2.0 REVIEW OF LITERATURE

21 CFR 320.1 defines bioavailability as “the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action”. This definition focuses on the processes by which the active ingredients or moieties are released from an oral dosage form and move to the site of action.

Bioequivalence is “the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study”. Where there is an intentional difference in rate (e.g., in certain extended release dosage forms), certain pharmaceutical equivalents or alternatives may be considered bioequivalent if there is no significant difference in the extent to which the active ingredient or moiety from each product becomes available at the site of drug action. This applies only if the difference in the rate at which the active ingredient or moiety becomes available at the site of drug action is intentional and is reflected in the proposed labeling, is not essential to the attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug (21 CFR 320.1).

The therapeutic effect of a solid drug product is assumed to be a function of the concentration of the active ingredient in the systemic circulation and is thus related to its bioavailability. The principles and methodology involved in estimating and comparing the bioavailability of drug products involves measurement of the concentration of the active ingredient in the systemic circulation, either directly in blood or indirectly through studies of urinary excretion. Certain drug products contain the same active moieties but do not have the same salt, ester or dosage form. These products, although similar, are not chemically equivalent but may be therapeutically equivalent. For example,
tetracycline hydrochloride tablets, capsules and syrup all may be expected to produce the same therapeutic effect as tetracycline phosphate capsules, but they are different in dosage or salt forms (MacDonald et al., 1969; Barr et al., 1972). Drug products which are not chemically equivalent but have the same therapeutic effect may be referred to as pharmaceutical alternatives but not as chemical or pharmaceutical equivalents.

The FDA’s designation of “therapeutic equivalence” indicates that the generic formulation is (among other things) bioequivalent to the innovator formulation and signifies the FDA’s expectation that the formulations are likely “to have equivalent clinical effect and no difference in their potential for adverse effects”. The assessment of “interchangeability” between the innovator and generic products is carried out by a study of “in vivo equivalence” or “bioequivalence” (Midhal et al., 2009).

The pertinent situations in which bioequivalence studies are required include:

i) when the proposed marketed dosage form is different from that used in pivotal clinical trials;

ii) when significant changes are made in the manufacture of the marketed formulation; and

iii) when a new generic product is tested against the innovator’s marketed product.

2.1 HISTORICAL PERSPECTIVE OF BIOEQUIVALENCE STUDIES

Generics have existed throughout the history of the pharmaceutical industry, but modern generic firms emerged only in the mid 1960s in the context of the shakeup of regulatory arrangements in the USA following the thalidomide tragedy. The 1984 US Drug Price Competition and Patent Restoration (Hatch-Waxman) Act was the decisive moment in the development of the generics industry in the world’s major pharmaceutical market. The Hatch-Waxman Act provided for facilitated market entry for generic versions of all post-1962 approved products, in exchange for an extension of the patent period (Congressional Budget Office, 1998).
This opened ‘the floodgates for generic competition of pharmaceutical products, creating the modern generic pharmaceutical industry’ (Barr Laboratories, 2002). In the first year after the introduction of the Hatch-Waxman Act, the FDA received more than 1,000 applications for approval of new generic drugs (Harnden, 1998). Since then, using the BE as the basis for approving generic drugs was established. Subsequently various criteria and approaches for conducting and reporting BE studies for generic products from various regulatory authorities have been progressing (Melethil S, 2005).

The concepts of BA and BE have gained considerable importance during the last three decades and have become the cornerstones for the approval of brand-name and generic drugs globally. Consequently regulatory authorities also started developing and formulating regulatory requirements for approval of generic drug products. It is encouraging to know that efforts by regulatory authorities and the scientific community at national as well as international levels are continuing, in order to understand and develop more efficient and scientifically valid approaches to assess BE of various dosage forms, including some of the complex special dosage forms.

Every country now has its own individual regulatory authority as well as regulatory guidance for BA/BE studies, and the magnitude of assessment of BE of drug product is influenced by the regulatory environment of the respective country of marketing. The magnitude of regulatory influence is often dictated by the availability of resources, expertise, and lack of regulation or its implementation. Thus there is a greater need to harmonize the regulatory environment globally for BE assessment as far as practicable so that the drug product marketed in different parts and regions of the world would have optimum drug product quality in terms of interchangeability.

With passage of the 1984 Drug Price Competition and Patent Term Restoration amendments to the Food, Drug and Cosmetic Act, BE took on added importance for generic drugs. As defined in implementing regulations, an applicant submitting an Abbreviated New Drug Application (ANDA) under Section 505(j) of the Act (excepting Suitability Petitions submitted under 505 (j) (2) (c) of the Act) must demonstrate both pharmaceutical equivalence (PE) and BE between the generic
product and listed innovator reference drug product. With acceptance of this documentation by FDA, along with other information, the generic product is deemed bioequivalent, therapeutically equivalent, and interchangeable with the listed reference drug product.

Table 2.1  A brief historical overview of Food and Drug Administration activities with respect to BA/BE studies

<table>
<thead>
<tr>
<th>Year</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1906</td>
<td>Food and Drug Act (Wiley Act)</td>
</tr>
<tr>
<td>1927</td>
<td>The Bureau of Chemistry is reorganized into two separate entities. Regulatory functions are located in the Food, Drug, and Insecticide Administration, and non-regulatory research is located in the Bureau of Chemistry and Soils.</td>
</tr>
<tr>
<td>1930</td>
<td>The name of the Food, Drug, and Insecticide Administration is shortened to Food and Drug Administration (FDA) under an agricultural appropriations act.</td>
</tr>
<tr>
<td>1970</td>
<td>FDA became interested in biological availability of new drugs and a drug bioequivalence study panel was formed by the Office of Technology Assessment (OTA) to understand the chemical and therapeutic equivalent relationship of drug products.</td>
</tr>
<tr>
<td>1970</td>
<td>On the basis of the recommendations from OTA, the FDA formulated regulations for the submission of bioavailability data. These regulations are currently incorporated in the 21st volume of Code of Federal Regulation, Part 320 (21CFR320).</td>
</tr>
<tr>
<td>1970</td>
<td>75/75 (or 75/75 – 125) rule was originally proposed in the late 1970s as an alternative means of testing the bioequivalence of two formulations of a pharmaceutical agent.</td>
</tr>
<tr>
<td>1980</td>
<td>Power Approach for statistical analysis.</td>
</tr>
<tr>
<td>1984</td>
<td>United States Congress passed the Drug Price Competition and Patent Term Restoration Act (Hatch-Waxman Act) that authorized FDA to approve generic drug products through BA and BE studies.</td>
</tr>
<tr>
<td>1986</td>
<td>Discontinuation of 75/75 rule and power approach.</td>
</tr>
<tr>
<td>1986</td>
<td>FDA conducted public hearing due to public concern about BE.</td>
</tr>
<tr>
<td>1986-1989</td>
<td>BE Task Force formed by FDA investigated the scientific issues raised at the public hearing.</td>
</tr>
<tr>
<td>Year</td>
<td>Event</td>
</tr>
<tr>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>1989</td>
<td>BE Task Force report was released Letter on the provision of new procedures and policies affecting the generic drug review process.</td>
</tr>
<tr>
<td>1991</td>
<td>Letter on the request for cooperation of regulated industry to improve the efficiency and effectiveness of the generic drug review process, by assuring the completeness and accuracy of required information and data submissions.</td>
</tr>
<tr>
<td>1993</td>
<td>Letter to all ANDA and AADA applicants about the Generic Drug Enforcement Act of 1992 (GDEA), and the Office of Generic Drugs intention to refuse-to-file incomplete submissions as required by the new law. Letter to regulated industry notifying interested parties about important detailed information regarding labeling, scale-up, packaging, minor/major amendment criteria, and bioequivalence requirements.</td>
</tr>
<tr>
<td>1994</td>
<td>Letter on incomplete Abbreviated Applications, Convictions Under GDEA, Multiple Supplements, Annual Reports for Bulk Antibiotics, Batch Size for Transdermal Drugs, Bioequivalence Protocols, Research, Deviations from OGD Policy.</td>
</tr>
<tr>
<td>1996</td>
<td>Structure and Content of Clinical Study Reports.</td>
</tr>
<tr>
<td>1999</td>
<td>ClinicalTrials.gov is founded to provide the public with updated information on enrollment in federally and privately supported clinical research, thereby expanding patient access to studies of promising therapies.</td>
</tr>
<tr>
<td>2000</td>
<td>Waiver of In Vivo Bioavailability and Bioequivalence Studies for</td>
</tr>
</tbody>
</table>
### 2.2 Purposes of Bioequivalence Studies

#### 2.2.1 BE for first entry products

BE studies may be useful during drug development and registration for a first entry product during the Investigational New Drug (IND) or New Drug Application (NDA) period to establish links between (i) early and late clinical trial formulations (ii) formulations used in clinical trial and stability studies, if different (iii) Clinical trial formulations and to be marketed drug products (iv) other comparisons as appropriate. In each comparison, the new formulation or new method of manufacture is the test product and the prior formulation or method of manufacture is the reference product.
2.2.2 BE for interchangeable multi-source products

BE studies are a critical component of Abbreviated New Drug Applications (ANDA). The purpose of these studies is to compare relative BA measures between a pharmaceutically equivalent multi-source test product and the corresponding reference pioneer product. The pioneer product is termed as reference listed drug (RLD). Together with the determination of pharmaceutical equivalence, demonstrating BE allows a regulatory conclusion of therapeutic equivalence and interchangeability between the test and reference product (CDER, 1999).

2.2.3 BE for post approval changes

Generally, specifications are adequate to assure product quality on the assumption that no important change occurs post-approval. In the presence of major changes in components and composition, and/or method of manufacture of a drug product after approval, BE may need to be re-demonstrated. For approved first-entry products, the drug product after the change should be compared to the drug product before change. For approved interchangeable multi-source products, the drug product after the change should be compared to the reference listed drug.

2.3 Assessment of bioequivalence

Relative BA studies are useful in comparing the systemic exposure profiles of different dosage forms and routes of administration. In this context, BA information, sometimes together with pharmacokinetic or pharmacodynamic and other data, can be used to demonstrate the similarity of two dosage forms and ensure comparable clinical outcomes (Schug et al., 1996). A goal in BA and BE studies is to assess rate and extent of drug absorption. Extent of absorption is readily measured by AUC either to the last sampled time point (AUC0-t) or following extrapolation to time infinity - AUC0-∞ (Tozer et al., 1996).

For most orally administered and other (e.g., transdermal) drug products, BA may be described by a systemic exposure profile obtained from measuring the blood or plasma concentration of active ingredient(s) and/or active moiety (ies)
over time after administration of the drug product. From a pharmacokinetic perspective, in addition to systemic exposure, BA studies may provide additional useful information about metabolism, transport, distribution, and elimination of the drug, dose proportionality, nutrient effects on drug absorption, etc.

BE assesses the relative BA of two drug products, and thus, focuses on comparative drug product performance (Sheiner L.B., 1997).

According to regulatory definitions, generic drug products need to be identical to their reference with respect to the active substance, the route of administration as well as quality standards. In contrast to innovator drugs, which have to demonstrate their clinical efficacy and safety, generics are considered therapeutically equivalent based on simple bioequivalence testing.

The assessment of BE of different drug products is based on the fundamental assumption that two products are equivalent when the rate and extent of absorption of the test/generic drug does not show a significant difference from the rate and extent of absorption of the reference/brand drug under similar experimental conditions as defined.

As per the different regulatory authorities, BE studies are generally classified as:

1. Pharmacokinetic studies.
2. Pharmacodynamic studies.
3. Clinical studies or Comparative Clinical trials.
4. In vitro dissolution studies.

The general descending order of preference of these studies includes pharmacokinetic, pharmacodynamic, clinical, and in vitro studies (Chen et al, 2001).

2.3.1 Pharmacokinetic studies

The statutory definition of BA and BE, expressed in rate and extent of absorption of the active moiety or ingredient to the site of action, emphasizes the use of pharmacokinetic measures to indicate release of the drug substance from the drug
product with absorption into the systemic circulation. This approach rests on an understanding that measurement of the active moiety or ingredient at the site(s) of action is generally not possible and that some predetermined relationship exists between the drug concentration at the site of action relative to that in the systemic circulation.

A typical BE study is conducted as a crossover study, in which clearance and physiologic variables (e.g., gastric emptying, motility, and pH) are assumed to have less interoccasion variability within an individual compared with variability between individuals. Where needed, a pilot study may be useful to validate analytic methodology, to assess intra- and intersubject variability in systemic exposure measures, and to optimize sample collection times.

A recent FDA guidance, therefore, has recommended that measures of systemic exposure be used to reflect clinically important differences between test and reference products in BA and BE studies. These measures include (i) total exposure (AUC$_{0-t}$ or AUC$_{0-\infty}$ for single-dose studies and AUC$_{0-t}$ for steady-state studies), (ii) peak exposure (Cmax), and (iii) early exposure (partial AUC to peak time of the reference product for an immediate-release drug product).

These studies are most widely preferred to assess BE for drug products, where drug level can be determined in an easily accessible biological fluid (such as plasma, blood, urine) and drug level is correlated with the clinical effect. Regulatory guidance recommends that measures of systemic exposure be used to reflect clinically important differences between test and reference products in BA and BE studies.

The two major pharmacokinetic methods are:

i) Plasma level-time studies.

ii) Urinary excretion studies.

### 2.3.1.1 Plasma level-time studies

Unless determination of plasma drug concentration is difficult or impossible, it is the most reliable method and method of choice in comparison to urine data. This
method is based on the assumption that two dosage forms that exhibit superimposable plasma level-time profiles in a group of subjects should result in identical therapeutic activity. The three parameters of plasma level-time studies, which are considered important for determining bioavailability, are:

1. $C_{\text{max}}$: The peak plasma concentration that gives an indication whether the drug is sufficiently absorbed systemically to provide a therapeutic response.

2. $T_{\text{max}}$: The time of peak plasma concentration corresponds to the time required to reach maximum drug concentration after drug administration. At $t_{\text{max}}$, absorption is maximized and the rate of drug absorption equals the rate of drug elimination. When comparing drug products, $t_{\text{max}}$ can be used as an approximate indication of the drug absorption rate.

3. AUC: The area under the plasma level-time curve that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation.

### 2.3.1.2 Urinary excretion studies

This method of assessing bioavailability is based on the principle that the urinary excretion of unchanged drug is directly proportional to the plasma concentration of drug. This method is particularly useful for drugs extensively excreted unchanged in the urine. The method involves collection of urine at regular intervals for a time span equal to 7-10 biological half-lives, analysis of unchanged drug in the collected sample and determination of the amount of drug excreted in each interval and cumulative amount excreted. The three major parameters examined in urinary excretion data obtained with a single dose study are:

1. $(\text{dx}_u/\text{dt})_{\text{max}}$: The maximum urinary excretion rate, is obtained from the peak of plot between rate of excretion versus midpoint time of urine collection period. It is analogous to $C_{\text{max}}$ derived from plasma level studies since the rate of appearance of drug in the urine is proportional to its concentration in
systemic circulation.

2. \((t_{u})_{\text{max}}\): The time for maximum excretion rate, is analogous to the \(t_{\text{max}}\) of plasma level data. Its value decreases as the absorption rate increases.

3. \(X_{u}\): The cumulative amount of drug excreted in the urine, is related to the AUC of plasma level data and increases as the extent of absorption increases.

Single dose studies to document BE are preferred because they are generally more sensitive in assessing in vivo release of the drug substance from the drug product when compared to multiple dose studies (Tahtawy et al, 1995 and 1998). The following are the circumstances that demand multiple-dose study/steady state pharmacokinetics:

- Dose- or time-dependent pharmacokinetics.
- For modified-release products for which the fluctuation in plasma concentration over a dosage interval at steady state needs to be assessed.
- If problems of sensitivity preclude sufficiently precise plasma concentration measurements after single-dose administration.
- If the intra-individual variability in the plasma concentration or disposition precludes the possibility of and this variability is reduced at steady state.
- When a single-dose study cannot be conducted in healthy volunteers due to tolerability reasons, and a single-dose study is not feasible in patients.
- If the medicine has a long terminal elimination half-life, and blood concentrations after a single dose cannot be followed for a sufficient time.
- For those medicines that induce their own metabolism or show large intra-individual variability.
- For combination products for which the ratio of plasma concentration of the individual substances is important.
- If the medicine is likely to accumulate in the body.
- For enteric coated preparations in which the coating is innovative. Under normal circumstances, blood should be the biological fluid sampled to measure drug concentrations.
Most drugs may be measured in serum or plasma; however, in some drugs (e.g., tacrolimus), whole blood may be more appropriate for analysis. If the blood concentrations are too minute to be detected and a substantial amount (>40%) of the drug is eliminated unchanged in the urine, the urine may serve as the biological fluid to be sampled (e.g., alendronic acid).

2.3.2 Pharmacodynamic studies

Locally acting drug products include oral inhalation drug products, such as metered dose inhalers and dry powder inhalers, and topically applied dermatologic drug products such as creams and ointments. These drug products deliver an active moiety or active ingredient to local sites of action where they exert their primary clinical effects. Pharmacokinetic studies measure systemic exposure but are generally inappropriate to document local delivery BA and BE. In such cases, BA may be measured, and BE may be established, based on a pharmacodynamic (PD) study, providing an appropriate PD endpoint is available, which can be studied with sufficient accuracy, sensitivity, and reproducibility (Juniper et al., 1991; Sterk et al., 1993). An essential component of a BA or BE study based on a PD response is documentation of a dose-response relationship. The dose-response curve should be characterized as part of the study.

Pharmacodynamic evaluation is measurement of the effect on a pathophysiological process, such as a function of time, after administration of two different products to serve as a basis for BE assessment. Regulatory authorities request justification from the applicant for the use of pharmacodynamic effects/parameters for the establishment of BE criteria.

These studies generally become necessary under two conditions:

1) if the drug and/or metabolite(s) in plasma or urine cannot be analyzed quantitatively with sufficient accuracy and sensitivity;

2) if drug concentration measurement cannot be used as surrogate endpoints for the demonstration of efficacy and safety of the particular pharmaceutical product.
The two pharmacodynamic methods involve determination of bioavailability from:

(i) Acute pharmacologic response

(ii) Therapeutic response

2.3.2.1 Acute pharmacologic response

In some cases quantitative measurement of a drug is difficult, inaccurate or non reproducible. In such cases an acute pharmacologic effect such as effect on pupil diameter, heart rate or blood pressure can be a useful index of drug bioavailability. Bioavailability can be determined by construction of pharmacologic effect-time curve as well as dose-response graphs. The method requires measurement of responses for at least 3 biological half-lives of drug in order to obtain a good estimate of AUC.

2.3.2.2 Therapeutic response

Theoretically the most definite, this method is based on observing the clinical response to a drug formulation given to patients suffering from disease for which it is intended to be used. Bioequivalent drug products should have the same systemic drug bioavailability and therefore the same predictable drug response. However, variable clinical responses among individuals that are unrelated to bioavailability might be due to differences in the pharmacodynamics of the drug. Various factors affecting pharmacodynamic drug behaviour may include age, drug tolerance, drug interactions and unknown pathophysiologic factors.

2.3.3 Clinical studies or Comparative clinical trials

In the absence of pharmacokinetic and pharmacodynamic approaches, adequate and well-controlled clinical trials may be used to establish BA/BE. The use of BE studies with clinical trial endpoints can be appropriate to demonstrate BE for orally administered drug products when measurement of the active ingredients or active moieties in an accessible biological fluid (pharmacokinetic approach) or pharmacodynamic approach is infeasible (FDA Guidance).
2.3.4 *In vitro* dissolution studies

In 1974, the Congressional Office of Technology Assessment’s Drug Bioequivalence Study Panel made eleven recommendations, one of which stated: “It is neither feasible nor desirable that studies of bioavailability be conducted for all drugs or drug products”.

Under certain circumstances, product quality BA and BE can be documented using *in vitro* approaches (21 CFR 320.24:b). For highly soluble, highly permeable, rapidly dissolving, and orally administered drug products, documentation of BE using an *in vitro* approach (dissolution studies) is appropriate based on the biopharmaceutics classification system.

Biopharmaceutics Classification System (BCS) has categorized drug substances as having either high or low solubility and permeability and drug products as exhibiting rapid dissolution (Amidon et al., 1995). According to this approach, drug substances may be classified into four primary groups:

i) highly soluble and highly permeable
ii) highly permeable and poorly soluble
iii) highly soluble and poorly permeable
iv) poorly soluble and poorly permeable.

Using this BCS approach, a highly permeable, highly soluble drug substance formulated into a rapidly dissolving drug product may need only *in vitro* dissolution studies to establish BE (Davit et al., 2008).

2.4 Criteria of bioequivalence

To establish bioequivalence, the calculated 90% confidence interval for AUC and Cmax should fall within the bioequivalence range, usually 80 – 125%. Tighter limits for permissible difference in bioavailability may be required for drugs that have:

i) A narrow therapeutic index
ii) A serious, dose related toxicity
iii) A steep dose/effect curve or
iv) A non-linear pharmacokinetics within the therapeutic dose range

2.5 DOSAGE FORMS AND DELIVERY SYSTEMS OF ORAL DRUGS:

Oral ingestion is the predominant and most preferable route for drug delivery mainly due to their convenience of administration, patient compliance and their suitability for delivery of drugs for systemic effects (Aher et al., 2011). Following oral administration most drugs have to be absorbed into the blood to produce therapeutic action. However certain drugs have a “region-specific absorption” or “absorption window”. The region specific absorption may be due to various reasons, such as poor solubility at different pH values, poor stability in some GI regions, presence or absence of absorptive or efflux transporters, and presystemic metabolism in the gut wall (Kagan and Hoffman, 2008).

The drug delivery system employed plays a vital role in controlling the pharmacological effect of the drug as it can influence the pharmacokinetic profile of the drug, the rate of drug release, the site and duration of drug action and subsequently the side-effect profile. An optimal drug delivery system ensures that the active drug is available at the site of action for the correct time and duration. The drug concentration at the appropriate site should be above the minimal effective concentration (MEC) and below the minimal toxic concentration (MTC). This concentration interval is known as the therapeutic range. Achieving the desired concentration of a drug is dependent on the frequency of dosing, the drug clearance rates, the route of administration and the drug delivery system employed (Florence and Attwood, 2009; Tozer et al., 2006).

In order to achieve an optimum response from any dosage form, a drug should be delivered to its site of action at a rate and concentration that both minimize its side effects and maximize its therapeutic effects (Chein Y.W, 1992).

The development of safe and effective drug dosage forms and delivery systems requires a thorough understanding of physicochemical principles that allow a drug to be formulated into a pharmaceutical dosage form. Design of the appropriate dosage form or delivery system depends on the:
Physicochemical properties of the drug, such as solubility, oil-to-water partition coefficient (Ko/w), pKa value, and molecular weight.

- Dose of the drug.
- Route of administration.
- Type of drug delivery systems desired.
- Pathologic condition to be treated.
- Desired therapeutic effect.
- Drug release from the delivery system.
- Bioavailability of the drug at the absorption site.
- Pharmacokinetics and pharmacodynamics of the drug.

Differentiating drug delivery systems according to their mechanism of drug release:

One of the systematic that can be used to differentiate drug delivery systems is according to the way the drug is released. Broadly, one can differentiate as follows:

1. Immediate release
2. Modified release: Modified release systems can be further classified as:
   i. Delayed release
   ii. Extended release: This can be further subdivided into
      o sustained release systems and
      o controlled release systems

Whilst immediate-release dosage forms are designed to give a fast onset of drug action, modifications in drug release are often desirable to increase the stability, safety and efficacy of the drug, to improve the therapeutic outcome of the drug treatment and/or to increase patient compliance and convenience of administration.

2.5.1 Immediate release dosage forms

Many dosage forms are designed to release the drug immediately or at least as quickly as possible after administration. This is useful if a fast onset of action is
required for therapeutic reasons. For example, a tablet containing a painkiller should disintegrate quickly in the gastrointestinal tract to allow a fast uptake into the body. The onset of action is very fast for intravenous injections and infusions and the pharmacological effect may be seen in a matter of seconds after administration.

Immediate-release dosage forms usually release (dissolve or disperse) the drug in a single action following a first-order kinetics profile. This means the drug is released initially very quickly and then passes through the mucosal membrane into the body, reaching the highest plasma level in a comparatively short time. Uptake through the mucosal membranes may be due to passive diffusion or by receptor-mediated active transport mechanisms. Once taken up into the body the drug is distributed throughout the body and elimination of the drug by metabolism and excretion occurs. The elimination process also usually follows first-order kinetics. Therefore the plasma levels measured over time after administration of an immediate-release dosage form basically are the sum of a first-order absorption and a first-order elimination process.

An important consideration for immediate-release dosage forms is that the time of action of the drug is limited to the time that the concentration of the drug is above the MEC. If the drug has a short biological half-life, this time interval may be short, requiring frequent dosing and potentially leading to low patient compliance and suboptimal therapeutic outcome.

### 2.5.2 Modified Release dosage forms

Modified release (MR) drug products are complex dosage forms designed to release drug in a controlled manner to achieve desired efficacy and safety profiles. These are designed to modify the release of the drug over a given time or after the dosage form reaches the required location (Sastry et al., 2000).

Modified Release Systems can be further classified as:

i) Delayed Release

ii) Extended Release
2.5.2.1 **Delayed Release**

Delayed-release dosage forms can be defined as systems which are formulated to release the active ingredient at a time other than immediately after administration (Kadhe and Arasan, 2002). Delayed-release systems can be used to protect the drug from degradation in the low pH environment of the stomach or to protect the stomach from irritation by the drug. In these cases drug release is delayed until the dosage form has reached the small intestine.

Often polymers are used to achieve this aim. The dosage form (for example, a tablet or the granules before tableting) can be coated with a suitable polymer (Fukui et al., 2000). The polymer dissolves as a function of pH, so when the dosage forms travel from the low-pH environment of the stomach to the higher-pH environment of the small intestine, the polymer coat dissolves and the drug can be released. Once this occurs, the release is again immediate and the resulting plasma concentration versus time curve is similar to the one for immediate release dosage forms.

Delayed release from oral dosage forms can control where the drug is released, e.g. Intestinal Release System (when the dosage form is released in the small intestine or the Colonic Release System (colon-specific dosage forms).

a) **Intestinal Release System of delayed release drug products:**

Site-specific, drug delivery of a therapeutic agent to the intestinal region can be readily accomplished by the application of an enteric coating on a solid dosage form (Porter S.C, 1995). A drug may be enteric coated for several known reasons such as to prevent gastric irritation, prevent destabilization in gastric pH, etc. Certain drugs are delivered to the distal end of small intestine for absorption via peyer’s patches or lymphatic system. Peyer’s patches are mucosal lymphoid tissues that are known to absorb macromolecules like proteins and peptides and antigens by endocytosis. Selective release of such agents to peyer’s patch region prevents them from getting destroyed/digested by the intestinal enzymes.

b) **Colonic Release System of delayed release drug products:**
The development of colon-specific drugs and dosage forms may be advantageous for the treatment of local and systemic diseases, including colorectal cancer and Crohn’s disease. Especially for peptide and protein drugs, this form of release may also be advantageous for systemic administration given the more favourable pH conditions in the colon compared to the stomach and the generally lower enzymatic activity compared to the small intestine.

The advantage is taken of the fact that pH sensitive bioerodible polymers like polymethacrylates release the medicament only at the alkaline pH of colon or use of divinylbenzene cross-linked polymer that can be cleaved only by the azoreductase of colonic bacteria to release free drug for local effect or systemic absorption (Robinson et al., 2009; Verma et al., 2002).

### 2.5.2.2 Extended release

Extended-release products are formulated to make the drug available over an extended period after ingestion (Khan M.G, 2001). This allows a reduction in dosing frequency compared to a drug presented as a conventional dosage form (e.g. as a solution or an immediate-release dosage form). A drug having biological half-life between 2 to 8 hours is best suited for oral extended release drug delivery system (Jain N.K, 2004; Brahankar et al., 1995; Maderuelo et al., 2011). Extended release can be achieved using sustained- or controlled-release dosage forms.

#### a) Sustained release

It includes any drug delivery system that achieves slow release of drugs over an extended period of time not particularly at a pre-determined rate. These preparations may provide an immediate dose required for the normal therapeutic response, followed by the gradual release of drug in amounts sufficient to maintain the therapeutic response for a specific extended period of time usually 8 – 12 hours (Pandey et al., 2003). The major advantage of this system is that, in addition to the convenience of reduced frequency of administration, it provides blood levels that are devoid of the peak and valley effect which are characteristics of the conventional intermittent dosage regimen (El-Halim et al., 2011). Sustained
release dosage forms are designed to complement the pharmaceutical activity of the medicament in order to achieve better selectivity and longer duration of action.

b) Controlled-release

It includes any drug delivery system from which the drug is delivered at a predetermined rate over a fixed period of time. The release kinetics is usually zero-order. In contrast to sustained-release systems, the dose in the therapeutic system is of less importance than the release rate from the therapeutic system. Controlled-release systems also offer a sustained-release profile but, in contrast to sustained-release forms, controlled-release systems are designed to lead to predictably constant plasma concentrations, independently of the biological environment of the application site. This Controlled release dosage forms enhance the safety, efficacy, reliability, and convenience of drug therapy by controlling the rate and duration of drug release to control drug actions and to reduce the frequency of drug administration, thus encouraging patient compliance.

2.6 PEPTIC ULCER DISEASE

Peptic Ulcer disease encompasses both gastric and duodenal ulcers, and are defined as breaks in the mucosal surface >5 mm in size with depth to the submucosa. Peptic Ulcer disease has a tremendous effect on morbidity and mortality until the last decades of the twentieth century, when epidemiological trends started to point to an impressive fall in its incidence. Since the introduction of Histamine H2 receptor antagonists, Proton Pump Inhibitors, Cyclooxygenase-2 selective antiinflammatory drugs and eradication of H. Pylori infection, the incidence of Peptic Ulcer Disease (PUD) and ulcer complications has decreased.

2.6.1 Epidemiology and Pathophysiology:

The time trends in the epidemiology of Peptic Ulcer reflect complex, multifactorial etiologies. Peptic Ulcer was rare before the 1800s. The pathology of gastric ulcer was first described in 1835; during the late 1800s, the prominent form was gastric ulcers in young women. Duodenal Ulcer was rare until about 1900 and then
became a prevalent condition during the first half of the twentieth century.

Historically, our understanding of the pathophysiology of peptic ulcer disease focussed on abnormalities in the secretion of gastric acid and pepsin, and on the suppression of acid as a treatment strategy. Today, gastric hypersecretion – associated with Zollinger Ellison syndrome, antral G-cell hyperplasia, an increase in parietal cell mass, and a physiological imbalance between the antagonistic gastric hormones- gastrin and somatostatin is still an important issue in peptic ulcer disease (Vachhani et al., 2009). Moreover it is known that cholinergic hypersensitivity and parasympathetic dominance are related to the stimulation not only of hydrochloric acid but also pepsin, which is often neglected as a cofactor in the development of erosive injury to the gastric mucosa. Psychologic stress, cigarette smoking, alcohol consumption, use of NSAIDs and an age related decline in prostaglandin level have all been shown to contribute to Peptic Ulcer disease (Yuan and Hunt, 2006).

The aetiology of peptic ulcer, for so long a matter for whimsical speculation, was suddenly illuminated in the early 1980s through the discovery by Barry Marshall and his colleagues in Perth, Western Australia, that a microorganism adhering to the mucosa of the stomach and duodenum was of major importance. The organism, Helicobacter pylori, was cultured from biopsy specimens and, when introduced into his own stomach, Marshall produced extremely unpleasant dyspeptic symptoms, which were relieved by appropriate antibiotic treatment (Marshall and Warren, 1984). The isolation of H. pylori and its identification as the most important cause of Peptic Ulcer disease led to the exploration of the role of inflammation and its associated cytokine cascade in gastric acid secretion. With the discovery of H. pylori infection, the causes, pathogenesis and treatment of Peptic Ulcer disease have been rewritten (Kumar et al., 2011). In patients without H. pylori infection, NSAIDs are the most common cause of peptic ulcer disease (Bytzer et al, 2001).

### 2.6.2 Clinical Presentation and Diagnosis

Symptoms of peptic ulcer disease commonly include epigastric pain, postprandial pain and nocturnal pain, pain that can wake the patient from sleep, and pain
relieved by food or antacids (Spiegelhalter et al., 1987). Less-common features include anemia caused by gastrointestinal blood loss, weight loss attributed to a reduced appetite caused by fear of pain, and vomiting associated with a gastric ulcer or pyloric stenosis. Especially in the elderly, PUD can present with ‘silent’ ulcer complications.

No specific symptom helps differentiate between *H. pylori*-associated or NSAID-associated ulcers, but a careful history can identify surreptitious NSAID users and an appropriate *H. pylori* test can detect infected individuals. Tests used to identify *H. pylori* includes Polymerase chain reaction (NIH Consensus Conference, 1994), Rapid urease testing (Abraham and Bhatia, 1997), Urea breath test (Tandon R., 2000), Serologic tests (Chiba et al., 1992), Stool antigen testing (Peterson et al., 1993).

Endoscopy is essential for an accurate diagnosis and differential diagnosis of peptic ulcer disease and ulcer complications (e.g. a gastric ulcer can be biopsied to exclude malignancy or to obtain tissue for an *H. pylori* diagnostic test).

2.6.3 Treatment

Before the discovery of *H. pylori*, the therapy of PUD was centered on the old dictum by Schwartz of "no acid, no ulcer." Although acid secretion is still important in the pathogenesis of PUD, eradication of *H. pylori* and therapy/prevention of NSAID-induced disease is the mainstay of treatment (Guimarães et al., 2006).

Commonly used drugs for treatment of acid peptic disorders are –

I. Acid-suppressing drugs

- **H₂ receptor antagonists**:
  - Cimetidine
  - Ranitidine
  - Famotidine
  - Nizatidine

- **Proton pump inhibitors**:
o Omeprazole
o Lansoprazole
o Rabeprazole
o Pantoprazole
o Esomeprazole

II. Mucosal protective agents

- Sucralfate
- Prostaglandin analogue: Misoprostol
- Bismuth-containing compounds: Bismuth subsalicylate

III. Eradication of *H. pylori*: Treatment duration is 10 to 14 days

*H. pylori* eradication therapies have mainly consisted of antimicrobial agents combined with antisecretory drugs. The first-line treatment should be triple therapy with a Proton Pump Inhibitor (Omeprazole 20 mg or Lansoprazole 30mg) twice daily plus clarithromycin 500 mg twice daily and either amoxicillin 1 g twice daily or metronidazole 500 mg twice daily for 7–14 days (Malfertheiner P, 2002). Treatment with PPIs twice daily is superior to treatment once daily (Vallve M et al., 2002).

Successful eradication with first-line treatments varies from 70%–95%, and 10-day and 14-day treatments are generally 7–9% more effective than the most commonly used 7-day regimens (Calvet X et al., 2000).

- Other Regimens used:
  - Ranitidine bismuth citrate 400 mg two times daily plus clarithromycin 500 mg two times daily or metronidazole 500 mg two times daily plus tetracycline 500 mg two times daily or amoxicillin 1 g two times daily
  - Levofloxacin 500 mg daily plus amoxicillin 1 g two times daily plus pantoprazole 40 mg two times daily
  - Bismuth subsalicylate 525 mg (two tablets) four times daily plus metronidazole 250 mg four times daily plus tetracycline 500 mg
four times daily plus H2 blocker for 28 days or proton pump inhibitor for 14 days (Talley et al., 2005).

2.7 PROTON PUMP INHIBITORS

Proton pump inhibitors (PPIs) have been widely used as acid inhibitory agents for the treatment of disorders related to gastric acid secretion for about 15 years. These agents provide the most rapid symptomatic control and best healing of acid related disorders amongst the available agents (Vault and Castell, 2005).

In 1989, the FDA approved the first proton pump inhibitor (PPI) in the United States, omeprazole (Prilosec™). Lansoprazole (Prevacid®) was the second FDA-approved PPI in 1995, followed by rabeprazole (Aciphex™) in 1999 and pantoprazole (Protonix®) in 2000. Esomeprazole (Nexium™) received FDA-approval in 2001.

2.7.1 Esomeprazole

2.7.1.1 Introduction

Omeprazole is a racemic mixture of S- and R- isomers. Esomeprazole, the S-isomer of Omeprazole is the first PPI to be developed as a single optical isomer. Esomeprazole has a better pharmacokinetic profile and provides greater acid suppression than that produced by other PPIs (Lindberg et al., 2003).

In clinical studies, the greater acid suppression produced by esomeprazole has translated into higher healing rates and more effective symptom relief when compared to Lansoprazole and Omeprazole in patients with Gastro Esophageal Reflux Diseases (Richter et al., 2001; Fennerty et al., 2005; Kendall MJ, 2003).

In addition, Esomeprazole treatment yields higher healing rates and provides sustained resolution of heartburn in erosive esophagitis patients more than any other PPIs (Miner et al., 2003; Edwards et al., 2006).

Chemically it is, bis (5-methoxy-2-[(S)-[(4-methoxy-3, 5-dimethyl-2- pyridinyl)methyl] sulfinyl]-1H benzimidazole-1-yl) magnesium trihydrate, Its molecular
formula is \((C_{17}H_{18}N_{3}O_{3}S)_{2} \text{Mg} \times 3 \text{H}_2\text{O}\) with molecular weight of 767.2 as a trihydrate and 713.1 on an anhydrous basis. The structural formula is:

![Esomeprazole Magnesium: Structural formula](image)

**Esomeprazole Magnesium: Structural formula**

### 2.7.1.2 Pharmacokinetics

**Absorption and distribution**

Esomeprazole is acid labile and is administered orally as enteric-coated granules. *In vivo* conversion to the R-isomer is negligible. Absorption of esomeprazole is rapid, with peak plasma levels occurring approximately 1-2 hours after dose. The Cmax increases proportionally when the dose is increased, and there is a three-fold increase in the area under the plasma concentration – time curve (AUC) from 20 to 40 mg. The absolute bioavailability is 64% after a single dose of 40mg and increases to 89% after repeated once-daily administration. For 20mg esomeprazole the corresponding values are 50% and 68%, respectively. The apparent volume of distribution at steady state in healthy subjects is approximately 0.22 L/kg body weight. Esomeprazole is 97% plasma protein bound.

Food intake both delays and decreases the absorption of esomeprazole although this has no significant influence on the effect of esomeprazole on intragastric acidity (Junghard et al., 2002).

**Metabolism and excretion**

Esomeprazole is completely metabolised by the cytochrome P450 system (CYP). The major part of the metabolism of esomeprazole is dependent on the polymorphic CYP2C19, responsible for the formation of the hydroxy- and
desmethyl metabolites of esomeprazole (Ishizaki et al., 1999) and they all exhibit polymorphic metabolism in humans (Andersson et al., 1993; Yasuda et al., 1995; Sohn et al., 1997). The remaining part is dependent on another specific isoform, CYP3A4, responsible for the formation of esomeprazole sulphone, the main metabolite in plasma.

Total plasma clearance of esomeprazole is about 17 L/h after a single dose and about 9 L/h after repeated administration. The plasma elimination half-life is about 1.3 hours after repeated once-daily dosing. The pharmacokinetics of esomeprazole has been studied in doses up to 40 mg b.i.d. The area under the plasma concentration-time curve increases with repeated administration of esomeprazole. This increase is dose-dependent and results in a more than dose proportional increase in AUC after repeated administration. This time- and dose-dependency is due to a decrease of first pass metabolism and systemic clearance probably caused by an inhibition of the CYP2C19 enzyme by esomeprazole and/or its sulphone metabolite. This parameters reflect mainly the pharmacokinetics in individuals with a functional CYP2C19 enzyme, extensive metabolisers (Tanaka et al., 1997; McColl and Kennerley, 2002).

Esomeprazole is completely eliminated from plasma between doses with no tendency for accumulation during once-daily administration. The major metabolites of esomeprazole have no effect on gastric acid secretion. Almost 80% of an oral dose of esomeprazole is excreted as metabolites in the urine, the remainder in the faeces. Less than 1% of the parent drug is found in urine.

The pharmacokinetics of esomeprazole are time and dose dependent (Mark et al., 2007).

2.7.1.3 Pharmacodynamics

Esomeprazole suppresses gastric acid secretion by specific inhibition of the H⁺/K⁺-ATPase in the gastric parietal cell. The S- and R-isomers of omeprazole are protonated and converted in the acidic compartment of the parietal cell forming the active inhibitor, the achiral sulphenamide. By acting specifically on the proton
pump, esomeprazole blocks the final step in acid production, thus reducing gastric acidity. This effect is dose-related up to a daily dose of 20 to 40 mg and leads to inhibition of gastric acid secretion.

The pharmacodynamic effect of esomeprazole, inhibition of gastric acid secretion and percentage of time with intragastric pH > 4.0, correlates with its area under the plasma concentration–time curve (Andersson et al., 2001).

**Effect on gastric acid secretion**

After oral dosing with esomeprazole 20 mg and 40 mg the onset of effect occurs within one hour (Berardi R.R, 2000). After repeated administration with 20 mg esomeprazole once daily for five days, mean peak acid output after pentagastrin stimulation is decreased 90% when measured 6 – 7 hours after dosing on day five. After five days of oral dosing with 20 mg and 40 mg of esomeprazole, intragastric pH above 4 was maintained for a mean time of 13 hours and 17 hours, respectively over 24 hours in symptomatic GORD patients. The proportion of patients maintaining an intragastric pH above 4 for at least 8, 12 and 16 hours respectively were for esomeprazole 20 mg 76%, 54% and 24%. Corresponding proportions for esomeprazole 40 mg were 97%, 92% and 56%.

**Therapeutic effects of acid inhibition**

Healing of reflux oesophagitis with esomeprazole 40 mg occurs in approximately 78% of patients after four weeks, and in 93% after eight weeks.

One week treatment with esomeprazole 20 mg b.i.d. and appropriate antibiotics, results in successful eradication of *H. pylori* in approximately 90% of patients. After eradication treatment for one week there is no need for subsequent monotherapy with antisecretory drugs for effective ulcer healing and symptom resolution in uncomplicated duodenal ulcers.

**Other effects related to acid inhibition**

During treatment with antisecretory drugs, serum gastrin increases in response to the decreased acid secretion.
An increased number of ECL cells possibly related to the increased serum gastrin levels, have been observed in some patients during long-term treatment with esomeprazole (Genta et al., 2000).

Decreased gastric acidity due to any means including proton pump inhibitors, increases gastric counts of bacteria normally present in the gastrointestinal tract. Treatment with proton pump inhibitors may lead to slightly increased risk of gastrointestinal infections such as Salmonella, Campylobacter and Clostridium difficile (Heidelbaugh et al, 2009).

### 2.7.1.4 Indications and Dosage

Esomeprazole tablets are indicated for:

- **Gastro-Oesophageal Reflux Disease (GORD)**
  - treatment of erosive reflux oesophagitis: 40 mg once daily for 4 weeks
  - long-term management of patients with healed oesophagitis to prevent relapse: 20 mg once daily
  - symptomatic treatment of gastro-oesophageal reflux disease (GORD)

- **In combination with an appropriate antibacterial therapeutic regimen for the eradication of Helicobacter pylori**
  - healing of Helicobacter pylori associated duodenal ulcer and
  - prevention of relapse of peptic ulcers in patients with Helicobacter pylori associated ulcers: 20 mg of esomeprazole magnesium delayed release tablets with 1 g amoxicillin and 500 mg clarithromycin, all twice daily for 7 days.

- **Patients requiring continued NSAID therapy**
  - Healing of gastric ulcers associated with NSAID therapy.
  - Prevention of gastric and duodenal ulcers associated with NSAID therapy, in patients at risk: 20 mg once daily for four to eight weeks.

- **Treatment of Zollinger Ellison Syndrome**
• The recommended initial dosage is 40 mg of esomeprazole twice daily. The dosage should then be individually adjusted and treatment continues as long as clinically indicated. Based on the clinical data available, the majority of patients can be controlled on doses between 80 and 160 mg esomeprazole daily. With doses above 80 mg daily, the dose should be divided and given twice-daily.

2.7.1.5 Safety overview

There is no special concern for safety with esomeprazole other than those encountered in clinical practice with the proton pump inhibitors in general. Adverse effects reported in clinical trials are mild and transient in nature and have generally been an extension of the pharmacological effects of the drug. The long-term safety profile of esomeprazole has been well established. No significant laboratory abnormalities have been reported. Esomeprazole is generally not recommended during pregnancy and lactation, since no data on exposed pregnancies are available and it is not known whether esomeprazole is excreted in human breast milk. Esomeprazole is extensively plasma protein bound and is therefore not readily dialyzable.