CHAPTER - 1
INTRODUCTION

Drug analysis is undertaken during various phases of pharmaceutical development [1], such as formulation and stability studies, quality control (QC) and toxicology and pharmacological testing in animals and man [2,3]. In hospitals, drug analysis is performed on patient’s samples in support of clinical trials, i.e. bioavailability and pharmacokinetic studies and in monitoring therapeutic drugs and drugs abuse [4–8]. All these investigations require reliable and validated analytical methods in order to measure drugs in complex media such as formulation and biofluids. Quality management in drug analysis covers a wide range of quality improving activities designed to ensure the reliability of the analytical data. These activities include ensuring that the samples are properly collected and preserved prior to analysis, that the analysis is carried out using the appropriate techniques and that the results are properly recorded and reported. Before applying the technique for analysis, guidelines on the quality management aspects of routine quality control (QC) work should be available [9]. Once the analytical method has been developed, it has to be validated before or during its use. Validation of the method establishes that its performance characteristics are adequate for the intended use. It builds quality and reliability into the method. In the pharmaceutical industry, validation of analytical methods is required in support of product registration application [10]. Validation is performed by conducting a series of experiments using the specific conditions of the method and the same type of matrix as the intended samples. The definitions and procedures used to calculate the parameters concerning the linearity range, recovery, etc., are adequately described in many publications related to pharmaceutical [10–20] and biomedical [21–28] analysis. The International Conference on Harmonization (ICH) has produced guidelines [29] on the validating of analytical procedures for pharmaceutical product registration applications. Validation does not imply that the method is free from errors. It only confirms that it is suitable for the purpose [30]. Any modification of a method
during its use requires its revalidation. For example, if a new instrument or a different type of electrode, etc., is brought into use, or the method is applied to a different type of sample, it will require revalidation. Some revalidation may also be required when transferring the method between laboratories or when changes are made in the manufacturing process for the drug. Other factors, which can be considered when validating a method, are the cost per analysis, the lack of difficulty, the rate of the operations and the potential for their automation. Once the method has been developed and validated, it is thus fully documented and approved for use. It should be then described in sufficient detail to allow any analyst to use it without difficulty. Tentative recommendations required for validation in drug electroanalysis [31]. The accuracy of a newly developed or modified method can be assessed by comparing the results obtained using it with these obtained using a reference method of known accuracy and precision using a linear regression analysis [32–34]. A reasonable number of samples (10–20) evenly spaced over a concentration range of interest must be analyzed by both the candidate method and the reference method. Results must be plotted as pointed with one axis (usually the abscissa) for the reference method and the other for the candidate method. Simple linear regression is a widely used statistical approach for assessing systematic and random errors associated with the new method. It involves relatively simple calculations and provides reliable estimates of intercept and slope. However, if an appropriate computer program is available for statistical calculations, it is more appropriate to use weighted linear regression since this compensates for the change in variance across the concentration range. Standard solutions of drugs in water or methanol must be used during many stages of analysis such as calibration, validation, etc. In bioanalytical work, although stock solutions can be prepared in water or methanol, standard solutions for calibration and other experiments should be prepared by dilution of the stock solutions with a relevant biological fluid. Indeed, behaviour of the drug in pure aqueous solutions can greatly differ from the behaviour in the complex biological fluids. Drug and reagent solutions must be
stored in such a way as to maintain their integrity. Prior to analysis their stabilities should be tested by comparison with freshly prepared solutions. In general, solutions of drugs and chemicals are more stable at low temperatures (4 or 20°C) than at room temperature. Samples to be analyzed must be handled in accordance with the approved procedures [35], since any deviance to the procedure will be a major contributor to measurement errors. The biofluids most commonly analyzed for drugs and/or metabolites are blood (plasma or serum) and urine. Blood should be centrifuged to retain either the plasma, if an anticoagulant such as heparin is added to the sample, or the serum, if the blood is coagulated. For urine, usually a midstream sample is collected for most analyses. However, in a urinary excretion study, sampling is performed quantitatively, i.e. the volume of urine is also measured at such collection. The laboratory in which analysis takes place must have a reliable system for the documentation of the samples, from sample receipt to the disposal of the sample excess. All analyses must be carried out in accordance with written procedures. Assays should preferably be performed in duplicate each time using a separate portion of the sample rather than repeating the determination on the final solutions, e.g. the repeated addition into the cell (in voltammetric analysis) or the repeated injection into the flow injection cell (in the FIA). This gives confidence in results and serves to check on the homogeneity of the sample and the random variation in the instruments response [36]. Quality control and laboratory accreditation are next steps in the quality management [37–45]. Many reviews related to environmental analysis [46], trace metal ions determination [46, 47], pharmaceuticals and biomedical analysis [48], chemical sensors for radiopharmaceuticals [49] have been reported in the literature. Application of polarography and/or voltammetry in analysis of drugs has been also reviewed in many citations [31, 48, 50–53]. The aim of the current introduction is to survey the voltammetric analysis of drugs. Voltammetry can be carried out using commercially available polarographic instruments employing the classical polarographic method (DC polarography) as well as pulse methods (e.g. DPP). Modern voltammetric instruments with
automatic timing of the individual operations are useful for controlling the individual steps in AdSV measurements (accumulation time, solution stirring, rest period, initiation of polarization); a computerized instrument is useful for this purpose. Square-wave voltammetry (SWV) has become more widely accessible. Voltammetry can be carried out practically at all types of electrodes designed for voltammetry and for which a completely reproducible constant surface area can ensure reproducible results over the whole measuring period or during a series of measurements. The working electrode is the electrode at which the reaction of interest occurs. Generally, the working electrode in voltammetry is characterized by its small surface area, which enhances polarization. Another reason for using very small electrodes is to minimize depletion (by electrolysis) of the analyte. The choice of the working electrode is very important for the sensitivity and reproducibility of stripping analysis. Stationary working electrodes used in stripping measurement fall into two large groups, mercury electrodes and inert solid electrodes. There are two types of mercury electrodes that have gained wide acceptance for stripping analysis: the hanging mercury drop electrode (HMDE) or static mercury drop electrode (SMDE) and the mercury-film electrode (MFE). There are several kinds of solid electrodes, such as glassy carbon electrode (GCE), graphite electrode, carbon paste electrode (CPE), platinum electrode (Pt), gold electrode used commonly in electroanalytical studies.

In drug analysis, adsorptive stripping voltammetry (AdSV) is popular because of the low limit of determination (reaching few ppb concentrations), its accuracy and precision, as well as low cost of instrumentation relative to other analytical methods of analysis. Adsorptive stripping voltammetry (AdSV) comprises a variety of electrochemical approaches, having a step of preconcentration onto the electrode surface prior to the voltammetric measurement. The major advantage of stripping voltammetry (SV) compared to direct voltammetric measurements is the preconcentration factor [54–57]. For trace analysis of organic compounds, the accumulation of the compound to be determined on the working
electrode will be followed by voltammetric oxidation of the accumulated substance (anodic stripping voltammetry, ASV) or by voltammetric reduction (cathodic stripping voltammetry, CSV). The stripping technique can be achieved by using different types of electrodes e.g. hanging mercury drop electrode (HMDE), static mercury drop electrode (SMDE) or the more recent mercury electrode called controlled growth mercury electrode (CGME). This accumulation step can also occur at many other types of solid electrodes, e.g. platinum electrodes, carbon paste electrode (CPE), glassy carbon electrode (GCE), wax-impregnated graphite electrode (WIGE) or the chemically modified electrode (CMCPES). The process at CME is not purely adsorptive accumulation, but also chemisorption through specific reactions at CME under controlled conditions. The applications of chemically modified electrodes (CME’s) to the determination of trace amount of organic analytes have been reviewed [58]. To achieve maximum sensitivity with AdSV method, optimum conditions for maximum adsorption should be utilized during the accumulation step. So, the measured peak height depends on many variables such as type of electrode materials, accumulation time, accumulation potential, solvent, surface properties of the compound, electrode area, ionic strength, pH and temperature [59, 60].

1.2. ELECTROCHEMISTRY IN CALCIUM CHANNEL BLOCKERS

1,4-dihydropyridines (1,4-DHP) antagonists of L-type calcium channels are widely used as therapeutics in the treatment of hypertension, angina, arrhythmias, congestive heart failure, cardiomyopathy, atherosclerosis, and cerebral and peripheral vascular disorders[61,62]. There exists considerable interest in the synthesis of new 1,4-dihydropyridines derivatives for their activity as calcium antagonists [63,64] and as candidates for the treatment of multidrug resistance (MDR) during cancer chemotherapy [65], as possible thromboxane synthetase inhibitors [66], PAF-acether antagonists and antithrombotic-antihypertensive agents [67]. The inclusion of a nitrophenyl group in the C4-position of the
1,4-dihydropyridine ring gave rise to several compounds with recognized therapeutic activity that are still used in the treatment of cardiovascular pathologies, i.e. nifedipine, nitrendipine, nicardipine [62,68]. Nevertheless, the presence of this group not only affects the pharmacological properties of this type of compounds, but also its redox properties. Thus, both the electrochemical reduction of the nitro group and the formation of intermediates, such as the nitro radical anions, have been investigated [69–73]. The electrochemistry of 4-nitrophenyl substituted 1,4-dihydropyridines has also been studied extensively in the last few years. The electrochemical reduction of nitrophenyl 1,4-DHP derivatives in aqueous media follows the general pattern of nitroaromatic compounds involving a single 4-electron step, producing the hydroxylamine derivative [74,75]. On the other hand, the electrochemical reduction of these derivatives is dramatically affected in mixed media [76–78]. A novel series of C-4 nitrosophenyl-1,4-dihydropyridines have been recently synthesized [79]. Their interest in the synthesis of this type of compounds lies on the fact that some commercial nitrophenyl-1,4-ihydropyridines undergo photolysis when exposed to short-wavelength (below 420 nm), visible or UVC (254 nm) radiation in aqueous solution, oxidizing to their nitroso derivative [80,81]. In general terms, the reduction of nitrosoaromatic compounds has received little attention [82–84] as seen by the low number of reports as compared with the reduction of nitroaromatic compounds. This may be partly due to its chemical instability and the difficulty to be synthesized. Mostly, literature has been devoted to nitrosobenzene [85, 86]. A good review about addition, reduction and oxidation reactions of nitrosobenzene was published some years ago by Zuman and Shah [87]. On the other hand, an electrochemical study about the reactivity of the nitroso radical anion electrochemically generated from nitrosobenzene with glutathione was recently reported [88]. The intermediary radicals in the chemical and electrochemical reductions of nitrobenzene in aqueous and non-aqueous solvent systems has been well-documented by electron spin resonance studies [89–92]. In another study [93], the electrochemical reductions of two nitroso derivatives, i.e.ortho- and meta-
nitrosotoluene were reported. In such a study, the UV–Vis and EPR spectroscopic characterization of the one-electron reduction product from these derivatives in aprotic media was also assessed. Nitroso derivatives constitute both a ubiquitous class of chemicals in nature and a prototype with interesting potential pharmacological and toxicological properties. Therefore, we consider that the knowledge of their redox properties is relevant. In another investigation, the authors studied systematically the electrochemical reduction of a series of synthesized C-4 nitrosophenyl-1,4-dihydropyridines and their parent nitroaryl-1,4-DHPs in which both the position of the reducible group in the aromatic ring (ortho-, meta- or para-position) and the bulk of the alkyl groups substituting the 3- and 5-positions on the dihydropyridine ring were modified. All solvents were of high-pressure liquid chromatography (HPLC) grade and all reagents were of analytical grade. Synthesis of 1,4-dihydropyridine derivatives was based on classical Hantzsch synthesis of 1,4-dihydropyridines [94,95]. To obtain the nitrosophenyl-1,4-dihydropyridine derivative, a chemical reduction was carried out for the nitro compound to the corresponding hydroxylamine and later oxidation to the final nitrosophenyl-1,4-dihydropyridine derivative. All the synthesized compounds were characterized by $^1$H NMR, $^{13}$C NMR spectroscopy using a 300 MHz spectrometer (Bruker, WM 300), infrared spectroscopy (FT-IR Paragon Spectrometer, 100PC) and Elemental analysis (Fisons).

1.2.1 Stripping Voltammetry for Pharmaceutical Analysis

It may be assumed that the application of stripping technique will grow, especially in connection with the pharmaceutical control. Further development of the method depends on basic research, which tends to be concentrated in several main areas. There are still many possibilities for the development of this relatively new method of trace analysis. A few of them are:

- Progress of the study of electrode reaction kinetics at the solid electrodes
• Adsorption phenomenon of electrochemistry in non-aqueous media and

• Development of chemical instrumentation which will enlarge the list of methods of monitoring the stripping process and will allow extensive automation of the method.

These voltammetric methods are applied in organic analysis most frequently in pharmaceutical chemistry and pharmacology, in polymer chemistry, in the foodstuff industry, in criminology and more recently in environmental research [96].

1.3. ELECTROANALYTICAL TECHNIQUES

Most of the applications of environmental analysis involve trace determination of the compounds, often at a ppb level or even lower. The techniques used in trace determinations must lead to high sensitivity, sufficient selectivity, precision and accuracy. These criteria are satisfied by electroanalytical techniques.

These methods are effective for environmental research because they enable immediate measurement of changes in the concentration of the compounds. Another advantage is that several compounds can be determined simultaneously. Continuous monitoring is also possible and systematic error caused by transport and storage of the sample could be avoided. The cost per sample analysis is also lesser compared to chromatographic methods. The choice of the method depends on the nature of the compound to be determined, as well as on the sensitivity and selectivity requirements.

The methods commonly used are voltammetry, polarography, potentiometry, coulometry and conductometry. The sensitivity limits of common electroanalytical methods are presented in the table 1.
### Table 1. Sensitivity limits of electroanalytical methods

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Techniques</th>
<th>Sensitivity limits (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AC polarography, Thin layer coulometry</td>
<td>10⁻⁴ to 10⁻⁵</td>
</tr>
<tr>
<td>2</td>
<td>Chronocoulometry, Classical polarography</td>
<td>10⁻⁵ to 10⁻⁶</td>
</tr>
<tr>
<td>3</td>
<td>Derivative polarography, Square wave polarography, Linear sweep voltammetry, Chemical stripping analysis</td>
<td>10⁻⁶ to 10⁻⁷</td>
</tr>
<tr>
<td>4</td>
<td>Pulse polarography, Amperometry, Conductivity</td>
<td>10⁻⁷ to 10⁻⁸</td>
</tr>
<tr>
<td>5</td>
<td>Anodic stripping with hanging mercury drop electrodes.</td>
<td>10⁻⁸ to 10⁻⁹</td>
</tr>
<tr>
<td>6</td>
<td>Anodic stripping with thin film electrodes or solid electrodes.</td>
<td>10⁻⁹ to 10⁻¹⁰</td>
</tr>
</tbody>
</table>

Hence electroanalytical techniques are applicable to a very large number of organic compounds encountered in many fields. The literature survey for the last 25 years shows that polarography and voltammetry have been used in the organic field, particularly for the pharmaceutical and biological fields [97]. Meites and Zuman have listed the polarographic behaviour of a large number of substances [98]. Many compounds that are neither reduced nor oxidized in the available potential range or for which the signals acquired are not suitable for the analytical purposes can be converted into electroactive substances via chemical or electrochemical methods and then they can be analysed [99,100].

### 1.3.1. Significance of Voltammetry

It has been established that voltammetry is a potent analytical tool in environmental trace studies. Hence, a suitable voltammetric method has become one of the preferred approaches in trace analysis. Voltammetry leads to extraordinary determination sensitivity with inherent high accuracy, i.e. small tendency of systematic errors. The following are the important advantageous features of voltammetry.
The simultaneous determination of several analytes by a single scan is often possible with voltammetric procedure.

It has a reasonable high determination rate. Voltammetry equals or even surpasses the analysis rate of atomic adsorption spectroscopy which is considered as a more sensitive and accurate method [101,102].

The present introduction of automation into voltammetry will further enhance convenience of application in routine analysis for the determination rate [103].

The instrument is very compact and is easily used in the field studies carried out in ships or in mobile terrestrial areas.

Voltammetry is essentially a substance-specific and not just an element-specific method like the other non-electrochemical methods.

1.3.2. Cyclic Voltammetry

Cyclic voltammetry is a technique that allows one to scan the potential of working electrode either in anodic or cathodic direction and observe peaks due to oxidation or reduction of the analyte. Then the potential scan is reversed in the cathodic or anodic direction. The peaks due to oxidation and reduction of intermediates formed during the forward scan may be observed. The electrode system in cyclic voltammetry is dictated by the nature of the medium as well as the process being studied. The commonly used electrodes are glassy carbon, planar platinum disks, and platinum wires, hanging mercury drop, graphite and carbon paste. It is a simple technique and provides a great deal of information about electrochemical behaviour. Hence it is considered as one of the most powerful electrochemical diagnostic tools. The potential may be swept anodically or cathodically and unlike polarographic waves, the curves obtained are peaks [104]. One of the outstanding features of cyclic voltammetry is its ability to generate a potential reactive species and then to examine it immediately by reversal [105], thereby providing an electrochemical overview for a reaction system.
The chief strengths of cyclic voltammetry are:

- Applicability to a wide range of electrode materials.
- A range of five orders of magnitude in scan rates.
- Great flexibility in setting up scan limits and reversal conditions.
- An intrinsic facility for highlighting the chemical conditions between various electroactive species present in the voltammogram.
- Highly developed theory.

The shape of the voltammogram depends strongly on the mechanism of the electrode process. Cyclic voltammetry can provide information about the number of electrons transferred in each peak. The diagnostic criteria for two important systems are discussed and others are presented in Table 2. The peak current for the reversible process at 25°C is given by Randles-Sevcik equation [106].

\[ i_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} C^{1/2} \]

Where \( i_p \) is the peak current in amperes, \( n \) is the number of electrons involved in the reaction, \( D \) is the diffusion coefficient of the oxidant or reductant in cm² sec⁻¹, \( A \) is the area of the electrode in cm² and \( v \) is the scan rate in Volt sec⁻¹.

The potential difference between \( E_p \) and \( E_p/2 \) is given by

\[ E_p - E_p/2 = 56.5/n \text{ mV at } 25^\circ \text{C} \]

The difference between \( E_p^a \) and \( E_p^c \) is given by

\[ E_p^a - E_p^c = 59/n \text{ mV at } 25^\circ \text{C} \]

For a reversible process, the anodic peak current \( i_{pa} \) is equal to cathodic peak current \( i_{pc} \) and hence \( i_{pa} / i_{pc} \) is unity and is independent of \( v \) [107].
Table 2. Diagnostic criteria for the charge transfer reactions

<table>
<thead>
<tr>
<th>System</th>
<th>Diagnostic criteria</th>
</tr>
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</table>
| Reversible                            | E<sub>p</sub> is independent of υ; E<sub>p</sub> = (E<sub>p</sub><sup>a</sup> − E<sub>p</sub><sup>c</sup>) / 2  
E<sub>p</sub><sup>c</sup> − E<sub>p</sub><sup>a</sup> = 59/n mV at 25<sup>0</sup> C and is independent of υ  
i<sub>p</sub>/υ<sup>1/2</sup> is independent of υ; i<sub>p</sub><sup>a</sup>/ i<sub>p</sub><sup>c</sup> is unity and independent of υ  
Wave shape is independent of υ                                                                 |

| Quasi reversible                      | E<sub>p</sub> shifts with υ  
E<sub>p</sub><sup>c</sup> − E<sub>p</sub><sup>a</sup> may approach 60/n mV at low υ but increases as υ increases  
i<sub>p</sub>/υ<sup>1/2</sup> is virtually only for α= 0.5                                                                 |

| Irreversible                          | No current response in reverse scan  
E<sub>p</sub> shifts cathodically by 30/αn mV per tenfold increase in υ  
The wave shape is determined by α and is independent of υ                                                                 |

| Preceding reversible chemical reaction | E<sub>p</sub> shifts anodically with an increase in υ  
i<sub>p</sub>/υ<sup>1/2</sup> decreases as υ increases                                                                 |

| Following reversible chemical reaction | E<sub>p</sub> shifts cathodically with an increase in υ  
i<sub>p</sub>/υ<sup>1/2</sup> virtually constant with υ  
i<sub>p</sub><sup>a</sup>/ i<sub>p</sub><sup>c</sup> decreases from unity as υ increases                                                                 |

| Charge transfer with catalytic regeneration | E<sub>p</sub> shifts anodically by a maximum of 60/n mV  
i<sub>p</sub>/υ<sup>1/2</sup> increases at low values of υ and becomes independent in higher υ  
i<sub>p</sub><sup>a</sup>/ i<sub>p</sub><sup>c</sup> is unity                                                                 |

| Following irreversible dimerisation reaction | E<sub>p</sub> shifts cathodically by 20/n mV per tenfold increase in υ and per tenfold decrease in initial concentration, C<sup>*</sup><sub>ox</sub>  
i<sub>p</sub>/υ<sup>1/2</sup> decreases a maximum of 20% from low to high υ  
i<sub>p</sub><sup>a</sup>/ i<sub>p</sub><sup>c</sup> increases with υ and decreases as C<sup>*</sup><sub>ox</sub> increases. |

The peak current and the peak potential for an irreversible process are given by

\[
i_p = 2.98 \times 10^5 \; n \; [\alpha n]^{1/2} \; AD^{1/2} \; C \; \nu^{1/2}
\]

\[
E_p = E^0 − RT/\alpha n F \left[0.78 − 2.3/2 \; \log \left(\alpha n F D / K^0 \nu R T \right)\right] − 2.3 \; RT/2\alpha n F \log \nu
\]

Hence, E<sub>p</sub> shifts with scan rate according to

\[
dE_p / d\log \nu = −30/\alpha n
\]
In cyclic Voltammetric experiments, no anodic or cathodic peak would be noticed in the subsequent cathodic or anodic sweep for an irreversible process. \( i_r/v^{1/2} \) is independent of scan rate while the peak shifts cathodically as the scan rate increases for an irreversible system.

Apart from cyclic voltammetry, other techniques used in electrochemical studies are differential pulse voltammetry, square wave voltammetry, chronocoulometry, controlled potential coulometry and stripping voltammetry.

1.3.3. Chronocoulometry

In this technique, the potential excitation function is stepped from an initial potential, where no redox reaction occurs to a final potential where the reaction of interest does occur, instead of measuring the current directly, it is integrated and the charge is measured.

It offers several advantages [108]. They are as follows:

- The later part of the response which is more accessible experimentally, is least destroyed by non-ideal potential rise and offers better signal to noise ratios while retaining the information from the early response,
- Integration eliminates random noise and
- Contributions from diffusional and interfacial components are easily separated.

The forward chronocoulometric response of diffusing reactants is described by the integrated Cottrell equation.

\[
Q_d = 2nFAD^{1/2}Ct^{1/2} / \pi^{1/2}
\]

Where \( Q_d = \) charge in coulombs, \( n = \) equivalent/mole, \( F = \) Faraday constant, 96485 c/equivalent, \( A = \) Area of the electrode, \( cm^2 \) \( D = \) Diffusion coefficient, \( cm^2/s \), \( C = \) concentration, \( mol/cm^3 \) and \( t = \) time, seconds.
1. 3. 4. Controlled potential coulometry

Controlled potential bulk electrolysis or coulometry is often referred to as a steady state technique which is used to determine the overall number of electrons involved in the reaction. It is used to prepare reaction products which are then identified by the application of conventional analytical techniques. A large electrode area to solution volume area is desirable for this technique.

On the basis of steady state or sweep voltammetry, a certain reaction potential under investigation will be at mass transport controlled rates. The current and its integral, the charge is monitored as a function of time, usually until the current drops to about 1.0% of its initial value. The most significant piece of information that is obtained in the coulometric experiment is the value of n, the number of electrons involved in the overall reaction.

\[ Q = nFN \]

\( Q = \) total charge consumed, \( F = \) Faraday constant, 96485 c, \( n = \) number of moles of electroactive species present and \( n = \) number of electrons involved.

1. 3. 5. Stripping Voltammetry

The electrochemical stripping analysis involves a preconcentration of the analyte on the working electrode prior to its determination by means of an electrochemical technique [109, 110]. It is a more important technique in trace analysis, since it has the lowest detection limit in trace analysis. The original method involves the cathodic electrodeposition of amalgam forming metals on a hanging mercury drop working electrode, followed by the anodic voltammetric determination of the accumulated metal during a positive signal potential scan [111]. In 1980s and 1990s several advances have been made in the development of alternative schemes which further enhanced the scope and power of stripping analysis [112, 113]. Consequently, numerous variants of stripping analysis exist currently which differ in their method of accumulation and measurements [114-117]. Stripping
voltammetry enables the determination of electroactive components in the concentration range from $10^{-6}$ to $10^{-9}$ M/dm$^3$.

Research on increasing sensitivity of electroanalytical methods has led to the development of the techniques of stripping voltammetry. The concentration step is carried out for a definite time under reproducible conditions and the stripping process in most cases is performed in some voltammetric scanning procedure. The resulting “stripping voltammogram” shows peaks, the heights of which are generally proportional to the concentration of the corresponding electroactive species and the potentials of which have the same qualitative meaning as their half-wave potentials in polarography. Dilute solutions in the range $10^{-6}$ to $10^{-9}$ M/dm$^3$ and less, are analysed with excellent precision and selectivity. Thus this technique extends the range of classical polarography by three to four times making possibly the analysis in nano range.

The important characteristics of stripping voltammetric peaks are its height, width and peak potential. They are affected by the type of electrodes and scan rate. The same electrode is used both in the concentration and stripping processes. The process is not disturbed by the presence of organic substances other than analyte, provided they are not adsorbed on the electrode surface.

Compared with other highly sensitive electroanalytical methods such as linear-sweep oscillographic polarography and square wave or pulse polarography, stripping voltammetry gives the same performance at lower cost.

1. 3. 5. 1. Factors influencing the pre-concentration

Electrochemical adsorption generally means the attachment of molecules or ions on the surface of the electrodes. The amount of adsorbate on a fully covered electrode surface depends on the size of the analyte. Less soluble analyte tends to promote strong accumulation. The stripping peak current mainly depends on the preconcentration time. Rate of mass transport by convection controls the peak current and detection limit [118]. The
mass-transport phenomena are greatly influenced by temperature [about 2% per °C] and hence the solution in the cell must be thermostated. Electrode materials and their geometry also affect the stripping analysis. At platinum electrodes, the presence of platinum oxide film hinders both deposition and stripping [119]. The organic surface-active substances as impurities in supporting electrolyte influence the double layer structure on the electrode [120]. The solution concentration should not be too high to avoid higher percentage of impurities. A rest period between the deposition and stripping process ensures the cease of convection in the solution [121].

1. 3. 5. 2. Factors influencing stripping process

The electrolytic stripping process depends on the experimental parameters of the electrodeposition process. The medium does not hinder the concentration process but has deleterious effects on the stripping process. The use of the same medium for concentration and stripping processes can render the analysis difficult [122] for the following reasons.

- The near coincidence of the half-wave potentials of two electroactive species hinders the stripping process and not the deposition process.
- In some cases, the medium for “concentration” is given; stripping cannot be carried out in such a medium where adsorption phenomena arise.
- In strongly acidic media, the stripping is hindered by relatively high residual current by the reduction of hydrogen ions. To overcome this difficulty, the medium is changed after the concentration process [123]. One may use sine wave [124], square wave [125] and pulse polarography to get a possible detection of $10^{-10}$ M/dm$^3$.

Trace analysis requires successful solution for (i) sensitivity (ii) selectivity and (iii) adsorption in very dilute solutions. Sensitivity can be increased by carrying out the
preconcentration in the system in which the stripping will be performed. This is the principle involved in electrochemical stripping method.

Current is measured as a function of changing electrode potential; peaks are formed on the polarization wave. Peak positions are the characteristic of the given substance and their heights are (or area) proportional to the concentration of the solution.

Voltammetric stripping processes are termed either cathodic or anodic in accordance with the functioning of the process (reduction or oxidation respectively). Differential pulse stripping voltammetry is primarily used for the determination of trace analysis. Detection limits are typically in the range of $10^{-8}$ to $10^{-10}$ M/dm$^3$.

Generally two approaches can be made in stripping voltammetry. In the first approach complete electrolysis of the substance in the solution and monitoring of the current density are necessary for complete dissolution of the deposit. This approach achieves high precision and accurate results under favourable conditions. But it is a lengthy process especially with large volumes. In small volumes, the depolariser is removed from the solution in a relatively short time [126].

The second approach involves pre-electrolysis under reproducible conditions for a certain time interval, so that the amount of substance deposited at the electrode is a reproducible fraction of the total initial amount of the substance in the solution (2 to 3%). In aqueous media, a potential range from +1.5 to -2.5V (vs. SCE) is available for stripping determination.
1. 4. WORKING ELECTRODES

Electrode material is an important factor in directing the course of the electrode reaction.

1. 4. 1. Glassy Carbon Electrode

A variety of carbon materials are now finding applications as electrode materials [127]. Among these, glassy carbon is a specific variety of synthetic carbon material, prepared by controlled heat treatment of phenol-formaldehyde resin up to 3000°C. The material was first prepared in 1962 [128]. It was found to have good electronic conductivity and hence found application as early as 1965 [129]. Simple methods of preparing glassy carbon electrodes have been described [130-132]. The electrochemical aspects GCE from time to time have also been reported in review articles [133-135]. From the earlier studies, it was realised that GCE materials prepared at high heat-treatment shows good electrochemical activity [136, 137].

Heat-treatment under vacuum is recommended for removing oxygen free functional groups [138]. It also decreases the chance of pitting of GCE during electrochemical polarisation [139]. The presence of fluoride ions in the electrolyte was found to improve the stability of GCE [140].

GCE is polished using alumina in the form of emery paper or powder, which activates it in electron transfer reactions. This behaviour has been thoroughly investigated using electrochemical as well as other microscopic techniques [141-145].

Recently a pre-treatment procedure for GCE that employs AC polarisation has also been recommended [146]. The most important pretreatment procedure involves cycling between selected anodic and cathodic potential regions at a fairly slow sweep rates, 10 mVs⁻¹ for 10-15 minutes [147].

GCE has the following advantageous features:

- Light weight and high mechanical strength
High resistivity to heat
High resistivity to chemicals
Absolute gas impermeability like glass
Excellent thermal and electrical conductivity
Less or no contamination on its fine impermeable structure

1.4.2. Modified Electrodes

1.4.2.1. Polypyrrole modified glassy carbon electrode (PPy / GCE)

Conducting polymers exhibit good electrical conductivity. The modification of electrodes with conducting organic polymers improves the electrode sensitivity and selectivity.

Among the conducting polymers so far produced, based on polyanilines, polypyrroles, polythiophenes, polyphenylenes and poly (p-phenylene vinylene) have attracted much attention. Recently, electrodes whose surfaces modified with conducting polymers especially polypyrrole, find extensive applications [148, 149].

1.4.2.2. Clay modified glassy carbon electrode

Clay minerals are cheap, widely available naturally occurring materials. Their well defined layered structures [150-152], flexible adsorption properties [153], and potential as catalysts and / or catalyst supports [154-158] make them interesting materials with which to modify electrode surfaces. They have also higher thermal and chemical stability than nafion and other polyelectrolytes.

Clay modified electrodes are prepared by the deposition of clay films on a conductive substrate. The aim is to take advantage of the adsorption and / or catalytic properties of these films to improve the selectivity or the sensitivity of the electrodes toward solution species. Clays are heterogeneous materials and individual clay has a range of different compositions and particle sizes. Moreover, clay films are imperfect stacks of clay layers. They contain
many defects such as holes and pores of various sizes. Hence they provide a number of sites for the adsorption of analyte [159]. Imperfections in the stacking of the clay layers result in holes, pores and other defects in the films where the adsorbed species could be found. With more defects, the probability that clay-bound cations have access to the electrode surface is increased.

Clay minerals used as modifiers belong to the class of phyllosilicates-layered hydrous aluminosilicates. Their layered structure is either formed from a sheet of SiO$_4$ tetrahedra and one sheet of AlO$_6$ octahedra (1:1 Phyllosilicates) or an Al-octahedral sheet is sandwiched between two Si-tetrahedral sheets (2:1 Phyllosilicates). A positive charge deficiency of layers is balanced by exchangeable cations (Na$^+$, K$^+$, NH$_4^+$ etc.) bound on the external surfaces for 1:1 phyllosilicates and also in the interlayer in the case of 2:1 phyllosilicates. A distance between the layers is an important characteristic of clay mineral and it depends on the number of intercalated water and exchangeable cations within the interlayer space. The important properties of phyllosilicate structure such as large specific surface, ion-exchange properties and ability to sorb and intercalate organic compounds (intercalation) predetermine phyllosilicates, especially a group of smectites for preparation of clay electrodes [160]. Montmorillonite (MM) is the most often used smectite. Its cation exchange capacity is typically 0.80-1.50 mmol g$^{-1}$; anion exchange is about four times lower. Thixotropy is a key physical feature that predetermines montmorillonite to be used as stable and adhesive clay film. HPMM/GCE gives better response only in the presence of surfactants. Sodium ion present in the clay matrix increases the conductivity. Hence, NaMM/GCE is employed as a modified electrode in the present investigation. The unit cell formula for sodium montmorillonite [161] is as follows:

\[ \text{[(Si}_{7.84}\text{Al}_{0.16}) (\text{Fe}^{3+}_{0.26}\text{Al}_{3.22}\text{Mg}_{0.4}\text{Fe}^{2+}_{0.12})\text{O}_{20} (\text{OH})_{4}\text{Na}_{0.68}] } \]

In general, the clay coated electrodes are more suitable for physicochemical studies, charge or ion transport of the clay membrane, photocatalysis, electrocatalysis etc.
1.4.2.3. MWCNTs modified Glassy carbon electrode

An important milestone in the history of carbon is the discovery of carbon nanotubes (CNTs) [162] having two distinct types of structures namely single walled and multiwalled. As a consequence of the excellent electronic and conducting properties of CNTs, electrodes modified with CNTs have demonstrated to improve the electroanalytical performance of different species. Due to their uniqueness, CNTs have received enormous attention for the preparation of electrochemical sensors as it was extensively reviewed [163-168]. The subtle electronic behavior of CNTs reveals that they have the ability to promote electron-transfer reaction when used as electrode materials. Recently CNT film coated electrodes have received increasing attention in analytical studies [169-172]. However a major barrier for developing the CNT modified electrode is the insolubility of CNTs in usual media [173] and many efforts have been made to disperse CNTs into suitable solvents such as DMF [174], acetone [175] and concentrated sulphuric acid [176]. Polymers like Nafion [177] and Chistosan [178, 179] were also used to disperse CNTs. Yunhua Wu et al. [180] dispersed MWCNTs in surfactants like dihexadecylphosphate, sodium dodecylbenzenesulphonate and used it to modify glassy carbon electrode for the electrochemical determination of lincomycin. Surfactants are a special kind of amphiphilic molecules, which can spontaneously adsorb at the interfaces or assemble into micelles in solutions, forming various regulated structures at electrode surfaces or in solutions. This resulted in extensive applications in electroanalysis [181]. Yuan-hai Zhu et al [182] functionalized MWCNTs using nitrating mixture and neutralized with dil.NaOH and modified MWCNTs to water soluble and used it for the determination of phenylephrine. In recent days, a noncovalent method [183] has been developed and ported for solubilizing MWCNTs functionalized with Congo red. Thus MWCNTs became a material with high solubility, high purity and does possess a special property of strong rebundling when dried, capable of forming uniform and
compact MWCNTs films with a 3D network structure of nanosizes on GCE. In the present work, we used anionic surfactant, sodium dodecyl sulphate (SDS) to disperse MWCNTs.

CNT modified electrode can impart strong electrocatalytic activity to some important biomolecules such as cytochrome c [184, 185], NADH [186], hydrogen peroxide [187, 188] and catecholamines such as dopamine [189] and ascorbic acid. It leads to a strong interfacial accumulation of the substrate that can serve as a preconcentration step for highly sensitive adsorptive stripping measurements.

Considering the importance of the above mentioned modified electrodes in the improvement of sensitivity, they are employed in the present study. This thesis consists of seven chapter in which the first chapter deals with the general introduction about the electranalytical studies and their importance. Gist about other chapter is presented below:

- Chapter 2 presents state of the art and scope of the present investigation
- Chapter 3 explains the experimental setup and procedure adopted for the present study.
- Chapter 4 deals with the electrochemical behaviour of six types of drugs on GCE.
- Chapter 5 explains the electrochemical behaviour of drugs on modified electrodes such as polypyrrole-coated electrodes, montmorilonite clay modified electrode and multiwalled carbon nanotubes modified electrodes.
- Chapter 6 deals with the stripping voltammetry determination of drugs using GCE and modified electrodes.
- Chapter 7 highlights the summary of the investigations carried out in the present study.