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Chapter I

Introduction

Analytical chemistry is the study of separation, identification and quantification of chemical components of natural and synthetic materials. In other words, it is concerned with the chemical characterization of matter, both qualitatively and quantitatively. It is important in nearly every aspect of our lives because chemicals make up everything we use [1]. Analytical chemistry is the measurement science consisting of a set of powerful ideas and methods that are useful in all fields of science and medicine. Modern analytical chemistry generally requires precise analytical measurements at very low concentrations, with a variety of instruments. Quantitative analytical measurements also play a vital role in many research areas in chemistry, biochemistry, biology, geology, physics, pharmaceutical chemistry, medicinal chemistry, biotechnology, environmental sciences and other sciences [2,3]. A few of the common terms used in analytical chemistry include the following:

Analyte

The chemical species that are the subjects of either qualitative or quantitative analysis are known as analytes. Analyte can be a pure substance or one constituent of a multi-component sample.

Analysis

It provides chemical or physical information about the constituent(s) in the sample or the sample itself. Analysis is a crucial servant of modern technology and it depends partly on modern technology for its functioning. Analysis has offered an important foundation for chemical development from the initial days of quantitative chemistry.

Assay

In commercial business with materials such as pharmaceutical products, the value of the product depends on its active pharmaceutical ingredient (API) content. Large amounts of the material are often involved, so that even a
minute variation in concentration can be of extensive commercial significance. Hence, accurate and reliable chemical analysis is essential.

Analytical methods are broadly classified as classical and instrumental methods, the former covers 'wet chemical' methods such as gravimetry and titrimetry. These methods involve the correlation of a physical property with the analyte concentration. A wide variety of parameters that may be measured are shown in Table 1. A general classification of important analytical techniques is shown below:

<table>
<thead>
<tr>
<th>Technique</th>
<th>Property measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravimetry</td>
<td>weight of a pure analyte or of a stoichiometric compound containing it</td>
</tr>
<tr>
<td>Volumetry</td>
<td>volume of standard reagent solution reacting with the analyte</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>intensity of electromagnetic radiation emitted or absorbed by the analyte</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>electrochemical property of the analyte</td>
</tr>
<tr>
<td>Radiochemical</td>
<td>intensity of nuclear radiation emitted by the analyte</td>
</tr>
<tr>
<td>Mass spectrometry</td>
<td>abundance of molecular fragments derived from the analyte</td>
</tr>
<tr>
<td>Chromatography</td>
<td>physico-chemical properties of individual analytes after separation</td>
</tr>
<tr>
<td>Thermal</td>
<td>physico-chemical properties of the sample as it is heated and cooled</td>
</tr>
</tbody>
</table>

The chromatographic, mass spectrometric and radiochemical techniques involve expensive instrumentation and are time consuming. The techniques such as gravimetric, titrimetric and thermal methods are less sensitive and are not suitable for analysis of pharmaceutical products. This prompted us to employ electrochemical (electroanalytical) methods in our investigation to develop simple, rapid and cost effective analytical methods for the analysis of bioactive compounds of pharmaceutical importance.
Electroanalytical methods

Electrochemistry can be generally defined as the study of charge-transfer phenomena. As such, the field of electrochemistry includes a broad range of different physical, chemical and biological phenomena. These areas include (but are not limited to) photosynthesis, battery chemistry, ion-selective electrodes, coulometry and many biochemical processes. Electrochemistry has found many practical applications in analytical measurements also. It utilizes the relationship between chemical phenomenon which involves the charge transfer (e.g. redox reactions, ion separation etc.) and electrical property. Electroanalytical techniques offer unique information on chemical, biochemical and physical systems. Both the instrumental basis and the theoretical fundamentals have been developed such that non-specialists can easily apply them. However, it is not broadly accepted except by those who have experience and training in electrochemistry [4].

The determination of drugs in bulk, pharmaceutical formulations and biological fluids is becoming increasingly important in pharmaceutical and biomedical sciences. Successful analysis involves sensitivity at ppm level or less, high selectivity and minimal interference from excipients and matrix contents. In order to bring out a drug product from the discovery stage to the commercial market, the analytical chemist develops methodology for quality control, stability testing, pharmacokinetics, identification and clinical studies. Till recently, pharmaceutical analysis rely predominantly on titrimetric, spectrophotometric [5], chromatographic, immunochemical methods [6] etc. The challenges in pharmaceutical assays include the achieving high specificity and determination of drugs with sub-nanogram detection limits especially in biological fluids.

Recent advances in the instrumentation make electroanalytical methods as interesting alternatives in pharmaceutical analysis [7]. Modern electrochemical techniques have exceptional limits of detection and a broad dynamic range [7]. Direct determination of pharmaceutical formulations and
complex biological matrices is necessary without the need for separation or pre-treatment whereas the use of spectroscopic and optical methods require preliminary separation in most of the cases. Additional advantages of electroanalytical methods include the need of small sample volumes (in microlitre range) coupled with low detection limits allowing analysis on sub-nanomolar levels of drug products and metabolites. On all these grounds besides their simplicity and rapidity, electroanalytical technique is uniquely suited for clinical and bioanalysis where small volumes of blood or urine are to be analyzed at low concentrations of drug products and metabolites.

Electroanalytical methods are concerned with the relationship between electricity and chemistry, namely, the measurement of electrical quantities such as current, potential or charge and its relationship with chemical parameters. Electrochemical processes take place at the electrode-solution interface compared to many other chemical measurements, which involve homogeneous bulk solutions. The type of electrical signal used for quantitation reveal the distinction among various electroanalytical techniques. Two major types of electroanalytical measurements are potentiostatic and potentiometric. Both types involve the use of at least two electrodes (conductors) and a sample solution, that constitutes the electrochemical cell. Thus, the electrode surface is a junction between an ionic conductor and an electronic conductor.

One of the two electrodes responds to the target analyte(s) and hence termed as the working electrode. The second one, the reference electrode, is of constant potential (i.e., independent of properties of the solution). In addition to these two, another electrode, termed as auxiliary electrode or counter electrode may be present. This passes the same current as the working electrode (although with an opposite sign) and therefore any electrochemical process on the working electrode will be accompanied by an opposite electrochemical process on the counter electrode. This is of course well known fact, though often tacitly ignored by customarily placing counter electrode away from the working electrode, often even in the separate compartment, to avoid the
electrolytic product generated on the counter electrode to enter vicinity of the working electrode [8]. Electrochemical cells may be classified as electrolytic or galvanic based on whether they consume electricity from an external source (former) or they are used to produce electrical energy (latter). The major types of electrochemical methods employed for chemical analysis include voltammetry, potentiometry, coulometry and conductometry.

**Voltammetry**

Voltammetry is a branch of electrochemistry, which was invented in 1922 by the Czech chemist, Jaroslav Heyrovsky, for which he received the 1959 Nobel Prize in chemistry. In 1942, Hickling built the first three electrodes potentiostat [9]. Earlier, voltammetric methods were hardly used for routine analytical applications owing to a number of practical obscurities. However, significant progress was made in all areas of voltammetry in 1960s and 70s (theory, methodology and instrumentation), which enhanced the sensitivity and expanded the range of analytical methods. The coincidence of these advances with the arrival of low-cost operational amplifiers also facilitated the rapid commercial development of relatively inexpensive instrumentation [10].

Voltammetry is a dynamic electrochemical technique, in which a time-dependent potential is applied to an electrochemical cell and measures the resulting current as a function of that potential. It can be used to study electron transfer reactions with solid electrodes. The resulting plot of electrical current response versus applied potential is called a voltammogram. It is the electrochemical equivalent of a spectrum in spectroscopy, providing quantitative and qualitative information about the species involved in the oxidation or reduction reaction [3]. Several types of voltammetric techniques include the following:

- Linear sweep voltammetry
- Squarewave voltammetry
- Anodic stripping voltammetry
- Adsorptive stripping voltammetry
- Staircase voltammetry
- Cyclic voltammetry
- Cathodic stripping voltammetry
- Alternating current voltammetry
Polarography  Rotated electrode voltammetry
Normal pulse voltammetry  Differential pulse voltammetry
Chronoamperometry

**Cyclic voltammetry**

Cyclic voltammetry (CV) is perhaps the most popular electrochemical technique for solid electrodes. The advantage of CV results from the fact that it has the ability to get reproducible results, at least for subsequent cycles, even on relatively badly defined electrode surfaces. The possibility to observe the reduction wave and oxidation wave simultaneously is an added advantage in the investigation of electrode processes. Analysis of cyclic voltammograms recorded at various scan rates can give insights into several electrode kinetics and electrosorption processes in detail.

In a cyclic voltammetric experiment, a cyclic potential sweep of triangular waveform (Figure 1) is imposed on the working electrode and the current response is observed. Analysis of the current response can give insights into thermodynamics and kinetics of electron transfer at the electrode solution interface, as well as the kinetics and mechanisms of solution chemical reactions initiated by the heterogeneous electron transfer [11].

![Figure 1. Typical waveform used in cyclic voltammetry](image-url)
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Theory

In a typical cyclic voltammetric experiment, the analyte is electrolyzed (oxidized or reduced or both) by placing its solution in an electrolyte in contact with the electrode surface and then making that surface sufficiently positive or negative in voltage in order to force electron transfer. In other words, the potential scan is started at a particular voltage with respect to a reference electrode such as Ag/AgCl or saturated calomel electrode (SCE), the potential of the working electrode is then linearly varied (Figure 1) to a higher or lower voltage and finally, the potential is changed back to the original value at the same rate, termed scan rate. When the surface becomes adequately negative or positive, the solution species may gain electrons from the electrode surface or transfer electrons to the electrode surface. This results in a computable current in the electrode circuitry, which is further amplified using external electronic circuits. The result of CV, termed as “voltammogram” is a cyclic plot between current and potential, potential on X axis and current on Y axis.

Typical cyclic voltammogram

Figure 2. A typical cyclic voltammogram

The voltammetric curve (Figure 2) can be detailed in the following way. The current rises as in a steady-state voltammogram on reaching a potential
where the electrode reaction begins. However, the supply of electroactive species begins to drop on continuing to sweep the potential from a certain value just before the maximum value of the current called the peak current. The current then begins to decay owing to depletion of electroactive species, following a profile which is proportional to $t^{1/2}$, similar to that after application of a potential step. A typical voltammogram is characterized by several important parameters such as the cathodic ($E_{pc}$) and anodic ($E_{pa}$) peak potentials, the cathodic ($I_{pc}$) and anodic ($I_{pa}$) peak currents, the cathodic half-peak potential ($E_{p/2}$) and the half-wave potential ($E_{1/2}$).

**Differential pulse voltammetry**

In differential pulse voltammetry (DPV), the applied waveform consists of small pulses of constant amplitude (10-100 mV) superimposed on a linearly changing base potential. In this technique, the current is measured twice in each pulse period, first at potential at the beginning of the applied pulse, and second at the end of the same pulse [4]. The instrumental output is actually the difference between the currents measured for each single pulse, referred to as differential pulse voltammogram. Therefore, the current measured in DPV enables one to obtain much higher sensitivity of DPV compared to CV and normal pulse voltammetry (NPV).

**Adsorptive stripping methods**

In adsorptive stripping voltammetry (AdSV), a small electrode, most commonly a hanging mercury drop electrode or a modified electrode consisting of a film of the modifier capable of adsorbing large quantities of analyte molecule is immersed in a stirred solution of the analyte for several minutes. In this step, accumulation of analyte on the electrode surface occurs mainly by physical adsorption rather than by electrolytic deposition. After accumulation of sufficient analyte, the stirring is discontinued, and the deposited material is determined by linear-scan or pulsed voltammetric measurements.
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Reversible reaction

A reversible reaction is one in which the current is directly proportional to concentration and increases with the square root of the sweep rate. Such dependence on the scan rate indicates that the electrode reaction is controlled by mass transport (semi-infinite linear diffusion). The criteria of reversibility (over a given range of conditions) are \( \Delta E_p = E_{pa} - E_{pc} = 57/n \text{ mV} \) and \( E_{p/2} - E_{pc} = 56.5/n \text{ mV} \) at 298 K. Ideally speaking, the ratio, \( I_{pa}/I_{pc} \) should be 1.0 for a reversible voltammetric response.

The following information is the characteristic of linear sweep and cyclic voltammogram of a reversible reaction:

- \( E_p \) independent of \( v \)
- \( I_p \propto v^{1/2} \)
- \( E_p - E_{p/2} = 56.5/n \text{ mV} \) and for cyclic voltammetry alone,
- \( E_{pa} - E_{pc} = 57/n \text{ mV} \)
- \( I_{pa}/I_{pc} = 1 \)

Irreversible reaction

Irreversible reaction of the type, \( O + n e^- \rightarrow R \) is characterized by the absence of a peak in the reverse scan in cyclic voltammogram. In the irreversible electrode process, the individual peaks are reduced in size and widely separated. A shift in the peak potential towards higher potentials with the scan rate is observed for a totally irreversible system.

Quasi-reversible reaction

The intermediate situation between reversible and irreversible is sometimes termed as 'quasi-reversible'. In case of a quasi-reversible system (with \( 10^{-1} > k^o > 10^{-5} \text{ cm/s} \)) both the charge transfer and mass transport control the peak current. The shape of the cyclic voltammogram is a function of \( k^o/(\pi n F D/RT)^{1/2} \). The process advances towards the reversible behavior as the ratio increases. The system exhibits an irreversible behavior for small values of
this ratio. Generally, the voltammograms of a quasi-reversible system are more
drawn out and exhibit a larger separation in peak potentials compared to that in
a reversible system.

The three electrode cell

In electrochemistry, the most common electrochemical cell setup used is
the three-electrode cell setup (Figure 3). Here, the flow of current is between
the counter electrode (CE) and the working electrode (WED). The potential
difference is controlled between the WED and the CE and measured between
the reference electrode (RE) and WED. The potential difference between RE
and WED is controlled all the time by controlling the polarization of the CE. It
is adjusted in such a way that the potential difference between the WED and
RE will be equal to the potential difference specified by the user. This three
electrode configuration allows the potential across the electrochemical interface
at the WED to be controlled precisely with respect to the RE.

Figure 3. Commonly used three electrode cell setup
Working electrode

WED signifies the most important component of an electrochemical cell. The selection of a WED material (or electrode modifier) is vital for success of an experiment. This will pass current to other species without being affected itself by that current. The most commonly used working electrode materials include platinum, gold, mercury and carbon. The biggest disadvantage of using platinum electrode besides its high cost, is that the presence of even small quantities of water or acid in the electrolyte leads to the reduction of proton to form hydrogen gas (hydrogen evolution) at fairly modest negative potentials. This reduction makes any useful analytical signal a vague. Gold electrodes work similar to platinum, but have limited use in the positive potential range due to the oxidation of its surface. It is very useful for the preparation of modified electrodes based on surface structures known as self-assembled monolayers (SAMs).

Carbon electrodes allow scans to more negative potentials than platinum or gold, as well as good anodic potential windows. The most common form of carbon electrode is glassy carbon electrode (GCE). Carbon paste electrodes are also useful in many applications. These electrodes are made from a paste of finely granulated carbon mixed with an oil substrate like paraffin oil. The paste is then firmly packed into cavity of an inert electrode tube. Carbon paste electrodes have the disadvantage of being prone to mechanical damage during use.

The counter or auxiliary electrode

The Counter or auxiliary electrode is the electrode that completes the current path. All electrochemistry experiments (with non-zero current) will have a WED-CE pair. In most experiments, the CE is simply the current source/sink. The only requirement of a counter electrode is, it should not dissolve in the electrolyte. Only noble metals and carbon fulfill this condition perfectly. Hence, noble metal like platinum is usually used as counter electrode in the form of foil, wire or a mesh.
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Reference electrodes

Since all investigations must be carried out in a complete cell system which involves two electrodes. It is common practice to employ a reference electrode as the other half of the cell. It is the reference for potential measurement. The major requirements of a reference electrode are— it should be easy to prepare and maintain, it should hold a constant potential during experiments etc. The concentration of any ionic species involved in the electrode reaction should be held at a fixed value. The easiest way to achieve this is to use an electrode reaction involving a saturated solution of an insoluble salt of the ion. Some commercially available reference electrodes include: silver/silver chloride (Ag/AgCl) (Figure 4), saturated calomel (Figure 4), mercury/mercury (mercurous) oxide, mercury/mercury sulfate, copper/copper sulfate etc.

![Saturated calomel electrode and Ag/AgCl electrode](image)

**Figure 4.** Typical Ag/AgCl and saturated calomel reference electrodes

The potentials of Ag/AgCl and saturated calomel electrodes have been determined against the hydrogen electrode very accurately [12].

Supporting electrolytes

Solutions containing a large excess of inert supporting electrolyte is generally required to perform electroanalytical experiments such as CV. The purpose of this supporting electrolyte is to ensure that the ionic strength of the solution is high and the oxidation or reduction of the analyte concerned does
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not perturb the homogeneous, near-zero electric field. It also helps to control reaction conditions by varying acid-base properties, complexing ability of the solution and changing the double-layer structure at the electrode surface. The important prerequisite of a supporting electrolyte is, it should not be easily oxidized or reduced; hence minimizing potential contamination or background contribution. The inert supporting electrolyte could be an inorganic salt, a mineral acid or a buffer.

Electrode processes

The phenomenon that occurs as a result of applying the voltage to an electrode, results in a current flow through that electrode. This is termed as the electrode process. Nature of the phase boundary of the working electrode and its related electrical double layer influence the course of an electrode process. Since, the potential applied to the cell exists only across the double layer of the working and reference electrodes, the importance of this region cannot be exaggerated when there is no current flow through the cell.

Most voltammetric and polarographic analyses utilize electrode processes in which electrons or ions are exchanged between the two phases; the electrode surface (solid phase) and the analyte molecule in solution phase. So, they are heterogeneous in nature. This reaction may take place in a single electron-transfer step or as a succession of two or more steps. The substances that receive and lose electrons are called the electroactive species. This process takes place within the very thin interfacial region at the electrode surface, and involves quantum-mechanical tunneling of electrons between the electrode and electroactive species. The work required to displace water molecules in hydration spheres of the ions constitutes part of the activation energy of the process [12]. As a result of which the analyte molecules will undergo either oxidation or reduction depending on the potential of the working electrode. Such an electrode process is referred to as a charge transfer reaction and produces a flow of charge (which is a current) through the electrode. This is pictorially shown below (Figure 5):
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Figure 5. Electron transfer at an anode

The transfer of charge involving a species in solution and an electrode surface constitutes a typical electrode process. The electrode reaction typically involves a sequence of steps:

1. Reactant moves towards the interface: this is called as mass transport

2. Quantum mechanical tunneling between the electrode and reactant close to the electrode (typical tunneling distances are less than 2 nm) can lead to the electron transfer.

3. The product moves away from the electrode surface into the bulk solution to allow fresh reactant to the surface of electrode.

Need for electrode modification

Whenever bare electrode surface does not accomplish the expected results, its modification may promise reasonably better results. During the last 3 decades, one of the most active areas of research interest in electrochemistry is the modification of the electrode surface for better results [13]. The ability to tune the performance of an electrode by chemically modifying its surface has provided a powerful tool in electroanalytical chemistry [14,15]. The electrode modification has provided a new direction in providing selectivity, anti-fouling properties, pre concentration of analyte species, improving electrocatalytic properties [16] and restraining interferences in a complex sample such as a
biological matrix [17]. Further, the modification of electrode surface has exhibited major impact for research dealing with energy conversion and storage, [18,19] corrosion studies [20], molecular electronics [21,22], electrochromic devices [23] and fundamental research of electrochemical processes [24].

Attachment of atoms, molecules or even nano particles to the surface allows one to shape the electronic and structural properties of a surface and consecutively, its functionality over a wide range. There are many ways in which analytical applications can gain advantages from the modification of the working electrode surface [25]. Generally, electrode modification is helpful to:

- accelerate the electron transfer reactions
- preferential accumulation
- lower redox potential
- enhance the surface area of the electrode
- enhance the peak current
- enhance the sensitivity
- increase the selectivity or stability on electrochemical devices

Chemically modified electrodes as electrochemical sensors

Electrochemical sensor is essentially an electrochemical cell which employs a two or three-electrodes arrangement. Electrochemical measurements can be made at steady-state or transient. The applied current or potential for electrochemical sensors may differ according to the method of operation, and the selection of the method is often anticipated to enhance the sensitivity and selectivity of a particular sensor. The general principles of electrochemical sensors have been extensively discussed in many electroanalytical references [26-28]. However, many electroanalytical methods are not used in routine sensing applications owing to their practical limitations and disadvantages. For example, dropping mercury electrode (DME) polarography is a well-established electroanalytical method; still its usefulness in sensor development, predominantly for in vivo sensing, is rather limited.
Based on sensing principle, electrochemical sensors generally can be categorized as conductivity/capacitance, potentiometric, amperometric and voltammetric sensors. Amperometric sensors can also be considered as a sub-class of voltammetric sensors.

**Voltammetric sensors**

The basis for voltammetric sensors is the current-potential relationship of an electrochemical cell. An important sub-class of voltammetric sensors includes amperometric sensors, which are also based on the current-potential relationship of the electrochemical cell. A fixed potential is applied to the electrochemical cell and the corresponding oxidation or reduction current is measured in the case of amperometric sensors. The quantification of the species involved in the reaction is based on this current. The point to be noticed in an amperometric sensor is that it operates at a fixed potential. A voltammetric sensor in contrast, can be operated by linear/cyclic voltammetric methods. In general, voltammetric sensors are based on the concentration effect of the analyte species on the current-potential characteristics of the reduction or oxidation reaction involved. The kinetics of the Faradaic or charge transfer reaction at the electrode surface and the mass transfer rate of the detecting species in the reaction onto the electrode surface directly affect the current-potential characteristics. The voltammetric sensors can be used for the analysis of various organic and inorganic analytes in various matrices, for electrode kinetics etc [29-34].

**Carbon nanotube based voltammetric sensors**

Recently, one of the most active areas of analytical research is the development of chemical sensors. A chemical sensor is a small device that can be used for direct measurement of the analyte in the sample matrix [25]. Ideally, such a device is capable of responding continuously and reversibly and does not alter the sample. By combining the sample treatment and measurement steps, sensors eradicate the need for sample collection and preparation. Chemical sensors consist of a transduction element covered with a
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classical chemical detection layer. This layer interacts with the target analyte molecule and the chemical changes resulting from this interaction are converted into electrical signals by the transduction element.

Carbon nanotubes (CNTs) have become the subject of intense investigation since their discovery [35] in 1991 by Sumio Iijima [36]. CNTs have gained considerable interest in potential applications based on their electronic transport and field emission properties [37,38], high mechanical strength [39] etc. One of the most important applications of CNTs in analytical chemistry is that in the electrochemical sensor field [32,40,41]. Further, they have been of great interest in the research of field emission devices [42], nanoscale transistors [43] or components for composite materials [44].

Different types of CNTs such as SWCNTs, DWCNTs and MWCNTs have been used for the fabrication of electrodes for electroanalytical applications. Recently, modification of the surfaces of the conventional electrodes with CNTs as well as their integration into the electrode membrane matrix (forming pastes or rigid composites with other membrane components) has also been reported.

CNTs exhibit an extraordinary richness in their surface chemistry. Their sidewalls show a more inert behavior, the edges being chemically and electrochemically more reactive. They (CNTs) show chemical and electrochemical anisotropy. Therefore, a high density of CNT edges or defects is anticipated to enhance the electrochemical performance of electrodes. Owing to their high surface area, they exhibit an enhanced current signal. Depending on the redox system under investigation, oxygenated surface functionalities can increase even further the electrochemical performance. The remarkable benefits of CNT-modified electrodes including low detection limits, increased sensitivity, decreased overpotentials and resistance to surface fouling have been widely reported from the point of view of electroanalysis [41,45-47].

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Graphene based electrochemical sensors

Graphene (Figure 6), a novel material that has emerged as a rapidly rising star in the field of material science, consists of a sp$^2$-bonded carbon structure, arranged in a honeycomb lattice which is one-atom-thick planar sheet with exceptionally high crystal and electronic quality [48]. Since its ground breaking discovery in 2004 [49], graphene has been making a great impact in many areas of research due to its remarkable physico-chemical properties. Graphene is the essential building block for zero dimensional spherical fullerenes, one-dimensional CNTs, three-dimensional graphite where stacks generally consist of more than ten graphene sheets [50]. Owing to its unique electrochemical and physical properties, it has already enhanced its use in specific technological fields, in spite of its relatively young age and also attracted incredible attention from many scientific communities [51,52].

**Figure 6. A single graphene sheet**

Graphene (Figure 6) is an ideal material and has great potential for providing new approaches and critical improvements over graphite or CNTs in the field of electrochemistry [53-56] due to its very large 2-D electrical conductivity, large surface area and low cost. Graphite oxide can accumulate...
the electroactive analyte species and facilitate their electron transfer (ET) at electrode surfaces owing to its favorable electron mobility and unique surface properties, like one-atom thickness and high specific surface area. A couple of advantages of graphene are noticeable compared to graphite and CNTs,

- Single-layer graphene is predicted to have a large surface area of 2630 m²/g [57] which is much higher than that of natural graphite (~10 m²/g), and is two times larger than that of CNTs (1315 m²/g) [53].
- Graphene does not contain metallic impurities as CNTs do [58]. Such impurities frequently dominate the electrochemistry of CNTs and lead to misinterpretations [59].
- The production of graphene is easy, since it is prepared from graphite, which is cheap and accessible.

**Graphene oxide-layered double hydroxides (clay) composites as sensing materials**

In the last decade, works devoted to the development of electrochemical devices suitable for the detection of bioactive compounds have gained growing interest as innovative alternatives in terms of environmental-friendly nature, on-site detection, sensitivity, rapid response, miniature size and low cost. Hence, clay or its composites modified electrodes are gaining great attention for electrochemical analysis of drugs or pollutants [60].

Delamination of layered solids such as graphite oxide and bentonite in appropriate solvents under suitable conditions results in colloidal dispersions of solvated, anisotropic two-dimensional nanosheets [61]. Reassembly of layers (graphene oxide and bentonite) resulting in reflocculation occurs owing to the fact that these colloidal dispersions of nanosheets are thermodynamically unstable [62]. Layered composites are formed by the costacking of layers from the colloidal dispersions of two different layered solids. We have chosen bentonite and graphite oxide as these are structurally unrelated (except for the
fact that both are layered solids) and composites based on these two solids are ubiquitous.

**Drug-human serum albumin interactions**

The word "protein" originated from the Latin word 'primarius' or from the Greek God 'Proteus' [63]. It is perhaps surprising to notice that the term "protein" was introduced nearly 170 years ago even after the huge accumulation of knowledge about proteins over the last 50 years. One early description of protein was done by G. J. Mulder in 1839 where his studies on the composition of animal matter, primarily fibrin, albumin and gelatin revealed the existence of carbon, hydrogen, oxygen and nitrogen. In addition, he recognized the presence of sulfur and phosphorus in 'animal substances' that contained huge numbers of atoms. In other words, he recognized that these 'animal substances' were macromolecules.

Proteins have various functions in biological systems such as DNA replication, formation of cytoskeletal structures and transportation of oxygen around the bodies of multicellular organisms. A protein is involved intimately in the biological process occurring in a living cell.

The study of proteins is particularly important since the protein structure and function have an impact on human endeavor like medicine. Understanding the nature of the drug-protein interaction is extremely significant in drug discovery. Majority of molecular complexes which make life feasible are due to interactions of proteins with other molecules. Therefore, interactions of this nature have been the subject of enormous theoretical and experimental investigations. In particular, the biotechnology and pharmaceutical industries have a considerable interest in computing the number and strength of ligand-protein interactions. Such computation requires information about the number of interacting molecular species (stoichiometry) and strengths of binding interactions (free energies). Fluorescence spectroscopic technique is extensively used in the study of ligand (drug)-protein interactions [64].
Importance of drug-HSA interaction studies

Human serum albumin (HSA) (Figure 7) is a helical single-chain, non-glycosylated polypeptide [65] with turns and extended loops, and resembles a heart shape, with approximate dimensions of $80 \times 80 \times 30$ Å [65,66].

In systemic circulation, drugs have two forms namely, bound or unbound to plasma proteins. The unbound drugs can passively pass through the barriers into the organs where they are metabolized, biliary excretion or glomerular filtration in kidney [67] occurs. Further, specific transport systems disperse them intracellularly. Therapeutic effects are produced only by interactions of free drug molecules (unbound) with therapeutic targets [68]. In most cases, the unbound concentration of the drug in the tissue depends on the unbound drug concentration in the plasma [67]. Therefore, drug-HSA interactions are a significant factor to realize the pharmacokinetics and pharmacological effects of drugs [69-71].

**Figure 7. Structure of HSA**

Being the most abundant protein of blood plasma, HSA has many vital physiological functions. Some of HSA’s functions include regulation of
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colloidal osmotic pressure, transportation of numerous endogenous compounds such as fatty acids (FA), hormones, bile acids, amino acids, metals and toxic metabolites [65,72,73]. In addition to this, there is a wide variety of bioactive compounds that are transported to their target organs/tissues by binding with HSA [65,67,74]. As a result, HSA not only protects the bound drugs against oxidation and influences the in vivo drug distribution, but also alters the pharmacokinetic and pharmacodynamic properties of drugs [67,75,76].

The interactions between ligands and HSA have been extensively studied for several years using a variety of methods [77-81]. These results provide salient information of the structural features that determine the therapeutic effectiveness of drugs, and hence become an important research field in chemistry, life sciences and clinical medicine.

REAGENTS/COMPONENTS USED IN THE PRESENT STUDY

The lists of compounds employed in the present study are shown below:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>Ranbaxy Fine chemicals Ltd., New Delhi, India</td>
</tr>
<tr>
<td>Methanol</td>
<td>Ranbaxy Fine chemicals Ltd., New Delhi, India</td>
</tr>
<tr>
<td>MWCNTs</td>
<td>Sigma Chemical Company, St Louis, USA</td>
</tr>
<tr>
<td>Human Serum Albumin</td>
<td>Sigma Chemical Company, St Louis, USA</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>sd fine-CHEM Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Ortho phosphoric acid</td>
<td>sd fine-CHEM Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>sd fine-CHEM Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>sd fine-CHEM Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Nitric acid</td>
<td>sd fine-CHEM Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Sulphuric acid</td>
<td>sd fine-CHEM Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>sd fine-CHEM Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Boric acid</td>
<td>sd fine-CHEM Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Bentonite</td>
<td>Sigma Chemical Company, St Louis, USA</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>sd fine-CHEM Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>sd fine-CHEM Ltd., Mumbai, India</td>
</tr>
</tbody>
</table>
The development of pharmaceuticals brought a revolution in human health. The rise in global drug consumption makes control over their determination, quality and therapeutic actions. The interaction of a drug with a protein is a matter of foremost importance in biological, chemical and pharmaceutical sciences. The structure of the drug is the starting point for the development of a suitable procedure for identification and quantitative analysis.
The following bioactive compounds of pharmaceutical importance have been selected in the present study:

**Mycophenolate mofetil** [Dr. Reddy’s Laboratories, Hyderabad, India]

**Methdilazine hydrochloride** [Glaxo Smith Cline Pharmaceuticals (India) Limited, Mumbai]

**Isothipendyl hydrochloride** [German Remedies Limited, Goa]

**Almotriptan malate** [Dr. Reddy’s Laboratories Ltd, Hyderabad, India]

**Valganciclovir hydrochloride** [Dr. Reddy’s Laboratories, Hyderabad, India]

**Linezolid** [Biocon Limited, Bangalore, India]

**Pramipexole dihydrochloride monohydrate** [Dr. Reddy’s Laboratories, Hyderabad, India]

The general drug profiles of the selected drugs are given below:

### 1. Mycophenolate mofetil (MMF)

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>2-morpholinoethyl((E))-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>Molecular formula</td>
<td>(C_{23}H_{31}NO_7)</td>
</tr>
<tr>
<td>Molecular mass</td>
<td>433.5</td>
</tr>
<tr>
<td>Description</td>
<td>A white or almost white, crystalline powder.</td>
</tr>
<tr>
<td>Solubility</td>
<td>Slightly soluble in water; sparingly soluble in alcohol; soluble in methyl alcohol; freely soluble in acetone.</td>
</tr>
<tr>
<td>Category</td>
<td>Immunosuppressant</td>
</tr>
</tbody>
</table>

Mycophenolate mofetil (MMF), an immunosuppressant and prodrug of mycophenolic acid, is used extensively in transplant medicine. It is a reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH) in purine (guanine) biosynthesis which is necessary for the growth of T cells and B cells. Other cells are able to recover purines via a separate salvage pathway and are thus able to escape the effect.
2. Methdilazine hydrochloride (MDH)

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>10-((1-methylpyrrolin-3-yl)methyl)-4a,10a-dihydro-10H-phenothiazine hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td><img src="image" alt="Structure of Methdilazine" /></td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C_{18}H_{23}ClN_{2}S</td>
</tr>
<tr>
<td>Molecular mass</td>
<td>334.91</td>
</tr>
<tr>
<td>Description</td>
<td>A light tan crystalline powder having a slight characteristic odor</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water</td>
</tr>
<tr>
<td>Category</td>
<td>Antihistamine</td>
</tr>
</tbody>
</table>

Methdilazine hydrochloride (MDH) is a phenothiazine compound with antihistaminic activity. It is used in the treatment of various dermatoses to relieve pruritus.

3. Isothipendyl hydrochloride (IPH)

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>1-(5a,9a-dihydro-10H-benzo[b]pyrido[2,3-e][1,4]thiazin-10-yl)-N,N-dimethylpropan-2-amine hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td><img src="image" alt="Structure of Isothipendyl" /></td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C_{16}H_{22}ClN_{3}S</td>
</tr>
<tr>
<td>Molecular mass</td>
<td>323.88</td>
</tr>
<tr>
<td>Description</td>
<td>A white crystalline powder</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water</td>
</tr>
<tr>
<td>Category</td>
<td>Antihistamine</td>
</tr>
</tbody>
</table>

Isothipendyl hydrochloride (IPH) is a first generation antihistamine and anticholinergic agent. It is used as an antipruritic. It is nowadays scarcely used in the first line relief of allergies due to the anticholinergic side effect of somnolence but does have some limited use through topical application in the relief of insect bites and related itching (pruritus).
4. Almotriptan malate (ALM)

Chemical name: \( N,N\text{-dimethyl-2-(5-((pyrrolidin-1-ylsulfonyl)methyl)-1H-indol-3-yl)ethan-1-amine 2-hydroxy succinate} \)

Structure:

Molecular formula: \( C_{21}H_{31}N_{5}O_{7}S \)

Molecular mass: 469.55

Description: A white crystalline powder

Solubility: Soluble in water

Category: Anti-migraine

Almotriptan malate (ALM) is a selective and potent serotonin 5-hydroxy tryptamine 1B/1D (5-HT 1B/1D) receptor agonist. ALM is used to treat severe migraine headaches. It is available in the market as conventional tablets (Axert).

5. Valganciclovir hydrochloride (VGCV)

Chemical name: 2-((2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy)-3-hydroxypropyl L-valinate

Structure:

Molecular formula: \( C_{14}H_{22}N_{6}O_{5} \)

Molecular mass: 354.37

Description: A white to off-white powder

Solubility: Freely soluble in water and alcohol

Category: Antivirals

Valganciclovir hydrochloride (VGCV) is an antiviral drug used for the treatment of cytomegalovirus infections. It is a prodrug for ganciclovir. Soon after oral consumption, it rapidly gets de-esterified to ganciclovir by intestinal and hepatic esterases.
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6. Linezolid (LIN)

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>(S)-N-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C₁₆H₂₀FN₃O₄</td>
</tr>
<tr>
<td>Molecular mass</td>
<td>337.35</td>
</tr>
<tr>
<td>Description</td>
<td>A white crystalline powder</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in organic solvents such as methanol, ethanol and DMSO</td>
</tr>
<tr>
<td>Category</td>
<td>Oxazolidinone antibiotic</td>
</tr>
</tbody>
</table>

Linezolid (LIN) is a synthetic antibiotic, the first of the oxazolidinone class, used for the treatment of infections caused by multi-resistant bacteria including *streptococcus* and methicillin-resistant *Staphylococcus aureus* (MRSA). The drug works by inhibiting the initiation of bacterial protein synthesis.

7. Pramipexole dihydrochloride monohydrate (PPX)

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>(S)-N⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine dihydrochloride hydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C₁₀H₂₁Cl₂N₃OS</td>
</tr>
<tr>
<td>Molecular mass</td>
<td>302.26</td>
</tr>
<tr>
<td>Description</td>
<td>A white to off-white powder substance</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water</td>
</tr>
<tr>
<td>Category</td>
<td>Dopamine agonist</td>
</tr>
</tbody>
</table>

Pramipexole (PPX) is a new non-ergot dopaminergic agonist used for the treatment of Parkinson’s disease and restless legs syndrome. The ability of PPX to alleviate the signs and symptoms of Parkinson’s disease is believed to be related to its ability to stimulate dopamine receptors in the striatum. However, the precise mechanism of action of PPX against restless legs syndrome is not known.
REFERENCES


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