CHAPTER - I

Introduction
The term drug is often used synonymously with the terms, medications, therapies, treatments, medicine, therapeutics or medicaements. Generally speaking, drugs are substances that influence the function of the human body. Drugs are molecules (called chemicals or compounds) that affect the body when they are ingested, inhaled, injected, or otherwise enter the body. Drugs are either substances that are used as medication such as pain relievers, or substances used for recreation (drugs of abuse) such as alcohol. Any new drug is usually administered in simple preliminary formulation by parenteral and oral routes to determine its pharmacological activity, its relative safety, toxicity, and dose response characteristics. The cost of drug discovery and development has become so high that research management is eagerly looking forward to have more systematic and process. The genealogy of quite recently introduced drugs however provide a good illustration of the role that serendipity, inhibition or even pure chance have played in drug discovery up until recently.

The measurement of drugs in biological fluids is required, to study the absorption, distribution, biotransformation and elimination characteristics of a drug substance, and is by far one of the most complex problems faced by the analytical biochemist. With the development of patented drugs of specific pharmacological action, the quantities required for maintenance of clinical effectiveness have dropped from gram and multiple gram amounts to milligram and submilligram amounts. Thus, the determination of both therapeutic and toxic concentration of drugs in biological media at these low dosages must routinely reach the nanogram level of sensitivity.

1.1 Drug Classification

Drugs can be classified according to various criteria including chemical structure or pharmacological action. The preferred classification is the latter one, which may be divided into main groups as follows:

a) Chemotherapeutic agents - used to cure infectious diseases and cancer. (sulfa drugs, antibiotics)
b) Pharmacodynamic agents - used in non-infectious diseases (cholinergic, adrenergic, hallucinogenic).

c) Miscellaneous agents (narcotic analgesics, local anesthetics)

1.2 Disease Classification

A disease is a condition of impaired health resulting from a disturbance in the structure or function of the body. Diseases may be classified into the following major categories:

(1) Infections caused by viruses, bacteria, fungi, protozoa and worms

(2) Allergic diseases caused by antigens and foreign substances

(3) Metabolic disorders caused by defects in the body's ability to carry out normal reactions - these may be hereditary, deficiency, and congenital defects

(4) Cancer

(5) Toxic diseases caused by poisons

(6) Psychosomatic and mental diseases

Chemotherapy is broadly defined as the treatment of any disease by chemicals including infectious and non-infectious diseases. The original definition applied only to drugs which were used in the treatment of infectious diseases. The proper term for the treatment of non-infectious diseases is pharmacodynamics.

1.3 Mode of Drug Action

It is important to distinguish between actions of drugs and their effects. Actions of drugs are the biochemical physiological mechanisms by which the chemical produces a response in living organisms. The effect is the observable consequence of a drug action. For example, the action of penicillin is to interfere with cell wall synthesis in bacteria and the effect is the death of the bacteria.
One major problem of pharmacology is that no drug produces a single effect. The primary effect is the desired therapeutic effect. Secondary effects are all other effects beside the desired effect which may be either beneficial or harmful. Drugs are chosen to exploit differences between normal metabolic processes and any abnormalities which may be present. Since the differences may not be very great, drugs may be nonspecific in action and alter normal functions as well as the undesirable ones and leads to side effects.

The biological effects observed after a drug has been administered are the result of an interaction between that chemical and some part of the organism. Mechanisms of drug action can be viewed from different perspectives, namely, the site of action and the general nature of the drug-cell interaction.

1.3.1 Killing Foreign Organisms

Chemotherapeutic agents act by killing or weakening foreign organisms such as bacteria, worms, viruses. The main principle of action is selective toxicity, i.e. the drug must be more toxic to the parasite than to the host.

1.3.2 Stimulation and Depression

Drugs are act by stimulating or depressing normal physiological functions. Stimulation increases the rate of activity while depression reduces the rate of activity.

1.4 Mechanism of action

In pharmacology, the term mechanism of action (MOA) refers to the specific biochemical interaction through which a drug substance produces its pharmacological effect. A mechanism of action usually includes mention of the specific molecular targets to which the drug binds, such as an enzyme substrate complex.

For example, the mechanism of action of aspirin involves irreversible inhibition of the enzyme cyclooxygenase, which suppresses the production of prostaglandins and throm boxanes, there by reducing pain and inflammation.
1.5 Pharmacokinetic

Pharmacokinetics, sometimes abbreviated as PK, (from Ancient Greek pharmakon "drug" and kinetikos "to do with motion") is a branch of pharmacology dedicated to the determination of the fate of substances administered externally to a living organism. In practice, this discipline is applied mainly to drug substances, though in principle it concerns itself with all manner of compounds ingested or otherwise delivered externally to an organism, such as nutrients, metabolites, hormones, toxins, etc. Pharmacokinetics is often studied in conjunction with pharmacodynamics. Pharmacodynamics explores what a drug does to the body, whereas pharmacokinetics explores what the body does to the drug. Pharmacokinetics includes the study of the mechanisms of absorption and distribution of an administered drug, the rate at which a drug action begins and the duration of the effect, the chemical changes of the substance in the body (e.g. by enzymes) and the effects and routes of excretion of the metabolites of the drug.1

1.6 Absorption, Distribution, Metabolism and Excretion (ADME)

Pharmacokinetics is divided into several areas which include the extent and rate of absorption, distribution, metabolism and excretion. This is commonly referred to as the ADME scheme. However recent understanding about the drug-body interactions brought about the inclusion of new term Liberation. Now Pharmacokinetics can be better described as LADME.

- Liberation is the process of release of drug from the formulation.
- Absorption is the process of a substance entering the body.
- Distribution is the dispersion or dissemination of substances throughout the fluids and tissues of the body.
- Metabolism is the irreversible transformation of parent compounds into daughter metabolites.
- Excretion is the elimination of the substances from the body. In rare cases, some drugs irreversibly accumulate in a tissue in the body.
Pharmacokinetics describes how the body affects a specific drug after administration. Pharmacokinetic properties of drugs may be affected by elements such as the site of administration and the concentration in which the drug is administered. These may affect the absorption rate.\(^2\)

### 1.6.1 Pharmacokinetic Analysis

Pharmacokinetic analysis is performed by noncompartmental (model independent) or compartmental methods. Noncompartmental methods estimate the exposure to a drug by estimating the area under the curve of a concentration-time graph. Compartmental methods estimate the concentration-time graph using kinetic models. Compartment-free methods are often more versatile in that they do not assume any specific compartmental model and produce accurate results also acceptable for bioequivalence studies.

### 1.6.2 Noncompartmental analysis

Noncompartmental PK analysis is highly dependent on estimation of total drug exposure. Total drug exposure is most often estimated by area under the curve methods, with the trapezoidal rule (numerical differential equations) the most common area estimation method. Due to the dependence on the length of 'x' in the trapezoidal rule, the area estimation is highly dependent on the blood/plasma sampling schedule. That is the closer the trapezoids are to the actual shape of the concentration-time curve.

### 1.6.3 Compartmental analysis

Compartmental PK analysis uses kinetic models to describe and predict the concentration-time curve. PK compartmental models are often similar to kinetic models used in other scientific disciplines such as chemical kinetics and thermodynamics. The advantage of compartmental over some noncompartmental analyses is the ability to predict the concentration at any time. The disadvantage is the difficulty in developing and validating the proper model. Compartment-free modeling based on curve stripping does not suffer this limitation. The simplest PK compartmental model is the one-compartmental PK model with IV bolus administration and first-order elimination. The most complex PK models (called PBPK models) rely on the use of physiological information to ease development and validation.
1.6.4 Bioanalytical methods

Bioanalytical methods are necessary to construct a concentration-time profile. Chemical techniques are employed to measure the concentration of drugs in biological matrix, most often plasma. Proper bioanalytical methods should be selective and sensitive.

1.6.4 Mass spectrometry

Pharmacokinetics is often studied using mass spectrometry because of the complex nature of the matrix (often blood or urine) and the need for high sensitivity to observe low dose and long time point data. The most common instrumentation used in this application is LC-MS with a triple quadrupole mass spectrometer. Tandem mass spectrometry is usually employed for added specificity. Standard curves and internal standards are used for quantitation of usually a single pharmaceutical in the samples. The samples represent different time points as a pharmaceutical is administered and then metabolized or cleared from the body. Much attention is paid to the linearity of the standard curve; however it is not uncommon to use curve fitting with more complex functions such as quadratics since the response of most mass spectrometers is less than linear across large concentration ranges.¹ ³

There is currently considerable interest in the use of very high sensitivity mass spectrometry for micro dosing studies, which are seen as a promising alternative to animal experimentation.⁶

1.7 Pharmaceutical formulation

Pharmaceutical formulation, in pharmaceutics, is the process in which different chemical substances, including the active drug, are combined to produce a final medicinal product. A pharmaceutical formulation ⁷ ¹¹ is aimed at ensuring drug delivery the site of action in order to produce the desired therapeutic effect.

Formulation studies involve developing a preparation of the drug which is both stable and acceptable to the patient. For orally taken drugs, this usually involves incorporating the drug into a tablet or a capsule. It is important to appreciate that a tablet contains a variety of other substances apart from the drug.
itself, and studies have to be carried out to ensure that the drug is compatible with these other substances.

Preformulation involves the characterization of a drug's physical, chemical, and mechanical properties in order to choose what other ingredients should be used in the preparation. In dealing with protein pre-formulation, the important aspect is to understand the solution behavior of a given protein under a variety of stress conditions such as freeze/thaw, temperature, shear stress among others to identify mechanisms of degradation and therefore its mitigation. Formulation studies then consider such factors as particle size, polymorphism, pH, and solubility, as all of these can influence bioavailability and hence the activity of a drug. The drug must be combined with inactive additives by a method which ensures that the quantity of drug present is consistent in each dosage unit e.g. each tablet. The dosage should have a uniform appearance, with an acceptable taste, tablet hardness, or capsule disintegration.

It is unlikely that formulation studies will be complete by the time clinical trials commence. This means that simple preparations are developed initially for use in phase I clinical trials. These typically consist of hand-filled capsules containing a small amount of the drug and a diluent. Proof the long-term stability of these formulations is not required, as they will be used (tested) in a matter of days. Consideration has to be given to what is called the drug load - the ratio of the active drug to the total contents of the dose. A low drug load may cause homogeneity problems. A high drug load may pose flow problems or require large capsules if the compound has a low bulk density.

By the time phase III clinical trials are reached, the formulation of the drug should have been developed to be close to the preparation that will ultimately be used in the market. Knowledge of stability is essential by this stage, and conditions must have been developed to ensure that the drug is stable in the preparation. If the drug proves unstable, it will invalidate the results from clinical trials since it would be impossible to know what the administered dose actually was. Stability studies are carried out to test whether temperature, humidity, oxidation, or photolysis (ultraviolet light or visible light) have any effect, and the preparation is analysed to see if any degradation products have been formed.
It is also important to check whether there are any unwanted interactions between the preparation and the container. If a plastic container is used, tests are carried out to see whether any of the ingredients become adsorbed on to the plastic, and whether any plasticizers, lubricants, pigments, or stabilizers leach out of the plastic into the preparation. Even the adhesives for the container label need to be tested, to ensure they do not leach through the plastic container into the preparation.

1. Oral formulations

The way a drug is formulated can avoid some of the problems associated with oral administration. Drugs are normally taken orally as tablets or capsules.

The drug (active substance) itself needs to be soluble in aqueous solution at a controlled rate. Such factors as particle size and crystal form can significantly affect dissolution. Fast dissolution is not always ideal. For example, slow dissolution rates can prolong the duration of action or avoid initial high plasma levels. Treatment of active ingredient by special way as spherical crystallization\textsuperscript{13} can have some advantages for drug formulation.

2. Tablet form

A tablet is usually a compressed preparation that contains:

- 5-10% of the drug (active substance)
- 80% of fillers, disintegrants, lubricants, glidants, and binders
- 10% of Compounds which ensure easy disintegration, disaggregation, and dissolution of the tablet in the stomach or the intestine.

The disintegration time can be modified for a rapid effect or for sustained release. Special coatings can make the tablet resistant to the stomach acids such that it only disintegrates in the duodenum as a result of enzyme action or alkaline pH. Pills can be coated with sugar, varnish, or wax to disguise the taste.

Some tablets are designed with an osmotically active core, surrounded by an impermeable membrane with a pore in it. This allows the drug to percolate out from the tablet at a constant rate as the tablet moves through the digestive tract.
3. Capsule form

A capsule is a gelatinous envelope enclosing the active substance. Capsules can be designed to remain intact for some hours after ingestion in order to delay absorption. They may also contain a mixture of slow- and fast-release particles to produce rapid and sustained absorption in the same dose.

4. Topical medication forms

- Cream – emulsion of oil and water in approximately equal proportions. Penetrates stratum corneum outer layer of skin well.

- Ointment - combines oil (80%) and water (20%). Effective barrier against moisture loss.

- Gel - liquefies upon contact with the skin.

- Paste - combines three agents - oil, water, and powder; an ointment in which a powder is suspended.

- Powder

1.8 Chemotherapy

Chemotherapy, in its most general sense, is the treatment of disease by chemicals especially by killing micro-organisms or cancerous cells. In popular usage, it refers to antineoplastic drugs used to treat cancer or the combination of these drugs into a cytotoxic standardized treatment regimen. In its non-oncological use, the term may also refer to antibiotics (antibacterial chemotherapy). In that sense, the first modern chemotherapeutic agent was Paul Ehrlich’s arsphenamine, an arsenic compound discovered in 1909 and used to treat syphilis. This was later followed by sulfonamides discovered by Domagk and penicillin discovered by Alexander Fleming.

The first use of drugs to treat cancer, however, was in the early 20th century, although it was not originally intended for that purpose. Mustard gas was used as a chemical warfare agent during World War I and was studied further during World War II. During a military operation in World War II, a group of people were
accidentally exposed to mustard gas and were later found to have very low white blood cell counts. It was reasoned that an agent that damaged the rapidly-growing white blood cells might have a similar effect on cancer. Therefore, in the 1940s, several patients with advanced lymphomas (cancers of certain white blood cells) were given the drug by vein, rather than by breathing the irritating gas. Their improvement, although temporary, was remarkable. That experience led researchers to look for other substances that might have similar effects against cancer. As a result, many other drugs have been developed to treat cancer, and drug development since then has exploded into a multibillion-dollar industry, although the principles and limitations of chemotherapy discovered by the early researchers still apply. Cancer is the uncontrolled growth of cells coupled with malignant behavior: invasion and metastasis. Cancer is thought to be caused by the interaction between genetic susceptibility and environmental toxins.

In the broad sense, most chemotherapeutic drugs work by impairing mitosis (cell division), effectively targeting fast-dividing cells. As these drugs cause damage to cells they are termed cytotoxic. Some drugs cause cells to undergo apoptosis (so-called "programmed cell death").

Scientists have yet to identify specific features of malignant and immune cells that would make them uniquely targetable (barring some recent examples, such as the Philadelphia chromosome as targeted by imatinib). This means that other fast-dividing cells, such as those responsible for hair growth and for replacement of the intestinal epithelium (lining), are also often affected. However, some drugs have a better side effect profile than others, enabling doctors to adjust treatment regimens to the advantage of patients in certain situations.

As chemotherapy affects cell division, tumors with high growth fractions (such as acute myelogenous leukemia and the aggressive lymphomas, including Hodgkin's disease) are more sensitive to chemotherapy, as a larger proportion of the targeted cells are undergoing cell division at any time. Malignancies with slower growth rates, such as indolent lymphomas, tend to respond to chemotherapy much more modestly.
Drugs affect "younger" tumors (i.e., more differentiated) more effectively, because mechanisms regulating cell growth are usually still preserved. With succeeding generations of tumor cells, differentiation is typically lost, growth becomes less regulated, and tumors become less responsive to most chemotherapeutic agents. Near the center of some solid tumors, cell division has effectively ceased, making them insensitive to chemotherapy. Another problem with solid tumors is the fact that the chemotherapeutic agent often does not reach the core of the tumor. Solutions to this problem include radiation therapy (both brachytherapy and teletherapy) and surgery.

There are a number of strategies in the administration of chemotherapeutic drugs used today. Chemotherapy may be given with a curative intent or it may aim to prolong life or to palliate symptoms. Combined modality chemotherapy is the use of drugs with other cancer treatments, such as radiation therapy or surgery. Most cancers are now treated in this way. Combination chemotherapy is a similar practice that involves treating a patient with a number of different drugs simultaneously. The drugs differ in their mechanism and side effects. The biggest advantage is minimising the chances of resistance developing to any one agent. In neoadjuvant chemotherapy (preoperative treatment) initial chemotherapy is designed to shrink the primary tumour, thereby rendering local therapy (surgery or radiotherapy) less destructive or more effective.

Adjuvant chemotherapy (postoperative treatment) can be used when there is little evidence of cancer present, but there is risk of recurrence. This can help reduce chances of developing resistance if the tumour does develop. It is also useful in killing any cancerous cells which have spread to other parts of the body. This is often effective as the newly growing tumours are fast-dividing, and therefore very susceptible. Palliative chemotherapy is given without curative intent, but simply to decrease tumor load and increase life expectancy. For these regimens, a better toxicity profile is generally expected.

All chemotherapy regimens require that the patient to be capable of undergoing the treatment. Performance status is often used as a measure to determine whether a patient can receive chemotherapy, or whether dose reduction is
required. Because only a fraction of the cells in a tumor die with each treatment (fractional kill), repeated doses must be administered to continue to reduce the size of the tumor.\textsuperscript{17} Current chemotherapy regimens apply drug treatment in cycles, with the frequency and duration of treatments limited by toxicity to the patient.\textsuperscript{18}

Although the word chemotherapy can mean the use of any drug (such as aspirin or penicillin) to treat any disease, to most people chemotherapy refers to drugs used for cancer treatment. Two other medical terms often used to describe cancer chemotherapy are antineoplastic (meaning anti-cancer) therapy and cytotoxic (cell-killing) therapy.

People treated with chemotherapy, particularly alkylating agents, may have an increased risk of developing leukemia several years after treatment. Some drugs, especially alkylating agents, cause infertility in some women and in most men who receive these treatments.

More than 100 drugs are used today for chemotherapy either alone or in combination with other drugs or treatments. As research continues, more drugs are expected to become available. These drugs vary widely in their chemical composition, how they are taken, their usefulness in treating specific forms of cancer, and their side effects. New drugs are first developed through research in test tubes and animals. Then the drugs are tested in clinical trials in humans to find out how safe they are and how well they work.

Chemotherapy is the first choice for treating many cancers. It differs from surgery or radiation in that it is almost always used as a systemic treatment. This means the drugs travel throughout the body to reach cancer cells wherever they may have spread. Treatments like radiation and surgery act only in a specific area such as the breast, lung, or colon, and so are considered local treatments.

1.8 Types of Chemotherapy Drugs

Chemotherapy drugs can be divided into several groups based on factors such as how they work, their chemical structure, and their relationship to another drug. Some chemotherapy drugs are grouped together because they were derived from the same plant. Because some drugs act in more than one way, they may
belong to more than one group. Knowing how the drug works is important in predicting side effects. This helps oncologists decide which drugs are likely to have synergic action. If more than one drug will be used, this information also helps them plan exactly when each of the drugs should be given (in which order and how often).

The majority of chemotherapeutic drugs can be divided into alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other antitumour agents. All of these drugs affect cell division or DNA synthesis and function in some way.

1. Alkylating agent

Alkylating agents are most active in the resting phase of the cell. These types of drugs are cell-cycle non-specific. There are several types of alkylating agents used in chemotherapy treatments:

Alkylating agents are so named because of their ability to add alkyl groups to many electronegative groups under conditions present in cells. Cisplatin and carboplatin, as well as oxaliplatin, are alkylating agents. The alkylating agents impair cell function by forming covalent bonds with the amino, carboxyl sulfhydryl and phosphate groups in biologically important molecules.

Other agents are mechlorethamine, cyclophosphamide, chlorambucil, ifosfamide. They work by chemically modifying a cell's DNA. Depending on the amount of dosage of this type of drugs there's also a risk of leukemia involved. The risk gets lower if the dose is lower, but if the doses are higher, are the high risks. The following are a few of the different types of alkylating agents. We'll mention the most common type which is nitrogen mustards.

Nitrogen mustards: The nitrogen mustards which include such drugs as chlorambucil, cyclophosphamide (cytoxan), ifosfamide, and melphalan.

Nitrosoureas: which include streptozocin, lomustine, and carmustine (BCNU). Drugs like cisplatin, carboplatin, and oxalaplatin are sometimes combined with alkylating agents because they destroy cells in a similar way. These drugs are also less likely to cause leukemia than the alkylating agents. The nitrosoureas are
distinguished by this high lipid solubility and chemical instability, cisplatin is an inorganic heavy metal complex that has activity typical of a cell cycle-phase – nonspecific alkylating agent.27

Metal salts: The platinum drugs (cisplatin, carboplatin, and oxalaplatin) are sometimes grouped with alkylating agents because they kill cells in a similar way. These drugs are less likely than the alkylating agents to cause leukemia.

- Ethylenimines: thiopeta and hexamethylmelamine.
- Alkylsulfonates: busulfan.
- Hydrazines and triazenes: altretamine, procarbazine, dacarbazine and temozolomide.
- Nitrosureas: carmustine, lomustine and streptozocin. Nitrosureas are unique because, unlike most types of chemo treatments, they can cross the blood-brain barrier. They can be useful in treating brain tumors.
- Alkyl sulfonates: busulfan.
- Triazines: dacarbazine (DTIC) and temozolomide (temodar®)
- Ethylenimines: thiopeta and altretamine (hexamethylmelamine

2. Plant Alkaloids

Plant alkaloids are chemotherapy treatments derived from certain types of plants. The vinca alkaloids are made from the periwinkle plant (catharanthus rosea). The taxanes are made from the bark of the Pacific Yew tree (taxus). The vinca alkaloids and taxanes are also known as antimicrotubule agents. The podophyllotoxins are derived from the May apple plant. Camptothecan analogs are derived from the Asian "Happy Tree" (Camptotheca acuminata). Podophyllotoxins and camptothecan analogs are also known as topoisomerase inhibitors, which are used in certain types of chemotherapy. The plant alkaloids are cell-cycle specific. This means they attack the cells during various phases of division.
• Vinca alkaloids: vincristine, vinblastine and vinorelbine.
• Taxanes: paclitaxel and docetaxel. These drugs have a novel 14 member ring the taxane.\textsuperscript{28}
• Podophyllotoxins: etoposide and teniposide.
• Camptothecan analogs: irinotecan and topotecan.
• Epothilones: ixabepilone.

3. Antitumor antibiotics

Antitumor antibiotics are chemotherapies made from natural products produced by species of the soil fungus Streptomyces. These drugs act during multiple phases of the cell cycle and are considered cell-cycle specific. The anthracycline\textsuperscript{29} antibiotics are products of the fungus streptomycetes percetus var caesius. There are several types of antitumor antibiotics:

• Anthracyclines: doxorubicin, daunorubicin, epirubicin, mitoxantrone and idarubicin.
• Chromomycins: dactinomycin and plicamycin.
• Other anti-tumor antibiotics include the drugs actinomycin-D, bleomycin and mitomycin-C.
• Bleomycin\textsuperscript{30} preferentially intercalates DNA at guanine-cytosine and guanine-thymine sequences, resulting in spontaneous oxidation and formation of free oxygen radicals that cause strand breakage.

Mitoxantrone is an anti-tumor antibiotic that is similar to doxorubicin in many ways, including the potential for damaging the heart. This drug also acts as a topoisomerase II inhibitor and can lead to treatment-related leukemia. Mitoxantrone is used to treat prostate cancer, breast cancer, lymphoma, and leukemia.

4. Antimetabolites

Antimetabolites are types of chemotherapy treatments that are very similar to normal substances within the cell. When the cells incorporate these substances into the cellular metabolism, they are unable to divide. Antimetabolites are cell-cycle
specific. They attack cells at very specific phases in the cycle. Antimetabolites are structural analogues of the naturally occurring metabolites involved in DNA and RNA synthesis. Antimetabolites are classified according to the substances with which they interfere.

- Folic acid antagonist: methotrexate.
- Pyrimidine antagonist: 5-fluorouracil, foxyuridine, cytarabine, capecitabine and gemcitabine.
- Purine antagonist: 6-mercaptopurine and 6-thioguanine.
- Adenosine deaminase inhibitor: cladribine, fludarabine, nelarabine and pentostatin.

5. Topoisomerase inhibitors

Topoisomerase inhibitors are types of chemotherapy drugs that interfere with the action of topoisomerase enzymes (topoisomerase I and II). During the process of chemo treatments, topoisomerase enzymes control the manipulation of the structure of DNA necessary for replication.

- Topoisomerase I inhibitors: irinotecan, topotecan.
- Topoisomerase II inhibitors: amsacrine, etoposide, etoposide phosphate, teniposide.

6. Corticosteroids

Steroids are natural hormones and hormone-like drugs that are useful in treating some types of cancer (lymphoma, leukemias, and multiple myeloma), as well as other illnesses. When these drugs are used to kill cancer cells or slow their growth, they are considered chemotherapy drugs. Corticosteroids are commonly used as anti-emetics to help prevent nausea and vomiting caused by chemotherapy, too. They are also used before chemotherapy to help prevent severe allergic reactions (hypersensitivity reactions). When a corticosteroid is used to prevent vomiting or allergic reactions, it is not considered chemotherapy. Examples include prednisone, methylprednisolone and dexamethasone.
7. Miscellaneous Antineoplastics

Several useful types of chemotherapy drugs are unique:

- Ribonucleotide reductase inhibitor: hydroxyurea.
- Adrenocortical steroid inhibitor: mitotane
- Enzymes: asparaginase and pegaspargase.
- Antimicrotubule agent: estramustine
- Retinoids: bexarotene, isotretinoin, tretinoin (ATRA)

Over the years, many people have been successfully treated with chemotherapy thanks to ongoing research into the use of these drugs. Yet despite the best treatments, some cancers are very difficult to control, and some will come back. Several exciting new uses of chemotherapy and other agents hold even more promise for curing or controlling cancer. New drugs, new combinations of drugs and new delivery techniques will improve medicine's ability to cure or control cancer and improve the quality of life for people with cancer. There are many expected advances in coming years:

- New classes of chemotherapy medicines and combinations of medicines are being developed.

- New ways to give the drugs are being studied, such as using smaller amounts over longer periods of time or giving them continuously with special pumps.

- Some new medicines, called targeted therapies, are specifically developed to attack a particular target on cancer cells. These drugs may have fewer side effects than standard chemotherapy drugs and may eventually be used along with them. Several are now under study. Some are already in use; for instance, lapatinib (tykerb\textsuperscript{\textregistered}) can be used along with other drugs to treat women whose breast cancer is positive for HER2/neu.

- Other approaches to targeting drugs more specifically at the cancer cells such as attaching drugs to monoclonal antibodies may make them more effective and cause fewer side effects. Monoclonal antibodies, which are special types of proteins made in the lab, can be designed to guide chemotherapy medicines
directly to the tumor. Mylotarg® (gemtuzumab ozogamicin) is a chemotherapy agent made of a monoclonal antibody attached to an anti-tumor antibiotic, calicheamicin. This drug is used to treat acute myelogenous leukemia. Monoclonal antibodies (without attached chemotherapy) can also be used as immunotherapy drugs, to strengthen the body's immune response against cancer cells. For instance, rituximab and alemtuzumab are directed at certain lymphoma cells, and are used to treat some types of non-Hodgkin lymphoma. More of these types of drugs are being developed.

- Liposomal therapy involves using chemotherapy drugs that have been packaged inside liposomes (synthetic fat globules). The liposome helps the drug penetrate the cancer cells more selectively and decreases possible side effects (such as hair loss and nausea and vomiting). Examples of liposomal medicines already in use are doxorubicin and daunorubicin.

- Chemoprotective agents are being developed to protect against specific side effects of certain chemotherapy drugs. For example, dexamethasone (zinecard®) helps prevent heart damage, amifostine helps protect the kidneys, and mesna protects the bladder.

Some new agents may be given along with chemotherapy to help overcome drug resistance. Cancer cells often become resistant to chemotherapy by developing the ability to pump the drugs out of the cells. These new agents inactivate the pumps, which allow the chemotherapy to remain in the cancer cells longer, hopefully making it more effective.

**Investigations of chemotherapeutic drugs**

Tamoxifen, (TX) is a synthetic compound of the non-steroidal antiestrogen used widely for the treatment of hormone dependent breast cancer. Moreover, the significant differences in activity elucidated by the tamoxifen in a variety of animal species are puzzling; tamoxifen is a pure estrogen agonist in the mouse uterus, a partial agonist/antagonist in the chick oviduct. The polarographic behavior of tamoxifen and other triphenylethylene derivatives was analyzed by Fijalek et al. who used it for determining these drugs in pharmaceutical tablets.
The oxidation of tamoxifen at a glassy carbon flow detector was exploited for its amperometric monitoring in chromatographic effluent. The potentiometric behavior at a glassy carbon electrode was examined by Wang et al. An unexpected selectivity of a propranolol derived molecular imprint for tamoxifen was studied by Martin et al. Induction of mutations in rats after treatment them with tamoxifen and hydroxyl tamoxifen was studied by Gamboa et al. A new screening assay for estrogens using an array type DNA glass slide was investigated by Sung et al.

The main challenge in developing a procedure for the measurement of any drug substances in high sensitivity associated with applications to real samples. Adsorptive stripping voltammetry has effectively been used for the determination of nanomolar or subnanomolar levels of several drugs. The adsorption of tamoxifen onto a hanging mercury drop electrode (HMDE) can be used as an effective preconcentration step before a voltammetric measurement. In this way, a highly sensitive, measurement of this drug at low level (found in serum after therapeutic doses of ng/ml) tamoxifen can be achieved. Such techniques for measurement of serum concentrations of TM are necessary in order to evaluate and toxic concentrations of such drugs.

Anthracycline, such as daunorubicin, continues to be widely used in the treatment of cancer, although they share the adverse effect of chronic, cumulative dose-related cardiotoxicity. The only approved treatment in prevention of anthracycline cardiotoxicity is dexrazoxane, a putative iron chelator. The interactions between daunorubicin and human serum albumin (HSA) were studied using the spectrofluorimetric method. The optimum conditions of fluorometric determination of daunorubicin were studied and the developed method was successfully applied to the determination of daunorubicin in serum samples. Capillary electrophoresis (CE) with laser-induced fluorescence detection was applied to quantify daunorubicin in plasma.

This study was designed to determine the pharmacokinetic of daunorubicin in plasma and tissues, including the heart. This study was designed to examine the interaction of daunorubicin with human serum albumin (HSA) for the first time by fluorescence spectroscopy in combination with UV absorption and molecular
modeling under simulative physiological conditions. They described a technique of separation and quantification of the liposomal and non-liposomal forms of daunorubicin in the plasma of patients treated with Daunorubicin, a liposomal formulation of daunorubicin. This technique is rapid, can be automated in order to handle large series of samples, and the plasma can be frozen after sampling by addition of glycerol. The recovery of liposomal daunorubicin as well as the precision, linearity and accuracy of the technique appear satisfactory for pharmacokinetic purposes.

Major problem of anthracycline anticancer treatment are the cardiotoxic side effects associated with drug therapy. Increased attention has recently been focused on the 13-hydroxy anthracycline metabolites which are formed by carbonyl reduction of the parent drug as contributing to cardiotoxicity. The prevention of these metabolites may represent a potential approach for enhancing the safety and efficacy of anthracycline chemotherapy. The stability of daunorubicin (DNR) in rabbit and human plasma, bile and urine and in rabbit faeces was studied in the presence or absence of light, and at body, room and cold room temperatures. Under each set of conditions, DNR fluorescence decreased with time. The describe the characterization of a DNA aptamer that displays high affinity and specificity for the anthracyclines daunomycin, is frequently used in chemotherapy.

This study was designed to examine the interaction of daunorubicin with human serum albumin (HSA) for the first time by fluorescence spectroscopy in combination with UV absorption. The study represents an attempt to establish the electrochemical reduction mechanism of DNR using electrochemical techniques and developed methods for its analysis by differential pulse polarography.

Doxorubicin is an anthracycline antibiotic isolated from streptomyacin paucities and has been clinically used in treatment of patients with leukemia's and tumors in the lung, or the breast. The anthracycline antibiotics are generally composed of the amino sugar linked to the anthraquinone aglycone. The sensitive and accurate techniques are required to control chemotherapy of doxorubicin, or other anticancer anthracyclines. For the assay of doxorubicin, employing HPLC with UV detector set 254 nm. The Korean official assay methods for doxorubicin include
the spectrophotometric method measured at 495 nm as well as a microbial assay using Bacillus subtilis ATCC 6633. Recently, the analysis of anthracycline contents in biological samples or in pharmaceutical preparations have been reviewed by Zagotto et al.\textsuperscript{61} Visible spectrophotometric methods by Sastry and Rao\textsuperscript{62}, and HPLC with fluorescence detector by Buehler et al.\textsuperscript{63} Alvarez-Cedron et al.\textsuperscript{64} have been employed for the determination of doxorubicin in pharmaceutical formulations, or in biological samples. Electrochemical detection combined with capillary zone electrophoresis (CE) by Hu et al.\textsuperscript{65} and with HPLC by Ricciarello et al.\textsuperscript{66} has been developed for the determination of anthracyclines in biological fluids. The detecting system connected to CE was a carbon disk working electrode with an applied potential of -0.95 V vs. an Ag/AgCl (3 M KCl), which measured anodic currents due to the oxidation of two phenolic hydroxyls in the aglycone of daunorubicin by Hu et al.\textsuperscript{67,68}

Electrochemical assay often offers selectivity and sensitivity due to the selective detection of electroactive species among the complex samples. The chemical structure of doxorubicin contains a electrochemically a reducible quinone moiety in the aglycone which prompted us to study its electrochemical behavior by using mercury electrodes, followed by developing the fast and sensitive square wave voltammetric (SWV) procedure for the determination of doxorubicin hydrochloride.

In modern voltammetry, different types of electrodes are applied for the determination of organic species in environmental samples as well as in biological samples.\textsuperscript{69} Nifedipine have been determination by HPLC,\textsuperscript{70} nifedipine by gas chromatography.\textsuperscript{71} Cathodic stripping voltammetry is a very sensitive technique that has been extensively used for determining pharmaceutical compounds. This technique is based on a pro-concentration step of the analytical species on a mercury electrode surface.\textsuperscript{72} The possibility of determining miconazole by stripping voltammetry was considered since the adsorption process detected in the electrochemical reduction could be used as an electro pre-concentration step prior to the voltammetric reduction of the drug.\textsuperscript{73} The CE methods enable precise and accurate quantification of daunorubicin and daunorubicinol in small sample volumes over a wide concentration range.\textsuperscript{74} Cathodic stripping voltammetric determination of losartan using hanging mercury drop electrode (HMDE) was described.\textsuperscript{75}
Determination of nifedipine and nimodifine in pharmaceutical formulations, urine and serum samples by differential pulse adsorption stripping voltammetry (DPAdV). Adrenaline is determined by adsorption stripping voltammetry using a carbon paste electrode in a base solution of 0.5 mol. L$^{-1}$ H$_2$SO$_4$. The electrode reaction of adrenaline is irreversible process with two electrons and two protons on the carbon paste electrode. Analytical techniques employed for minocycline determination includes chromatography. The optimum conditions of fluorimetric determination of daunorubicin were studied and the developed method was successfully applied to the determination of daunorubicin in serum samples.


High-performance liquid chromatographic analysis of tamoxifen, toremifene and their major human metabolites, pharmacokinetic analysis of high-dose toremifene in combination with doxorubicin, quantitation of toremifene and its major metabolites in human plasma by high performance liquid chromatography following fluorescent activation, selected reaction monitoring LC-MS determination of idoxifene and its pyrrolidinone metabolite in human plasma using robotic high-throughput, sequential sample injection. Atmospheric pressure photoionization liquid chromatographic–mass spectrometric determination of
idoxifene and its metabolites in human plasma. Comparison between liquid chromatography-time-of-flight mass spectrometry and selected reaction monitoring liquid chromatography-mass spectrometry for quantitative determination of idoxifene in human plasma, HPLC analysis of raloxifene hydrochloride and its application to drug quality control studies. Fluorimetric determination of oxamniquine in biological fluids was reported by Rizk et al.


In previous reports, nisoldipine in human plasma has been mainly determined using liquid or gas chromatography with mass spectrometry, following a liquid–liquid extraction. On the basis of the previous excellent work by Polson et al. and developed a simple, sensitive and accurate method for determining nisoldipine in human plasma using liquid chromatography with tandem mass spectrometry following a simple protein precipitation with a mixture of an organic solvent and a metal ionic solution. While a liquid–liquid extraction method was used for the purification of nisoldipine from plasma in the previous study nisoldipine was extracted with ethyl acetate following alkalization. At the very beginning, methanol or acetonitrile were only used for the precipitation. New spectrophotometric method for the determination of nifedipine in pharmaceutical formulations.
Investigated the analytical response of daunomycin, an effective drug used for cancer chemotherapy, at different types of electrodes. The following five different materials for three electrodes have been compared: hanging mercury drop, gold, gold/bismuth, gold/mercury and silver mercury. Especially at the hanging mercury drop electrode and also at the silver/mercury and gold/mercury amalgam electrode, nicely shaped cyclic voltammograms with nearly no peak separation could be found whereas both the gold and the gold/bismuth electrode showed a significant peak separation. Organic compounds such as organic nitro compounds have been determined by means of differential pulse voltammetry and amperometry. Daunomycin (DM), an anthracycline antibiotic, is an effective drug used for cancer chemotherapy. In electroanalytical chemistry it is also widely used as an indicator of DNA. They most commonly used working electrode material in this method is mercury. The advantages of the hanging mercury drop electrode (HMDE) include renewable surface and high hydrogen over potential. Analytical methods based on amalgam electrodes have been reviewed. A traditional assay for the detection of these compounds is HPLC (High Performance Liquid Chromatography) with fluorescence detection. They found that daunomycin strongly adsorbs on bismuth electrodes, which can be used for trace detection of this compound by means of adsorptive stripping voltammetry.

An extensive literature survey was carried out, and it is evident that ondansetron is official in the British Pharmacopoeia and the United State Pharmacopoeia. Many HPLC methods and of spectrophotometric methods are available in the literature for the determination of ondansetron in pharmaceutical formulations.

The electrochemical behavior of CPT at glassy carbon electrode was investigated by cyclic, linear sweep and differential pulse voltammetry (DPV). A well defined reduction peak of CPT at $4 \times 10^{-5}$ M was observed in Britton-Robinson (BR) buffer of pH 2.5. A differential pulse voltammetry method with good precision and accuracy was developed for the determination of CPT in pharmaceutical formulations. The peak currents were found to be linearly dependent on the concentration range of $8 \times 10^{-7}$ to $5 \times 10^{-3}$M CPT. The limit of detection (LOD) and limit of quantification (LOQ) were noticed to be $1.13 \times 10^{-7}$ and $3.78 \times 10^{-7}$.
10⁻⁷ M, respectively.¹²⁸ Few analytical methods viz., HPLC, LC–MS and LC–MS/MS have been reported for the assay of CPT¹²⁹–¹³⁵ in biological samples. Redox properties of a drug can give insights into its metabolic fate or its in vivo redox processes or pharmaceutical activity.¹³⁶–¹³⁸

There have been intensive studies to apply modern voltammetric methods in nucleic acid research and DNA analysis¹³⁹–¹⁵⁰ since the electroactivity of deoxyribonucleic acid (DNA) was first discovered.¹⁵¹ The interactions of some anticancer drugs with DNA have been studied with a variety of techniques¹⁵²–¹⁵⁵ and in recent years, there is a growing interest in the electrochemical investigations of interactions between anticancer drugs and other DNA-targeted molecules.

Spectrophotometric methods including colour reactions¹⁵⁶,¹⁵⁷ and UV-measurements¹⁵⁸ have been described. The NMR absorption and Fourier Transisssion Infrared Resonance (FTIR) spectroscopic studies on the complexes of cisplatin with some amino acids (such as cysteine, glutathione, oxidized glutathione and methionine or ascorbic acid) have been reported.

In electrochemistry the carbonyl groups is extensively used and is strongly dependent on the media and acid –base characteristic of the substrate and supporting electrolytes.¹⁵⁹,¹⁶⁰ Nitro group containing drugs have got a good biological system for their proper growth and metabolic function. Though nitro group reduces easily under certain conditions. The electrochemistry of nitro group is complicated due to dimerisation, coupling, tautomerisation and various intermediate that are formed during a electrochemical processes. Hence, the electrochemical mechanisms and analytical investigation of nitro group compounds are very important. Slikata and co-workers¹⁶¹–¹⁶³ described the reduction of nitro group at a dropping mercury electrode in aqueous solution. The behaviour of aromatic nitro compounds in aqueous systems is some what different.¹⁶⁴–¹⁶⁶ Neutral or alkaline solution, only the four electron process occurs with substituted hydroxylamine as the final product. In acidic solution, both reactions occur with a total of six electrons per molecules transference.
Compounds with molecules containing the carbon carbon double bonds grouping have been known for a long time and these are continuously attracting attention owing to the great possibilities they offer in synthesis and for pharmaceutical purposes. Hormone or hormone blocking agents having carbon carbon double bonds groups either corticosteroid manipulate the hormone environment is hormone response tumours. For example the antiandrogenic agent, flutamide. Similarly the antiestrogenic agent, tamoxifene, binds to infra cellular estrogen receptors then enters the nucleus where the tamoxifene estrogen receptor complex inhibiting DNA and developed the electrochemistry of the antiestrogen like raloxifene\textsuperscript{167} and flutamide.\textsuperscript{168}

The functional groups which show excellent voltammetric properties including nitro compounds and their voltammetric activity can be deduced from the functional groups.\textsuperscript{169} A numer of chemical and physical properties of such compounds are unexpected owing to the reason that electronic and steric factors are mutually influenced. The study of these factors in one of the most important problems in modern theoretical organic chemistry.\textsuperscript{170}

In the present investigation, some of the widely used chemotherapeutic drugs containing carbonyl groups such as zileuton, lenalidomide and prednisone, nitro group containing chemotherapeutic drugs like nisoldipine and oxamansquale and carbon carbon double bond groups containing chemotherapeutic drugs like toremifene and idoxifene are extensively studies by various electrochemical methods in order to understand the electrochemical reduction mechanism and electrode kinetics of carbonyl, nitro and carbon carbon double bond groups. The various techniques used to carryout these studies are cyclic voltammetry, differential pulse polarography, differential pulse stripping voltammetry, controlled potential electrolysis and millicoulometry. The universal buffer and Britton -Robinson buffer solutions of pH ranging from 2.0-12.0 are used as supporting electrolytes and they are prepared by using 0.2M boric acid, 0.05M citric acid, and 0.1M trisodiumorthophosphate and 0.04M acetic acid, 0.04M orthophosphoric acid, 0.04M boric acid and pH were adjusted by using 0.2M sodium hydroxide solution respectively. Methanol, dimethylformide and triple distilled water served as solvents.
In the present investigation, studies of the electrode process associated with chosen carbonyl group, nitro group and carbon-carbon double bond group containing chemothepeutic drugs are systematically carried out and the peculiarities of their behaviour at the DME and HMDE arising from their molecular structure are discussed. The advanced electrochemical techniques such as cyclic voltammetry, differential pulse polarography, differential pulse cathodic stripping voltammetry, controlled potential electrolysis and millicoulometry have been employed:

1. To get detailed information of intermediates, adsorption complications, electrode kinetics and electrode reaction mechanism of species took up in the present work.

2. To evaluate the kinetic parameters such as transfers co-efficients, diffusion co-efficients and heterogeneous forward rate constants of the species.

3. To develop analytical procedures for the quantitative determination of chemothepeutic drugs.

4. To carry out the analysis of chemothepeutic drugs in pharmaceutical formulation and biological samples and

5. To estimate the magnitude of chemothepeutic drug residues in order to evaluate their maximum tolerance limits.
References


27. Farrell and Nicholas, methods and investigation of biological systems, 32 (1996), 622.


34. Lerner L, Jordan V. Cancer Res. 50 (1990) 4177.


57. Propper, Dirk; Maser, Edmund, Pharmacology & Toxicology. 80(5) (1997) 40.


60. Feng-Ling Cui, Li-Xia Qin, Gui-Sheng Zhang, Xiao-Jun Yao and Juan Du, International Journal of Biological Macromolecule, 42(3) (2008) 221.


84. Liu, Qing ; Farley, Katherine L BS; Johnson, Amy J P; Muthusamy, Natarajan ; Hofmeister, Craig C ; Blum, Kristie A ; Schaaf, Larry J ; Grever, Michael R ; Byrd, John C ; Dalton, James T; Phelps, Mitch A, Therapeutic Drug Monitoring, 30 (5)(2008) 620.


