before it reaches to site of action. The metabolite of organic nitrates are much less effective than the parent compounds. Therefore, the rate of ingestion must exceed the rate of metabolism by the liver in order to reach its therapeutic levels, in blood. Frequently, this requires 50 to 150 mg or more of drug per day.

Like the other organic nitrate, its mechanism is not coronary vasodilation but rather peripheral vasodilation which decreases cardiac work by decreasing peripheral resistance. After sublingual administration the onset of action is seen within 2 to 3 minutes and the duration of the action is 2 hours, even though the biological half life is about 8 hours. The onset of action after oral administration is observed within 30 minutes and duration of action is 4 to 6 hours. Thus, isosorbide dinitrate is indicated only for the prophylaxis of attack of angina in situations in which attack can be anticipated.
Pentaerythritol tetranitrate (PETN) slowly releases nitrite ions in the body. The nitrite ions thus formed induce vasodilation and reduces cardiac work. PETN has much longer duration of action than nitroglycerin but it has slower onset of effect. Hence it is useful in the prophylaxis of attack of angina pectoris, but not in the management of the acute attack.

Inorganic nitrites too, are vasodilators, but their potency is about one hundredth that of nitroglycerin. Inorganic nitrites have property of relaxing smooth muscles including that of blood vessels. In moderate doses, they have little effect on recumbent blood pressure but can produce marked postural hypotension and syncope due to the relaxation of veins. Nitrites do not produce a useful lowering of the blood pressure without the simultaneous occurrence of disturbing postural hypotension. Nevertheless, they were used extensively during the first four decades of this century. Before the discovery of reserpine, sodium nitrite was a major but not really effective drug for hypertension. They are still included in number of proprietary antihypertensive mixtures but their effect, after oral administration, is limited. The amounts of inorganic nitrites are usually too low to produce either a significant decrease in blood pressure or troublesome side effects.
1.3 Pharmacological Properties:

Anginal pain strikes when the myocardial need for oxygen exceeds the amount of oxygen available from the coronary blood flow. Since vasodilatory effect of nitroglycerin and amyl nitrite was known even before these drugs were used on angina patients, it was natural to attribute the relief of pain to their ability to relax coronary arteries. The dilation of arteries would increase the blood flow and alleviate the pain.\(^{13,28}\) This view was not seriously challenged until about 30 years ago. Some physicians found it difficult to accept that a drug shown to relax healthy vessels could also dilate the structurally abnormal, hardened arteries of heart. In 1959 Gorlin et al. did not agree with the dilation hypothesis. They observed a two fold increase in the coronary blood flow after the administration of nitroglycerin in normal subjects but in angina patients there was no change in flow. Neither was there any change in the coronary vascular resistance of these patients.\(^{29,30}\) Therefore, it is obvious that nitroglycerin somehow must lower the cardiac output and cut down oxygen requirement.

The work done by the heart depends on the pulse rate, the contractile state of heart muscles and the stress on the vascular walls, i.e. force of contraction.\(^{31-34}\) Organic nitrates do not change the pulse rate (in large dose, organic nitrates may increase the pulse rate as a result of the relax compensation of the effect of the drug) or the state of contractility, but they lower the
ventricular stress. They accomplish this by dilating both venous and arterial vessels. \textsuperscript{13,31,35-41}

Even though the total cardiac flow remains unchanged by organic nitrates, probably the flow within the myocardium is altered. Normally, a considerable portion of the blood flow in the cardiac muscle is short circuited through artery-vein by passes without being utilized for its oxygen or nutritional content. Thus, relaxation of the vascular tone in selected region of the heart muscle may redistribute the flow and provide the ischemic area with fresh blood.\textsuperscript{31,39,42,43} Recent use of nitroglycerin on patients with myocardial infarction is based on this assumption. It is hoped that prompt administration of the drug to the victim channels the blood flow to the area of infarct and keeps it from spreading.\textsuperscript{44} The basic pharmacological action of nitrites is to relax smooth muscle. The relaxation is non-specific and affects all smooth muscles irrespective of its innervation or the nature of its responses to adrenergic, cholinergic or other types of antagonists. However, nitrite ion does not prevent cells from responding to an appropriate stimulus, and its effect can be antagonized by any drug that can activate the smooth muscle under consideration. Thus, nitrite is a functional antagonist of norepinephrine, acetylcholine, histamine and many other agents. The response can vary from maximal contraction to maximal relaxation, depending on the relative concentration of the members of any such pair. This antagonism must be kept in mind when assessing
overall responses to a nitrite. An increase in vascular resistance does not mean that nitrite does not act on the vessels in question. Its effect is simply overcome by compensatory sympathetic activity.

1.4 Metabolism and side effects:

Organic nitrates undergo a stepwise denitration in the liver with the production of a nitrite ion in each step. Only compounds capable of denitration relax muscles. The reaction requires glutathione. The reaction is catalysed by an enzyme called organic nitrite reductase.

\[
\begin{align*}
\text{CH}_2\text{O} & \quad \text{NO}_2 \\
\text{CH}_2\text{ONO}_2 & + 2\text{-Glu-Cys-Gly} \quad \text{Organic nitrite reductase} \\
\text{CH}_2\text{ONO}_2 & \quad \text{Reduced glutathione}
\end{align*}
\]

Trinitroglycerin

\[
\begin{align*}
\text{CH}_2\text{ONO}_2 & \quad \text{CH}_2\text{ONO}_2 \quad \text{V-Glu-Cys-Gly} \\
\text{CH}_2\text{ONO}_2 & \quad \text{or} \quad \text{CHOH} + S + \text{NO}_2^- + H_2O \\
\text{CH}_2\text{OH} & \quad \text{CH}_2\text{ONO}_2 \quad S \\
& \quad \text{V-Glu-Cys-Gly}
\end{align*}
\]

1,2-Dinitroglycerin 1,3-Dinitroglycerin

Dinitrates of glycerol undergo further denitration at much slower rates in the liver yielding glycercylmononitrate and inorganic nitrite.

\[
\begin{align*}
\text{CH}_2\text{O} & \quad \text{NO}_2 \\
\text{CH}_2\text{O} & \quad \text{NO}_2 \\
\text{CH}_2\text{O} & \quad \text{NO}_2 \\
\text{CH}_2\text{OH} & \quad \text{CH}_2\text{O} & \quad \text{in liver} \quad \text{CH}_2\text{OH} + \text{NO}_2^- \\
\text{Glyceryl dinitrate} & \quad \text{Glyceryl mononitrate}
\end{align*}
\]
Glyceryl mononitrate appears as a major metabolite of nitroglycerin in the urine. Dinitrate esters are also excreted by kidneys, but none of the parent molecule finds its way to the urinary tract.46-48

Although a good portion of glyceryl mononitrate appears in the urine, some of it seems to undergo further degradation because when labelled nitroglycerin is given to rats, 17% of the radioactive carbon shows up as CO₂, while about 1% of 14C eventually enters the structure of glycogen, lipids, proteins and even RNA and DNA.49,50

The metabolism of the other organic nitrates follows a parallel course to nitroglycerin. They also undergo stepwise denitration with the production of nitrite.19,48,49,51

The critical point is that all parent compounds and their pharmacologically active metabolites, if any, are rapidly denitrated by the glutathione-organic nitrate reductase system in the liver. For example, pentaerythritol tetranitrate metabolites are rapidly denitrated. Pentaerythritol and its mononitrate are the major metabolites found in urine. Similarly glycercylyl and isosorbide mononitrates are the major circulating metabolites and excretory products of nitroglycerin and isosorbide dinitrate, respectively. Denitration appears to be limited by the amount of endogenous hepatic glutathione
and the circulation of large amounts of organic nitrate through the liver can deplete glutathione and slow greatly inactivation.\textsuperscript{50,52,52} Denitration enhances the water solubility of the metabolites and thus, facilitates their elimination from the body. Interestingly, as the water solubility of an organic nitrate increases, its effectiveness as antianginal agent diminishes. For example, glyceryl dinitrate has about 10% of the potency of nitroglycerin and isosorbide mononitrate is only 1/30 to 1/100 as active as isosorbide dinitrate.\textsuperscript{57} In fact, the solubility in fat seems to be a prerequisite for vasodilatory action.\textsuperscript{2,20,58} The receptors for nitrates and nitrites seem to be located in lipophilic environments.

In excessive doses, organic nitrates may lead to cyanosis, a condition, characterised by a bluish grey or a purple discolouration of the skin.\textsuperscript{27,58} What makes amyl nitrite, an antidote for cyanide poisoning, is also responsible for the cyanosis.

Methemoglobinemia rarely arises from the therapeutic uses of the nitrate drugs, but it occurs in cases of over dose of drug or accidental ingestion of the nitrite ion.\textsuperscript{60,61} Nitrite ion readily oxidizes hemoglobin to methemoglobin both in vitro and vivo. Severe poisoning and even death can result from the ingestion of nitrate by infants who are particularly sensitive to nitrites or nitrates.\textsuperscript{62,63} Nitrate poisoning in infants has reported from the use of bismuth subnitrate as an antidiarrheal
agent and from the ingestion of well water with a high nitrate content. The safe limit of nitrate in water for infants under 10 weeks age is probably not more than 10 ppm which is at par with the nitrate content of some famous brands of mineral water.

A good deal of controversy has recently attended the use of sodium nitrate and sodium nitrite as preservatives for meat because of the suspected nitrosamine production. However, nitroglycerin has more than 100 years of history of extensive therapeutic and industrial use with no hint of carcinogenicity.
2. Drug profile of Nitroglycerin

2.1 Synonyms:
Nitroglycerin, glyceryl trinitrate, trinitroglycerin, 1,2,3-propanetriol trinitrate

Formula: \( C_3H_5N_3O_9 \)

Structure:

\[
\begin{align*}
H & \quad | \\
H-C-O-NO_2 & | \\
H-C-O-NO_2 & | \\
H-C-O-NO_2 & | \\
H & 
\end{align*}
\]

Molecular weight: 227.09

2.2 Description:
It occurs as pale yellow, odorless, oily liquid with a sweet, burning taste.

2.3 Solubility:
Nitroglycerin has an aqueous solubility of 1.73 and 2.46 mg/ml at 20° and 60°, respectively. In ethanol it dissolves 375 mg/g at 0° and 540 mg/g at 20°. Its solubility in methanol and carbon disulphide is 56 mg/g and 8.3 mg/g respectively. In hot ethanol, it is miscible in all proportions. It is miscible with acetone, ether, glacial acetic acid, ethyl acetate, benzene, toluene, phenol, nitrobenzene, chloroform, ethylene chloride, nitric esters, pyridine and ethylene bromide, but sparingly soluble in petroleum ether, liquid petrolatum and glycerin.
2.4 Physical properties:
(a) IR spectrum shows absorption bond at 1650, 1280, 850 cm$^{-1}$
(b) Mass spectrum gives m/e 76, 46, 43, 30, 29 and 28.
(c) Vapour pressure: Vapour pressure of nitroglycerin at 20°, 25° and 37° is 2.6x10$^{-5}$, 5.5x10$^{-5}$ and 2.2x10 Torr, respectively.
(d) Boiling/melting point: Pure nitroglycerin boils at 145° (with decomposition).
At low temperature nitroglycerin exists in two crystalline forms. It freezes to form a stable dipyramidal polymorph melting at 13.2°. Under certain conditions, an unstable triclinic crystal may form which on standing is converted into the more stable form.
(e) Density: The density of nitroglycerin is 1.001 at 15°.
(f) Viscosity:

<table>
<thead>
<tr>
<th>Viscosity (cP)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35.5</td>
<td>20</td>
</tr>
<tr>
<td>21.0</td>
<td>30</td>
</tr>
<tr>
<td>9.4</td>
<td>50</td>
</tr>
<tr>
<td>6.8</td>
<td>60</td>
</tr>
</tbody>
</table>

2.5 Stability:
2.5.1 Chemical Stability:

Hydrolysis:
The stability of nitroglycerin in alcoholic solution as a function of pH has been studied by Amshler.\textsuperscript{66} It was found to be relatively stable in neutral and weakly acidic solutions, but degrades very rapidly in alkaline medium.\textsuperscript{67,68}
2.5.2 Photolytic Stability:

Although, it was suggested earlier that nitroglycerin is susceptible to photolysis, however, its aqueous solution on exposure to light does not lead to accelerated degradation of nitroglycerin.

2.5.3 Thermal degradation:

The thermal decomposition of nitroglycerin is highly dependent on the ratio of amount of nitroglycerin to the volume of the reaction vessel. Within the temperature range of 140° to 160°, and mass to volume ratio of 3.5x10^-3 gm cm^-3, vapour degradation follows first order kinetics. However, deviation from first order kinetics is observed in the liquid phase. This may probably due to autocatalytic effect. Below 140°, the decomposition reactions are also affected by autocatalyst.

2.5.4 Physical Stability:

Instability of nitroglycerin in dosage forms can generally be attributed to two processes: (i) volatization leading to loss of drug to the atmosphere, and (ii) sorption of drug to plastic containers. The appreciable volatibility of nitroglycerin at room temperature has been shown to be a major cause of loss of potency and inter-tablet migration of the drug during storage of unstabilized sublingual tablets. This problem has been somewhat overcome by the addition of polyethylene glycol 400 and povidone as stabilizers. The loss of drug due to sorptive phenomenon has been implicated when nitroglycerin tablets are
2.6 Metabolism:

The metabolism of nitroglycerin and other organic nitrates has been reviewed extensively.\(^{81,82}\)

The metabolic fate of nitroglycerin in rat can be schematically summarised as follow:

\[
\begin{array}{c}
\text{Nitroglycerin} \\
\text{Glycerol dinitrate} \\
\text{Glyceryl mononitrate} \\
\text{Glycerol} \\
\text{Glycogen (Expired Proteins \text{ air})} \\
\text{Lipids} \\
\text{RNA \& DNA} \\
\end{array}
\begin{array}{c}
\rightarrow \text{Glyceryl-1,2-dinitrate glucuronide} \\
\rightarrow \text{Glyceryl-1,3-dinitrate glucuronide} \\
\rightarrow \text{Glyceryl mononitrate glucuronide} \\
\rightarrow \text{CO}_2 \\
\rightarrow \text{Urine} \\
\rightarrow \text{Bile} \\
\rightarrow \text{Polar Components}
\end{array}
\]

Only glyceryl mononitrates have been found as a major urinary metabolite of nitroglycerin in man.\(^{83}\)

Heppel and Hilmo\(^e\)\(^{84}\) observed that the reaction between nitroglycerin and glutathione is catalyzed in presence of hog liver microsomal enzyme. Subsequent investigation showed that
the liver enzymes from rat and guinea pig consisted of two
distinct fragments with different activity for nitroglycerin
and other organic nitrates.  

Polynitric esters are rapidly metabolized by liver
organic nitrate reductases, in order of mannitol hexanitrate >>
erythritol tetranitrate >> nitroglycerin. In vitro studies
have demonstrated that the metabolism of nitroglycerin in
liver homogenates can be enhanced or depressed upon pretreat-
ment of the experimental animals with barbiturates or
bromobenzene.  

Under physiological conditions, rat serum hydrolyses
nitroglycerin to dinitrate and mononitrate, but at a much
slower rate. The effects of concentration, temperature,
red blood cell hydrolysis and silver nitrate on nitroglycerin
stability in human and rat have been examined. Depending
upon the temperature, nitroglycerin is degraded 10-50 times
faster in rat plasma compared to human plasma.

2.7 Methods of Analysis:
The various procedures of analysis are reviewed in
Chapter 4.
3. DRUG PROFILE OF ISOSORBIDE DINITRATE

3.1 Synonyms:

1,4:3,6-Dianhydro-d-glucitol dinitrate, 1.4:3,6-dianhydro-d-glucitol-2,5-dinitrate, dinitrosorbide, glucitol, 1,4:3,6-dianhydro "dinitrate, D", Isordil, Isorbid, Vascardin, Carvamil, Isosorbide dinitrate.

Formula:

\[
\begin{align*}
\text{M.W.} & = 236.14 \\
\end{align*}
\]

Molecular Weight: 236.14

3.2 Description:

Isosorbide dinitrate occurs as a white, odorless, crystalline powder. Diluted isosorbide dinitrate with lactose, mannitol or other inert excipients is an ivory white powder. The mixture usually contains about 25%-40% of isosorbide dinitrate.

3.3 Solubility:

It is soluble in hexane and freely soluble in ethanol, acetone, ether and chloroform, but sparingly soluble in water.
3.4 Physical Properties:

(a) Melting Point:

The melting range reported for isosorbide are 50.5-52, 70-71.5° and 70-71°.92,93

(b) Optical Rotation:

The specific rotation $[\alpha]^D_{25}$ of isosorbide dinitrate was determined to be +137°. The Merck Index reports $[\alpha]^D_{25}$ of +135°, Jackson and Hayward90 found the $[\alpha]^D_{20}$ to be +141° and Goldberg91 +134°.

(c) IR Spectrum shows absorption peaks at 2950-2850, 1665, 1635, 1460, 1285-1270, 1100 and 865 cm$^{-1}$.

(d) Mass spectrum gives m/e 273, 190, 144, 125.

3.5 Stability:

3.5.1 Chemical Stability:

In acidic medium isosorbide dinitrate hydrolyses in stepwise forming isosorbide-2-mononitrate and isosorbide-5-mononitrate as intermediates. The final products are isosorbide and inorganic nitrate.94 Degradation in alkali is somewhat more rapid than in acidic medium.94

Jackson and Hayward90 studied the decomposition of isosorbide dinitrate in pyridine and found that a polymer, nitrogen oxides, and pyridinum nitrate are formed when heated above 50°C.
3.5.2 Thermal stability in solid form:

Isosorbide dinitrate in solid form is stable at 45° for 12 months and at room temperature for a period of 60 months.  

3.6 Metabolism:

Dietz reported that isosorbide dinitrate is almost completely metabolized in man and in dog. Less than 1% of the dose was recovered as isosorbide-2-mononitrate and isosorbide-5-mononitrate and the rest of the drug as isosorbide which is the major metabolite.

Sherber et al. demonstrated that 80% of isosorbide dinitrate, administered intravenously, is cleared from rabbit blood within 90 seconds.

In a later study, Reed et al. observed after oral administration of 14C isosorbide dinitrate it was excreted in the urine of dogs during the first 24 hours. Some 20 to 30% of the carbon skelaton of isosorbide dinitrate was excreted mainly as isosorbide, isosorbide-2-mononitrate and isosorbide-5-mononitrate and their monoglucuronide of isosorbide.

Sisenwine and Ruelius have found that the initial biotransformation occurs by denitration of isosorbide-2-mononitrate and isosorbide-5-mononitrate. As the both the
mononitrate esters disappear, a small amount of isosorbide appears. Further transformation of isosorbide molecule does not take place. The disappearance of isosorbide-5-mononitrate from plasma is probably caused by transformation of the molecule to glucuronide and other conjugates. A postulated biotransformation scheme is illustrated as below. These investigators did not find any isosorbide-2-mononitrate in dog urine. Contrary to the findings of Dietz and Reed et al.

Metabolic Pathways of Isosorbide Dinitrate
3.7 Methods of Determination:

The methods for determination of isosorbide dinitrate are summarized in Chapter V.
4. DRUG PROFILE OF PENTAERYTHRITOL TETRANITRATE

4.1 Synonyms: Nitropentaerythrite, Pentrit, Penta, PETN.

Formula:

\[
\begin{align*}
\text{CH}_2\text{-}0\text{-}\text{NO}_2 \\
\text{O}_2\text{NO}\text{-CH}_2\text{-C}\text{-CH}_2\text{-}0\text{-}\text{NO}_2 \\
\text{CH}_2\text{-}0\text{-}\text{NO}_2
\end{align*}
\]

Molecular Weight: 316.14

4.2 Description:

It occurs as colorless, prismatic needles which crystallizes from water as tetragonal crystals. It is non-hydroscopic when exposed to an atmosphere of 90% relative humidity at 30°.

4.3 Melting Point:

It melts at 141-3°

4.4 Density:

Its sp. gr. is 1.765 at 25.

4.5 Solubility:

It is sparingly soluble in water, methanol, ethanol, ether, benzene, toluene and carbon tetrachloride but soluble in acetone and methyl acetate.
4.6 Stability:

4.6.1 Physical Stability:

Pentaerythritol tetranitrate melts and burns quietly. Though its explosion-temperature test value is nearer to that of glycercyl trinitrate, it is less sensitive to impact and friction than glycercyl trinitrate. When rubbed in a rough porcelain mortar, it crackles but does not explode.

4.6.2 Chemical Stability:

It is hydrolyzed much more slowly than cellulose nitrate on boiling with 2.5% solution of sodium hydroxide. At 50°C, a solution of sodium sulfide decomposes pentaerythritol tetranitrate slowly, however, a boiling solution of ferrous chloride decomposes it more rapidly.

4.7 Metabolism:

The absorption, biotransformation and excretion of pentaerythritol tetranitrate was studied after oral administration of $^{14}$C-PENT tablets. The total $^{14}$C excreted in 48 hours was approximately 92% of dose. Drug radioactivity was detected in the blood within 15 minutes and peak levels were obtained from 4 to 8 hours after administration of Pentaerythritol tetranitrate, Pentaerythritol dinitrate and Pentaerythritol mononitrate were found in blood, urine and faces as major metabolites.
The metabolism of pentaerythritol tetranitrate\textsuperscript{99,100} can be summarized as follows (Scheme I).

\begin{equation}
\begin{array}{c}
\text{Pentaerythritol tetranitrate} \\
\text{Pentaerythritol trinitrate} \\
\end{array}
\end{equation}

\begin{equation}
\begin{array}{c}
\text{Pentaerythritol dinitrate} \\
\text{Pentaerythritol mononitrate} \\
\text{Pentaerythritol} \\
\end{array}
\end{equation}

\textbf{Scheme-I : Metabolic Fate of Pentaerythritol tetranitrate}

\textbf{4.8 Analysis :}

Pentaerythritol tetranitrate is analysed by volumetric, colorimetric, gas chromatographic, HPLC and IR spectroscopic procedures (Chapter 6).
5. AIM AND PLAN OF THE PRESENT WORK

The organic nitrates are employed as cardiovascular drugs particularly for the relief of pain of angina pectoris. Nitroglycerin, isosorbide dinitrate and pentaerythritol tetranitrate are widely used in present day therapy. Among those, isosorbide dinitrate and pentaerythritol tetranitrate have long duration of action, while the effect of nitroglycerin is of short duration, but its onset of action is quick and is used in emergency treatment of angina pectoris.

The aim of present work is to establish a simple, accurate and precise method for the estimation of nitroesters in bulk drugs, dosage forms and in biological fluids.

The literature survey on analysis of organic nitrates reveals that number of procedures are suggested for their determination. Among them, colorimetric procedures involving diazotization of the primary aromatic amine by the nitrite ion produced by hydrolysis of nitroesters is widely employed. Since the method is carried out in aqueous medium, it is suitable for the analysis of nitroesters in biological fluids without prior extraction of these drugs.

However, the method has limited sensitivity and requires large sample size of blood. So it was thought to investigate
the various reaction conditions and reagents in order to increase the sensitivity of the test (Chapter II).

Prior to their estimation, the nitroesters should be hydrolyzed to form nitrite ion. Various parameters of hydrolysis are studied. The effect of catalysts is investigated for the rapid hydrolysis under ambient reaction conditions (Chapter III).

The method is applied to the analysis of trinitroglycerine, isosorbide dinitrate and pentaerythritol tetranitrate in bulk powder and their dosage forms and in biological fluids. (Chapter IV, V, & VI)