2.1. SELECTION OF DRUGS

Electroanalysis of organic substances belonging to drugs containing various basic electroactive units have been the subject of interest for many electroorganic chemists and electroanalysts. Perusal of literature revealed that little work has so far been carried out in the electrochemical aspects of the drugs such as Calcium Channel Blockers that is Amlodipine Felodipine, Lercanidipine, Nifedipine, Nimodipine and Nitrendipine on modified electrodes. These calcium channel blockers are important life saving drugs and their quantity assumes significance in the treatment because their presence in excess would result in danger. Hence the analysis of these drugs becomes important and that to sensitive methods are always in demand. This led to the selection of these drugs and the development of sensitive analytical method for the present investigation.

Analysis of drugs composition and purity occupies the central position in quality control laboratories of pharmaceutical industries. The application of electrochemical techniques in pharmaceutical analysis opened a new dimension in the pharmaceutical industries and R & D laboratories. This was chiefly based on potentiometric titrations, DC polarography and stripping analysis. The electrochemical studies on the pharmaceutical samples now-a-days lead to a greater importance and interests. The study of electrochemical behaviour of the calcium channel blockers also led to analytical applications. Hence the main objective of the present investigation is to study the electrochemical behaviour of selected drugs on GCE and modified electrodes using cyclic voltammetry and the development of electroanalytical method for the determination of these drugs. This paves way to probe the cyclic voltammetric behaviour and stripping analysis of pharmaceutically important anti hyper tension drugs in the present investigation. The table 2.1 presents the structure of the six calcium channel blockers selected for the present investigation.
Table 2.1. Structure and properties Calcium Channel Blockers

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Physical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amlodipine (AMLD)</strong>&lt;br&gt;CAS No. 88150-42-9</td>
<td><img src="image1" alt="Amlodipine Structure" /></td>
<td>Molecular Formula: C_{20}H_{25}Cl N_{2}O_{5}&lt;br&gt;Physical State: white to off-white crystalline powder, Melting point: 195 - 204 °C, Solubility In water: Slightly soluble, Stability: Stable under ordinary conditions.</td>
</tr>
<tr>
<td><strong>Felodipine (FELD)</strong>&lt;br&gt;CAS No. 72509-76-3</td>
<td><img src="image2" alt="Felodipine Structure" /></td>
<td>Molecular Formula: C_{18}H_{16}Cl_{2}NO_{4}&lt;br&gt;Physical State: White or Light yellow solid, Melting point: 142 - 145 °C, Solubility In water: Insoluble, Stability: Stable under ordinary conditions.</td>
</tr>
<tr>
<td><strong>Lercanidipine (LERD)</strong>&lt;br&gt;CAS No. 100427-26-7</td>
<td><img src="image3" alt="Lercanidipine Structure" /></td>
<td>Molecular Formula: C_{36}H_{41}N_{3}O_{6}&lt;br&gt;Physical State: Light yellow powder, Melting point: 118-120 °C, Solubility: soluble in chloroform and methanol, practically insoluble in water</td>
</tr>
<tr>
<td><strong>Nifedipine (NIFD)</strong>&lt;br&gt;CAS No. 21829-25-4</td>
<td><img src="image4" alt="Nifedipine Structure" /></td>
<td>Molecular Formula: C_{17}H_{18}N_{2}O_{6}&lt;br&gt;Melting point: 172-174 °C, Physical State: Yellow powder, Solubility: (g/L, at 20°C) in the following solvents: acetone, 250; methylene chloride, 160; chloroform, 140; ethyl acetate, 50; methanol, 26; ethanol, 17</td>
</tr>
<tr>
<td><strong>Nimodipine (NIMD)</strong>&lt;br&gt;CAS No. 66805-59-4</td>
<td><img src="image5" alt="Nimodipine Structure" /></td>
<td>Molecular Formula: C_{21}H_{26}N_{2}O_{7}&lt;br&gt;Physical State: light yellow, Melting point: 7 °C (45 °F), Solubility In water: Insoluble,</td>
</tr>
<tr>
<td><strong>Nitrendipine (NITD)</strong>&lt;br&gt;CAS No. 39562-70-4</td>
<td><img src="image6" alt="Nitrendipine Structure" /></td>
<td>Molecular Formula: C_{18}H_{20}N_{2}O_{6}&lt;br&gt;Physical State: light yellow, Melting point: 156-160 °C, Solubility In water: Insoluble</td>
</tr>
</tbody>
</table>

2.1.1. Calcium Channel Blockers

Calcium channel blockers are a class of drugs and natural substances that disrupt the conduction of calcium channels [190]. It has effects on many excitable cells of the body,
such as cardiac muscle, i.e. heart, smooth muscles of blood vessels, or neurons. The main clinical usage of calcium channel blockers is to decrease blood pressure. It is for this action that they are used in individuals with hypertension. Most calcium channel blockers decrease the force of contraction of the myocardium (muscle of the heart). This is known as the negative inotropic effect of calcium channel blockers. It is because of the negative inotropic effects of most calcium channel blockers that they are avoided (or used with caution) in individuals with cardiomyopathy.

Many calcium channel blockers also slow down the conduction of electrical activity within the heart, by blocking the calcium channel during the plateau phase of the action potential of the heart (see: cardiac action potential). This results in a negative chronotropic effect resulting in a lowering of the heart rate and the potential for heart block. The negative chronotropic effects of calcium channel blockers make them a commonly used class of agents in individuals with atrial fibrillation or flutter in whom control of the heart rate is an issue. Pharmacologic Beta blockade is superior to Calcium channel blockade regarding chronotropic properties of the myocardium. Titration of a Beta Blocker to a desired heart rate is decidedly easier than titration of a non dihydropyridine CCB.

Calcium channel blockers work by blocking voltage-gated calcium channels (VGCCs) in cardiac muscle and blood vessels. This decreases intracellular calcium leading to a reduction in muscle contraction. In the heart, a decrease in calcium available for each beat results in a decrease in cardiac contractility. In blood vessels, a decrease in calcium results in less contraction of the vascular smooth muscle and therefore an increase in arterial diameter (CCB's do not work on venous smooth muscle), a phenomenon called vasodilation. Vasodilation decreases total peripheral resistance, while a decrease in cardiac contractility decreases cardiac output. Since blood pressure is determined by cardiac output and peripheral resistance, blood pressure drops. With a relatively low blood pressure, the afterload on the
heart decreases; this decreases the amount of oxygen required by the heart. This can help ameliorate symptoms of ischemic heart disease such as angina pectoris. Unlike β-blockers, calcium channel blockers do not decrease the responsiveness of the heart to input from the sympathetic nervous system. Since moment-to-moment blood pressure regulation is carried out by the sympathetic nervous system (via the baroreceptor reflex), calcium channel blockers allow blood pressure to be maintained more effectively than do β-blockers.

However, because calcium channel blockers result in a decrease in blood pressure, the baroreceptor reflex often initiates a reflexive increase in sympathetic activity leading to increased heart rate and contractility. A β-blocker may be combined with a calcium channel blocker to minimize these effects. Ionic calcium is antagonized by magnesium ions in the nervous system. Because of this, dietary supplements of magnesium oxide and other magnesium preparations may increase or enhance the effects of calcium channel blocker. Dihydropyridine calcium channel blockers are often used to reduce systemic vascular resistance and arterial pressure, but are not used to treat angina (with the exception of amlodipine and nifedipine, which carry an indication to treat chronic stable angina as well as vasospastic angina) because the vasodilation and hypotension can lead to reflex tachycardia. This CCB class is easily identified by the suffix ",-dipine".

2.2. AMLODIPINE

Amlodipine (as besylate, mesylate or maleate) \(((RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate) is a long-acting calcium channel blocker (dihydropyridine class) used as an anti-hypertensive and in the treatment of angina. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle. Amlodipine is marketed as Dailyvasc in the Philippines by Xeno Pharmaceuticals, and by Pfizer as Norvasc.
in North America, Australia and some European countries, and as Istin in the United Kingdom and Ireland. Generic brands (sold under names such as Perivasc in Australia) are also available. In the United Kingdom tablets of amlodipine from different suppliers may contain different salts. The strength of the tablets is expressed in terms of amlodipine base, i.e., without the salt. Tablets containing different salts are therefore considered interchangeable. The efficacy and tolerability of a fixed-dose combination of amlodipine 5mg and perindopril 4mg, an angiotensin converting enzyme (ACE) inhibitor, has recently been confirmed in a prospective, observational multicenter trial 1250 hypertensive patients [191]. Amlodipine is almost entirely metabolised to inactive metabolites. Ten per cent of the parent substance and 60% of the metabolites are excreted in urine.

2.3. FELODIPINE

Felodipine (3-ethyl 5-methyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) is a calcium channel blocker (calcium antagonist), a drug used to control hypertension (high blood pressure). It is marketed with the brand name Plendil by AstraZeneca. The formulation patent for the substance expired in 2007.

AstraZeneca dropped Plendil from its support and AZ&Me free Rx access program in October 2008. Recent findings have suggested that felodipine in combination with grapefruit juice can cause toxic effects. Oral administration of felodipine is first metabolized in the gastrointestinal tract and liver by the enzyme CYP3A4. Grapefruit juice contains bergamottin which is found to have an inhibiting effect over this enzyme and as a result the bioavailability of the drug increases, raising the risk for abnormal side effects[192].

2.4. LERCANIDIPINE

Lercanidipine (2[(3,3-diphenylpropyl)(methyl)amino]-1,1-dimethylethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) is a calcium channel
blocker of the dihydropyridine class. For the treatment of Hypertension and management of angina pectoris and Raynaud's syndrome. Lercanidipine, a dihydropyridine calcium-channel blocker, is used alone or with an angiotensin-converting enzyme inhibitor, to treat hypertension, chronic stable angina pectoris, and Prinzmetal's variant angina. Lercanidipine is similar to other peripheral vasodilators. Lercanidipine inhibits the influx of extra cellular calcium across the myocardial and vascular smooth muscle cell membranes possibly by deforming the channel, inhibiting ion-control gating mechanisms, and/or interfering with the release of calcium from the sarcoplasmic reticulum. The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased afterload. By deforming the channel, inhibiting ion-control gating mechanisms, and/or interfering with the release of calcium from the sarcoplasmic reticulum, Lercanidipine inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cell membranes. The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased afterload.

2.5. NIFEDIPINE

Nifedipine (3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) is a dihydropyridine calcium channel blocker. Its main uses are as an antianginal (especially in Prinzmetal's angina) and antihypertensive, although a large number of other uses have recently been found for this agent, such as Raynaud's phenomenon, premature labor, and painful spasms of the esophagus in cancer and tetanus patients. It is also commonly used for the small subset of pulmonary hypertension patients whose symptoms respond to calcium channel blockers. Nifedipine rapidly lowers blood pressure, and patients
are commonly warned they may feel dizzy or faint after taking the first few doses. Tachycardia (fast heart rate) may occur as a reaction. These problems are much less frequent in the sustained-release preparations of nifedipine (such as Adalat OROS). A more novel release system is GITS (Gastro-Intestinal Therapeutic System), which - according to Bayer - provides 24-hour continuous release through an osmotic push system. Recent trials with GITS include INSIGHT (for blood pressure)[193] and ACTION (for angina)[194]. Extended release formulations of nifedipine should be taken on an empty stomach, and patients are warned not to consume anything containing grapefruit or grapefruit juice, as they raise blood nifedipine levels. There are several possible mechanisms, including the lowering of CYP3A4 activity [195]. The approved uses for nifedipine are the long-term treatment of hypertension (high blood pressure) and angina pectoris. In hypertension, recent clinical guidelines generally favour diuretics and ACE inhibitors, although calcium channel antagonists are still favoured as primary treatment for older black patients [196]. Sublingual nifedipine has previously been used in hypertensive emergencies. This was found to be dangerous, and has been abandoned. Sublingual nifedipine causes blood-pressure lowering through peripheral vasodilation. It can cause an uncontrollable decrease in blood pressure, reflex tachycardia, and a steal phenomenon in certain vascular beds. There have been multiple reports in the medical literature of serious adverse effects with sublingual nifedipine, including cerebral ischemia/infarction, myocardial infarction, complete heart block, and death. As a result of this, the FDA reviewed all data regarding the safety and efficacy of sublingual nifedipine for hypertensive emergencies in 1995, and concluded that the practice should be abandoned because it was neither safe nor efficacious[197-198]. Nifedipine has been used frequently as a tocolytic (agent that delays premature labor). A Cochrane review has concluded that it is comparable with magnesium sulfate and beta-agonists (such as ritodrine) with fewer side-effects[199]. Its role vis à vis atosiban is not established. Raynaud's phenomenon is often treated with nifedipine. A 2005 meta-analysis showed modest benefits
(33% decrease in attack severity, 2.8-5 reduction in absolute number of attacks per week); it does conclude that most included studies used low doses of nifedipine[200]. Topical nifedipine has been shown to be as effective as topical nitrates for anal fissures[201]. Nifedipine is also used in high-altitude medicine to treat high altitude pulmonary edema[202]. Oral nifedipine has also been found to cause iron loss in the urine of small animals [203]. A NIH NIDDK study [204] is currently seeing if the drug can increase the removal of iron into the urine in humans as well, thus becoming a possible treatment for iron overload. Nifedipine (initially BAY a1040) was developed by the German pharmaceutical company Bayer, with most initial studies being performed in the early 1970s [205]. The use of nifedipine and related calcium channel antagonists was much reduced in response to 1995 trials that mortality was increased in patients with coronary artery disease who took nifedipine [206]. This study was a meta-analysis, and demonstrated harm mainly in short-acting forms of nifedipine (that could cause large fluctuations in blood pressure) and at high doses of 80 mg a day and more [207].

2.6. NIMODIPINE

Nimodipine (3-(2-methoxyethyl) 5-propan-2-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) is a dihydropyridine calcium channel blocker originally developed for the treatment of high blood pressure. It is not frequently used for this indication, but has shown good results in preventing a major complication of subarachnoid hemorrhage (a form of cerebral hemorrhage) termed vasospasm; this is now the main use of nimodipine. The regular dosage is 60 mg tablets every four hours. If the patient is unable to take tablets orally, it was previously given via intravenous infusion at a rate of 1–2 mg/hour (lower dosage if the body weight is <70 kg or blood pressure is too low)[208] but since the withdrawal of the IV preparation, administration by nasogastric tube is an alternative. Because it has some selectivity for cerebral vasculature, nimodipine's main use is in the prevention of cerebral vasospasm and resultant ischemia, a complication of subarachnoid
hemorrhage (a form of cerebral bleed), specifically from ruptured intracranial berry aneurysms irrespective of the patient's post-ictus neurological condition[209]. Its administration begins within 4 days of a subarachnoid hemorrhage and is continued for three weeks. If blood pressure drops by over 5%, dosage is adjusted. There is still controversy regarding the use of intravenous nimodipine on a routine basis [210]. A 2003 trial (Belfort et al.) found nimodipine was inferior to magnesium sulfate in preventing seizures in women with severe preeclampsia[211]. While nimodipine is not used in head injury currently, it has shown promise in clinical studies. A 2009 study found that patients with severe head trauma who were given nimodipine, via peripheral vein injection, along with the standard procedures had significantly higher cerebral perfusion pressure and jugular venous oxygen saturation, while intracranial pressure, jugular lactate and jugular glucose were lower. The study concluded that Glasgow outcome score values were higher, and that the cerebral metabolism was improved[212]. In humans, it is administered primarily orally and reaches peak plasma concentrations within one and a half hours. Patients taking enzyme-inducing anticonvulsants have a lower concentrations, while patients taking sodium valporate were markedly higher[213]. Nimodipine is metabolized in the first pass metabolism. The dihydropyridine ring of the nimodipine is dehydrogenated in the hepatic cells of the liver, a process governed by Cytochrome P-4503A (CYP3A). This can be completely inhibited however, by troleandomycin (an antibiotic) or ketoconazole (an antifungal drug) [214]. Studies in non-human mammals using radioactive labeling have found that 40-50% of the dose is excreted via urine. The residue level in the body was never more than 1.5% in monkeys. There were some metabolites of the drug found in the milk of lactating rat subjects [215]. Nimodipine binds specifically to L-type voltage-gated calcium channels. There are numerous theories about its mechanism in preventing vasospasm, but none are conclusive [216]. Nimodipine is associated with low blood pressure, flushing and sweating, edema, nausea and other gastrointestinal problems, most of which are known characteristics of calcium channel
blockers. It is contraindicated in unstable angina or an episode of myocardial infarction more recently than one month. While nimodipine was occasionally administered intravenously in the past, the FDA released an alert in January 2006 warning that it had received reports of the approved oral preparation being used intravenously, leading to severe complications; this was despite warnings on the box that this should not be done [217]. The FDA has classified the side effects into groups based on dosages levels at q4h. For the high dosage group (90 mg) less than 1% of the group experienced adverse conditions including itching, gastrointestinal hemorrhage, thrombocytopenia, neurological deterioration, vomiting, diaphoresis, congestive heart failure, hyponatremia, decreasing platelet count, disseminated intravascular coagulation, deep vein thrombosis.

2.7. NITRENDIPINE

Nitrendipine (ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) is a pyridine calcium channel blocker. For the treatment of mild to moderate hypertension Nitrendipine, a dihydropyridine calcium-channel blocker, is used alone or with an angiotensin-converting enzyme inhibitor, to treat hypertension, chronic stable angina pectoris, and Prinzmetal's variant angina. Nitrendipine is similar to other peripheral vasodilators. Nitrendipine inhibits the influx of extra cellular calcium across the myocardial and vascular smooth muscle cell membranes possibly by deforming the channel, inhibiting ion-control gating mechanisms, and/or interfering with the release of calcium from the sarcoplasmic reticulum. The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased after load. By deforming the channel, inhibiting ion-control gating mechanisms, and/or interfering with the release of calcium from the sarcoplasmic reticulum, Nitrendipine inhibits the influx of extra cellular calcium across the myocardial and vascular smooth muscle cell membranes The
decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased after load.

2.8. SCOPE OF THE INVESTIGATION

2.8.1. Electrochemical Studies

Cyclic voltammetry is often used as a first electrochemical technique to study the electrochemical reactions. It is an important technique because of its ability to generate potentially reactive species which enables us to examine them immediately by reversal. Its experimental simplicity leads to its ready usage and a variety of systems are extensively studied. Electroorganic chemists have used this technique for the following purposes.

- To characterise the surface and interfacial structures
- To evaluate the electron transfer reactions
- To determine reaction mechanisms and substituent effects
- To compare electrochemical reaction system with bio-systems and
- To evaluate the effects on the redox potential of the central metal ion in complexes and multi nuclear clusters.

The usefulness and importance of this technique has led to the selection of cyclic voltammetry for this investigation. To substantiate and supplement cyclic voltammetric results other electrochemical methods such as chronocoulometry (CC) and controlled potential coulometry (CPC) have been chosen for this investigation. Now a days, coated electrode systems / modified electrode systems are very much employed to characterise organic compounds. Hence conducting polymer modified glassy carbon electrode such as polypyrrole (PPy/GCE), sodium montmorilonite clay modified glassy carbon electrode
(NaMM/GCE) and multiwall carbon nanotube modified glassy carbon electrode (MWCNT/GCE) are utilised here to study electrochemical behaviour of the selected drugs.

The major objectives of voltammetric studies of the selected six drugs are as follows:

- To study the electrochemical behaviour of selected drugs
- To study the effect of pH on the reduction/oxidation of the drugs
- To find out the diffusion coefficients through chronocoulometry
- To determine the number of electrons transferred from controlled potential coulometry
- To investigate possible redox mechanisms
- To find out the electrochemical behaviour in modified electrode systems
- To find out the suitable electrode system for electroanalytical determination and
- Optimisation of differential pulse stripping voltammetric procedures.