Chapter - II
Chapter II

Introduction

2.1 Introduction

A drug may be defined as a chemical compound that is used in the prevention, diagnosis, treatment, or cure of disease, for the relief of pain, or to control or improve any physiological or pathological disorder in humans or animals. Drugs do not create new body functions, but rather modify or improve existing ones. The drug will remain active within the body to produce a desired effect; the average dose of most drugs will have a duration of action lasting between 4 to 6 hours. Most of the drugs will be metabolized and excreted from the body by the liver and kidneys. Average or therapeutic dose of drug is the amount of drug necessary to produce a desired effect in the body with a minimum of side effects. Dosage range should consist of the smallest and largest dose of a drug that will have a desired therapeutic effect on a patient without causing severe side effects.
The study of pharmacology does not lie in its purely scientific aspects but in its relation to alleviation of disease [1]. Pharmacology is a science, which deals with the understanding of the preparation of various drugs and their application in diagnosis, prevention and mainly in the treatment and cure of diseases. Pharmacotherapeutics deals with the use of drugs in the prevention and treatment of diseases. The relation between the dose of a drug given to a patient and the utility of that drug in treating the patient is described by two basic areas of pharmacology.

1. Pharmacokinetics: which can be defined as what the body does to the drug.

2. Pharmacodynamics: which can be defined as what the drug does to the body.

The rate of drug absorption in the body is decided by the route of administration. The common routes in the increase order of rapidity of absorption in man are oral, subcutaneous, intramuscular, inhalation and intravenous. The drug administration is always followed by the expected reaction. It is many times associated with other side reactions; considered to be toxic reactions. This may range from a mild skin rash through more serious blood dyscrasias and liver damage may occur. The characteristic action of a drug is intimately related to its chemical structure [2] and this relationship has led to the synthesis of many valuable drugs. The adverse effect of a drug is called toxicity. The toxic effect of the pharmacological agents employed in therapy also draws our attention to the general principles applicable to the prevention, recognition and treatment of drug poisoning of any causes [3-5].

The physiological activity of drugs has been found to depend upon the presence of particular functional groups or structural units. Such a part of the drug, which causes the actual physiological effect, is known as pharmacophore. When a pharmacophore is introduced
into biological inactive compound, this makes the compound biologically active many times. Thus, it is possible to make the compounds biologically active but less toxic by introducing various pharmacophores. Some examples of pharmacophores are alkyl, hydroxyl, aldehyde or ketone, halogens and unsaturated lipids.

The drugs are of many types, which may be defined as

1. **Antibiotics:** Antibiotics are specific chemical substances derived from or produced by living organisms which in small concentrations are capable of inhibiting the life process of micro-organisms.

2. **Antibacterial:** These drugs are used in the treatment of infections caused by bacteria.

3. **Antimycobacterial:** Antimycobacterial agents are the drugs used in the treatment of infections caused by mycobacteria. Antituberculous and antilepral agents are antimycobacterial agents

4. **Antifungal:** These drugs are used against the infection caused by fungi. They can be either fungistatics or fungicides.

5. **Anti-inflammatory:** These drugs modify the inflammatory response to diseases but are not curative and do not remove the underlying cause of the disease.
6. Central nervous system (CNS) drugs: These drugs produce depressing effects on the central nervous system as their principle pharmacological action. These include general anaesthetics, hypnotics, sedatives and tranquilizers. Central nervous system is subjected to depression by these drugs in the following order depending upon dosage.

Sedation → Hypnosis → Anaesthesia → Coma → Death

7. Cardio-vascular drugs: These drugs influence heart's mechanism; they produce direct action on the heart or on the other parts of the vascular (blood vessels) system. These drugs affect heart muscles.

8. Anti-viral drugs: They are selective inhibitors of one or more unique steps of the replicate cycle of viruses. They improve antibody formation and activity. They are selectively active against either RNA containing or DNA containing viruses.

9. Anticancer drugs: These drugs are used for the treatment of cancer in combination; they interfere with cell division. Cancer is a form of abnormal development, transforming normal cells into cancerous cells. It is a tumour, which means a
usual amount of growth or enlargement of tissue due to unlimited and uncontrolled repeated division of cells.

10. **Metabolic diseases and endocrine functions**: This group comprises drugs for treatment of inflammation, diabetes, disorders of lipid metabolism, atherosclerosis, as well as sex hormones and peptide hormones.

11. **Vitamins**: These are simple organic compounds, which are required in small quantities by animals for their maintenance and normal growth of life. Vitamins are not synthesized by the body but are supplied through the food. These are used for eye diseases, scurvy, beriberi, rickets etc.

12. **Hormones**: These are chemical substances produced in certain specific parts of the body called ductless glands, also known as endocrine glands. The deficiency of a hormone causes a disease called physiological disease.

The various factors that govern the action of drugs at the site may be due to **structurally specific and non-specific drugs**. A number of compounds that possess remarkable pharmacological actions are essentially the structurally specific drugs. Though the physical
characteristics of the drug play an important role in the biological activity, the chemical properties do exert their justified influence on the activity. The structurally non-specific drugs include general anaesthetics, hypnotics together with a few bactericidal compounds and insecticides. However, it is important to note here that the biological characteristic of such drugs is solely linked with the physical properties of the molecules rather than the chemical feature. Structurally, non-specific action is usually due to the accumulation of a drug in an important part of a cell, which possesses dominant lipid characteristics. Substances like alkanes, alkenes, alkynes, ketones, amides, chlorinated hydrocarbons, ethers and alcohols display narcotic activity, which is directly proportional to the partition co-efficient of each individual substance.

There are certain vital factors that govern the ability of a drug to reach the active site soon after its administration through various modes known to us. These factors essentially include absorption, distribution, biotransformation (metabolism) and elimination. However, in all these instances, the drug molecule has to cross a few biological membranes in one form or the other.

1. Absorption: Biological membranes play a vital role towards the absorption of a drug molecule. Soon after a drug is taken orally, it makes its way through the gastrointestinal tract, cross the various membranes and finally approach the site or cell where it exerts its desired pharmacological action

2. Distribution: As soon as finds its way into the blood stream, the drug tries to approach the site of biological action. Hence, the distribution of
drug is markedly influenced by such vital factor as tissue
distribution and membrane penetration, which largely depends
on the physico-chemical characteristics of the drug.

3. Excretion: Excretion of drugs from their sites of action is of paramount
importance and may be effectively carried out with the help of a
number of processes, namely renal excretion, biliary excretion,
excretion through lungs and above all by drug metabolism.
Drugs are mostly water soluble or get metabolized gradually
and eliminated through the kidneys.

4. Metabolism (Biotransformation):
When a drug molecule gets converted into the body to an
altogether different form, which may be either less or more
active than the parent drug; the phenomenon is termed as
biotransformation. Mostly, the drug metabolism occurs in liver.
In fact, a number of pathways are genuinely responsible for
carrying out various divers metabolism reactions in the body.
The metabolism of a drug may have a profound effect on its
pharmacological behavior and hence clinical activity. This
frequently varies in different animal species. The degree of
metabolism will change even from patient to patient and can
influence the rate of elimination of drug and thus its duration of
The biological activity and toxicity of drug is therefore, greatly dependent upon and is governed by its metabolic profile.

The drug formulations contain not only the drug but also the binding material etc., associated with it. Because of the fact that newly developed drugs are often more physiologically active, they can be administered in smaller amounts and hence more sensitive analytical methods are needed for the monitoring of these drugs. The speed of the analysis is also important as large number of samples is to be analysed particularly at the production stage.

Analysis of drugs and pharmaceutical formulations includes spectrophotometric, chromatographic, colorimetric and electrochemical methods.

Spectrophotometric technique for the simultaneous determination of furazolidone and nifuroxime in pharmaceutical formulations has been carried out by Lila et al [6]. A visible spectrophotometric method for cephalosporins and penicillins are described by Fogg et al [7] after derivatization with indophenol. Two papers describing spectrophotometric methods for cephalosporinics were also published [8,9]. Chlorpromazine and its sulfoxide impurity in dosage forms is characterized by UV spectroscopy [10]. Morgan et al [11] developed flow injection analysis with the fourier transform IR detection for many vitamins. Hoffman et al [12] employed mass and IR spectra with chromatographic technique for the determination of benzimidazoles. Roy [13] has reviewed the use of mass fragmentometry in drug research. Hydrozone products were identified by using IR and Mass spectra [14]. Turczan et al [15]
used NMR spectroscopy for the determination of bisulphate in tablets. Gamot et al [16] exploited Raman spectroscopy for use in toxicological investigations of crystalline and aqueous solutions of cocaine. Halzebecher et al [17] and Bond et al [18] have determined uranium by employing atomic absorption spectrometry and X-ray fluorescence spectrometry. Perry et al [19] has developed spectrophotometric method for the determination of Theophylline in biological samples. Valentin et al [20] reported the determination of antihypertensive drug prazosin in urine and formulations by UV spectrophotometry. Determination of Indomethacin in pharmaceutical formulations and biological fluids has been reported by spectrophotometry [21-24]. An antiepileptic drug phenytoin has been assayed in pharmaceutical preparations, spectrophotometrically using first and second derivative spectra [25,26]. Vilchez et al [27] reported the determination of an antibacterial drug travofloxacin in human serum and urine samples by solid phase spectrofluorimetric method. An helminthic carbamate compounds have been investigated using traditional method of spectrophotometry [28]. Ultraviolet spectrophotometric method has been reported by Emara et al [29] for the determination of nifuroxazide. Determination of nimesulide in raw material and in pharmaceutical dosage forms has been carried out by spectrophotometry [30,31].

Chromatography is an important analytical method for the separation of molecular mixtures. Chromatographic techniques are classified into various categories based on the nature of mobile phase as well as stationary phase. These include paper chromatography, column chromatography, gas chromatography and high performance liquid chromatography.
General aspects of chromatography as applicable to the development of a new drug [32-34] as well as specific aspects of compound analysis by capillary gas chromatography [35] and high performance liquid chromatography [36] were reported. Several reviews of liquid chromatographic methods for antibiotics have appeared [37, 38]. Douglas et al [39] developed reverse-phase column which is useful in separations involving drugs and metabolites. Mazza [40] analysed anthocyanins and related compounds in methanol-formic acid mixtures by using paper chromatography. Haque and Stewart reported a direct serum injection method for HPLC determination of selected non-steroidal anti-inflammatory drugs (NSAIDS) using RAM columns [41]. A clinical pharmacokinetics study on laranclidipine, which was based on data obtained by HPLC-UV detection has been, published [42]. Indomethacin a non-steroidal anti-inflammatory agent has been determined in pharmaceutical formulations and biological fluids by HPLC [43-52]. Torasemide and its metabolites have been determined in biological fluids using HPLC with ultraviolet detection [53-55].

Metabolites of nandralone were determined in the urine of several sportsmen, sedentary and post-menopausal woman by capillary gas chromatography-mass spectrometry quadrupole (GC-MS) and capillary gas chromatography mass–mass spectrometry ion trap (GC-MS-MS) methods [56]. Evaluation and optimisation of separation buffers for the determination of corticosteroids with micellar electrokinetic capillary chromatography (MECC) have been reported [57]. Noe et al [58] developed a method for the determination of prednisolone in serum by using solid-phase extraction and micellar electrokinetic chromatography. A liquid chromatography combined to mass spectrometry (LC-MS/MS) method to confirm the presence of 1q1 synthetic glucocorticoids in cattle liver [59] and for the analysis of
glucocorticoids in urine samples [60-63] has been reported. Thin layer chromatography technique (TLC) has been applied for the analysis of phenytoin in pharmaceutical formulations [64].

A variety of colorimetric methods have been published for the analysis of analgesics [65-67], anti-inflammatories [68-71], furazolidone and furaludone [72]. Colorimetric and fluorometric methods were described by karam et al [73] for the determination of acrivastine in dosage forms using acid-dye and charge-transfer complexation techniques. El-Shabourie et al [74] reported the determination of theophylline in biological samples by colorimetric method.

The determination of oxidation – reduction potentials of biological significance has long been a research area of vital interest. Although actual biological redox system may be of extreme complexity due to enzyme interaction, much information can be obtained in the laboratory with regard to general levels of oxidation – reduction intensity. As hydrogen ions play an important part in almost all organic redox reactions, studies of the redox potentials as a function of the acid – base equilibria involved are important.

Many of the early measurements involved interaction of the biological system with a highly colored redox system. Although this method has distinct limitations, it is a valuable technique in certain situations. The schools of Michaelis and Clark developed potentiometric measurements at a high degree in their classical work on biological redox systems [75,76].
Polarography with the dropping mercury electrode has found some use, especially with redox systems of relatively low potentials.

Electroanalytical techniques and particularly polarography were applied very early to problems of pharmaceutical interest because the samples were well defined and contained only one active compound in relatively large amounts. Furthermore, most of these physiologically active compounds were shown to be electroactive. The situations is now quite different, the requirements of pharmaceutical analysis is becoming more demanding in process control and in the analysis of drugs in complex media such as in biological fluids or in tissues after administration of the compound. The great diversity of electroanalytical methods, along with the refinements realized over the last 10 years in electronics, computers and in electrode design and handling has allowed to fulfill these requirements.

In early thirties and forties, the sensitivity of polarographic method of analysis enabling determination of electroactive species was up to $10^{-9}$ M solutions. In fifties and sixties, more organic compounds (Drugs and Pesticides) used lower concentration levels, which requires more sensitive analytical method. This was achieved by developing pulse techniques in which charging current is minimised which allows determination of electroactive species at $10^{-8}$ M solutions. In seventies, eighties and nineties of this century, the above sensitivity was often insufficient for the analysis of species in biological samples. This leads to development of stripping techniques by which we can determine the electroactive species up to $10^{-10}$ M to $10^{-11}$ M. Among the voltammetric techniques, differential pulse
polarography and stripping voltammetry are of great importance for the estimation of drugs.

In general, voltammetric techniques are useful in the

- Quantitative determination of organic and inorganic compounds in aqueous and non-aqueous solutions
- Measurement of kinetic rates and constants.
- Determination of adsorption process on surfaces.
- Determination of electron transfer and reaction mechanisms.
- Fundamental studies of oxidation and reduction processes in various media.
- Quantitative determination of pharmaceutical compounds.
- Determination of redox potentials.

The specific features and versatility of advanced polarographic and voltammetric techniques qualify them as 'solvers' of real analytical problems in the industrial laboratories. Some of the main features of modern polarography and voltammetry of importance for the analytical chemistry are

- The techniques are very rapid and time saving.
- Simultaneous determination of several metal ions and multi species in single run.
- A large useful concentration range for reducible or oxidisable organic species over the range $10^{-3} - 10^{-10}$ M or even less.
- Polarography applicable over a much wider range of concentration than most other instrumental methods, due to the linearity of the plots of signal versus concentration.
The cost of instrument is usually small fraction of instruments used for chromatographic techniques such as HPLC and GC.

Voltammetric methods offer information about the oxidation state of analyte and also their chemical forms.

- Multi element, multi species determination in a single run.
- A large array of solvents (aqueous, mixed, aprotic) can be used.

The voltammetric methods of analysis for organic compounds are attributable to their simplicity and rapidity. An extremely large number of organic compounds are either directly reducible or oxidisable at hanging mercury drop electrode, dropping mercury electrode or solid electrodes or can be readily derivatised chemically to yield derivatives. Some of the functional groups, which show excellent voltammetric properties, include \(-\text{NO}_2, >\text{C}=\text{O}, >\text{C}=\text{N}, >\text{C}=\text{C}<, -\text{CHO}, \) and hydroquinolones etc, respectively [77].

Voltammetric methods [78-82] have been successfully applied for the analysis of drugs in dosage forms and in biological fluids at highest sensitivities. In the analysis of drugs in dosage forms, electrochemistry has shown to be an exceptional method, and quite superior to classical wet methods or spectrophotometric methods. For the most part this is due to great ease of sample preparation and lack of interference’s from incipients in the dosage form. Dosage forms are typically pulverised (if necessary), dissolved in an aqueous solvent, filtered and analysed by one of the electrochemical methods. More recently, fast scan and differential pulse polarography (DPP) have become extremely useful for the measurement of drugs in pharmaceutical formulations and in biological fluids. This can be directly attributed to the
introduction of highly sensitive commercial instrumentation, which yields easily interpretable data for routine quantification at low levels. Enhanced precision and reproducibility of measurements and also simple control of complicated time sequences have contributed towards a renewed interest in electrochemical methods of analysis.

Stubauer et al [83] reported the determination of trace levels of migallopine in urine and blood by adsorptive stripping voltammetry at a hanging mercury drop electrode. Polarographic determination of benzaldehyde in benzyl alcohol and sodium diclofenac injection formulations was carried out by Kazemifard et al [84]. Sturm et al [85] described voltammetric study of ketorolac and its differential pulse polarographic determination in pharmaceuticals. Jayaseelan et al [86] developed a method for trace determination of dexamethasone sodium phosphate in pharmaceutical formulations by differential pulse polarography. Vela et al [87] studied the electrochemical behavior of sertraline at a hanging mercury drop electrode and its determination in pharmaceutical formulations. Square-wave voltammetric determination of cefoperazone in a bacterial culture, pharmaceutical drug, milk and urine was carried out by Bilova et al [88]. The electro-reduction and electro oxidation of cefoperazone as the active component of the clinical drug cefobid have been investigated at mercury electrodes and carbon paste electrodes using either polarography or voltammetry in connection with the linear or pulse sweep modes, including the stripping voltammetric techniques [89-93]. Quite recently application of electroanalytical techniques in the analytical chemistry of cephalosporines has been reviewed [94].
Polarography and adsorptive stripping voltammetry have been successfully used for the fluorinated antibacterial quinolone derivative fleroxacin in quinodis tablets [95] and some other quinolone derivatives [96-102]. Botta et al. [103], have carried determination of the antibiotic drug peflloxacin in bulk form, tablets and human serum using square wave cathodic adsorptive stripping voltammetry. Electroanalytical techniques have been used for the determination of fluoroquinolones of the first and second generations such as Norfloxacin [104], ciprofloxacin [105], enoxacin [106], ofloxacin [107] and lomefloxacin [109, 110]. Furlanetto et al. [111] studied voltammetric method for kynurenic acid determination in human urine. Determination of ceftriaxone by differential pulse adsorptive stripping voltammetry has been reported [112-114], and in aqueous humour and serum samples [115]. Use of solid – phase extraction cartridges with differential pulse cathodic stripping voltammetry at a hanging mercury drop electrode for the determination of nedocromil sodium and pentamidine isethionate in urine was reported by Valnice et al. [116].

Polarographic behavior of 8-chlorotheophylline and its determination in dosage forms was carried out by Gil et al. [117]. Obendorf et al. [118] described the adsorptive stripping voltammetry of nicardipine at a HMDE; determination of trace level nicardipine in blood and urine. Polarographic method for the quantitative determination of acrivastine was developed on the reduction of the drug at the dropping mercury electrode by Ablize et al. [119].

Temirt et al. [120] described the differential pulse and square-wave cathodic stripping voltammetry of xanthine and xanthosine at a mercury electrode. A differential pulse polarographic method is described for detection and trace determination of benzophenone (the
main impurity) in phenytoin powder [121]. Design and optimisation of the variables in the adsorptive stripping voltammetric determination of riflloxacin in tablets, human plasma and urine has been described by Furlanetto et al [122]. Tens polarography, differential, normal pulse polarography, differential pulse adsorptive cathodic stripping voltammetry, differential pulse adsorptive anodic stripping voltammetry and square-wave adsorptive cathodic stripping voltammetric determination of anti-inflammatory indomethacin drug in tablets and human serum at a mercury electrode have been reported [123-127]. Electrochemical characterisation of nefazodone hydrochloride and voltammetric determination of the drug in pharmaceuticals and human serum has been reported [128]. A polarographic method for the determination of ranitidine is described by Richter et al [129], based on the reduction of the nitro group at a dropping mercury electrode in pharmaceutical formulations and urine. Designing experiments to optimise and validate the adsorptive stripping voltammetric determination of nimesulide was reported by Furlanetto et al [130]. Polarographic behavior of nifuroxazide [131-133], determination in human serum by adsorptive stripping voltammetry was reported [134]. Voltammetric analysis of Alfuzosin HCl in pharmaceuticals, human serum and simulated gastric juice has been described by Bengi Uslu [135]. The basic D.C. polarographic behavior of nalidixic acid at dropping mercury electrode was first studied by Starosak et al [136], later Ibrahim et al [137] carried cathodic adsorptive stripping voltammetric determination of nalidixic acid in pharmaceuticals, human urine and serum.

Jayarama Reddy et al have utilized these voltammetric techniques extensively for the determination of several organic systems and pharmaceutical formulations, some of the investigations include, voltammetric behaviour and measurement of Nimorazole [138],
electrochemical behaviour of Nitrofurantoin and Nitrofurazone and assay of its formulations [139, 140], voltammetric determination of Niclosamide [141], electrochemical reduction behaviour and determination of Nicoumalone [142], differential pulse polarographic determination of Diazepam in pharmaceutical formulations [143], voltammetric determination of Oxazepam and Lorazepam [144], Clonazepam [145], Alprazolam [146] and Famotidine [147] and their assay in pharmaceutical formulations.

2.2 Chemically modified electrodes:

Chemically modified electrodes have attracted considerable interest over the past three decades as researchers have attempted to exert more direct control over the chemical nature of an electrode. The ability to manipulate the molecular architecture of the bulk matrix of an electrode and its surface in particular has led to a wide range of analytical applications of chemically modified electrodes and created powerful opportunities for electroanalysis.

For electroanalytical purpose, a chemically modified electrode can be designed [148-151] as a powerful (predominantly, voltammetric, amperometric, potentiometric and also impedimetric and microgravimetric) sensing device, by deliberate modification of the surface or bulk matrix material of the electrode with a selected agent (monomeric or polymeric) that governs its electrochemical properties. Such manipulations of the molecular composition of the electrode aims at improving sensitivity, selectivity and /or stability allowing for tailoring its response in order to meet analytical needs. Traditionally, conventional bulky electrodes (solid, carbon paste) are used as a support for the preparation of chemically modified electrodes and testing new reagents and detection schemes [152-154]. Compared to the
conventional electrodes, chemically modified electrodes offer unique well-recognized advantages especially in the field of electrocatalysis and surface sciences [155-157]

Applications of modified electrodes are very important for the study of chemicals in biological and environmental matrices [158-159]. For this purpose, different types of electrodes are in use. Among these, the use of the carbon paste electrodes (CPE) has been almost exponential due to the fact that it has following advantages [160]

- The CPE can be advantageously modified in order to improve its selectivity and sensitivity.
- It is cheap, easy to prepare and use. It exhibits good stability and reproducibility.
- It has smaller residual currents, which is smaller than at solid electrodes.
- The primary advantage is its application to anodic oxidation
- It has low background currents over the whole anodic range.

The base of modified carbon pastes is usually a mixture of graphite powder and non-electrolytic binder [152,160,161]. Modifier can be dissolved directly in the binder [152,162,163] or admixed homogenization [164,165]. It is also possible to soak graphite particles with a solution of a modifier, and after evaporating the solvent, use so impregnated carbon powder [166,167]. Finally, already-prepared pastes can be modified in situ [168]. Where as directly modifications obviously provide special sensors for one purpose use, consideration in situ approaches offer a possibility to employ the same carbon paste for respective modifications with different agents.
Kalcher [160] has made a classification of four possible functions of modifiers that can be summarized in this way

- Preferential entrapment of desired species (e.g., Preconcentration in stripping analysis).
- Mediation of electrode reaction via immobilized or their fragments.
- Acting in catalytic phenomena (Catalytic electrochemical responses).
- Alteration of the surface characteristics of the carbon paste electrode.

Most of the modifiers currently employed are synthetic catalysis, polymers, clays, zeolites, β-cyclodextrins, complexing agents etc. Dong and Wang et al [169] reviewed recent advances in the modification of electrode surfaces in electroanalysis. Recommended terminology and definitions concerning chemically modified electrodes were introduced [170, 171]. Labuda Jan et al reviewed the state-of-art of various types of chemically modified electrodes [172-177]. Complexation abilities and analytical applications of working electrodes with attached cyclodextrins (CDs) are reviewed by Ferencova et al [178]. The cyclodextrins adsorbed on a mercury drop electrode forming compact layers [179,180], for instance the complexation of phenylglyoxalic acid and stereoselectivity of its reduction were investigated using cyclodextrin modified mercury electrode [179]. Walcarius et al [181] evaluated screen-printed carbon paste electrodes modified with zeolites for the determination of the herbicides paraquat and diquat is described and compared to the corresponding zeolite-modified carbon paste electrodes.
Navratilova et al. [182] described the determination of gold using clay modified carbon paste electrode. Performance of the polyion-coated mercury film electrode in the determination of heavy metals in samples containing surfactants has been reported [183]. The electrochemical behavior of redox-active probe species on clay modified electrodes has been extensively explored for fundamental and practical purposes [184-194]. Gadzekpo et al. [195] described the voltammetric detection of the polycation protamine by use of electrodes modified with self-assembled monolayers of thioctic acid. The use of a zeolite modified electrode for the study of the methyl viologen-sodium ion exchange in zeolite Y has been reported [196].

The performance of modified carbon paste electrodes is good when looking into analysis of several pesticides [197-204]. The increase in sensitivity was enabled by adsorptive accumulation of the analyte on silica modified carbon paste electrode [197-200] or by formation of host-guest complexes [201,202]. These complexes enabled the determination of selected pesticides even in partial samples like soil or natural waters with limits of determination in the concentration range of $10^{-6}$ M or even lower.

The group of pharmaceuticals is very well suited for analysis using chemically modified electrodes [205-213]. Wang et al. [214] described a novel method for determination of metoclopramide by second derivative adsorptive anodic stripping voltammetry with a polyion modified glassy carbon electrode. Cheng et al. [215] reported square-wave voltammetric detection of apomorphine on a polyion film modified glassy carbon electrode.
Carbon paste electrode modified with \( \beta \)-cyclodextrin has been developed by Yanez et al \[216\] for the determination of nitrendipine. A sensitive voltammetric method was developed by Arranz et al \[20\] for the determination of prazosin using a nafion modified carbon paste electrode. Moane et al \[217\] developed a method for the determination of clenbuterol in bovine urine using differential pulse voltammetry, based on the electrochemical behavior of clenbuterol at a nafion modified carbon paste electrode. An electrochemical study of three important \( \beta \)-agonist drugs at nafion modified carbon paste electrode has been reported \[218\].

A differential pulse adsorption voltammetry for the determination of procaine hydrochloride at a pumice modified carbon paste electrode in pharmaceutical preparations and urine samples has been reported by Wang et al \[219\]. Differential pulse cathodic stripping voltammetric determination of nedocromil sodium at a poly-L-lysine modified hanging mercury drop electrode was carried out by Pirzad et al \[220\]. Carbon paste electrode modified with \( \beta \)-cyclodextrin have been investigated by Feranceova et al \[221\] for the differential pulse voltammetric determination of tricyclic antidepressants – imipramine, trimipramine and thioridazine. Tenax-modified carbon paste electrode has been developed by Arranz et al \[222\] for the determination of Doxazosin in urine and pharmaceutical tablets. Determination of codeine in urine and drug formulations using a clay-modified screen-printed carbon paste electrode has been reported by Shih et al \[223\].

Cyclodextrins (cyclic oligopyranose oligomers) are important and widely studied examples of host molecular reactions because of their great affinity for hydrophobic molecules in aqueous media \[224-226\]. Cyclodextrins form inclusion complexes with a great
variety of analytes [227]. Many of the potential analytical applications of host–guest system requires immobilization of the host molecule. Thus several works concerning immobilized thiolated cyclodextrin on gold [228-230] and on silver [231] have been published. The use of cyclodextrins to modify carbon paste electrode is a totally new challenge in the field of the modified electrodes. There are few publications revealing carbon paste electrodes modified with β-cyclodextrin [216,221,232].

Carbon paste electrodes modified with clays can easily be prepared and can be used for the oxidation or reduction of electroactive groups in adsorbed compounds. Such electrodes can be used for the determination of organic compounds, which are difficult to determine electrochemically with mercury, carbon or metal electrodes, on account of signals that are difficult to interpret owing to capacitive or residual currents, which may mask the analytical signal. Such modified carbon-paste electrodes have been used for determination of various organic compounds with out prior separation [223,233-239].

Nafion is a perfluorinated ion-exchange material composed of carbon-fluorine backbone chains and perfluoro side chains containing sulfonic acid groups. These perfluorinated polymers are chemically and thermally inert, non-electroactive, and insoluble in water, thus making them suitable for preparation of modified electrodes. Nafion polymers are negatively charged which renders them capable of preconcentrating cationic analytes from sample solution and discriminating against anionic interferents. Nevertheless, the preconcentration is mainly based on the electrostatic binding of the sulfonate group on the polymer backbone with the cations in solution and in some cases due to a hydrophobic
interaction. The nation modified electrodes have been used for the determination of various organic, inorganic compounds and metal ions [183,214,215,217,218,240-249].

Electrochemical mechanistic and analytical investigation of drugs with electroactive groups such as >C=N-, >C=O, -NO₂, -NH₂, have received prominent position in the field of electrochemistry. The polarographic activity of cephalosporins is attributed to the reduction of >C=N- of the cephem nucleus, which might help in understanding the action of the drug in vivo. Polarography has been widely used to study the reduction mechanism of azomethine derivatives in order to understand the biological degradation of the compound. Nitrogroup containing drugs and pharmaceuticals have got a good biological importance and these are essential for biological systems for their proper growth and metabolic functions. Though nitro group reduces easily under any conditions, the electrochemistry of nitro group is complicated by possibility of dimerisation, coupling, tautomerisation and various intermediates formed during the electrochemical process. Carbonyl group containing drugs have been known for a very long time and these are continuously attracting attention owing to the great possibilities they offer in synthesis and for application in the field of medicine. Amin group containing drugs under goes oxidation process, and this group of drugs is active ingredient of many pharmaceutical preparations. Hence extensive study on the electrochemistry of such compounds under varied conditions and with newly developed electrochemical techniques might provide much helpful data from the point of view of understanding drug action and reaction pathways in vivo, besides providing opportunities to work out their analytical determination at trace levels in pharmaceutical formulations and so on.
In the present investigation, the studies of electrode process associated with selective group containing drugs are treated systematically and their behaviour at mercury, β-cyclodextrin, clay and nafion modified electrodes are described. The electroanalytical techniques such as cyclic voltammetry (cv), differential pulse polarography (dpp), adsorptive stripping voltammetry (adsv), anodic stripping voltammetry (asv), controlled potential electrolysis (cpe) and mili coulometry (mc) are employed which may help in the following:

1. To study detailed information of electrode mechanism, electrode kinetics, reactive intermediates and adsorption complications of the species under investigation.

2. To measure the kinetic parameters such as transfer co-efficient, diffusion co-efficients and heterogeneous forward rate constant.

3. To study the behavior of selected drugs by using mercury, β-cyclodextrin, clay, nafion modified electrodes.

4. To develop suitable electroanalytical procedures for the analysis of different drugs and pharmaceuticals in trace quantities, which may contribute to the wide use of electroanalytical techniques in drug control studies at the manufacturing stage.

5. To carry out the analysis of drugs in pharmaceutical formulations, urine and serum samples.
2.3 The scope of the present investigation

A drug is a preparation intended for the prevention, alleviation, or cure of disease in man or other animals. Due to continuous increase in growth in demand for the prevention and treatment of diseases, drugs and pharmaceuticals play an important role in our daily life. The characteristic action of a drug is intimately related to its chemical structure. The requirement of pharmaceutical analysis is becoming more demanding due to very potent drugs that are now being developed. The identification of these pharmacologically important compounds present in the body fluids at very low levels, the knowledge of their mode of action and their evaluation in biological media have acquired great importance. It is therefore necessary to develop sensitive trace analytical methods for the analysis of the drugs. Electroanalytical techniques are well adopted to help one investigate and elucidate the process occurring with in biological systems and in environmental matrices. The main advantage that voltammetric methods offer in this present area is the possibility to use them often with out preliminary separations for analysis of complex biological materials as well as environmental matrices. These methods are selective, reproducible, fast, convenient, sensitive and inexpensive and could be applied for the analysis of drugs.

The cephalosporines having the azomethine group linkage are of an important class of antibiotic agents. The electrochemistry of azomethine group containing drugs is of special interest to electrochemist from the point of elucidation of mechanistic aspects and pharmaceutical analysis. Carbonyl group containing drugs are of interest in the sense that fluoroquinolones are important antibacterials developed in recent years, which have wide applications in veterinary and human medicine. Nitro group containing drugs are of interest in
the sense that the biological activity changes for nitro group compounds depending on the moiety to which the nitro group is attached. Amine group containing drugs exhibit significant local anaesthetic properties. Electroanalytical techniques such as polarography and voltammetry occupy the central position due to the many advantages embedded in these techniques for the analytical estimations of electroactive species.

The thesis consists of the results of the following i) azomethine group containing drugs and pharmaceuticals studied at mercury electrode by employing the electroanalytical techniques, cyclic voltammetry, differential pulse polarography, milliamperemetry and controlled potential electrolysis iii) carbonyl group containing drugs studied at β-cyclodextrin modified carbon paste electrode, nitro group containing drugs studied at clay modified carbon paste electrode and amine group containing drugs studied at nafion modified glassy carbon electrode by employing cyclic voltammetry, differential pulse voltammetry, adsorptive stripping voltammetry and anodic stripping voltammetry. The following compounds are chosen for the study

1. Cefixime

![Cefixime molecular structure]
2. Cefpodoxime proxetil

3. Sparfloxacin

4. Ofloxacin
5. Norfloxacin

6. Gatifloxacin

7. Lomefloxacin
8. Nifedipine

![Nifedipine structure](image)

9. Nimodipine

![Nimodipine structure](image)

10. Benzocaine

![Benzocaine structure](image)

11. Butacaine

![Butacaine structure](image)
Britton Robinson (BR) buffer of pH ranging from 2.0 - 12.0 were used as supporting electrolytes and was prepared using 0.04 M boric acid, 0.04 M orthophosphoric acid, 0.04 M acetic acid solutions and were mixed with 0.2 M NaOH to obtain desired pH. Triple distilled water was used for measurements. Cyclic voltammetry was specially employed for the identification of intermediates if any and also used for adsorption studies. The numbers of electrons involved in the electrode process were confirmed with the aid of millicoulometry, controlled potential electrolysis has been employed for the collection of the reduced products and the products were suitably identified with the help of IR spectra. Kinetic parameters such as heterogeneous forward rate constant, diffusion co-efficient and transfer co-efficient values were evaluated for cyclic voltammetry and differential pulse polarography to understand the electrode kinetics at various pH values. The electrochemical behavior and determination of some selected drugs has been studied by using β-cyclodextrin; clay and nafion modified electrodes with the aid of cyclic voltammetry, differential pulse voltammetry, adsorptive stripping voltammetry and anodic stripping voltammetry.

Analytical procedures were worked out for the determination of drugs under study. The developed assay procedures will certainly be of useful to monitor the levels of these drugs in the body fluids of the patients to whom these drugs are given. The estimated procedures are applied to the analysis of the pharmaceutical formulations of these drugs by using differential pulse polarography/voltammetry and adsorptive stripping voltammetry.
Chapter - III