Chapter 1

INTRODUCTION TO BIOPHARMACEUTICS AND PHARMACOKINETICS

1.1. INTRODUCTION

Since 1960s till date, most **Pharmaceuticals** right from the ***generic*** **Analgeric Tablet** being used extensively in the ***Community Pharmacy*** to the ***state-of-the-art*** **Immunotherapy** being practiced in ***super-specialized*** **Hospitals**, invariably subjected to a vigorous research and development (R&D) episode prior to the ultimate approval by the **United States-Food and Drug Administration [US-FDA].**

Importantly, the meticulous systematic well-defined ***in-vivo*** overall *performance, safety*, and *efficaciousness* of the **Drug Products** (or **Dosage Forms**) are ascertained and established due to: **" the various *Physiochemical Characteristics* of the so-called Active Pharmaceutical Ingredient (API) or Drug Substance, and also the precise and exact route of administration (*viz.*, IV, IM, SC, CSF) are regarded to be the most reliable and critical determinants perceptively."**

In addition, the characteristic features of the '**drug substance'** and its respective '**dosage form'** are being engineered with utmost care and precision and tested to yield a fairly '**stable drug product'** that:

" on being administered to the respective patients does afford the so-called desired *Therapeutic Response* perceptively".

NOTE **Hence, both *Pharmaceuticals Scientist* and *Pharmacist* should thoroughly understand and comprehend these inherent *'Complex Relationships'* in the use and development of Pharmaceuticals for the benefit of the patients.**

Critical Importance of Drug Substance and Drug Formulation

In order to fully understand and illustrate the critical importance of **Drug Substance** and **Drug Formulation** upon these most *vital*, *prevalent*, and *important* aspects, such as:

- **Absorption Pattern of Drug**, and
- **Distribution Profile of Drug *in-vivo*,**

one would certainly take cognizance of the so-called:

" sequence of events that essentially precede elicitation of a *drug's therapeutic effect* precisely".

Following are the ***four*** **sequential stages** that need to ascertain the **Drug's Therapeutic efficacy** predominantly:

- ***First*** the **Drug** in its ***Dosage Form*** (or **Drug Product**) is administered in the patient *via* **oral, IV, SC, transdermal route of administration.**
- ***Secondly*** the **Drug** gets subsequently released right from the ***Dosage Form*** (or **Drug Product**) precisely in a **predictable and characterizable modality.**
- ***Thirdly*** certain fraction of the **Drug** gets duly **absorbed from the site of administration** – critically into:
  - **Surrounding tissues**, or
  - **Various parts of the body*.**
- ***Fourthly*** the **Drug** gains an access to the **site-of-action.** Thus, in a situation when the **drug concentration at the site-of-action** does exceed categorically, at:

  "**Minimum Effective Concentration (MEC)",**

one may observe a positive **pharmacologic response**.

Remarks: Importantly, it is quite necessary to ascertain the ***actual dosing regimen*** (*viz*., **Dose, Dosage Form, Dosing Interval**) very meticulously in the **Clinical Trials** so as to obtain the accurate and precise **drug concentration at the very site-of-action.**

NOTE **Amazingly, the ensuring *sequence-of-events* gets affected profoundly, at times seem to be *orchestrated* by the respective design of the Dosage Form, Drug itself, or even both.**

This chapter deals specifically with ***two*** **extremely important aspects,** namely *Biopharmaceutics* and **Pharmacokinetics**. These would be treated individually and briefly in the sections that follow.

Definition and Scope: **Biopharmaceutics** refers to the study of relationship existing *physical, chemical and biological characteristic features* of matter in relation to **drugs**, **drug products, drug availability and actions.**

1.2. HISTORICAL PERSPECTIVE OF BIOPHARMACEUTICS

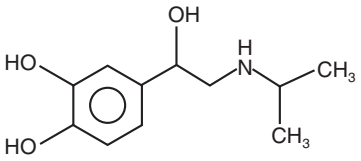
Historically, the **Pharmaceutical Scientists** made a genuine effort towards the *systematic evaluation* of the *relative availability* of '**Drug**' to the human or animal body in vivo after due administration of the respective **Dosage Form** (or **Drug Product**) and ultimately comprising the following ***three*** **aspects**:

- **Particular pharmacologic activity profile,**
- **Clinical responses**, and
- **Possible toxic activity feature.**

Example

Isoproterenol-α, β-adrenergic agonist used as a **Bronchodilator** represents a typical example that shows different pharmacologic activity with different routes of administration, such as:

* That is, quite similar to the **'Oral Dosage Forms'.**



Isoproterenol

Intravenous (IV) administration causes an enhancement in the heart rate. Oral administration shows practically little effect on heart rate.

Interestingly, the **Degree of Bioavailability** also varies appreciably from one particular drug product (**manufactured by 'A'**) to another product (**manufactured by 'B'**) containing the same '**Drug**' even though the route of administration remains the same. Nevertheless, the actual observed difference in the **Bioavailability** of the drug substance may be shown by carefully examining the difference in the **specific therapeutic effectiveness of the drug products or the dosage forms.**

It may, however, be concluded that the following ***three*** **important aspects,** namely:

- **precise nature of the drug molecule**.
- **actual route of administration of the dosage form**, and
- **critical formulation of the drug product may accurately establish and determine whether the 'administered secondary pahramaceutical product' is deemed to be**
  - (a) **effective therapeutically,**
  - (b) **toxic in nature,** and
  - (c) **devoid of any apparent pharmacologic effect.**

As to date, **Biopharmaceutics** has virtually emerged as the most versatile and well-developed scientific discipline that actually looks at closely the prevailing inter-relationship among the following ***three*** **critical aspects,** such as:

- **drug's physicochemical features,**
- **drug product (dosage form) in which medicament is administered**, and
- **route of drug administration [*i.e.*, intramuscular (IM), intravascular (IV), subcutaneous (SC)] exerting its effect upon the ensuing rate as well as degree of systemic drug absorption.**

In other words, **Biopharmaceutics** predominantly gets influenced by ***four*** **cardinal circumstances**, which essentially contributes towards:

- **actual stability of the drug within the dosage form,**
- **actual release pattern of the drug from the dosage form,**
- **actual rate of dissolution or release profile of the drugs at the site of absorption (*in vivo*),** and
- **actual absorption of the drug systematically.**

Fig:1.1. illustrates, the detailed **Block-Diagram** of the so-called '***General Scheme'*** that elaborates comprehensively the **dynamic** relationship prevailing explicitly amongst the **Drug-Dosage Form- Pharmacological- and/or Clinical Pharmacokinetic-Response** predominantly.

Remarks: The everexpanding discipline of **Biopharmaceutics** critically focuses upon the following ***two*** **aspects** generously:

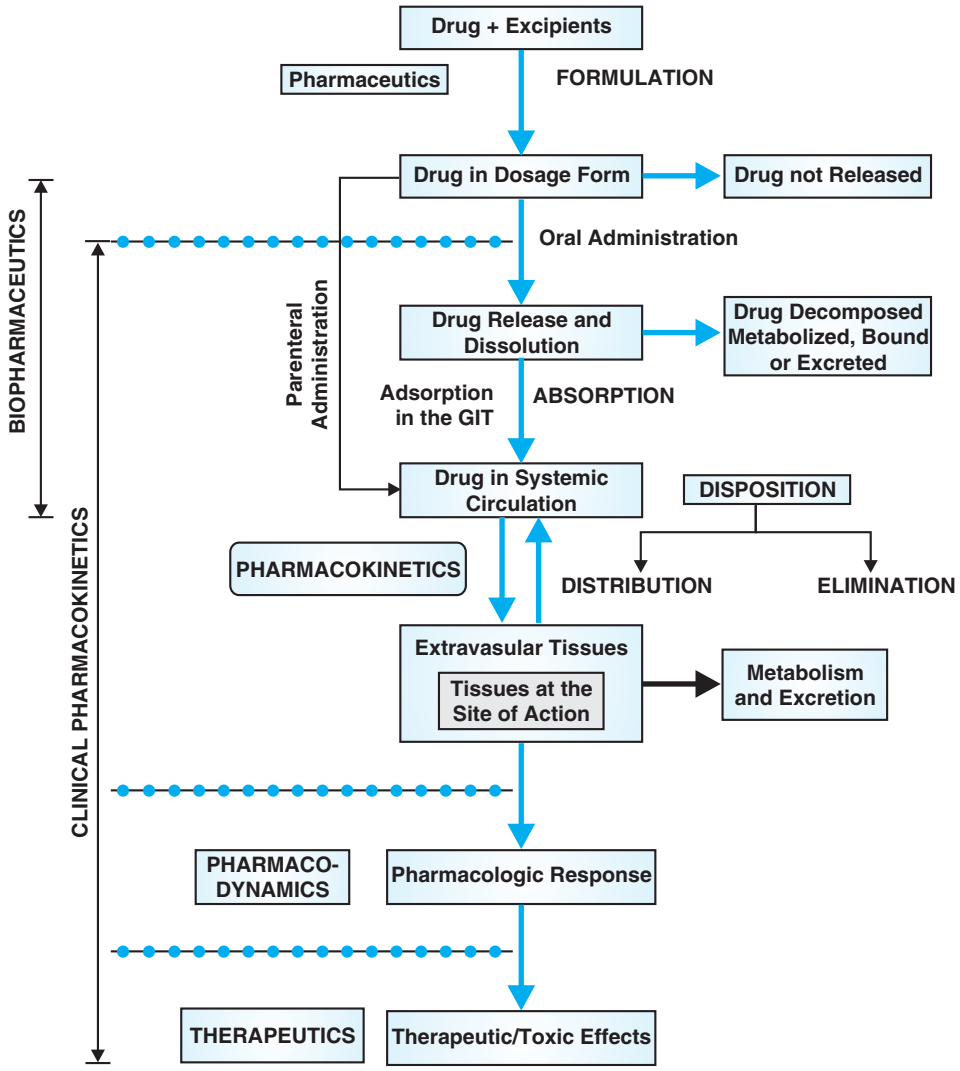


Fig. 1.1: Diagrammatic Representation of a 'General Scheme' Demonstrating Evidently the Dynamic Relationship Existing Among Drug Dosage Form Pharmacologic or Clinical Pharmacokinetic Response.

- **Fundamental Scientific Principles;** and
- **Detailed *in-vivo* Investigative Studies**.

Thus, we may have the following glaring scientific revelations:

***In vitro* investigative studies** : Essentially comprise an array of pharmacologic test apparatus, *e.g.*, **analgesiometer activity cage, rota-rod apparatus, conditioned-avoidance response apparatus**; and

***In vivo* investigative studies** : Essentially includes rather complex evaluations engaging **healthy human subjects** or even **healthy laboratory** animals (*with known strains*).

Undoubtedly, the **'Realm of Biopharmaceuticals'** critically evaluates the following much-needed valuable information as/stated under:

- **Physical–chemical properties of drugs,**
- **Commercial production of drugs,**
- **Large scale production of the dosage forms based solely upon the actual observed biologic response of the active constituent (drug) in a typical physiological environment**,
- **Drugs with a preplanned (anticipated) therapeutic applications**, and
- **Establish the specific 'route of administration' of the drug product.**

1.3. EXTRAVASCULAR ADMINISTRATION OF A DRUG

A **drug** on being administered to a subject by an **Extravascular Route** (*i.e.*, **outside the vessel**) should by all means be transferred right from the **Dosage Form** (*viz*. **tablet, capsule, injection**) to the **'Circulating Blood'** so as to remain **Bioavailable**. It is, therefore, suggested emphatically that **Bioavailability** involves as a necessary consequence the following ***two*** **vital and important aspects,** namely:

- **Exact and precise quantum of drug entering the blood stream,** and
- **Rate at which drug gains entry to blood stream**

Biopharmaceutics, therefore, may be defined as **'the critical study of such factors that eventually influence the bioavailability*** **and the subsequent utilization of this accumulated knowledge in order to optimize the ultimate clinical success of the Drug Products'.**

Earlier, it was believed that the actual prevailing **Therapeutic Response of a Drug** is predominantly by virtue of its **Intrinsic Pharmacologic Activity.**** Nevertheless, as to date, it is reasonably understood and overwhelmingly conceptualized that the **'dose-response relationship'** duly accomplished after the administration of a drug *via* different routes, such as: **oral, SC** and **parenteral**, do not show the same response. In addition, there are certain obvious noticeable variations in the critical anticipated response when the 'same drug' is being administered either at **different dosage forms** or **identical dosage forms** (*produced by several manufacturers*), which exclusively depend, in turn, on a host of **cardinal governing factors**, namely:

- **Physiochemical parameters of the drug,**
- **Presence of several excipients in the dosage form,**
- **Method of formulation adopted,** and
- **Actual route of administration of a drug.**

Bearing in mind the importance and impact of the above-cited **intricacies, technicalities,** and **therapeutic evaluations of a Dosage Form** necessitated the development and evolution of an altogether new and separate **'discipline'** termed as **Biopharmaceutics** so as to take care of all important criteria/factors, which ultimately exert a gainful and visible response upon the **therapeutic effectiveness of a drug.**

---

* **Bioavailability**: It refers to the rate and extent of absorption of a drug from a dosage form into the inner compartments of the body.

** **Intrinsic Pharmacologic Activity**: It refers to the pharmacologic activity with respect to these degree of response initiated as a result of a drug-receptor interaction.

1.4. SCOPE OF BIOPHARMACEUTICS

In the recent past, the scope of **Biopharmaceutics** has attained a glorious new height. Impotantly, almost all **Pharmaceuticals** ranging from the simple **Generic Analgesic Dosage Forms** [*viz*. **Acetaminophen Tablets (USA), Paracetamol Tablets (Asia)** and its branded counterparts: **Tylenol (McNeil consumer), Valadol (Squibb), Tapar (Parke-Davis)**] that are being sold either as **'Over-the-Consumer (OTC) Drugs'** or in the **Community Pharmacy'** across the globe in comparison to the highly sophisticated state-of-the-art medicaments invariably used for **immunotherapy** in modern specialized hospitals [*viz*., **Mithracin (Pfizer-Roaring, Dome)-Antineoplastic Agent, Harmony] (Abbott)- antipsychotics**] are being subjected to both meticulous development and extensive research before getting the due approval from the **US-FDA***:

Besides, there are quite a few **critical determinants** pertaining to the ***in vivo* performance, efficiency and safety of the Dosage Form or Drug Product** or **Secondary Pharmaceutical Product** that are entirely dependent upon such crucial factors as given below:

- **Active Pharmaceutical Ingredient(s) (APIs) in relation to their inherent physiochemical properties,**
- **Dosage Form (*i.e.*, Formulated Product) itself**, and
- **Route of administration of medicament.**

Biopharmaceutics Classification Systems (BCS)- Interestingly, as an integral segment of :

- **Safety and**
- **Efficacy**

The overall assessment of a **Generic Drug Product**, the recognized ***Regulatory Bodies*** do critically need an elaborated and comprehensive **Bioavailability (BA) Study** (see also *section 1.10*) together with a **Bioequivalence (BE) study** *vis-à-vis* the research-based **Innovator Product** perceptively, whose actual **Safety** as well as **Efficacy aspects** would have been adequately ascertained *via* the so-called stipulated expensive ***Clinical Trials*** [*i.e.,* an absolute mandatory conditionalities laid down by **US-FDA** for approved marketing the respective **Dosage Forms** globally].

Besides, such investigative studies do need an ***in-vivo* comparisons** of the resulting **Plasma-Drug Concentration** in the ***healthy human subjects*** perceptively between:

- **Actual Test and**
- **Reference Products**

Consequently, the **BCS** was eventually introduced and promulgated along with certain specified guidelines from the **US-FDA** ultimaltely.

NOTE **The Regulatory Objective of the BCS is to render the *Drug Development and Review Phenomenon* by recommending a clear strategy for replacing certain *BE studies* with *Surrogate in-vitro* Dissolution Tests.**

The progress in the domain of **Pharmaceuticals Technology** has made it possible to preplan about the characteristic features of both the **Drug** (*e.g*., through **Prodrugs**) and the **Secondary Pharmaceutical Product** via carefully engineered means, thereby producing a fairly stable drug

---

* **US-FDA:** United States Food and Drug Administration.

product, which on being administered to a patient gives rise to appropriate and desired therapeutic response significantly.

In short, it may be added that both the **Pharmacist** and the **Pharmaceutical Scientist** may have to play a big, pivotal and responsible job with utmost concerned efforts to understand thoroughly these intricate, transcrucial and complex relationships so as to apprehend the appropriate ***usage*** **and** ***meaningful*** **development of Pharmaceuticals of the Future.**

1.5. VARIANTS IN BIOPHARMACEUTICS

Biopharmaceutics refers to the science that:

"**Examines critically the inherent relationship of the so-called *Physicochemical characteristic features* of the Drug substance, the *Dosage Form* in which the drug is being administered, and finally the route of administration upon the rate and the *degree of systemic absorption of the Drug*".**

The enormous scientific revelations and literature survey that the discipline **Biopharmaceutics** essentially involves are the following ***four*** **cardial aspects** perceptively, such as:

(a) **Stability of the *Drug* within the *Drug Product* (or Dosage Form);**
(b) **Release pattern of the *Drug* from the *Drug Product*;**
(c) **Rate of Dissolution/Release profile of the *Drug* at the *absorption site;*** and
(d) **Critical *Systemic Absorption* of the *Drug*.**

Fig:1.2 depicts a *general scheme* between the **Drug Substances** and the **Drug Product** *vis-a-vis* the resulting **Pharmacologic Effect.**

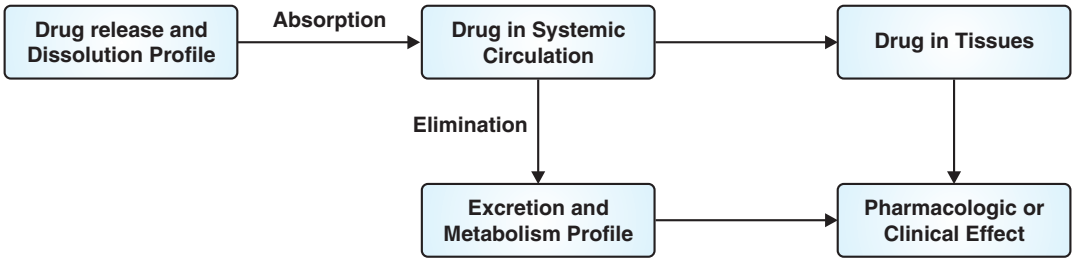


Fig. 1.2: Representation of the Scheme Depicting Dynamic Relationship between the Drug and the Drug Product *vis-a-vis* the Pharmacologic Effect.

Having mustered a fairly sufficient understanding of the **Basics of Biopharmaceutics**, it is indeed a prime requirement to have a closer look at the various **Variants in Biopharmaceutics**, such as:

- **Pharmacokinetics**,
- **Clinical Pharmacokinetics,**
- **Pharmacodynamics**,
- **Toxicokinetics and Clinical Toxicology,** and
- **Bioavailability**.

which shall now be discussed briefly in this introductory chapter so as to familiarize the readers with these above-mentioned terminologies. The objective is to recognize the inherent importance

of these factors in the *design, formulation,* and *large-scale production of drug products* across the globe to help ultimately the mankind to lead a **'better quality of life'**.

1.6. PHARMACOKINETICS

Pharmacokinetics refers to –'**the study of how compounds (or *chemical entities*) are absorbed, distributed, metabolized, and eliminated (ADME) by the body. (*i.e.*, the study of how the body acts upon the drug)'.**

Alternatively, **Pharmacokinetics** relates to '**the study of the quantitative relationships pertaining to the rates of drug absorption, distribution, and elimination processes *i.e.*, the data used to establish the specific dosage amount and frequency for the desired therapeutic response'**.

In true sense, the volume of data so generated may be used both intelligently and judiciously to:

- **obtain and ascertain the precise and exact '*dosage regimen*',** and
- **establish perceptively the frequency of a drug to accomplish the so-called desired and intended therapeutic response predominantly.**

Pharmacokinetics may be further studied, explored, and expatiated under the following ***three* heads**, namely:

- ***Modus Operandi,***
- **Salient Features of Pharmacokinetics**, and
- **Experimental Aspects of Pharmacokinetics,**

which shall now be discussed individually in the sections that follows:

1.6.1. *Modus Operandi*

The **Extravascular Administration of Drugs** may be achieved in a variety of mean and ways, such as:

- **Buccal**,
- **Intramuscular (IM),**
- **Intravenous (IV),**
- **Intraocular (IO),**
- **Intraoperative**,
- **Intraparietal**,
- **Intraperitoneal,**
- **Intrarenal,**
- **Intraspinal**,
- **Intrathecal**,
- **Intravesical**,
- **Intraventricular**,
- **Rectal**,
- **Subcutaneous (SC),** and
- **Topical**,

and the results thus obtained when plotted between the **Observed Plasma Concentration and Time (in hours),** distinctly show an initial increase followed by a subsequent decrease, as illustrated in Figure 1.3. However, the **observed drug plasma concentration evidently passes *via* a maximum value C_{max} at time T_{max}.**

It is, however, pertinent to state here that the administered **'Drug'** should be released right from the **Dosage Form** (*viz*. **Tablet, Capsule, Injection**) *via* several **Physiological Barriers** (*viz*. **Biological Membranes, Body Fluid**) in order to gain its legitimate entry into the circulating blood, as depicted explicitly in Fig. 1.4.

In fact, an elaborated study of the aforesaid phenomenon should usually comprise the following ***two* aspects**, namely:

- **Total quantum of drugs absorbed *in-vivo*,** and
- **Rate of absorption of drug.**

1.6.2. *Salient Features of Pharmacokinetics*

These essentially include the following ***five* important** aspects, namely:

1. **Experimental segment of pharmacokinetics includes *three* cardinal points**:
   - **Critical development of newer biological sampling techniques,**
   - **Latest analytical techniques [*e.g.*, liquid chromatography–mass spectrometry, gas chromatography and mass spectrometry, high performance liquid chromatography (HPLC), reversed-phase HPLC, differential scanning calorimetry]: for the assay of drugs and metabolites*,** and
   - **Various procedures, which categorically facilitate data collection and manipulation.**

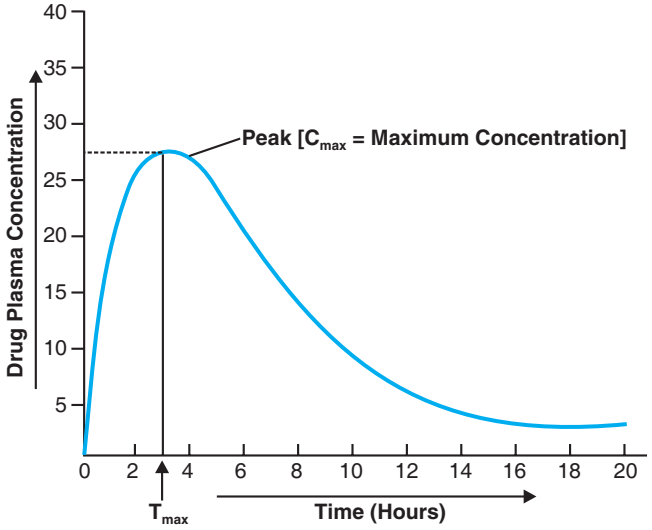


Fig. 1.3: A Plot between Drug Plasma Concentration vs. Time (hours) Following Administration of Drug *via* Extravascular Route.

* Kar A: **Pharmaceutical Drug Analysis,** 3rd ed., New Age International, Pvt Ltd, New Delhi, 2016.

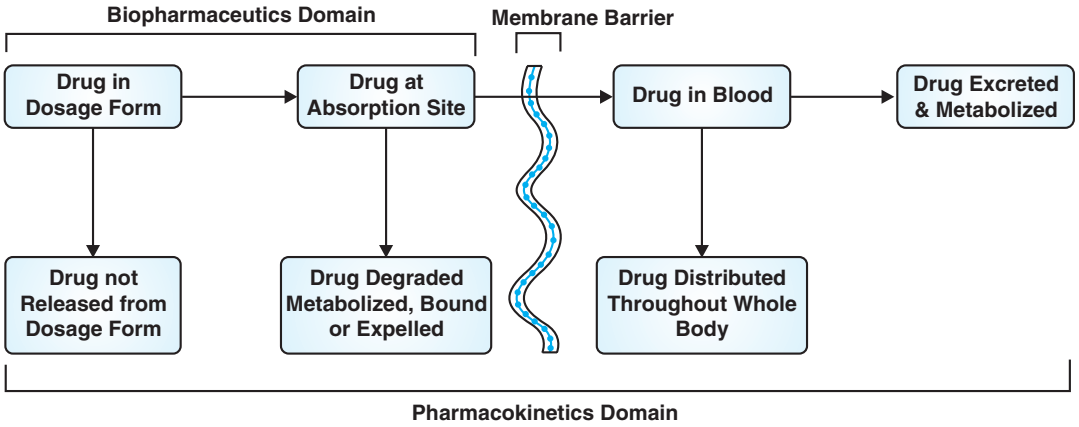


Fig. 1.4: Drug in Dosage form Shown in Pharmaceutics Domain and in Pharmacokinetics Domain.

2. Theoretical segment of **Pharmacokinetics** embraces critically the meticulous and development of an array of **Sophisticated Pharmacokinetics Models** which may be responsible for the precise prediction of drug deposition soon after drug administration *via* a known route.
3. **Biostatistics: Biostatistics** refers to '**the application of statistical processes and methods to the analysis of biological data'.** It essentially forms an integral part of the **Pharmacokinetics Studies** to determine **vital aspects,** namely:
   - **Pharmacokinetics Parameter,**
   - **Data Interpretation,**
   - **Predict the Actual Designing of 'Optimal Dosing Regimen' for Individuals or Groups of Patients**, and
   - **Applicable to Various Pharmacokinetics Models to Determine both**
     (a) **Data Error and**
     (b) **Structural Model Deviations.**
4. **Computational and Mathematical Techniques** based on the ***theoretical aspects*** pertaining to a host of **Pharmacokinetics Methods.**
5. Exercising and implementing of **'Classical Pharmacokinetics'**, which critically deals with the study of **Absolute Theoretical Models** that solely forms the centre of activity with respect to the following ***two*** **features**, such as:
   - **Model Development**, and
   - **Parameterization.**

1.6.3. *Experimental Aspects of Pharmacokinetics*

The **experimental aspects of Pharmacokinetics** essentially involves the following ***three*** **vital and important** features, such as:

- **Specific Development of Biologic Sampling Methods,**
- **Analytical Techniques for the Measurement of Drugs and Metabolites, and**
- **Typical Procedures that Essentially Facilitate Data Collection and Manipulation.**

Besides, the **application of statistics** does form an integral component of most **Pharmacokinetic Investigative Studies.** However, the **Statistical Procedures** are being used solely for:

- **Pharmacokinetics Parameter Estimation**, and
- **Data Interpretation,**

so as to accomplish finally the very **purpose of designing and predicting the so-called optimized dosing regimens for either individuals or groups of patients.**

Advantages of Statistical Methods: These are exclusively applied to the **Pharmacokinetics Models** to estimate precisely both the ensuring **Data Error** and **Structural Model Deviations** perceptively. Also the intelligent usage of **mathematical and computational procedures** do critically form the so-called **Theoretical Basis of a Host of Pharmacokinetics Methodologies.**

1.7. CLINICAL PHARMACOKINETICS

Clinical Pharmacokinetics refers to 'the application of Pharmacokinetics principles for the Rational Design of an individualized dosage regimen'.

However, the ***two*** **major objectives** are as given below:

- **Maintenance of maximum drug concentration at the receptor-site in order to accomplish the desired therapeutic response for a pre-determined period, and**
- **Manipulation of any Advance Drug Response (ADR) or Toxic Effects of the Drug being tested.**

Another school of thought relates the application of **Pharmacokinetics** to the safe and effective therapeutic management of an individual patient exclusively.

Interestingly, the necessity related to a '**Drug Therapy**' more or less designates, an unpredictable, hit-and-miss, absolute gamble; nevertheless, the gainful optimization of the ensuing '**Benefit-to-Risk**' ratio is the ultimate reward. In fact, the factual statement entirely based upon the so-called population averages crucially refer to as one's acclaimed knowledge on drug action(s).

In actual practice, while expressing critically the '**Normal Dosage Regimen**' of a **drug product,** the so-called anticipated results are invariably projected as a '**Statistical Most Probable Best Guess**' that may be termed as follows:

- **Usual dosage of a drug** and
- **Actions and adverse drug reactions.**

As-to-date, medical practitioners or clinicians do have a tendency to recognize that each and every individual subject is altogether '**different**'; and, therefore, the usual '**Practice of Medicine'** predominantly tries to;

- **Predict and avoid 'Non average Complications',** or
- **Readjust therapeutic approach solely based on the specific observations pre-emptively.**

The influx of **Newer Drug Products** in the past couple of decades have urgently necessitated the particular active and vigilant consideration(s) being more important than the prevailing characteristics features pertaining to the ensuing **drug-patient interaction that may eventually enhance the** possibility and likelihood of attaining a rather more definite **favourable benefit-to-risk ratio perceptively.**

The survey of literature reveals that there are several excellent examples of drug substance that obviously illustrates and expatiate the fundamental clinical usage of the **Principles of Pharmacokinetics** in accomplishing a positive improvement in the prospects related to the **magnificent success in drug therapy.**

Thus, one may come across *individualized* **'Pharmacokinetics Variations'**, which may categorically come into play in such ***in vitro*** processes, namely **ADME**. Consequently, the aforesaid biological phenomena are duly influenced, affected and guided by disease conditions, age, drugs and the like.

Clinical Pharmacokinetics in true sense makes use of the **well-defined, recognized and accepted methodologies so as to counteract or negate these influences by intelligently and skillfully affording individualization of drug therapy.** It has been duly observed and established that two major situations invariably come into being on account of either **intra-individual variation or inter-individual variation, for instance:**

- **Subtherapeutic response of a drug product, wherein the concentration of the drug stands below the minimum effective concentration (MEC), or**
- **Toxic response of drug product, wherein the concentration of the drug remains above the minimum toxic concentration (MTC),**

which would perhaps predominantly need an immediate adjustment to the prevailing dosing regimen. Thus, **Clinical Pharmacokinetics** predominantly involves the broad application of various **Pharmacokinetic Methodologies** in the domain of **drug therapy**. It will be worthwhile to impress at this point in time that '**Clinical Pharmacokinetics**' critically embraces a purely multidisciplinary approach with respect to the individually optimized dosing strategies that are exclusively based upon the patient's actual disease condition *vis-a-vis* patient-specific related careful thoughts.

In the light of the aforesaid statement of facts and observations, one may safely come to a meaningful conclusion. That the valuable and informative inputs derived from the extensive and intensive studies pertaining to the **Clinical Pharmacokinetics of drug products in various disease conditions do require input from medical research as well as pharmaceutical research.**

Based on the **National Vital Statistics Report (2003)*** of the **rate of death**** from 10 most glaring causes of death in the United States, which has been duly provided in Table 1.1.

1.7.1. *Therapeutic Drug Monitoring (TDM) and Pharmacokinetics*

Quite recently, the tentacles of **Pharmacokinetics** have been duly explored, evaluated and utilized effectively in most versatile and urgently needed **Therapeutic Drug Monitoring (TDM)**. Importantly, TDM is generally applicable to extremely **potent drug substances,** *viz*. **Aminoglycosides: Streptidine, Spectinamine** and **Anticonvulsants: Sulthiame, Valproic Acid**, usually having a narrow therapeutic range in order to accomplish the following **two main objectives:**

- **To optimize efficacy of drug product,** and
- **To check and prevent any possible adverse toxicity.**

Therefore, in order to carry out **TDM** in a methodical manner, it is absolutely necessary to place the patient under constant vigil for monitoring in ***two*** **accepted means**, namely:

* National Vital Statistics Report (USA): **52** (3), 2003.

** Age-adjusted death rates by male-female ratio.

- **Plasma-Drug concentration levels (*e.g.*, Theophylline, Hydrocortisone),** and
- **Particular Pharmacodynamic end point, *viz.* prothrombin-clotting time (*e.g.*, Warfarin, Heparin)**

Table 1.1. Observed Ratio of Age-adjusted Death Rates Obtained by Male-Female Ratio Derived from 10 Leading Causes of Death in the United States (2003)

S. No.	Disease Conditions	Rank	Male-Female Ratio
1	Heart disease	1	1.5
2	Malignant neoplasms	2	1.5
3	Cerebrovascular disease	3	4
4	Chronic liver respiration disease	4	1.4
5	Accidents and others	5	2.2
6	Diabetes mellitus	6	1.2
7	Pneumonia and influenza	7	1.4
8	Alzheimer	8	0.8
9	Nephritis, nephritic syndrome	9	1.5
10	Septicaemia	10	1.2

NOTE **The clinical Pharmacokinetics service attached to modern hospitals does provide requisite urgent pharmacokinetics and drug-analysis services that are more or less indispensable for 'safe-drug monitoring'.**

1.8. PHARMACODYNAMICS

Pharmacodynamics relates to '**the study of the physiological effects and mechanisms of action of a chemical entity (compound), and how this varies with either concentration/ dosage or the study of how the drug acts upon the body'.**

Alternatively, Pharmacodynamics refers to 'the study of absorption, distribution, metabolism, and excretion (ADME) drug *i.e.*, explaining the mechanism of action and its biochemical and physiological effects perceptively'.

Pharmacodynamics turns to the information of the in-depth study of **ADME of a drug with specific reference to its:**

- **Mechanism of action,**
- **Biochemical reaction,** and
- **Physiological effect.**

In other words, **Pharmacodynamics** designates '**the study of drugs and their subsequent actions upon the living organism'**.

Pharmacodynamics invariably described the character of the specific concentration of a drug at the very site of action *vis-à-vis* its ensuing relationship to the magnitude of observed physiological effects.

In a rather much simpler version, **Pharmacodynamics** deals with what the drug actually does to the body very much in contrast to **'Pharmacokinetics',** which is essentially a study of what the body does to the drug.

Another school of thought defines **Pharmacodynamics** as '**refurbishing and surrogating the ensuing relationship between the concentration of the drug at the 'Receptor Site'** (*i.e.*, **site of action**) and **the corresponding pharmacologic response,** *i.e.*, **the various biochemical and physiological effects**, **which ultimately are responsible for the interaction of a 'drug molecule with the receptor**'.

In this manner, the interaction of a drug molecule with a receptor initiate a remarkable sequence of molecular event that may give rise to either a **pharmacological response or a toxic response**.

The skillful and judicious design of the so-called **Pharmacokinetics-Pharmacodynamic Model,** in fact, offers an excellent bridge of relationship between ***two*** **important entities**:

- **Plasma-drug level,** and
- **Drug concentration at the receptor site,**

thereby establishing and ascertained the intensity as well as time course of the drug under investigation.

1.9. TOXICOKINETICS AND CLINICAL TOXICOLOGY

Toxicokinetics refers to the critical and specific application of pharmacokinetic principles to the so-called:

"**Design, Conduct, and Interpretations of drug safety evaluation studies in a comprehensive manner**".*

The above findings were used in validating the dose-related exposure to animals subsequently. Importantly, the resulting **Toxicokinetic Data** does help in a big way in the critical interpretations of the ensuing **toxicological findings** in the animals. Thus, the results were carefully extrapolated *vis-a-vis* the resulting data to humans perceptively.

Obviously, the **Toxicokinetic Investigative Studies** are invariably carried out in healthy laboratory animals in the course of the so-called **Proclinical Drug Developmen**t episodes, which may even be extended beyond to maintain the continuity profile after '**drug**' has been subjected to the **test in the Clinical Trials.****

Clinical Toxicology relates **to 'the study of the Adverse Drug Effects (ADE) and the respective toxic substances (*poisonou*s) in the body'**.

Observations: They essentially comprise

1. The '**Pharmacokinetics'** of a drug usually in an **over-medicated** (*intoxicated*) patient may

* Leal M *et al*: **Use of Toxiokinetic Principles in Drug Development: Bridging Preclinal and Clinical Studies**, In: Yakobi A *et al* (Eds.): *Integration of Pharmacokinetics, Pharmacodynamics, and Toxicokinetics in Rational Drug Development*, Plenum Press, New York, pp: 55-67, 1993.

** Clinical Trial: It refers to a carefully designed and executed investigation of the 'drug' being adminstered to human subjects. The main goal is to define the clinical efficiency and pharmacological effects (e.g., toxicity, side-effects, incompatibilities or interactions). US-FDA requires strict testing of all new drugs before their approval for use as therapeutic agents.

be very different *vis-a-vis* the **Pharmacokinetics** of the *same drug* being given in relatively **lower-therapeutic dose levels.**

2. However, at **very high-doses** the observed drug concentration present in the human body may saturate the enzymes that are involved perceptively in:
   - **Absorption**
   - **Biotransformation** and
   - **Active Renal Excretions Mechanisms,**
     - thereby altering the ensuing **Pharmacokinetic profiles from linear to non-linear Pharmacokinetics ultimately.**

1.10. BIOAVAILABILITY

Bioavailability refers to '**the precise proportion of a medicine (drug product or dosage form) reaching the systemic circulation soon after a specific-predetermined-route of administration'.**

First-Pass Metabolism-It relates to the most prevalent and significant factor in the determination of **Bioavailability** profile of a **Drug Product (Medicine).** Furthermore, it critically ascertains the so-called **presystemic metabolism** taking place either at the intestine or the liver perceptively.

Examples: A few classical examples essentially comprise:

- An array of **lipid-soluble medicines** *viz*., **β-Blockers***[**metaprolol®**], a few ***Tricyclic* Antidepressant Drugs,** and an altogether different ***opiate* Analgesics** are affected quite severely.
- Besides, the food may also affect the **bioavailability *via*** several proven and established means and ways, such as:
  - **Modification of Gastric Emptying Process,** and
  - **Hence slowing Medicine Absorption Profile.**

NOTE **Ingested Calcium may specifically combine with Medicines (drug substances) *viz*., *Tetracyclines* further reduce the absorption of Ca^{2+} systematically.**

US-FDA defines **'Bioavailability'** as **'the rate and extent to which the drug substances gets absorbed and becomes available at the site of action'.**

Based on the extensive and intensive survey of literature reveals that there are ***four* distinct and important Functional Terminologies for Bioavailability,** namely:

- **Absolute Bioavailability,**
- **Relative Bioavailability,**
- **Quantifying Absolute Availability,** and
- **Quantifying Relative Availability,**

which shall now be discussed individually in the sections that follows:

---

* **β-Blockers:** It is also known as: **Beta-Adrenoreceptor Blocking Drugs** or **Beta Blockers** (**Metapcolol®**]-that eventually inhibit the effects caused by the stimulation of **β_1- and β_2-adrenergic neurons**.

1.10.1. *Absolute Bioavailability*

Bioavailability refers to '**a comparison of the extent of a drug being absorbed *in vivo via* altogether different means, using IV administration as a standard procedure**.* That is without considering the rate of absorption. Nevertheless, the '**absolute bioavailability**' is namely guided by such vital and important characteristics features, such as:

- **Active substance (*i.e.*, drug),** and
- **Absorption receptor site.**

1.10.2. *Relative Bioavailability*

In true sense, the critical importance for the evaluation of the **Secondary Pharmaceutical Products** (or) **Dosage Forms** actually looked at from a **formulator's point of view** in an event when a newer pharmaceutical formulation is being crafted and designed. In actual practice, one may eventually come across ***two* possible options,** namely:

- ***First***, that exclusively deals with the so-called **Comparative Bioavailability** when the rate and degree of absorption are duly subjected to comparison by a '**generic equipment**' *vis-a-vis* an '**Innovative-Researched Product**'. As a result, one would favourably expect that the ensuing **Pharmacokinetic Parameters** must agree very intimately for the aforesaid ***two* formulations** (*viz*. **generic/researched product**). Thus, the ***two* altogether different drug products** are regarded overwhelmingly to be **Bioequivalent.**
- ***Second,*** that critically demands to alter and modify the existing **Pharmacokinetic profile** of the so-called **Patent Formulation,** *e.g.*, **modified drug-release dosage form(s).** obiviously, in such an instance one may make use of the **Pharmacokinetic profile** of the '**Patent Formulation**' **as an ideal means of comparison to access and record the extent of absorption.**

Interestingly, the most relevant and important definitions of their relationships are intelligently pieced together and depicted in Figure 1.5

In short, it may be added that while '**Clinical Pharmacokinetics**' studies are found to be useful in determining the safety and efficacy of **Dosage Forms** (or **Drug Product**), and the **Bioavailability studies** are meant to define the overall ensuing effect of changes taking place in the **physiochemical characteristics** of.

- **Drug Substance,** and
- **Effect of Drug Product (or Dosage form),**

upon the **Pharmacokinetics of the Drug.**

1.10.3. *Qualifying Absolute Availability*

The **Absolute Availability of a Drug** represents precisely the **Systemic Availability** of a **Drug** after an **extravascular administration** (*viz*. **oral, rectal, SC, transdermal**) *vis-a-vis* **IV dosing alternatively.**

In general, the **Absolute Availability of a Drug** is measured by comparing meticulously two vital aspects, namely:

- **Respective Area Under Curves (AUCs) after oral, rectal, SC, transdermal administrations,** and
- **Corresponding IV administration.**

---

* That is without considering the rate of absorption.

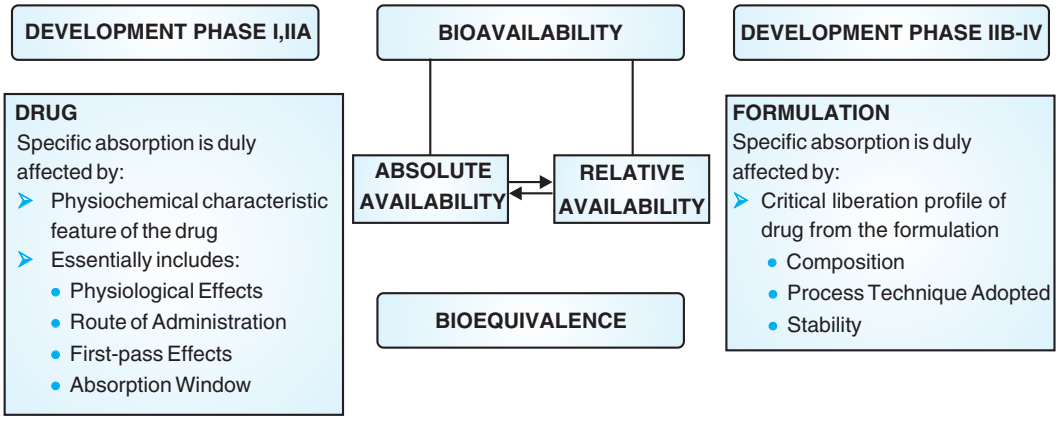


Fig. 1.5: Bioavailability and Related Terminologies.

However, the measurement of **Absolute Availabilit**y may be carried out so far as both 'V_d*' and ***k*** ** remain grossly independent of the route of administration.

Importantly, one may obtain the **Absolute Availability,** after due oral administration by making use of the ensuing '**Plasma Concentration Data**' as given below:

$$\textbf{Absolute availability } F = \frac{(AUC)_{PO}/Dose_{PO}}{(AUC)_{IV}/Dose_{IV}} \qquad \textbf{(a)}$$

Besides, the **Absolute Availability** may also be represented as a fraction or as a percent by multiplying **F×100.** On the basis of the **Urinary Drug Excretion Data,** one may express the **Absolute Availability** as stated below:

$$\textbf{Absolute availability} = \frac{(DU)^{\infty}_{PO}/Dose_{PO}}{(DU)^{\infty}_{IV}/Dose_{IV}} \qquad \textbf{(b)}$$

Important points: Following are four **important points** pertaining to **Absolute Availability.**

1. It is equal to 'F' *i.e.*, **the critical fraction of the dose or the bioavailable dose**.
2. It is variably expressed as a percent, *i.e.*, **F = 1 or 100%**
3. Intravascularly administered drugs get absorbed almost completely, for instance, IV Bolus.*** Injection, wherein **F = 1**, because almost complete absorption occurs.
4. Extravascularly administered drugs, *e.g.*, **oral route (PO)**, the observed absolute bioavailability '**F**' perhaps may not exceed beyond **100% (*i.e.*, F > 1).**

Equations **(a)** and **(b)** given above actually determine the **Absolute Bioavailability 'F'**, where **PO** designated an oral route, and other **Extravascular Route(s)** of drug administration, such as:

- **SC route,**
- **Intraperitoneal route,** and
- **Rectal route.**

---

* V_d**:** Volume of distribution.

** ***k*****:** Overall drug elimination rate constant [$k = k_e + k_m$], where k_e = excretion rate constant [first order] k_m = metabolism rate constant [first order].

*** It refers to the **volume of drug given rapidly by IV route 1.**

1.10.4. *Quantifying Relative Availability*

Importantly, the **Relative Availability** (or apparent) refers to the availability of a drug right from a dosage form (or drug product) *vis-a-vis* another '**Recognized Standard Product**'.

It has been proved beyond any reasonable doubt that the exact and precise fraction of a dose available systematically from an oral product is rather difficult to ascertain. Invariably, a **standard solution of the pure drug duly evaluated in a 'Crossover Study'** may be accomplished effectively by determining the availability of drug in the **Dosage Form** (*i.e.*, **Formulation**) *vis-a-vis* the availability of drug in a Standard Dosage Formulation'.

In this manner, the relative availability of two **Dosage Forms** (or **Drug Products**) administered at the identical dosage level and by the identical route of administration may be obtained by the help of the following expression:

$$\text{Relative availability} = \frac{(AUC)_A}{(AUC)_B} \qquad \textbf{(c)}$$

where, **A** = drug in a dosage form and

B = recognized reference standard.

The resulting fraction when multiplied by 100 gives rise to the corresponding **Relative Availability.**

In a situation, when different dosage levels are duly administered, a necessary correction as applicable for the exact '**size of the dose**' needs to be incorporated as shown in the following equation:

$$\text{Relative availability} = \frac{(AUC)_A/\text{Dose}_A}{(AUC)_B/\text{Dose}_B} \qquad \textbf{(d)}$$

Alternatively, one may even make use of the '**Urinary Excretion Data**' in order to measure the relative availability, so far as one maintains to collect the '**Total Quantum of Intact Drug**' excreted in the urine. Thus, it is possible to determine the per cent **Relative Availability** by making use of the **Urinary Excretion Data** as given in the following expression:

$$\text{Percent relative availability} = \frac{(Du)^{\infty}_A}{(Au)^{\infty}_B} \times 100 \qquad \textbf{(e)}$$

where, **(Du) = Total amount of drug excreted in the urine.**

1.11. BIOEQUIVALENCE

Bioequivalence relates to a specific instance when ***two*** **medicines** (**Drug Products or Dosage Form**) are said to be **bioequivalent** when they contain the same amount of an identical active chemical entity (compound); and when their **bioavailability** remains the same on being administered in equal doses under similar parameters.

Bioequivalent (**Biological Equivalent**) relates to '**those equivalents that when administered in the same quantum do provide the same biological or physiological availability profile –as measured precisely by blood-levels and urine-levels**'.

Bioequivalence designates a relative term that essentially denotes that the drug present in two or more *identical dosage forms* (or **drug product**), critically reaches the ensuing systemic circulation usually:

- **At the same relative rate,** and
- **At the same relative degree.***

Obviously, the critical appearance and observations of the statistically significant differences in the *two* or *three* or even more dosage form do indicate the **Bioequivalence.**

1.11.1. *Bioequivalence: The Canadian Regularly Perspective*

Canada enjoys the reputation of being one of the pioneers in the critical application of the '**Concept of Bioequivalence'**, by virtue of the imposition of **compulsory and mandatory 'Licensing Legislation',** which with effect from **1969 to 1988** (almost a span of two decades) not only promulgated but also facilitated the gainful entry of the '**Generic Drug Product****'.

- **Westlake (1973)*** ** postulated an artitrary standard of almost 80% extant of bioavailability with respect to a reference product, The **Exprt Advisory Committee on Bioavailability** was duly established in 1974, which eventually carried out and examined several approaches for the in-house stydies due to the urgent and crucial lack of the much-needed statistical procedures for both **Bioavailability** and **Bioequivalence.**

Examples

Phenylbutazone Equivalence Study: **McGilveray *et al.* (1978)** specifically studied the **Plasma Concentration Derived Data**, for instance:

- **Area under the concentration-time curve (AUC),** and
- **Maximum observed concentration (C_{max}).**

which were adequately examined after

(a) **Transformation to corresponding log value, and**

(b) **Confidence intervals (CI) up to 95%.**

Table: 1.2 records the different categories of complicated drug substances in the critical and precise assessment of the **Bioequivalence Values** perceptively.

The **Canadian Approach** and **Suggestion to Bioequivalence** predominantly helps in the judicious and logical classification of **Orally Administered Drugs** into ***two* main categories**, namely:

---

* That is, their ensuing plasma concentration-time profiles shall be more or less quite identical without any appreciable observed '**statistical differences**'.

** **Generic Drug Product**s: It essentially refers to a chemically equivalent copy of a brand-name drug whose patent has expired legally.

*** Westlake (1973): Use of Statistical Methods in Evaluation of *in vivo* Performance of Dosage Forms, *J Pharm Sci.*, **62**: 1579-1589, 1973.

- **Uncomplicated Class**, and
- **Complicated Class.**

Table 1.2. Complicated Drug *vis-a-vis* Their Variants for Bioequivalence Evaluation Studies

S. No.	Complicated Variants
1	Sustained (or modified) drug release secondary pharmaceutical products
2	Drugs having either variable or complicated pharmacokinetics profiles, namely: (a) Nonlinear kinetics (*viz*. those having an appreciable first-pass effect say > 40%) (b) Variable kinetics on account of different 'genetic phenotypes'* (c) Stereochemical effects, *viz*. inversion of configuration in vivo and (d) Prolonged plasma half-life (t_{12})
3	Drugs for which the exact 'time of onset of the effect' as well as the precise 'rate of absorption' are equally of prime importance
4	Highly toxic drugs as well as those with a narrow therapeutic range
5	Unabsorbable drug substances and whose therapeutic remains active locally in the GI tract.
6	Typical drugs having no clear cut known measurable technique either sensitive enough or dependable enough to estimate blood concentration up to at least terminal plasma half-lives
7	Combination dosage forms (or drug products)
8	Most biological products, *e.g*., typhoid vaccine, γ-globulin

Very much in time and in line with the nature and degree of commonly encountered **Bioequivalence problems**.** Various researchers making dedicated contributions across the globe on crucial bioequivalence problems thought it worthwhile to ponder over such cardinal aspects thoughtfully as:

- **Strictly adhere to 'one basic protocol' *viz*. single-dose crossover,** and
- **A critical predetermined universally acceptable decision protocol *viz*. CI 90% pertaining to the 'relative mean parameter' necessarily falling within the range of 80-120% or 80-125% may not be suitable, practical and feasible for most drug variants, *e.g*., α-lactoms, metronidazole, chloroquine and sulpha drug.**

Interestingly, from a close look of the contents given in Table 1.2, we may find the various types of drugs (or even their respective drug products) that are regarded to be explicitly and overwhelmingly complicated in terms of their critical '**Bioequivalence Assessment**'.

1.11.2. *Uncomplicated Drugs*

The **Uncomplicated Drugs** invariably refer to the various '**Oral Drug Product**' duly marketed as the common conventional formulations, which do not find their place under single or several complicated categories (Table 1.2).

---

* **Genetic Phenotype:** the expression of the genes (pertaining to reproduction) duly presented in an individual.

** **Expert Advisory Committee on Bioavailability**: Report on bioavailability of oral dosage formulations of drug used for systemic effects. Drugs with uncomplicated characteristics, drug Directorate, Health Protection Branch, Health and Family Welfare Canada, 1990.

1.11.3. *Guidelines for Equivalence Studies*

It is, however, pertinent to state here that while making for formulating the guidelines for **Bioequivalence Studies** for the actual conduct and careful analysis, the following array of pivotal factors must be considered, such as:

- **Physiochemical characteristic features,**
- **Pharmacokinetics profile of the drug,**
- **Vital and important clinical aspect,** and
- **A host of methodological aspects,**

for both classification of aforesaid drugs (*viz*. **Complicated ones and Uncomplicated ones as well**).

1.11.4. *Plasma-Drug Concentration Versus Time Curve*

The **Plasma-Drug Concentration** *versus* time curve is usually obtained by determining precisely the **Drug Concentration in Plasma Samples** meticulously drawn at predetermined/ programmed time interval (in hours) soon after a **Drug Product** is duly administered to a patient.

1.11.5. *Modus Operandi*

The various steps that are essentially involved in the preparation of plasma-drug concentration versus time curve are enumerated as follows:

1. The actual concentration obtained after a single oral dose of a drug product being analyzed in each plasma sample is duly plotted on a rectangular coordinate graph paper by taking:
   - **Plasma-drug concentration along *Y*-axis,** and
   - **Time interval* (in hours) along *X*-axis, to obtain a typical curve, as illustrated in Figure 1.5.**
2. Figure 1.6 distinctly elaborates the various observed **Pharmacokinetic** and **Pharmacodynamic** parameters.

Explanations

These essentially comprise:

The various vital and important aspects of the **Plasma-Drug Concentration *Vs* Time Curve** are explained duly as under:

1. Administered drug does have a remarkable tendency to reach the general (systemic) circulation, thereby giving rise to the attainable drug concentration in plasma to a maximum level.
2. Invariably, one may observe critically that the absorption of a drug is found to be more fast and rapid *vis-a-vis* its elimination phenomenon.

---

* **Time Interval**: It refers to the corresponding 'time' at which the 'plasma sample' was collected duly from the patient after the administration of the drug product.

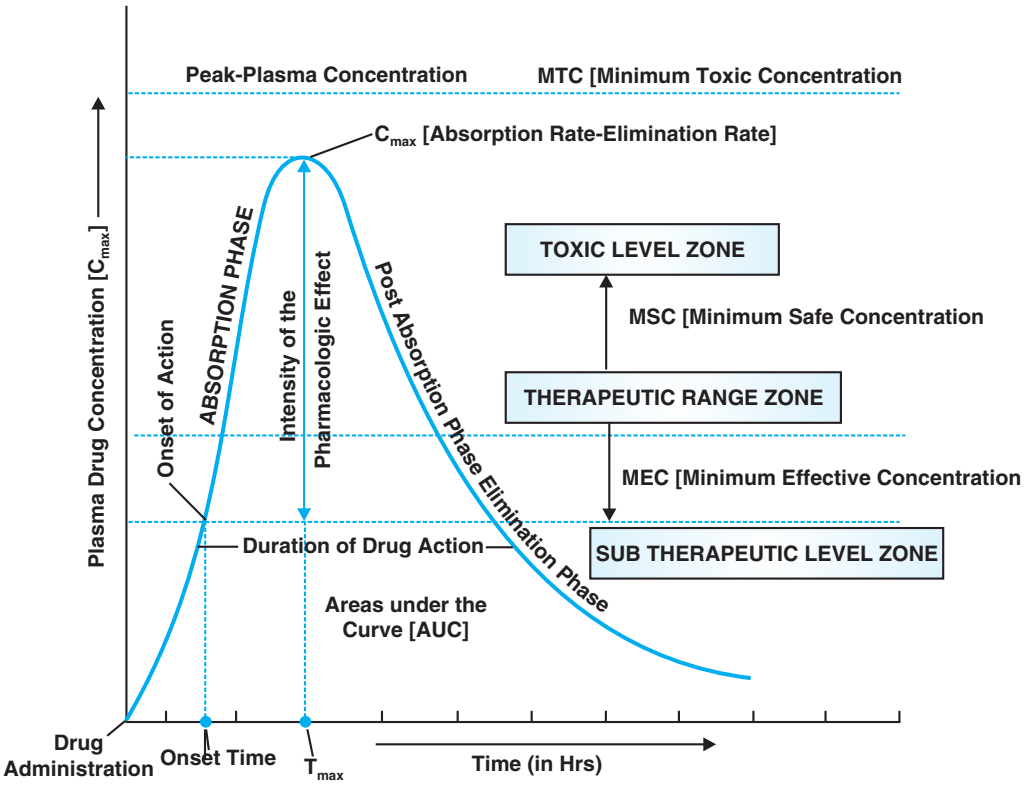


Fig. 1.6: Diagrammatic Representation of a Typical Plasma-Drug Concentration *vs* Time Curve Showing Explicitly Pharmacokinetics Pharmacodynamic Parameters accomplish after Oral Administration of a Single Dose.

3. Importantly, the drug soon after its absorption right into the systemic circulation gets eventually distributed to most of the tissues in the body and ultimately, undergoes elimination simultaneously.
4. Interestingly, the process of 'elimination' may be gainfully accomplished by
   - excretion,
   - biotransformation or
   - a combination of both processes.
5. There are ***three*** **distinct cut-off points along the *Y*-axis,** representing **Plasma-Drug Concentration** (C_{max}) in Figure 1.5, namely:

   MTC: Minimum toxic concentration,

   MSC: Maximum safe concentration, and

   MEC: Minimum effective concentration.

   MEC: Such drugs that act specifically upon the **Autonomus Nervous System**, namely:
   - **Norepinephrine-acting on the sympathetic system,** and
   - **Acetylcholine-acting on the parasympathetic system.**

It is absolutely necessary and useful to know the actual concentration of 'drug substance' which would just afford a bare **minimum level of pharmacologic effect.** At this point in time, let us assume that the prevailing concentration of the drug substance duly presented in the plasma remains to be in perfect equilibrium status with the tissue; the ensuing **MEC** duly designates the minimum concentration of drug required actually at the receptor to exhibit the anticipated pharmacologic effect.

MTC: Likewise, **MTC** designates the prevailing drug concentration required to cause just a bare minimum extent of toxic effect.

6. The intensity of the **pharmacologic effect (as shown in the apex segment of Fig.1.5) is observed to be proportional to the number of drug receptor occupied predominantly**. Nevertheless, this specific pharmacologic action is distinctly reflected in the observation that the higher plasma-drug concentrations do exhibit a greater pharmacologic response up to a maximum degree.
7. The **duration of drug action (as shown in the lower segment of AUC in Fig. 1.5) is shown by the difference between the onset time and the time needed for the drug to decline back to MEC.**
8. A**UC***: In fact, the wisdom and skill of a **Pharmacokinetics** may also elaborate and describe the respective **Plasma-Drug Level Concentration** Vs T**ime Curve** of the following ***three*** **Pharmacokinetics terms,** namely:
   - **Peak plasma level,**
   - **Time for peak plasma level,** and
   - **AUC**
9. **AUC:** It is virtually guided by ***three*** **cardinal factors,** such as:
   - **Absorption phase,**
   - **Postabsorption phase,** and
   - **Elimination phase.**
10. **Average rate of drug absorption:** It is represented by the **Time of Peak Plasma Level *vis-à-vis* Time of Maximum Drug Concentration recorded in the plasma.**
11. **Peak Plasma Concentration**: The **Peak plasma Concentration** or **Maximum Drug Concentration i**s intimately dependent upon the following ***three*** **cardinal factors,** such as:
    - **Directly related to the dose**
    - **Rate constant for absorption,** and
    - **Elimination constant of the drug.**

A Few extra terms you must know!

Dosage Regimen: It is the method that should be used to administer the appropriate quantity of the drug.

Drug Disposition: Distribution and elimination play a role in the therapeutic activity of a drug and are together referred to as drug disposition.

---

* **AUC:** It is related to the actual quantum of drug being absorbed systemically.

Drug Distribution: The relative motion of drug between different compartments of the body (*e.g.*, blood and extravascular tissue) is known as drug distribution.

Elimination: This is the process in which of drug activity is terminated (through biotransformation) and drug metabolites are removed from the body (excretion).

ADME: Absorption, distribution, metabolism and excretion.

KADME: Kinetics of ADME or pharmacokinetics.

SUGGESTED READINGS

Gibaldi M : **Biopharmaceutics and Clinical Pharmacokinetics** 4th ed., Pharma Book Syndicate, Hyderabad, 2006.

Lutz R and Dedrick: R : **Physiological Pharmacokinetics: Reference to Human Risk Assesment,** In: Li AP (Ed): *Toxicity Testing: New Applications and Applications in Human Risk Assessment,* Raven, New York, pp: 129-149, 1985.

Rowl and N and Tozor T : **Clinical Pharmacokinetics: Concepts and Applications**, 3rd ed., Lea & Febiger, Philadelphia, PA, (USA), 1995.

Shargel L *et al.* : **Applied Pharmaceutics & Pharmacokinetics,** 5th ed., McGraw Hill, New Delhi, 2005.

Tipnis HP : **Bioavailability and Bioequivalence**, New Age International Publishers, New Delhi, 1996.

Welling P and Tse E : **Pharmacokinetics**, Applied Therapeutics, Marcel Dekker, New York, 1993.

Winters ME : **Basic Clinical Pharmacokinetics,** Applied Therapeutics, Vancouver, WA, 1994.