|| *Jay Ravechi Maa* ||

*A Thesis*

# *Study of Computational Method, Biopharmaceutics, Pharmacokinetics and Develop a Integrated Computational Prototype as Bioinformatics Application*

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*Submitted by*

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# Guide's Certificate

*I hereby certify that Mr. Harshad B. Bhadka has completed his thesis for doctorate degree entitled "Study of Computational Method, Biopharmaceutics, Pharmacokinetics and Develop a Integrated Computational Prototype as Bioinformatics Application". I further certify that the research work done by him is of his own and original and is carried out under my guidance and supervision. For the thesis that he is submitting, he has not been conferred any degree, diploma or distinction by either the Saurashtra University or any other University according to best of my knowledge.*

*Place:*
*Date:*

DR. N. N. Jani

# Researcher Certificate

*I certify that the developed model for Drug Dissolution Parameters Computations and strategies derived by analysis and described in the thesis has been based on the literature survey, bibliographical references, and research papers from International journals & National journals, various conferences proceedings and through study literature available on various websites in respect of related areas.*

*Apart from these, all the analysis, hypothesis, inferences and interpretation of data and strategy have been my own and original creation. The model has been prototyped to a domain, which is my own and original creation. Moreover, I declare that for the work done in the thesis, either the Saurashtra University or any other University has not conferred any degree, diploma or distinction on me before.*

*Place:*
*Date:*

H. B. Bhadka

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| SNo. | Word | Description |
|---|---|---|
| *1* | *CLS* | *Computational & Life Science* |
| *2* | *BNF* | *Backus-Naur Form* |
| *3* | *WFF* | *Well-Formed Formulas* |
| *4* | *MATLAB* | *Matrix Laboratory* |
| *5* | *CADD* | *Computer-Aided Drug Design* |
| *6* | *StarDOM* | Self-defining Text Archival and Retrieval – Document Object Model |
| *7* | *BLAST* | Basic Local Alignment Search Tool |
| *8* | *EMBOSS* | European Molecular Biology Open Software Suite |
| *9* | *HTS* | *High-Throughput Screening* |
| *10* | *HMM* | *Hidden Markov Models* |
| *11* | *ADME* | Absorption, Distribution, Metabolism, Excretion |
| *12* | *USP* | United States Pharmacopoeia |
| *13* | *AUC* | Area Under Curve |
| *14* | *CL* | Clearance |
| *15* | *Conc* | Concentration |
| *16* | *ROA* | Routes Of Administration |
| *17* | *Cs* | Drug Solubility |
| *18* | *VD* | Volume of Distribution |
| *19* | IVIVC | In Vitro–In Vivo Correlation |
| *20* | SIF | Simulate Intestinal Fluid |
| *21* | SGF | Simulate Gastric Fluid |
| *22* | pH | Phosphate Buffer |
| *23* | RPM | Resolution Per Minute |
| *24* | HPLC | High-Performance Liquid Chromatography |
| *25* | IR | Infrared Spectroscopy |
| *26* | FT | Fourier transform |
| *27* | DF | Dilution Factor |
| *28* | CPR | Cumulative Percentage Release |
| *29* | GMP | Good Manufacturing Practice |
| *30* | EP | European Pharmacopeia |
| *31* | GUI | Graphical User Interface |
| *32* | MRT | Mean Residence Time |
| *33* | ADC | Analog to Digital Converter |
| *34* | INTR | Interrupt |
| *35* | DE | Dissolution Efficiency |
| *36* | CDER | Center for Drug Evaluation and Research |
| *37* | FDA | Food and Drug Administration |
| *38* | AUMC | Area Under Mean Concentration |
| *39* | LOD | Limit of Detection |
| *40* | LOQ | Limit of Quantification |

# Chapter 1: COMPUTATIONAL METHODS & LIFE SCIENCES

## 1.1. Introduction:

The convergence of Life Sciences and Informatics/Computer Science is rapidly transforming our ability to understand and positively influence our lives and the world around us. The intertwining of basic science with applied fields such as biomedical engineering and robotics is the first stage of a knowledge revolution that will lead to remarkable advances of great societal importance [1].

### 1.1.1 The Three Pillars of Computational and Life Sciences (CLS)

CLS seeks to capture new intellectual frontiers by integrating three diverse pillars of modern scientific discovery [2-4]:

#### 1.1.1.1 Computational Science & Informatics:

Computational Science & Informatics modeling, simulation, high-end computing/data analysis, for information-based knowledge discovery and synthesis. Algorithms, database theory, statistics, numerical methods, and systems design form core elements of CLS.

#### 1.1.1.2 Synthetic Sciences:

It's combining design, construction and engineering in physical sciences with molecular biology leads naturally to a synthetic biology; an approach that spans synthetic chemistry and condensed matter physics to exploit adaptive evolutionary principles for the generation of new functional materials, molecular machines and therapeutics.

#### 1.1.1.3 Systems Biology:

It holistic exploration of living systems across multiple scales, from molecular to cellular, organ, individual and population. High-throughput, quantitative technologies will underpin a network-level understanding of interacting components, enabling a predictive science that unifies and enriches CLS.

## 1.2 Computational Science:

It is the field of study concerned with constructing mathematical models and numerical solution techniques and using computers to analyze and solve scientific, social scientific and engineering problems. In practical use, it is typically the application of computer simulation and other forms of computation to problems in various scientific disciplines.

The field is distinct from computer science (the mathematical study of computation, computers and information processing). It is also different from theory and experiment which are the traditional forms of science and engineering. The scientific computing approach is to gain understanding, mainly through the analysis of mathematical models implemented on computers.

Scientists and engineers develop computer programs, application software, which model systems being studied and run these programs with various sets of input parameters. Typically, these models require massive amounts of calculations (usually floating-point) and are often executed on supercomputers or distributed computing platforms. Numerical analysis is an important underpinning for techniques used in computational science [5].

### 1.2.1 Applications of Computer Science:

Problem domains for computational science/scientific computing include[6-7]:

#### 1.2.1.1 Numerical Simulations:

Numerical simulations have different objectives depending on the nature of the task being simulated:

- Reconstruct and understand known events (e.g., earthquake, tsunamis and other natural disasters).

- Predict future or unobserved situations (e.g., weather, sub-atomic particle behavior).

#### 1.2.1.2 Model Fitting and Data Analysis:

Appropriately tune models or solve equations to reflect observations, subject to model constraints (e.g. oil exploration geophysics, computational linguistics). Use graph theory to model networks, especially those connecting individuals, organizations, and websites.

#### 1.2.1.3 Optimization:

Optimize known scenarios (e.g., technical and manufacturing processes, front end engineering).

Programming languages commonly used for the more mathematical aspects of scientific computing applications include Fortran, MATLAB, GNU Octave, and PDL.

Computational science application programs often model real-world changing conditions, such as weather, air flow around a plane, automobile body distortions in a crash, the motion of stars in a galaxy, an explosive device, etc. Such programs might create a 'logical mesh' in computer memory where each item corresponds to an area in space and contains information about that space relevant to the model.

For example in weather models, each item might be a square kilometer; with land elevation, current wind direction, humidity, temperature, pressure, etc. The program would calculate the likely next state based on the current state, in simulated time steps, solving equations that describe how the system operates; and then repeat the process to calculate the next state.

The term computational scientist is used to describe someone skilled in scientific computing. This person is usually a scientist, an engineer or an applied mathematician who applies high-performance computers in different ways to advance the state-of-the-art in their respective applied disciplines in physics, chemistry or engineering. Scientific computing has increasingly also impacted on other areas including economics, biology and medicine.

Computational science is now commonly considered a third mode of science, complementing and adding to experimentation/observation and theory [1, 8].

# 1.3 Formal Methods:

In computer science, formal methods are mathematically-based techniques for the specification, development and verification of software and hardware systems. The use of formal methods for software and hardware design is motivated by the expectation that, as in other engineering disciplines, performing appropriate mathematical analyses can contribute to the reliability and robustness of a design. However, the high cost of using formal methods means that they are usually only used in the development of high-integrity systems, where safety or security is important [9-11].

## 1.3.1 Taxonomy

Formal methods can be used at a number of levels:

### 1.3.1.1 Level 0:

Formal specification may be undertaken and then a program developed from this informally. This has been dubbed formal methods life. This may be the most cost-effective option in many cases.

### 1.3.1.2 Level 1:

Formal development and formal verification may be used to produce a program in a more formal manner. For example, proofs of properties or refinement from the specification to a program may be undertaken. This may be most appropriate in high-integrity systems involving safety or security.

### 1.3.1.3 Level 2:

Theorem proverb may be used to undertake fully formal machine-checked proofs. This can be very expensive and is only practically worthwhile if the cost of mistakes is extremely high (e.g., in critical parts of microprocessor design).

#### 1.3.1.4 Lightweight Formal Methods:

Some practitioners believe that the formal methods community has overemphasized full formalization of a specification or design. They contend that the expressiveness of the languages involved, as well as the complexity of the systems being modeled, makes full formalization a difficult and expensive task. As an alternative, various lightweight formal methods, which emphasize partial specification and focused application, have been proposed. Examples of this lightweight approach to formal methods include the Alloy object modeling notation, Denney's synthesis of some aspects of the Z notation with use case driven development, and the CSK VDM Tools.

### 1.3.2 Uses:

Formal methods can be applied at various points through the development process. For convenience, we use terms common to the waterfall model, though any development process could be used. [12-15].

#### 1.3.2.1 Specification:

Formal methods may be used to give a description of the system to be developed, at whatever level(s) of detail desired. This formal description can be used to guide further development activities; additionally, it can be used to verify that the requirements for the system being developed have been completely and accurately specified.

The need for formal specification systems has been noted for years. In the ALGOL 60 Report, John Backus presented a formal notation for describing programming language syntax (later named Backus normal form or Backus-Naur form (BNF)); Backus also described the need for a notation for describing programming language semantics. The report promised that a new notation, as definitive as BNF, would appear in the near future; it never appeared.

#### 1.3.2.2 Development:

Once a formal specification has been developed, the specification may be used as a guide while the concrete system is developed (i.e. realized in software and/or hardware). Examples:

If the formal specification is in an operational semantics, the observed behavior of the concrete system can be compared with the behavior of the specification (which itself

should be executable or simulateable). Additionally, the operational commands of the specification may be amenable to direct translation into executable code.

If the formal specification is in an axiomatic semantics, the preconditions and post conditions of the specification may become assertions in the executable code.

#### 1.3.2.3 Verification:

Once a formal specification has been developed, the specification may be used as the basis for proving properties of the specification (and hopefully by inference the developed system).

##### Human-Directed Proof:

Sometimes, the motivation for proving the correctness of a system is not the obvious need for re-assurance of the correctness of the system, but a desire to understand the system better. Consequently, some proofs of correctness are produced in the style of mathematical proof: handwritten (or typeset) using natural language, using a level of informality common to such proofs. A "good" proof is one which is readable and understandable by other human readers.

Critics of such approaches point out that the ambiguity inherent in natural language allows errors to be undetected in such proofs; often, subtle errors can be present in the low-level details typically overlooked by such proofs. Additionally, the work involved in producing such a good proof requires a high level of mathematical sophistication and expertise.

##### Automated Proof:

In contrast, there is increasing interest in producing proofs of correctness of such systems by automated means. Automated techniques fall into two general categories:

- Automated theorem proving, in which a system attempts to produce a formal proof from scratch, given a description of the system, a set of logical axioms, and a set of inference rules.
- Model checking, in which a system verifies certain properties by means of an exhaustive search of all possible states that a system could enter during its execution.

Neither of these techniques work without human assistance. Automated theorem provers usually require guidance as to which properties are "interesting" enough to pursue; model checkers can quickly get bogged down in checking millions of uninteresting states if not given a sufficiently abstract model.

Proponents of such systems argue that the results have greater mathematical certainty than human-produced proofs, since all the tedious details have been algorithmically verified. The training required to use such systems is also less than that required producing good mathematical proofs by hand, making the techniques accessible to a wider variety of practitioners.

Critics note that such systems are like oracles: they make a pronouncement of truth, yet give no explanation of that truth. There is also the problem of "verifying the verifier"; if the program which aids in the verification is itself unproven, there may be reason to doubt the soundness of the produced results.

### 1.3.3 Criticisms:

The field of formal methods has its critics. At the current state of the art, proofs of correctness, whether handwritten or computer-assisted, need significant time (and thus money) to produce, with limited utility other than assuring correctness. This makes formal methods more likely to be used in fields where the benefits of having such proofs, or the danger in having undetected errors, make them worth the resources. Example: in aerospace engineering, undetected errors may cause death, so formal methods are more popular than in other application areas.

At times, proponents of formal methods have claimed that their techniques would be the silver bullet to the software crisis. Of course, it is widely believed that there is no silver bullet for software development, and some have written off formal methods due to those overstated, overreaching claims [16].

## 1.4 Formal System:

In logic and mathematics, a formal system consists of two components, a formal language plus a set of inference rules or transformation rules. A formal system may be formulated and studied for its intrinsic value, or it may be intended as a description (i.e. a model) of external phenomena.

Each formal system has a formal language, which is composed by primitive symbols. These symbols act on certain rules of formation and are developed by inference from a set of axioms. The system thus consists of any number of formulas built up through finite combinations of the primitive symbols—combinations that are formed from the axioms in accordance with the stated rules. Formal systems in mathematics consist of the following elements [17-18]:

- A finite set of symbols (i.e. the alphabet), that can be used for constructing formulas (i.e. finite strings of symbols).
- A set of axioms: each axiom must be well-formed formulas (WFF).
- A set of inference rules.
- A grammar, which tells how WFF are constructed out of the symbols in the alphabet. It is usually required that there be a decision procedure for deciding whether a formula is well formed or not.
- A formal system is said to be recursive (i.e. effective) if the set of axioms and the set of inference rules are decidable sets or semi decidable sets, according to context.

### 1.4.1 Formal Proofs:

Formal proofs are sequences of WFFs. For a WFF to qualify as part of a proof, it might either be an axiom or be the product of applying an inference rule on previous WFFs in the proof sequence. The last WFF in the sequence is recognized as a theorem.

The point of view that generating formal proofs is all there is to mathematics is often called formalism. David Hilbert founded metamathematics as a discipline for discussing formal systems. Any language that one uses to talk about a formal system is called a metalanguage. The metalanguage may be nothing more than ordinary natural language, or it may be partially formalized itself, but it is generally less completely formalized than the formal language component of the formal system under examination, which is then called the object language, that is, the object of the discussion in question.

Once a formal system is given, one can define the set of theorems which can be proved inside the formal system. This set consists of all WFFs of which there is a proof for. Thus all axioms are considered theorems. Unlike the grammar for WFFs, there is no guarantee that there will be a decision procedure for deciding whether a given wff is a theorem or not. The notion of theorem just defined should not be confused with theorems

about the formal system, which, in order to avoid confusion, are usually called metatheorems.

### 1.4.2 Formal Language:

In mathematics, logic, and computer science, a formal language is a language that is defined by precise mathematical or machine processable formulas. Like languages in linguistics, formal languages generally have two aspects:

- the syntax of a language is what the language looks like (more formally: the set of possible expressions that are valid utterances in the language)
- the semantics of a language are what the utterances of the language mean (which is formalized in various ways, depending on the type of language in question)

A special branch of mathematics and computer science exists that is devoted exclusively to the theory of language syntax: formal language theory. In formal language theory, a language is nothing more than its syntax; questions of semantics are not included in this specialty.

### 1.4.3 Formal Grammar:

In computer science and linguistics a formal grammar is a precise description of a formal language: a set of strings. The two main categories of formal grammar are that of generative grammars, which are sets of rules for how strings in a language can be generated, and that of analytic grammars, which are sets of rules for how a string can be analyzed to determine whether it is a member of the language. In short, an analytic grammar describes how to recognize when strings are members in the set, whereas a generative grammar describes how to write only those strings in the set.

## 1.5 Computational Visualistics:

Images take a rather prominent place in contemporary life. Together with language, they have been connected to human culture from the very beginning. For about one century – after several millennia of written word's dominance – their part is increasing again remarkably. Steps toward a general science of images, which we may call 'general

Visualistics' in analogy to general linguistics have only been taken recently. So far, a unique scientific basis for circumscribing and describing the heterogeneous phenomenon "image" in an interpersonally verifiable manner has still been missing while distinct aspects falling in the domain of Visualistics have predominantly been dealt with in several other disciplines, among them in particular philosophy, psychology, and art history. Last (though not least), important contributions to certain aspects of a new science of images have come from computer science.

In computer science, too, considering pictures evolved originally along several more or less independent questions, which lead to proper sub-disciplines: computer graphics is certainly the most "visible" among them. Only just recently, the effort has been increased to finally form a unique and partially autonomous branch of computer science dedicated to images in general. In analogy to computational linguistics, the artificial expression computational Visualistics is used for addressing the whole range of investigating scientifically pictures "in" the computer [2, 7, 19-20].

## 1.5.1 Areas Covered:

For a science of images within computer science, the abstract data type »image« (or perhaps several such types) stands in the center of interest together with the potential implementations. There are three main groups of algorithms for that data type to be considered in computational visualistics:

### 1.5.1.1 Algorithms from »image« to »image«:

In the field called image processing, the focus of attention is formed by the operations that take (at least) one picture (and potentially several secondary parameters that are not images) and relate it to another picture. With these operations, we can define algorithms for improving the quality of images (e.g., contrast reinforcement), and procedures for extracting certain parts of an image (e.g., edge finding) or for stamping out pictorial patterns following a particular Gestalt criterion (e.g., blue screen technique). Compression algorithms for the efficient storing or transmitting of pictorial data also belong into this field.

#### 1.5.1.2 Algorithms from »image« to “not-image”:

Two disciplines share the operations transforming images into non-pictorial data items. The field of pattern recognition is actually not restricted to pictures. But it has performed important precursory work for computational Visualistics since the early 1950’s in those areas that essentially classify information in given images: the identification of simple geometric Gestalts (e.g., “circular region”), the classification of letters (recognition of handwriting), the “seeing” of spatial objects in the images or even the association of stylistic attributes of the representation.

That is, the images are to be associated with instances of a non-pictorial data type forming a description of some of their aspects. The neighboring field of computer vision is the part of AI (artificial intelligence) in which computer scientists try to teach – loosely speaking – computers the ability of visual perception. Therefore, a problem rather belongs to computer vision to the degree, to which its goal is “semantic”, i.e., the result approximates the human seeing of objects in a picture.

#### 1.5.1.3 Algorithms from “not-image” to »image«:

The investigation of possibilities gained by the operations that result in instances of the data type »image« but take as starting point instances of non-pictorial data types are performed in particular in computer graphics and information visualization. The former deals with images in the closer sense, i.e., those pictures showing spatial configurations of objects (in the colloquial meaning of ‘object’) in a more or less naturalistic representation like, e.g., in virtual architecture.

The starting point of the picture-generating algorithms in computer graphics is usually a data type that allows us to describe the geometry in three dimensions and the lighting of the scene to be depicted together with the important optical properties of the surfaces considered. Scientists in information visualization are interested in presenting pictorially any other data type, in particular those that consist of non-visual components in a “space” of states: in order to do so, a convention of visual presentation has firstly to be determined – e.g., a code of colors or certain icons. The well-known fractal images form a borderline case of information visualization since an abstract mathematical property has been visualized.

# 1.6 Computational Problem:

In theoretical computer science, a computational problem is a mathematical object representing a question that computers might want to solve. For example, "given any number x, determine whether “x is prime” is a computational problem. Computational problems are one of the main objects of study in theoretical computer science, because nearly any task one would want to accomplish is an example of a computational problem. In the field of algorithms, we study methods of solving computational problems; in the complementary field of computational complexity theory, we organize computational problems based on how difficult they are to solve [21-23].

## 1.6.1 Problems and Instances:

A computational problem encodes a general problem, independent of its specific input. A problem with a specific set of inputs is called an instance. For example, "Given any two numbers x and y, find the sum of x and y" is a computational problem. A specific instance of that computational problem would be "What is the sum of 13 and 28?".

## 1.6.2 Types of Computational Problems:

Computational problems are organized in many different ways. They can be organized by how they are defined, and by how many computational resources are needed to compute an answer. Computational problems that intuitively look very similar can vary wildly in the amount of resources needed to compute them, and some computational problems are noncomputable, meaning that no possible algorithm could solve every instance.

A computational problem which only returns a yes-or-no answer is called a decision problem. Examples of decision problems include "given an integer n, determine whether n is prime" and "given two numbers x and y, determine whether x evenly divides y". Decision problems are often used in computational complexity theory, because they are easier to study than other problems.

Computational problems that are not restricted to yes-or-no answers are called function problems. Examples of function problems include "given an integer n, list the prime factorization of n" and "given two numbers x and y, output x divided by y" [3, 24-25].

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# Chapter 2: BIOINFORMATICS: CONCEPTS & APPLICATIONS

## 2.1. Introduction:

Bioinformatics is an exciting new discipline which applies computational techniques to store, organize, generate, retrieve, analyze and share genomic, biological and chemical data to support the drug discovery process. With the high cost of drug discovery, rising costs to the consumers, the product recalls being done and adverse side effects, all this topped with more awareness and education on the part of the consumers, the industry image has been battered. Thus it is imperative that the sector finds ways of reducing the costs of drug discovery as well as the time it takes to get the medicine from the laboratory to the patient and at the same time producing drugs which are target specific and with minimal side effects. Bioinformatics is one of the tools that industry has recently engaged to aid in the drug discovery process as well as to cut costs and the time-lines and indeed it was time that the industry caught on with information technology. Computer-aided drug discovery (CADD) is an exciting and diverse discipline where various aspects of applied and basic research merge and stimulate each other [1-5].

Bioinformatics is a field which uses computers to store and analyze molecular biological information. Using this information in a digital format, bioinformatics can then solve problems of molecular biology, predict structures, and even simulate macromolecules. In a more general sense, bioinformatics may be used to describe any use of computers for the purposes of biology, but the molecular biology specific definition is by far the most common [6-9]. Bioinformatics is emerging trends and that helps in various fields like Drug Development, Gene Therapy, Molecular Medicine, Waste Cleanup, Forensic Analysis of Microbes, Biotechnology, Personalized medicine, Antibiotic resistance, Bio-weapon creation, Insect resistance, Veternity science, Crop Improvement and many others. This category of IT tool uses computers to store, organize, generate, retrieve, analyze and share genomic, biological and chemical data [3, 10-11].

## 2.2. What is CADD?:

CADD stands for Computer-Aided Drug Design.

The field of drug discovery is extremely complex and ever changing, with new methodologies and technologies being introduced constantly. CADD methods are dependent on bioinformatics tools, applications and databases. The great aim of research in bioinformatics is to understand the functioning of living organisms in order to "*improve the quality of life*" [18-20].

Recently, one of the key trends in the pharmaceutical industry has been the integration of what have traditionally been considered 'development' activities into the early phases of drug discovery. The aim of this paradigm shift is the prompt identification and elimination of candidate molecules that are unlikely to survive later stages of discovery and development [21].

## 2.3. Hub of Bioinformatics:

Bioinformatics can be behaving as a central place that work with several branches, like scientific and support.

On the scientific side of the Bioinformatics, bioinformatics methods are used extensively in molecular biology, genomics, proteomics, and other emerging areas and in CADD research. On the support side of the Bioinformatics, Information Technology, Information Management, Software applications, databases and computation resources all provide the infrastructure for bioinformatics.

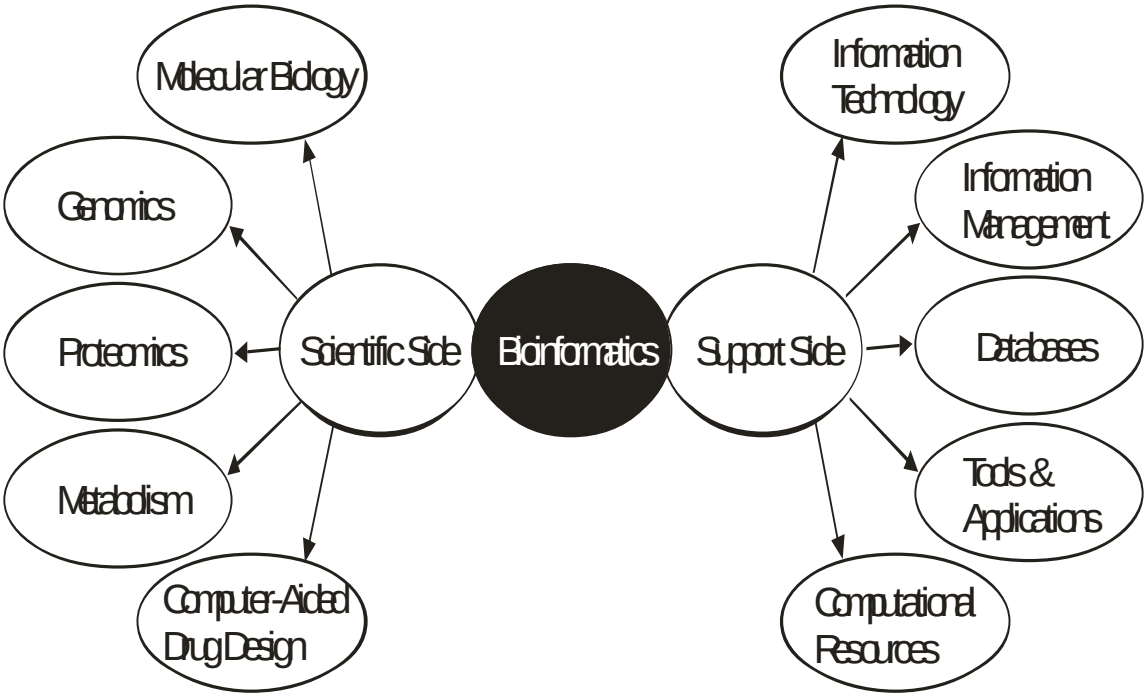


*Figure 2.3(1) Hub of Bioinformatics*

## 2.4. Objective of Bioinformatics:

An objective of bioinformatics is to extract useful knowledge from the flood of data, including biological texts, for the purpose of further analysis leading ultimately to drug discovery; in short, turning the flood of new bio-information into useable knowledge. But the data mining and knowledge management technologies that are being deployed today to assist researchers and regulators are unsuited to this task, for three reasons:

### 2.4.1. Ever-Expanding Information:

The results of groundbreaking research are being published every day, at a rate faster than any researcher or regulator can keep up with [79].

### 2.4.2. Ever-Expanding Sources:

To make matters worse, updated bio-information resides in many different locations: print journals, web-based journals, chat rooms, and various intra- and internet

data resources. With the sheer amount of new information out there, more than ever it is imperative to return search results that are relevant, so that time is not wasted sorting through irrelevant information [80].

### 2.4.3. Bioinformation Language:

The language used in bioinformation texts presents unique challenges to any information technology because of its proliferation of special terminology and symbols [81].

### 2.4.4. Conventional Search Technology:

The technology underlying typical knowledge management applications is not up to the task of dealing with these unique challenges, much less recognizing information that is relevant to a particular researcher or research program [82].

## 2.5. Tools of Bioinformatics:

There are no standard protocols or formats to cross-examine or exchange biological data. For this reason, there are various tools developed for different platforms in different programming languages. Databases of existing sequencing data can be used to identify homologues of new molecules that have been amplified and sequenced in the lab [22].

Examples of bioinformatics tools

- Biotechnix 3d: The software offers standard tools for primary sequence analysis, including DNA restriction, PCR primer selection, base composition, DNA to protein translation and ORF identification and display. Secondary sequence analysis includes amino acid composition, estimations of hydrophobicity, flexibility, local structure [22].
- StarDOM (Self-defining Text Archival and Retrieval – Document Object Model)
  StarDOM is a software package to transform data provided in the Self Defining Text Archival and Retrieval (STAR) format into XML. This opens new possibilities for visual editing, archiving, parsing and structured queries of structural biology data [18].
- FASTA
  A database search tool used to compare a Nucleotide or peptide sequence to a sequence database [14].
- BLAST (Basic Local Alignment Search Tool)

For comparing gene and protein sequences against others in public databases. Specialized BLASTs are also available for human, microbial, malaria and other genomes [15-16].

- EMBOSS (The European Molecular Biology Open Software Suite)
  EMBOSS is "The European Molecular Biology Open Software Suite". EMBOSS is a free Open Source software analysis package specially developed for the needs of the molecular biology (e.g. EMBnet) user community. The software automatically copes with data in a variety of formats and even allows transparent retrieval of sequence data from the web [19].
- RasMol:
  RasMol is a molecular graphics program intended for the visualization of proteins, nucleic acids and small molecules [20].

## 2.6. Applications of Bioinformatics:

Applications of Bioinformatics are given below:

### 2.6.1. BioJava

BioJava is an open-source project dedicated to providing a Java framework for processing biological data. It include objects for manipulating sequences, file parsers, DAS client and server suport, access to BioSQL and Ensembl databases, and powerful analysis and statistical routines including a dynamic programming toolkit [77].

### 2.6.2. BioPerl

Bioperl is a collection of Perl modules that facilitate the development of Perl scripts for bioinformatics applications. [78].

## 2.7. Drug Discovery Pipeline:

The process by which a new drug is brought to market stage is referred to by a number of names – most commonly as the development chain or “Pipeline” and consists of a number of distinct stages. Drug discovery involves different stages, including: basic

exploratory biology on target identification and validation; assay development; lead identification, which usually requires access to high-throughput screening; medicinal chemistry and pharmaceutical lead optimization; and drug candidate selection [23-26]. Following a successful Investigational New Drug application, the compound can undergo its first entry into humans (Phase I). The next stage is Phase II, which focuses on determining the optimal dosing of the product, followed by a pivotal Phase III study against a comparator product [27-29].

The significance of modern medicinal chemistry in both identifying an appropriate lead molecule and the iterative process of lead optimization is particularly poorly understood outside the pharmaceutical industry and needs to be continuously emphasized. Optimization for pharmaceutical properties (adsorption, distribution, metabolism and excretion, lack of overt toxicity, as well as for efficacy against the target organism, are crucial[30-31]. Drug candidates fail to achieve registration for several reasons: biopharmaceutical properties such as oral bioavailability and formulation issues are responsible for about 39% of failures, whereas toxicity constitutes about 21%. These factors are as important as lack of efficacy, which is responsible for about 29% of failures [31-32].

## 2.8. Bioinformatics Key Areas:

### 2.8.1. High-Throughput Screening (HTS) in Drug Discovery:

For those engaged in drug design, such as medicinal and computational chemists, the research phase can be broken down into two main tasks: identification of new compounds showing some activity against a target biological receptor, and the progressive optimization of these leads to yield a compound with improved potency and physicochemical properties *in vitro*, and, eventually, improved efficacy, pharmacokinetics, and toxicological profiles *in vivo* [33-34]. In recent years, this approach of *structure-based design* has had a major impact on the rational design and optimization of new lead compounds in those cases where the receptor structure is well-characterized [35-36]. HTS is now an essential component to identify leads [34, 37]. Virtual screening is increasingly gaining acceptance in the pharmaceutical industry as a cost-effective and timely strategy for analyzing very large chemical data sets [33].

### 2.8.2. Homology Modeling and Sequence Analysis:

The basic Hidden Markov Models (HMM) quite rare in other fields but ubiquitous in bioinformatics; some recent papers are a fully Bayesian approach to sequence alignment [38] and a novel accuracy-based a posteriori decoding method [39]. The second area is the use of probabilistic models for evolutionary tree analysis, concerning which there is a long-established research interest; some recent papers include the integration of an HMM with tree methods [40]. Some recent papers discuss the exciting ability to push into more-context-dependent grammars [41].

Frequently, the quality of the target- template sequence alignment is non-uniform along the sequence: parts can be modeled with a high confidence, whereas other parts differ strongly from the template. [42-43] one needs to evaluate a large number of possible conformations.

MODELLER [44] is used for homology or comparative modeling of protein three-dimensional structures [42]. MODELLER implements comparative protein structure modeling by satisfaction of spatial restraints [42-43], and can perform many additional tasks, including de novo modeling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures [42, 45-46].

### 2.8.3. Sequence Similarity Searching:

A natural question is whether there are other related sequences that share the same pattern. The most widely used tools for sequence similarity search allow matching between arbitrary regions of the query and database sequences [47-51]. In contrast, many motif-based search methods seek database sequences that match a pre-specified pattern [52-58].

### 2.8.4. Drug Lead Optimization:

When a promising lead candidate has been found in drug discovery program, the next step is to optimize the structure and properties of the potential drug. This usually involves a series of modifications to the primary structure and secondary structure of the compound. This process can be enhanced using software tools that explore related compounds to the lead candidate [59]. OpenEye's WABE is useful for exploring the chemical space around a lead compound to elucidate candidates with improved drug profiles [60].

### 2.8.5. PhysicoChemical Modeling:

The large number of components, the thermodynamic interactions among these components, the nonlinearity of the kinetic properties, the different time scales of various processes, and the spatial organization of the cellular environment are some of the qualitative similarities between the biological processes and processes whose study is central to chemical engineering [61-62]. SWISS-PDB is a powerful modeling tool. It has the capability to Searches of protein sequence databases, Mutation of side chains, and other structure alterations, Molecular dynamics minimizations, Calculation of electrostatic potential surfaces [63].

### 2.8.6. Drug Bioavailability:

The key characteristics for drugs are Absorption, Distribution, Metabolism, Excretion, Toxicity and Efficacy–in other words Bioavailability and Bioactivity. Although these properties are usually measured in the lab, they can also be predicted in advance with bioinformatics software [64-65]. Most drug candidates fail in phase III clinical trials after many years of research and millions of money has been spent on them. And most fail because of toxicity or problems with metabolism.

### 2.8.7. High-Throughput Massively Parallel Applications:

Recent advances in genomic and proteomic techniques, coupled with significant resources devoted to this research, have resulted in the production of enormous amounts of data. The analysis and interpretation of these data have become an important and time-limiting step in biological research. The most popular way to handle these data analysis issues is to use massively parallel programs. Two general approaches exist for adapting a computational biology tool to function as a massively parallel program: transform the tool into a genuine parallel application or use it with a parallel wrapper program. Three very popular tools BLAST and HMMER have been parallelized using the second approach, while the protein structure prediction program, LOOPP, is an example of rewriting the code and/or altering algorithms to create a genuine parallel application [10-11].

Bioinformatics seen as an emerging field with the potential to significantly improve how drugs are found brought to clinical trials and eventually release to the marketplace.

CADD methods are heavily dependent on bioinformatics tools, applications and databases. CADD and Bioinformatics together are a powerful combination in drug research and development. Bioinformatics clearly may be the answer to solve the drug discovery and cost woes of the pharmaceutical industry. Use of Bioinformatics in research in order to “improve the quality of life”. By eliminating potential drug failures early on during the process, it also helps cut the time; scientists take to get a drug from the laboratory to the chemist’s shop as they only concentrate their efforts on the leads which hold the greatest potential only. This improvement will be achieved by many means including drug design, identification of genetic risk factors, gene therapy, and genetic modification of food, crops and animals. Clearly what is needed is a next-generation technology that is intelligent enough to read unstructured text and return only relevant information. In short, a technology that is intelligent enough to turn simple information into knowledge that researchers can use confidently in the development of new bio-technologies. A challenge for computer scientists who are involved in research in bioinformatics is to achieve results that make a contribution to computer science.

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# Chapter 3: BIOPHARMACEUTICS AND PHARMACOKINETICS

## 3.1. Introduction:

### 3.1.1 Biopharmaceutics:

In the world of drug development, the meaning of the term “Biopharmaceutics” often evokes confusion, even among scientists and professionals who work in the field. “Pharmaceutics” narrowly defined is a field of science that involves the preparation, use, or dispensing of medicine. Addition of the pre- fix “bio,” coming from the Greek “bios,” relating to living organisms or tissues, expands this field into the science of preparing, using, and administering drugs to living organisms or tissues. Inherent in the concept of Biopharmaceutics as discussed here is the interdependence of biological aspects of the living organism (the patient) and the physical–chemical principles that govern the preparation and behavior of the medicinal agent or drug product.

#### 3.1.1.1 Biopharmaceutics Classification System:

The Biopharmaceutics Classification System is guidance for predicting the intestinal drug absorption provided by the U.S. Food and Drug Administration. This system allows restricting the prediction using the parameters solubility and intestinal permeability. The solubility classification is based on a United States Pharmacopoeia (USP) aperture. The intestinal permeability classification is based on a comparison to the intravenous injection. All those factors are highly important, since 85% of the most sold drugs in the USA and Europe are orally administered. According to the Biopharmaceutics Classification System, drug substances are classified as follows:

Class I - High Permeability, High Solubility:

Those compounds are well absorbed and their absorption rate is usually higher than excretion.

Class II - High Permeability, Low Solubility:

The bioavailability of those products is limited by their salvation rate. A correlation between the in vivo bioavailability and the in vitro salvation can be found.

Class III - Low Permeability, High Solubility:

The absorption is limited by the permeation rate but the drug is solvated very fast. If the formulation does not change the permeability or gastro-intestinal duration time, then class I criteria can be applied.

Class IV - Low Permeability, Low Solubility:

Those compounds have a poor bioavailability. Usually they are not well absorbed over the intestinal mucosa and a high variability is expected.

### 3.1.2 Pharmacokinetics:

A young child given an injection might ask "How will that 'ouch' get from there to my sore throat?". The answer to this question is the basis of pharmacokinetics. That is, how drugs move around the body and how quickly this movement occurs. Pharmacokinetics is the study of the time course of the drug concentration in the body, i.e., "what the body does to the drug".

Pharmacokinetics is a branch of pharmacology dedicated to the determination of the fate of substances administered externally to a living organism. In practice, this discipline is applied mainly to drug substances, though in principle it concerns itself with all manner of compounds ingested or otherwise delivered externally to an organism, such as nutrients, metabolites, hormones, toxins, etc. Pharmacokinetics is often divided into several areas including, but not limited to, the extent and rate of Absorption, Distribution, Metabolism and Excretion. This sometimes is referred to as the ADME scheme.

Absorption is the process of a substance entering the body. Distribution is the dispersion or dissemination of substances throughout the fluids and tissues of the body. Metabolism is the transformation of the substances and its daughter metabolites. Excretion is the elimination of the substances from the body. In rare cases, some drugs irreversibly accumulate in a tissue in the body. Pharmacokinetics is sometimes abbreviated as "PK". Pharmacokinetics is often studied in conjunction with pharmacodynamics. So while

pharmacodynamics explores what a drug does to the body, pharmacokinetics explores what the body does to the drug.

Pharmacokinetics is often studied using mass spectrometry because of the complex nature of the matrix (often blood or urine) and the need for high sensitivity to observe low dose and long time point data. The most common instrumentation used in this application is LC-MS with a triple quadrupole mass spectrometer. Tandem mass spectrometry is usually employed for added specificity. Standard curves and internal standards are used for quantitation of usually a single pharmaceutical in the samples. The samples represent different time points as a pharmaceutical is administered and then metabolized or cleared from the body. Blank or t=0 samples taken before administration are important in determining background and insuring data integrity with such complex sample matrices. Much attention is paid to the linearity of the standard curve; however it is not uncommon to use curve fitting with more complex functions such as quadratics since the response of most mass spectrometers is less than linear across large concentration ranges.

## 3.2 One Compartment I.V. Bolus:

Pharmacokinetics is the study of drug and/or metabolite kinetics in the body. It deals with a mathematical description of the rates of drug movement into, within and exit from the body. It also includes the study of drug metabolism or biotransformation rates. The body is a very complex system and a drug undergoes many steps as it is being absorbed, distributed through the body, metabolized or excreted (ADME).

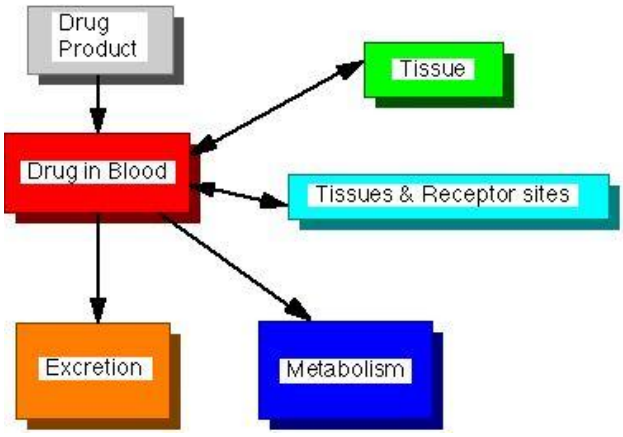


*Figure 3.2 (1) Drug Disposition*

The drug also interacts with receptors and causes therapeutic and/or toxic responses. Although the details of drug kinetics are complicated it is fortunate that we can often approximate drug kinetic processes using "simple" mathematical models.

## 3.2.1 Assumptions:

According to this model we will consider the body to behave as a single well- mixed container. To use this model mathematically we need to make a number of assumptions.

### 3.2.1.1 One Compartment:

The drug in the blood is in rapid equilibrium with drug in the extravascular tissues. The drug concentration may not be equal in each tissue or fluid however we will assume that they are proportional to the concentration of drug in the blood at all times. This is not an exact representation however it is useful for a number of drugs to a reasonable approximation.

### 3.2.1.2 Rapid Mixing:

We also need to assume that the drug is mixed instantaneously in blood or plasma. The actual time taken for mixing is usually very short, within a few of minutes, and in comparison with normal sampling times it is insignificant. We usually don't sample fast enough to see drug mixing in the blood.

### 3.2.1.3 Linear Model:

We will assume that drug elimination follows first order kinetics. First order kinetics means that the rate of change of drug concentration by any process is directly proportional to the drug concentration remaining to undertake that process. If we have a linear if we double the dose, the concentration will double at each time point.

## 3.2.2 First-Order Kinetics:

To illustrate first order kinetics we might consider what would happen if we were to give a drug by iv bolus injection, collect blood samples at various times and measure the plasma concentrations of the drug. We might see a steady decrease in concentration as the drug is eliminated, as shown in below figure.

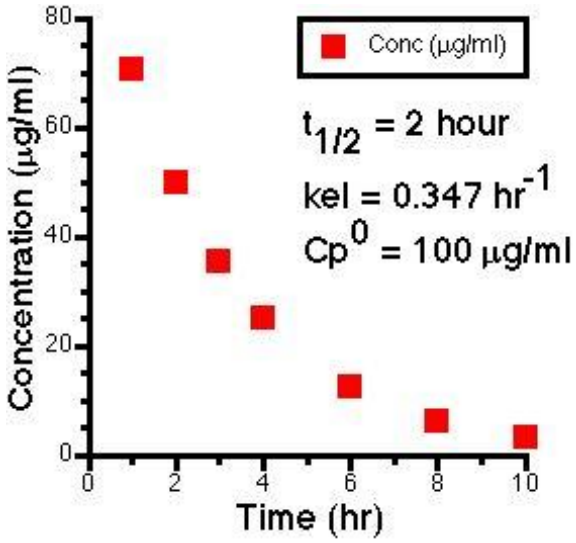


*Figure 3.2.2 (1). Linear Plot of Concentration versus Time*

### 3.2.2.1 Rate versus Cp:

If we measure the slope of this curve at a number of times we are actually measuring the rate of change of concentration at each time point represented by the straight line tangents in below figure.

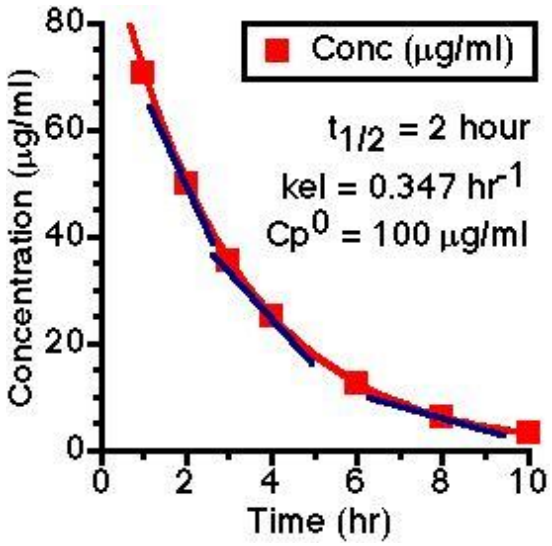


*Figure 3.2.2.1 (1). Rate versus Cp*

Now if we plot this rate of change versus the plasma concentration, for each data point, we will get a straight line when first order kinetics is obeyed.

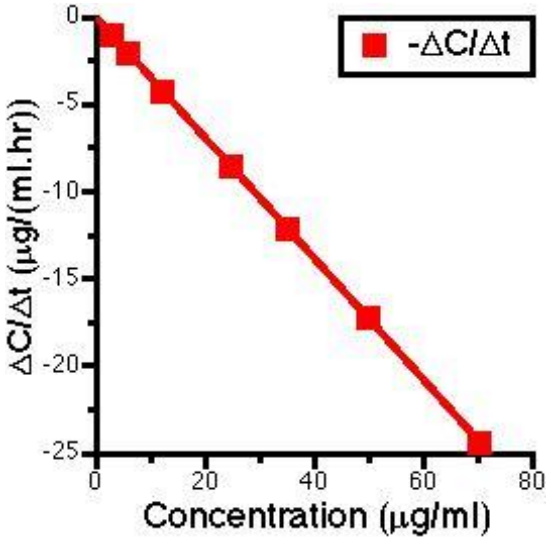


*Figure 3.2.2.1 (2). Plot of ΔCp/ Δt versus Cp for first order plot*

This behavior can be expressed mathematically as:-

$$\text{Rate of Change of Cp versus time} = -\frac{\Delta Cp}{\Delta t} = kel \bullet Cp$$

Change in Cp

Change in time

Proportionality constant called the elimination rate constant, kel

## 3.2.3 Plasma Data:

### 3.2.3.1 Scheme:

The one compartment pharmacokinetic model can be represented schematically as:

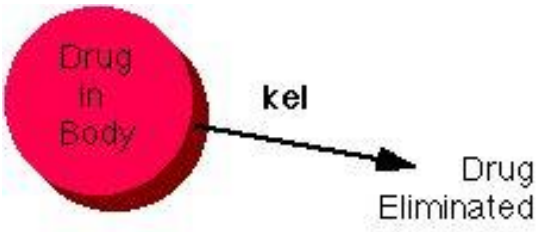


*Figure 3.2.3.1 (1). Scheme for a One Compartment Model, Intravenous Bolus Administration*

### 3.2.3.2 Differential Equation:

Going back to the slope calculations, if we measure the slope over small intervals we are calculating the tangent to the line. We can now say that the rate of elimination is the differential of the concentration with respect to time as $\Delta t \rightarrow 0$ then $\Delta Cp/\Delta t \rightarrow dCp/dt$ which gives: $\frac{dCp}{dt} = -kel \cdot Cp$

It is a differential equation for the one-compartment model after IV bolus administration. With the calculus term dCp/dt we are going from the gross or large time interval term $\Delta Cp/\Delta t$ to the continuously varying dCp/dt term.

### 3.2.3.3 Elimination Rate Constant, kel:

We should now distinguish between the elimination rate and the elimination rate constant. The rate (tangent or slope, dCp/dt) changes as the concentration changes, however, for a linear model the rate constant (kel) is constant, it does not change.

## 3.2.4 Area under the Curve:

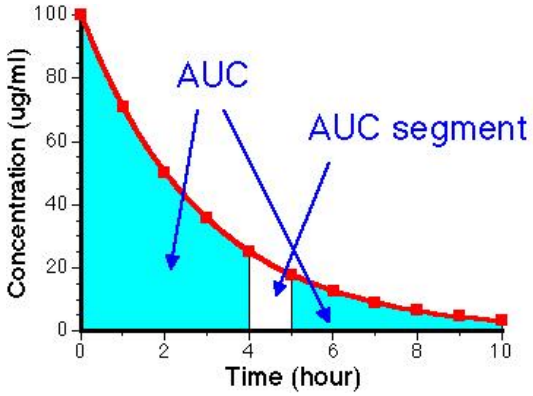


*Figure 3.2.4 (1). Linear Plot of Cp versus Time showing AUC and AUC segment*

We can continue by looking at two more parameters. These are the area under the plasma concentration time curve (AUC) and the half-life of elimination. First AUC. The AUC value is very useful for calculating the relative efficiency of different drug products. It will also give us another method of calculating the apparent volume of distribution, not just from $Cp^0$.

The AUC is calculated by adding these segments together. Each very narrow segment has an area = Cp*dt. Thus the total area has the area,

$$AUC = \int_{t=0}^{t=\infty} Cp^t \cdot dt$$

calculus uses very narrow segments

$$\text{Since} \quad Cp^t = Cp^0 \cdot e^{-kel \cdot t} = \frac{DOSE}{V} \cdot e^{-kel \cdot t}$$

then

$$AUC = \frac{DOSE}{V} \cdot \int_{t=0}^{t=\infty} e^{-kel \cdot t} \cdot dt$$

$$= \frac{DOSE}{V} \cdot \left[ \frac{e^{-kel \cdot t}}{-kel} \right]_{t=0}^{t=\infty}$$

at t = 0, $e^{-kel*t} = 1$

at t = $^{\infty}$, $e^{-kel*t} = 0$

Therefore,

$$AUC = \frac{DOSE}{V} \cdot \left[ 0 - \frac{1}{-kel} \right]$$

or

$$AUC = \frac{DOSE}{V \cdot kel} = \frac{Cp^0}{kel}$$

Analytical integration (exact solution, given exact values for V and kel).

Note: From t = 0 - > t = [[infinity]], AUC = $Cp^0/kel$ and for t = t - > t= [[infinity]] , AUC = $Cp^t/kel$]

Or

$$V = \frac{DOSE}{AUC \cdot kel}$$

Usually we don't and have to calculate AUC directly from the Cp versus time data. We need then a different approach, which is called the trapezoidal rule.

Trapezoidal Rule:

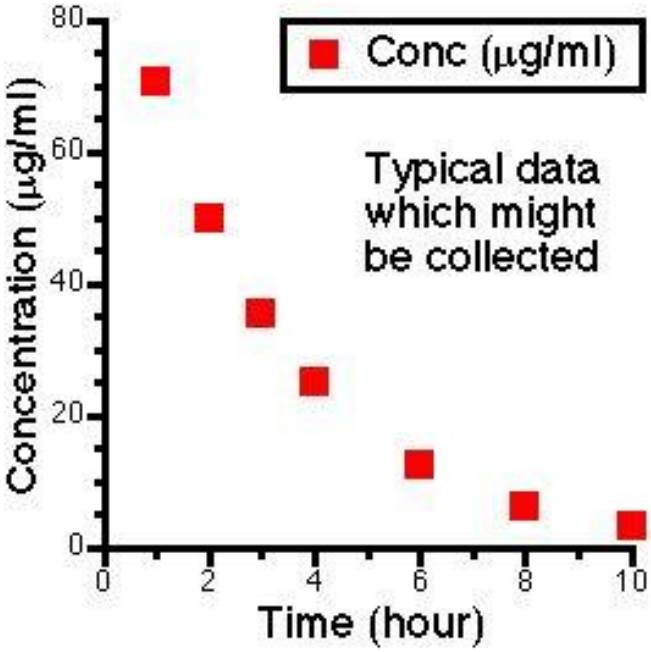


*Figure 3.2.4 (2). Linear Plot of Cp versus Time showing Typical Data Points*

We can calculate the AUC of each segment if we consider the segments to be trapezoids. [Four sided figures with two parallel sides].

The area of each segment is then given by the average concentration x segment width.

$$\text{Therefore} \quad AUC^{2-3} = \frac{Cp^2 + Cp^3}{2} \cdot (t^3 - t^2)$$

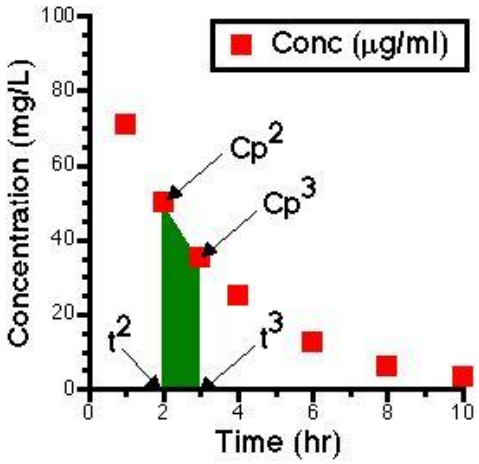


*Figure 3.2.4 (3). Linear Plot of Cp versus Time showing One Trapezoid*

The area from the first to last data point can then be calculated by adding the areas together.

$$AUC^{1-n} = \sum \left\{ \frac{Cp^1 + Cp^2}{2} \cdot (t^2 - t^1) \right\}$$

$$+ \left\{ \frac{Cp^3 + Cp^2}{2} \cdot (t^3 - t^2) \right\} + \ldots$$

Note: Summation of data point information (non-calculus)

This gives:

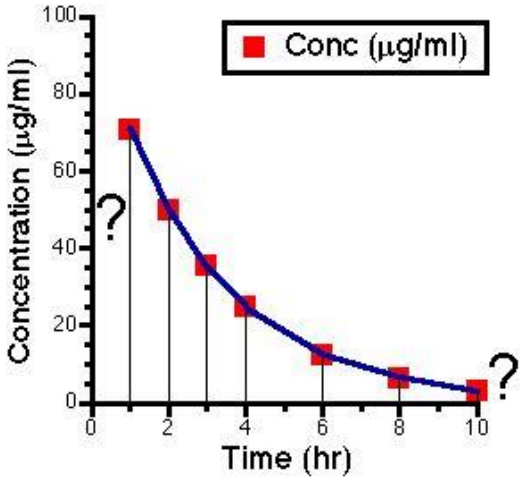


*Figure 3.2.4 (3). Linear plot of Cp versus time showing areas from data 1 to data n*

We now have two more areas to consider. The first and the last. First if we give a rapid I.V. bolus the zero plasma concentration $Cp^0$ can be determined by extrapolation.

Thus

$$AUC^{0-1} = \left\{ \frac{Cp^0 + Cp^1}{2} \times t^1 \right\}$$

The final segment can be calculated if we go back to the mathematical equation.

$$AUC^{t(last)-\infty} = \int_{t=t(last)}^{t=\infty} Cp \cdot dt$$

$$= \frac{Cp^{last}}{kel}$$ can be derived

Therefore the total AUC can be calculated as :-

$$AUC^{0-\infty} = AUC^{0-t(last)} + AUC^{t(last)-\infty}$$

$$= \sum \left[ \frac{Cp^0 + Cp^1}{2} \cdot t^1 \right] + \left[ \frac{Cp^1 + Cp^2}{2} \cdot (t^2 - t^1) \right] + \quad \ldots + \frac{Cp^{last}}{kel}$$

| Time (hr) | Concentration (µg/ml) | delta AUC | AUC (µg.hr/ml) |
|---|---|---|---|
| 0 | 100 | | |
| 1 | 71 | 85.5 | 85.5 |
| 2 | 50 | 60.5 | 146.0 |
| 3 | 35 | 42.5 | 188.5 |
| 4 | 25 | 30.0 | 218.5 |
| 6 | 12 | 37.0 | 255.5 |
| 8 | 6.2 | 18.2 | 273.7 |
| 10 | 3.1 | 9.3 | 283.0 |
| Total | | 8.9 | 291.9 |

*Table 3.2.4 (1). Calculation of AUC*

## 3.2.5 Half-life:

Another important property of first order kinetics is the half-life of elimination, $t^{1/2}$. The half-life is the time taken for the plasma concentration to fall to half its original value. Thus if Cp = concentration at the start and Cp/2 is the concentration one half-life later then:

$$\ln\frac{Cp}{2} = \ln Cp - kel \cdot t_{1/2}$$

$$\ln\left[\frac{Cp}{2} \cdot \frac{1}{Cp}\right] = -kel \cdot t_{1/2}$$

ln 2 = kel * t1/2

or

$$t_{1/2} = \frac{0.693}{kel}$$

OR

$$kel = \frac{0.693}{t_{1/2}}$$

These equations can be used as an approximate method of calculating kel. If we look at a plot of Cp versus time on semi-log graph paper.

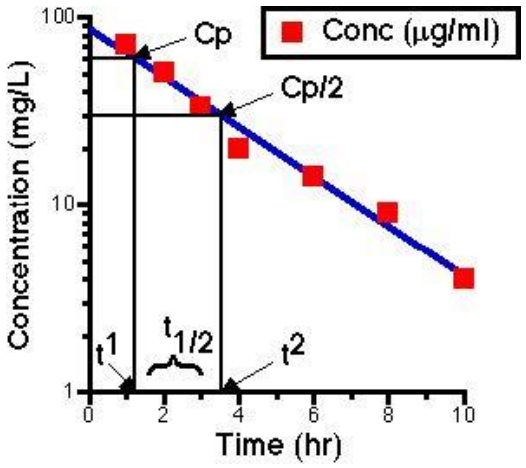


*Figure 3.2.5(1). Semi-log Plot of Cp versus Time with Some Data and a Line*

The steps to take

1) Draw a line through the points (this tends to average the data)
2) Pick any Cp and $t^1$
3) Determine Cp/2 and $t^2$
4) Calculate $t_{1/2}$ as ($t^2$ - $t^1$)

   Then kel = 0.693/$t^{1/2}$

Also consider determining Cp/4 or Cp/8 for two half-lives or three half-lives, respectively. The line smooths out the bumps. There may be bad data points, so by putting in a line you 'sort of' average the data. The half-life is the same whether going from 40 to 20 or from 10 to 5 mg/L. This is a property of the first order process.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cp | - | > | Cp/2 | in | 1 | half-life | i.e. | 50.0 | % | lost | 50.0 % |
| Cp | - | > | Cp/4 | in | 2 | half-lives | i.e. | 25.0 | % | lost | 75.0 % |
| Cp | - | > | Cp/8 | in | 3 | half-lives | i.e. | 12.5 | % | lost | 87.5 % |
| Cp | - | > | Cp/16 | in | 4 | half-lives | i.e. | 6.25 | % | lost | 93.75 % |
| Cp | - | > | Cp/32 | in | 5 | half-lives | i.e. | 3.125 | % | lost | 96.875 % |
| Cp | - | > | Cp/64 | in | 6 | half-lives | i.e. | 1.563 | % | lost | 98.438 % |

Cp - > Cp/128 in 7 half-lives i.e. 0.781 % lost 99.219 %

Thus over 95 % lost or eliminated in 5 half-lives. Typically considered the completion (my definition unless told otherwise) of the process, although in theory it takes an infinite time. Others may wish to wait 7 half-lives where over 99% of the process is complete.

# 3.3 Analysis of Urine Data:

So far we have looked at most of the information we can get from plasma data following a rapid intravenous dose of a drug using a one compartment model. There is another part of the model which can be sampled. Sometimes it is not possible to collect blood or plasma samples but we may be able to measure the amount of drug excreted into urine.

- we may not want to take repeated blood samples from certain patient populations, for example pediatrics;
- the apparent volume of distribution maybe so large that plasma concentrations are too small to measure.

If we collect data for amount of drug excreted into urine it may be possible to determine the elimination rate constant or half-life and other pharmacokinetic parameters.

## 3.3.1 Excretion Unchanged = No Metabolism:

First we'll consider the case in which the drug is excreted totally as the unchanged drug into urine. The scheme is then:

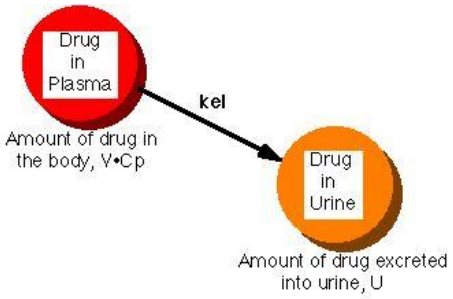


*Figure 3.3.1 (1), Scheme for drug excreted into urine, one compartment*

### 3.3.1.1 Differential Equation:

Since the rate of elimination from plasma is the same as the rate of appearance of drug into urine.

$$\frac{dU}{dt} = -\frac{V \cdot dCp}{dt} = +kel \cdot Cp \cdot V$$

Since according to this model, the drug is either in the body or has been eliminated into urine. Dose = Amount in body + Amount excreted = V * Cp + U

## 3.3.2 Metabolism and Excretion – Parrallel Pathways:

So far we have considered elimination by excretion into urine only. Usually drugs are eliminated by excretion AND metabolism, with possibly more than one metabolite involved. Schematically this can be represented as:-

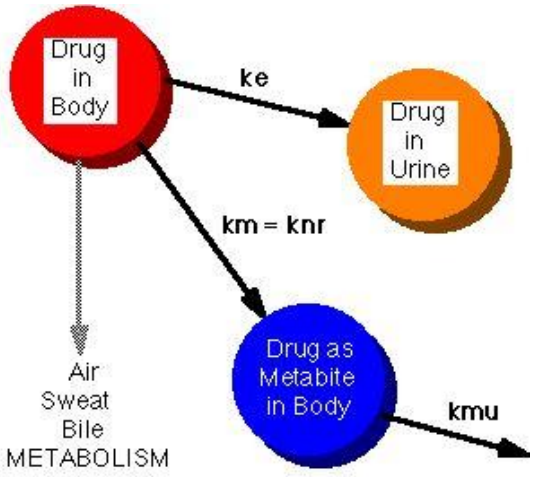


*Figure 3.3.2(1). Schematic for drug eliminated by excretion and metabolism*

ke is the excretion rate constant and km is the metabolism rate constant. Here we have two parallel pathways for elimination (with others as a shadow). We can write the differential equations for the three components shown in this diagram. There could be more pathways. It may be appropriate to specify excretion by exhalation, in sweat, or as is commonly the case, more than one metabolite.

## 3.3.3 Clearnace, CL:

At this point we can define another pharmacokinetic parameter, clearance. Clearance can be defined as the volume of plasma which is completely cleared of drug per unit time. The symbol is CL and the units are ml/min, L/hr, i.e. volume per time. Another way of looking at Clearance is to consider the drug being eliminated from the body ONLY via the kidneys.

The amount cleared by the body per unit time is dU/dt, the rate of elimination (also the rate of excretion in this example). To calculate the volume which contains that amount we can divide by Cp. That is the volume = amount/concentration. Thus:-

$$CL = \frac{dU}{dt} \cdot \frac{1}{Cp}$$

Since $\frac{dU}{dt} = kel \cdot V \cdot Cp$

$$CL = \frac{kel \cdot V \cdot Cp}{Cp} = kel \cdot V$$

As we have defined the term here it is the total body clearance. We have considered that the drug is cleared totally by excretion in urine. Below we will see that the total body clearance can be divided into a clearance due to renal excretion and that due to metabolism.

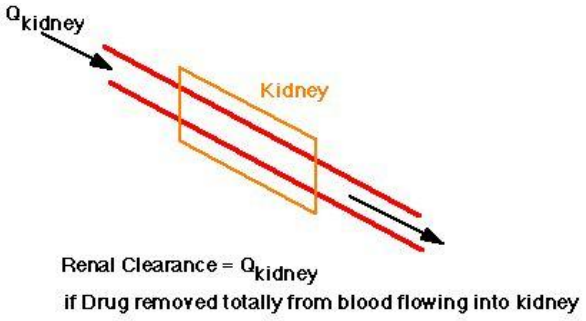


*Figure 3.3.3(1). Representation of Renal Clearance*

Clearance is a useful term when talking of drug elimination since it can be related to the efficiency of the organs of elimination and blood flow to the organ of elimination. It is useful in investigating mechanisms of elimination and renal or hepatic function in cases of reduced clearance of test substances. Also the units of clearance, volume/time (e.g. ml/min) are easier to visualize, compared with elimination rate constant (units 1/time, e.g. 1/hr).

Total body clearance, CL, can be separated into clearance due to renal elimination, CLr and clearance due to metabolism, CLm.

CLr = ke * V (renal clearance)

and

CLm = km * V (metabolic clearance)

NOTE

CL = CLr + CLm

ANOTHER METHOD of calculating CL can be derived

Integrating

$$\frac{dU}{dt} = ke \cdot V \cdot Cp = CL_r \cdot Cp$$

gives

$$U^{\infty} = CL_r \cdot \int_0^{\infty} Cp \cdot dt = CL_r \cdot AUC$$

thus

$$CL_r = \frac{U^{\infty}}{AUC} \quad \text{also} \quad CL_m = \frac{M^{\infty}}{AUC} \quad \text{and} \quad CL = \frac{DOSE}{AUC}$$

This equation uses the DATA only (without fitting a line through the data or modeling the data) using the trapezoidal rule. Thus this is a model independent method.

$$\frac{dU}{dt} = CL \cdot Cp$$

thus a plot of dU/dt versus Cp will give a straight line through the origin with a slope equal to the clearance, CL.

# 3.4 Intravenous Infusion:

Commonly in a hospital setting a patient will receive a drug by intravenous infusion. The inconvenience of administering the drug over a long time is not a real problem with bedridden patients. Some may already be receiving intravenous fluids. If a drug is chemically stable and is compatible with the intravenous fluid it may be added to the fluid and thereby be given by slow infusion. Some drugs cannot be given by rapid intravenous injection. Therefore they may be given by slower IV infusion over 15 or 30 minutes.

## 3.4.1 Continuous Infusion – Steady State:

Giving the drug by infusion will alter the kinetics of the drug. Schematically:

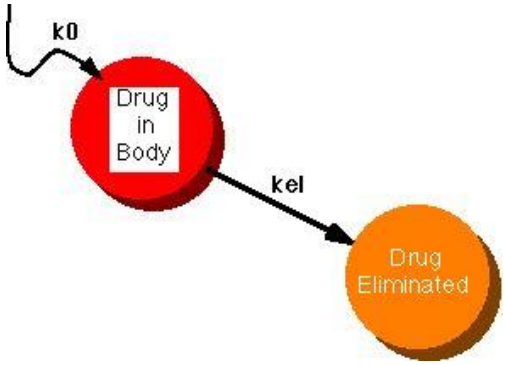


*Figure 3.4.1(1). Scheme for one compartment Intravenous Infusion*

Here we have added an infusion rate constant, k0 to the diagram presented earlier. This is a zero order process so the units of k0 would be amount per time; for example 25 mg/min.

## 3.4.2 Combined Infusion and bolus administration:

One reason we give a drug by IV infusion of course is because we need a quick therapeutic response. One way to achieve a therapeutic concentration more quickly is to give a loading dose by rapid intravenous injection and then start the slower maintenance infusion.

For example, theophylline again.

To achieve $Cp^{ss}$ = 14.1 mg/L; k0 = 60 mg/hr; V = 25 L; kel = 0.17 $hr^{-1}$.

A loading dose can be calculated from

$$Cp^0 = \frac{DOSE}{V}$$ (Equation VI-4)

thus

$DOSE = V * Cp^0 = 25 \times 14.1 = 353$ mg

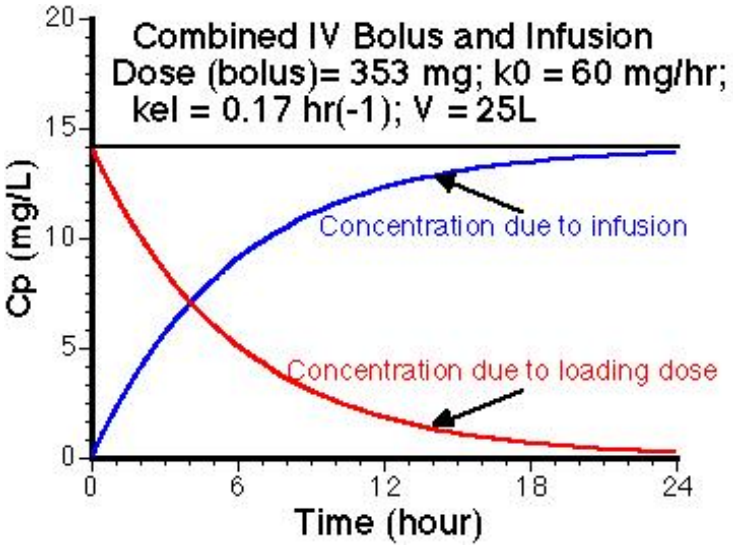


*Figure 3.4.2(1). Linear Plot of Cp versus Time Showing Bolus, Infusion, and Combined Curves*

An IV bolus and maintenance infusion is one way to achieve a steady state plasma concentration rapidly and maintain it. However, we may not be able to give a bolus dose intravenously…

## 3.4.3 Combined Slow and Fast infusion:

Alternately we can give a loading dose by rapid infusion and then give a slower maintenance infusion once the plateau concentration is achieved.

For example. Using the previous data

For theophylline, kel = 0.17 $hr^{-1}$; V = 25 L; with a required Cp = 14.1 mg/L

If we wish to give a loading infusion over 30 minutes we need to give the infusion at a rate which will produce Cp = 14.1 mg/L at 30 minutes. Therefore:-

$Cp^{30\ min}$ = 14.1 mg/L

$$Cp = \frac{k0}{V \cdot kel}\left[1 - e^{-kel \cdot t}\right]$$

(Equation VI-2 above)

$$14.1 = \frac{k0}{0.17 \times 25} \cdot \left[1 - e^{-0.17 \times 0.5}\right]$$

thus

k0 = 735 mg/hr

Therefore we need to give a dose of 367 mg over 30 minutes to achieve a plasma concentration of 14.1 mg/L at 30 minutes. It is important to realize what the steady state plasma concentration would be if we didn't turn this fast infusion off.

$$Cp^{ss} = \frac{k0}{kel \cdot V} = \frac{735}{0.17 \times 25} = 173\ mg/L$$

which would be quite toxic.

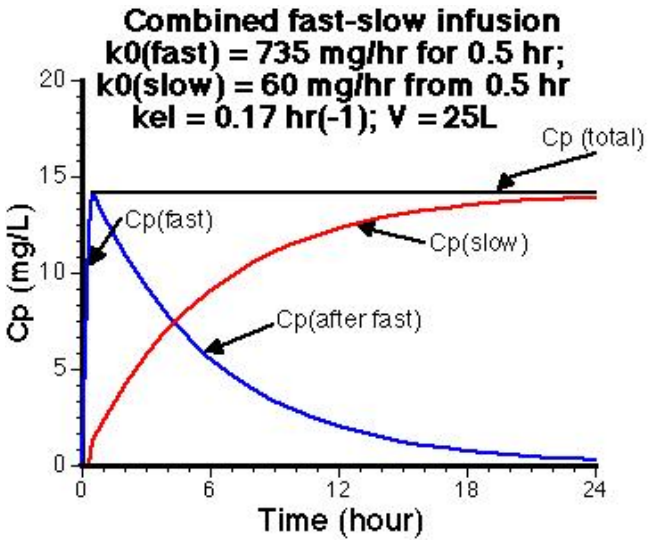


*Figure 3.4.3(1). Linear Plot of Cp versus Time showing Combined and Separate Curves for Both Infusions*

Consequently we would need to ensure that at 30 minutes the rapid infusion rate was slowed from 735 mg/hr to 60 mg/hr. One way to do this is to only provide 367 mg (or 360 mg) in the infusion syringe at first.

The dosing regimen (or controlled sequence of drug administration) to achieve the desired plasma concentration is:-

a) a loading dose by IV infusion of 367 mg/30 minutes followed by

b) a maintenance IV infusion of 60 mg/hr

## 3.4.4 Post infusion:

Before moving on to discussing various routes of drug administration, we can look at the equation for plasma concentration after an infusion is stopped. Remember that the equation for plasma concentration versus time during an IV infusion is:-

$$Cp = \frac{k0}{V \cdot kel} \cdot \left[1 - e^{-kel \cdot t}\right]$$

If the infusion is continued indefinitely then the plasma concentration approaches a steady state plasma concentration.

$$Cp^{ss} = \frac{k0}{kel \cdot V}$$

If however the infusion is stopped the plasma concentration can be expected to fall.

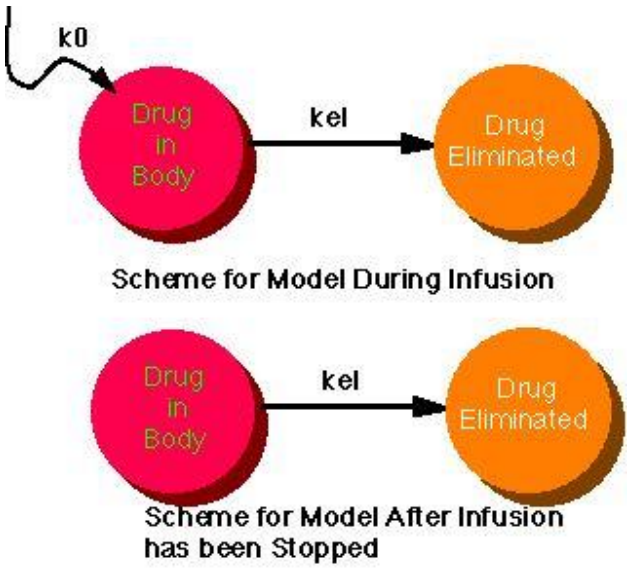


*Figure 3.4.4(1). During and After an IV Infusion - One Compartment Model*

The scheme shown to represent 'after the infusion is stopped' is the same as that for the bolus injection.

# 3.5 Routes of Drug Administration:

An alternate method of classifying these routes of administration is ENTERAL and PARENTERAL. Enteral means to do with the GI tract and includes oral, buccal, and rectal. Parenteral means not through the alimentary canal and commonly refers to injections such as IV, IM, and SC; but could also include topical and inhalation. We can also distinguish IV from the rest, as with all others at least one membrane must be crossed, thus an absorption process is involved in the administration and the pharmacokinetics.

## 3.5.1 Buccal / Sublingual:

Some drugs are taken as smaller tablets which are held in the mouth or under the tongue. These are buccal or sublingual dosage forms. Buccal tablets are often harder tablets [4 hour disintegration time], designed to dissolve slowly. Nitroglycerin, as a softer sublingual tablet [2 min disintegration time], may be used for the rapid relief of angina. This ROA is also used for some steroids such as testosterone and oxytocin. Nicotine containing chewing gum may be used for cigarette smoking replacement.

### 3.5.1.1 Advantages:

First pass - The liver is by-passed thus there is no loss of drug by first pass effect for buccal administration. Bioavailability is higher.

Rapid Absorption - Because of the good blood supply to the area absorption is usually quite rapid.

Drug Stability - pH in mouth relatively neutral (cf. stomach - acidic). Thus a drug may be more stable.

### 3.5.1.2 Disadvantages:

Holding the dose in the mouth is inconvenient. If any is swallowed that portion must be treated as an oral dose and subject to first pass metabolism.

Small doses only can be accommodated easily.

## 3.5.2 Rectal:

Most commonly by suppository or enema. Some drugs given by this route include aspirin, theophylline, chlorpromazine and some barbiturates.

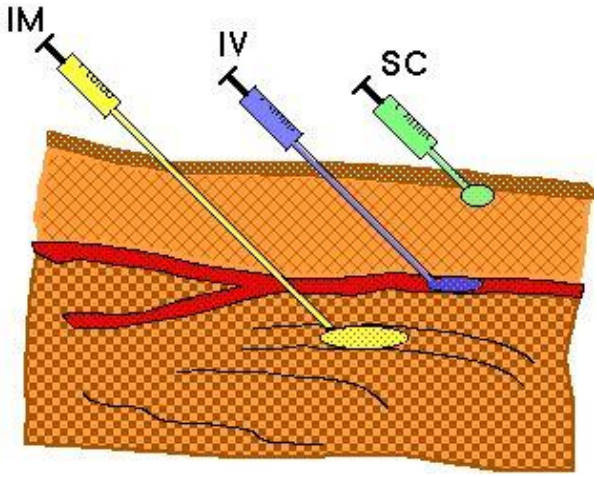


*Figure 3.5.2(1). Showing IV, IM, and SC Injection*

Drugs may be given into a peripheral vein over 1 to 2 minutes or longer by infusion. Rapid injections are used to treat epileptic seizures, acute asthma, or cardiac arrhythmias. Drugs may be given into a peripheral vein over 1 to 2 minutes or longer by infusion. Rapid injections are used to treat epileptic seizures, acute asthma, or cardiac arrhythmias

### 3.5.2.1 Advantages:

By-pass liver - Some of the veins draining the rectum lead directly to the general circulation, thus by-passing the liver. Reduced first-pass effect.

Useful - This route may be most useful for patients unable to take drugs orally or with younger children.

### 3.5.2.2 Disadvantages:

Erratic absorption - Absorption is often incomplete and erratic. However for some drugs it is quite useful. There is research being conducted to look at methods of improving the extent and variability of rectal administration.

### 3.5.3 Itravenous:

Drugs may be given into a peripheral vein over 1 to 2 minutes or longer by infusion. Rapid injections are used to treat epileptic seizures, acute asthma, or cardiac arrhythmias.

#### 3.5.3.1 Advantages:

Rapid - A quick response is possible.

Total Dose - The whole dose is delivered to the blood stream. Large doses can be given by extending the time of infusion.

Veins Relatively Insensitive - to irritation by irritant drugs at higher concentration in dosage forms.

#### 3.5.3.2 Disadvantages:

Suitable Vein - It may be difficult to find a suitable vein.

Maybe Toxic - Because of the rapid response, toxicity can be a problem with rapid drug administrations, could then give as an infusion, monitoring for toxicity.

Requires Trained Personnel - Trained personnel are required to give intravenous injections.

Expensive - Sterility, pyrogen testing and larger volume of solvent means greater cost for preparation, transport and storage.

### 3.5.4 Subcutaneous:

This involves administration of the drug dose just under the skin.

#### 3.5.4.1 Advantages:

It can be given by patient, e.g. in the case of insulin.

Absorption slow but usually complete.

Improved by massage or heat.

Vasoconstrictor may be added to reduce the absorption of a local anesthetic agent, thereby prolonging its effect at the site of interest.

#### 3.5.4.2 Disadvantages:

It can be painful.

Irritant drugs can cause local tissue damage

Maximum of 2 ml injection thus often small doses limit use.

## 3.5.5 Intramuscular:

### 3.5.5.1 Advantages:

Larger volume, than sc, can be given by IM.

A depot or sustained release effect is possible with IM injections, e.g. procaine penicillin

### 3.5.5.2 Disadvantages:

Trained personnel required for injections. The site of injection will influence the absorption, generally the deltoid muscle is the best site

Absorption is sometimes erratic, especially for poorly soluble drugs, e.g. diazepam, phenytoin. The solvent maybe absorbed faster than the drug causing precipitation of the drug at the site of injection.

## 3.5.6 Inhalation:

Local effect – bronchodilators.

Systemic effect - general anesthesia.

Rapid absorption, by-passing the liver.

Absorption of gases is relatively efficient, however solids and liquids are excluded if larger than 20 micron and even then only 10 % of the dose may be absorbed. Cromolyn is taken as a powder with 50 % of the particles within the range of 2 to 6 micron. Larger than 20 micron and the particles impact in the mouth and throat. Smaller than 0.5 micron and they aren't retained.

## 3.5.7 Topical:

Local effect - eye drops, antiseptic, sunscreen, callous removal, etc.

Systemic effect - e.g., nitroglycerin ointment.

Absorption through the skin, especially via cuts and abrasions but also intact, can be quite marked. This can be a real problem in handling toxic materials in the laboratory or pharmacy.

### 3.5.8 Other ROA's:

Other routes of administration include: intra-nasal, some systemic absorption has been demonstrated for propranolol and some low dose hormones; intra-arterial for cancer chemotherapy to maximize drug concentrations at the tumor site; and intrathecal directly into the cerebrospinal fluid.

## 3.6 Pharmacokinetics of Oral Administration:

So far we have considered the pharmacokinetics of intravenously administered drugs, either as a bolus or by infusion. If we know kel and V for a particular patient we can calculate appropriate doses or dosing rates (infusion rates) to produce the necessary therapeutic concentrations.

Most of the routes of administration were extravascular; for example IM, SC, and most importantly oral. With this type of drug administration the drug isn't placed in the central compartment but must be absorbed through at least one membrane. This has a considerable effect on drug pharmacokinetics and may cause a reduction in the actual amount of drug which is absorbed. Most commonly the absorption process follows first order kinetics. Even though many oral dosage forms are solids, which must dissolve before being absorbed, the overall absorption process can often be considered to be a single first order process. At least that's the assumption we will use for now. Schematically this model can be represented as:

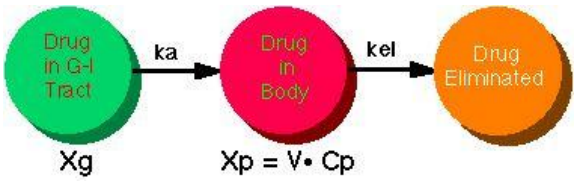


*Figure 3.6(1). Representing Oral Administration, One Compartment Pharmacokinetic Model*

Where Xg is the amount of drug to be absorbed, Xp is the amount of drug in the body, and ka is the first order absorption rate constant.

### 3.6.1 Differential Equation:

The differential equation for Xg is :-

$$\frac{dXg}{dt} = -ka \cdot Xg$$

This is similar to the equation for $\frac{dCp}{dt}$ after an IV bolus administration.

The integrated equation is:-

$Xg = Xg^0 * e^{-ka * t} = F * Dose * e^{-ka * t}$

where F is the fraction of the dose which is absorbed, the bioavailability.

We could therefore plot Xg (the amount remaining to be absorbed) versus time on semi-log graph paper and get a straight line with a slope of -ka.

For Xp ( = V * Cp) the amount of drug in the body, the differential equation is :-

$$\frac{dXp}{dt} = \frac{V \cdot dCp}{dt} = ka \cdot Xg - kel \cdot V \cdot Cp$$

The first term --> ka * Xg absorption

The second term --> kel * V * Cp elimination

Even without integrating this equation we can get an idea of the plasma concentration time curve.

At the start Xg >> V * Cp therefore the value of $V \cdot \frac{dCp}{dt}$ is positive, the slope will be positive and Cp will increase. With increasing time Xg will decrease, while initially Cp is increasing, therefore there will be a time when ka * Xg = kel * V * Cp. At this time $V \cdot \frac{dCp}{dt}$ will be zero and there will be a peak in the plasma concentration. At even later times Xg --> 0, and $V \cdot \frac{dCp}{dt}$ will become negative and Cp will decrease. The plasma concentration time curve will look like below figure:

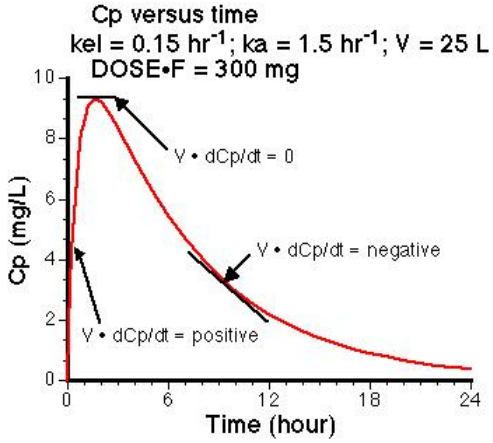


*Figure 3.6.1(1). Linear Plot of Cp versus Time after Oral Administration Showing Rise, Peak, and Fall in Cp*

## 3.6.2 Integrated Equation:

We can calculate the line using the integrated form of the equation. Starting with the differential equation we can substitute Xg = Xg *$e^{-ka * t}$. The integration process won't be described here but a reference, which describes the Laplace transform method of integration can be presented. This method makes integration as *easy* as the logarithmic transform makes multiplication and division easier.

If we use F * DOSE for $Xg^0$ where F is the fraction of the dose absorbed, the integrated equation for Cp versus time is :-

$$Cp = \frac{F \cdot DOSE \cdot ka}{V \cdot (ka - kel)} \cdot \left[ e^{-kel \cdot t} - e^{-ka \cdot t} \right]$$

CONSTANT x Difference between two exponential terms

Notice that the right hand side of this equation is a constant multiplied by the difference of two exponential terms. We can plot Cp as a constant times the difference between two exponential curve. If we plot each exponential separately.

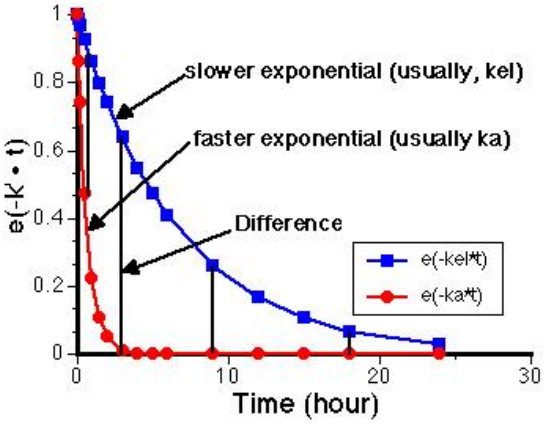


*Figure 3.6.2(1). Linear Plot of $e^{-ka \times t}$ versus Time for Two Exponential Terms*

Notice that the difference starts at zero, increases, and finally decreases again.

Plotting this difference by $\frac{F \cdot DOSE \cdot ka}{V \cdot (ka - kel)}$ gives Cp versus time.

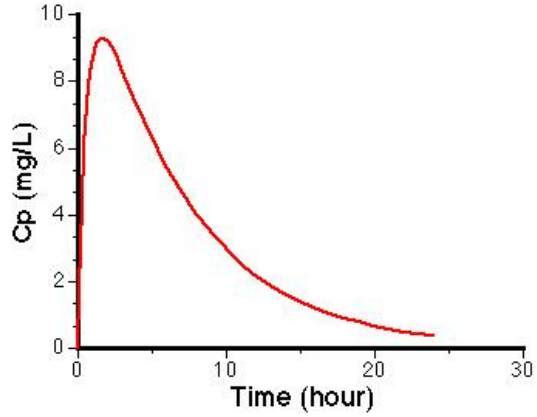


*Figure 3.6.2(2). Linear Plot of Cp versus Time*

We can calculate the plasma concentration at anytime if we know the values of all the parameters of Equation.

F, DOSE, ka — Dose and dosage form parameters

kel, V — Drug and patient parameters

We can also calculate the time of peak concentration using the equation:-

$$t_{peak} = \frac{1}{(ka - kel)} \cdot \ln\left(\frac{ka}{kel}\right)$$

As an example we could calculate the peak plasma concentration given that F = 0.9, DOSE = 600 mg, ka = 1.0 $hr^{-1}$, kel = 0.15 $hr^{-1}$, and V = 30 liter.

$$t_{peak} = \frac{1}{(1-0.15)} \times \ln\left(\frac{1}{0.15}\right) = 2.23 \text{ hour}$$

$$Cp = \frac{0.9 \times 600 \times 1}{30 \times (1 - 0.15)} \times \left[ e^{-0.15 \times 2.23} - e^{-1 \times 2.23} \right]$$

= 21.18 x [ 0.7157 - 0.1075] = 12.9 mg/L

As another example we could consider what would happen with ka = 0.2 $hr^{-1}$ instead of 1.0 $hr^{-1}$

$$t_{peak} = \frac{1}{(0.2-0.15)} \times \ln\left(\frac{0.2}{0.15}\right) = 5.75 \text{ hour}$$

$$Cp = \frac{0.9 \times 600 \times 0.2}{30 \times (0.2 - 0.15)} \times \left[ e^{-0.15 \times 5.75} - e^{-0.2 \times 5.75} \right]$$

= 72 x (0.4221 - 0.3166) = 7.6 mg/L lower and slower than before

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## 3.6.3 Absorption Rate Constant, ka:

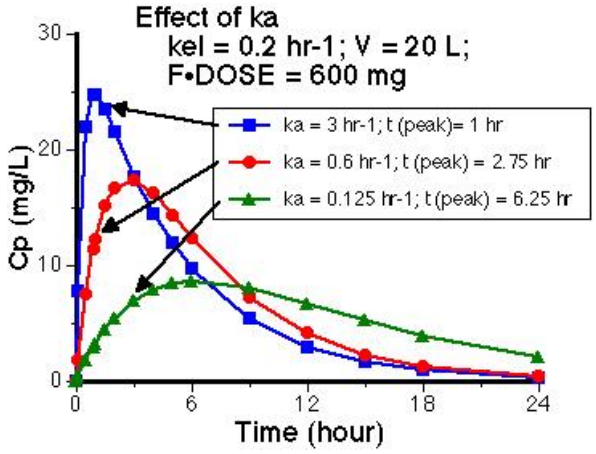



*Figure 3.6.3(1). Linear Plot of Cp versus Time with ka = 3, 0.6, or 0.125 hr sup>-1*

Before going on to calculate the parameters ka, kel, and F from data provided we can look at the effect different values of F and ka have on the plasma concentration versus time curve.

tpeak = 1, 2.75, 6.25 hour.

Notice that with higher values of ka the peak plasma concentrations are higher and earlier.

## 3.6.4 Extent of Absorption:

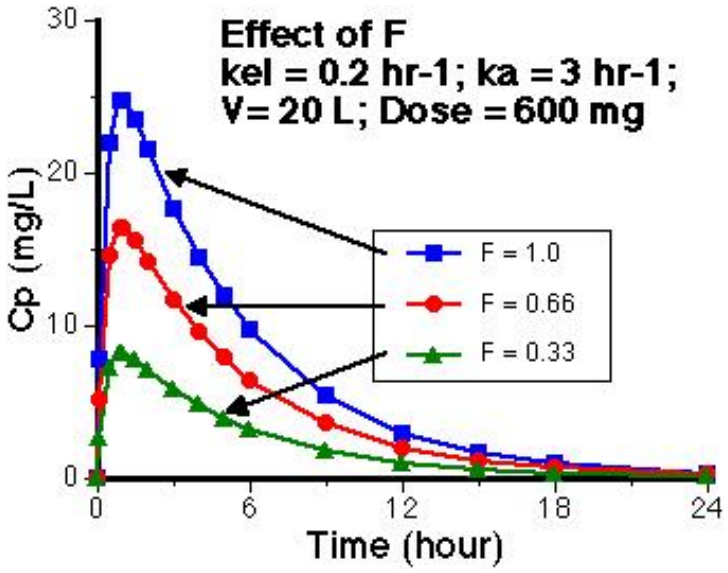



*Figure 3.6.4(1). Linear Plot of Cp versus Time with F = 1, 0.66, or 0.33*

Changing F values is equivalent to changing the dose. Thus the higher the F value the higher the concentration values at each time point. Since the values of kel and ka are unchanged the time of peak plasma concentration is unchanged.

Thus, tpeak = 1, 1, and 1 hour. The same in each case.

## 3.6.5 Calculation of Bioavailability Parameters:

On most occasions you will be able to get the parameter values from tables and references. However, you should also know how to get these values from the data. The two parameters we will concentrate on are ka and F. These values can be used to compare dosage forms or brands. In the previous Chapter we saw the effect changing ka or F has on the plasma concentration time curve.

## 3.6.6 Calculation of ka and F:

Starting with the equation for Cp versus time

$$Cp = \frac{F \cdot DOSE \cdot ka}{V \cdot (ka - kel)} \cdot \left[e^{-kel \cdot t} - e^{-ka \cdot t}\right]$$

this can be written as $Cp = A * e^{-kel * t} - A * e^{-ka * t}$

where $A = \frac{F \cdot DOSE \cdot ka}{V \cdot (ka - kel)}$

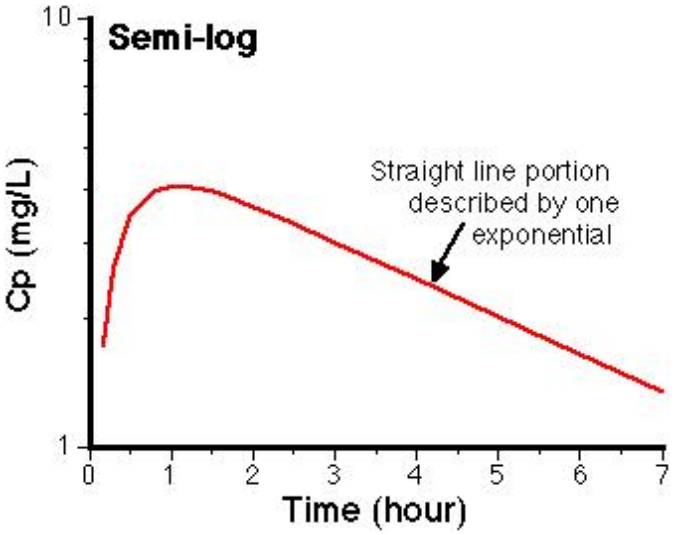



*Figure 3.6.6(1). Semi-log Plot of Cp versus Time Showing a Straight Line at Longer Time*

If one of the rate constants (ka or kel) is much larger than the other, the method works best if the difference is at least five times, then the faster differential will approach zero more quickly, and at later times can be ignored. If we plot Cp versus time on semi-log graph paper we will see that the slope will approach a straight line.

The equation for this straight line portion can be obtained from the equation for Cp by setting the faster term (usually $e^{-ka*t}$) to zero:

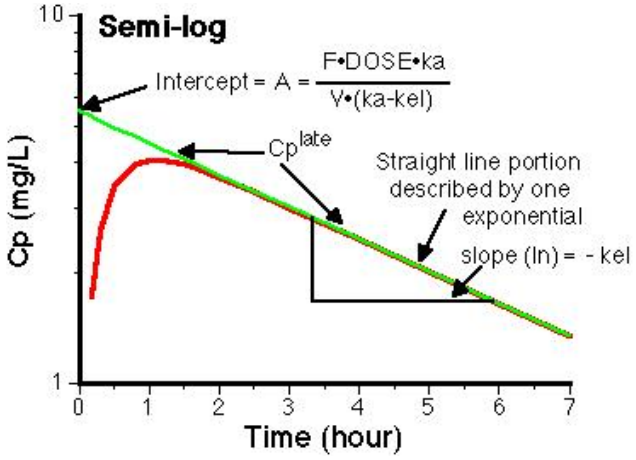



*Figure 3.6.6(2). Semi-log Plot of Cp versus Time Showing Cplate, Slope, and Intercept*

Then $Cp^{late} = A * e^{-kel * t}$ and plotting $Cp^{late}$ *versus* time gives a straight line on semi-log graph paper, with a slope (ln) = -kel and intercept = A.

Now

$$Cp = A \cdot e^{-kel \cdot t} - A \cdot e^{-ka \cdot t}$$

$Cp^{late}$

therefore

$$Cp^{late} - Cp = A \cdot e^{-ka \cdot t}$$

Difference or Residual

Plotting the ln (Residual) versus time should give another straight line graph with a slope (ln) equal to - ka and the same intercept as before, i.e. A

ln (Residual) = ln (A) - ka * t

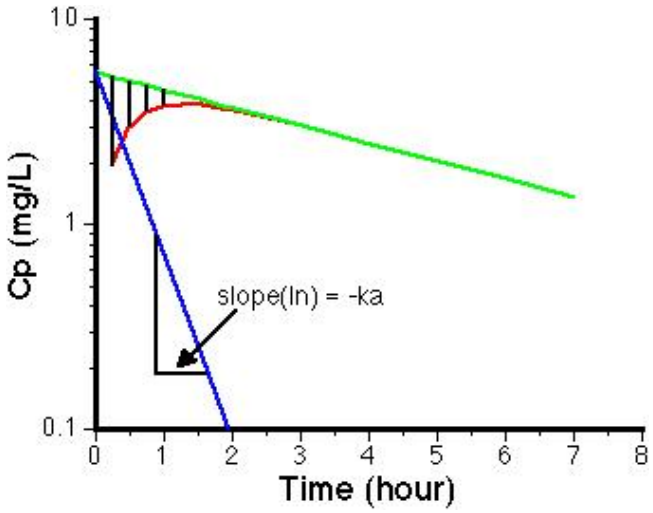



*Figure 3.6.6(3). Semi-log Graph of Cp versus Time Showing Residual Line*

This is the method of residual or "feathering". It can give quite accurate values of kel, ka, and V/F if :

- One rate constant is at least five times larger than the other and
- Both absorption and elimination are first order processes.

| Time (hr) | Plasma Concentration (mg/L) | Cp(late) (mg/L) | Residual [Col3 - Col2] (mg/L) |
|---|---|---|---|
| 0.25 | 1.91 | 5.23 | 3.32 |
| 0.5 | 2.98 | 4.98 | 2.00 |
| 0.75 | 3.54 | 4.73 | 1.19 |
| 1.0 | 3.80 | 4.50 | 0.70 |
| 1.5 | 3.84 | 4.07 | 0.23 |
| 2.0 | 3.62 | 3.69 | 0.07 |
| 3.0 | 3.04 | | |
| 4.0 | 2.49 | | Residual |
| 5.0 | 2.04 | $Cp^{late}$ | $= 5.5 * e^{2.05 * t}$ |
| 6.0 | 1.67 | $= 5.5 * e^{0.2 * t}$ | |
| 7.0 | 1.37 | | |

*Table 3.6.6(1). Cp and Residual versus Time*

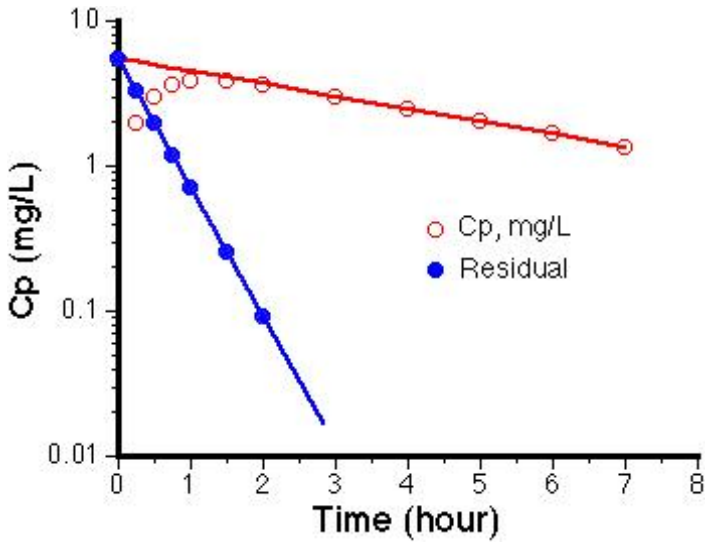



*Figure 3.6.6(4). Plot of Cp and Residual versus Time*

# 3.7 Bioavailability Studies:

Studies, which are carried to evaluate different dosage forms, are the topic of this Chapter. These studies called bioavailability or drug product evaluation studies might compare:-

i) one type of dosage form with another, e.g. regular tablet with sustained release tablet. For this type of tablet ka values should be slower; but F values should be similar or

ii) two (or more) dosage forms made by two different manufacturers.

Second brand or generic drug manufacturers are required to prove that their product is equivalent to previously marketed products which have demonstrated clinically efficacy. For most drugs, the second and subsequent manufacturer must show that their product is bioequivalent, i.e. same ka and F, as the product(s) on the market. During the development of new drugs and drug products, the original manufacturer will also perform bioavailability studies on new products, probably comparing the product to be marketed with an intravenous dosage form if at all possible.

## 3.7.1 Definition:

### 3.7.1.1 Bioavailability:

It indicates a measurement of the rate and extent (amount) of therapeutically active drug which reaches the general circulation.

### 3.7.1.2 Bioequivalent Drug Products:

*Bioequivalent Drug Products* means pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose of the therapeutic moiety under similar experimental conditions, either single dose or multiple doses.

Some pharmaceutical equivalents or pharmaceutical alternatives may be equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on chronic use, or are considered medically insignificant for the particular drug product studied.

### 3.7.1.3 Bioequivalence Requirement:

*Bioequivalence Requirement* means a requirement imposed by the Food and Drug Administration for the in vitro and/or in vivo testing of specified drug products which must be satisfied as a condition of marketing.

### 3.7.1.4 Brand Name:

*Brand Name* is the trade name of the drug.

### 3.7.1.5 Chemical Name:

*Chemical Name* is the name used by the organic chemist to indicate the chemical structure of the drug.

### 3.7.1.6 Drug Product:

*Drug Product* means a finished dosage form, e.g., tablet, capsule, or solution, that contains the active drug ingredient, generally, but not necessarily, in association with inactive ingredients.

### 3.7.1.7 Generic Name:

*Generic Name* is the established, non proprietary or common name of the active drug in a drug product.

### 3.7.1.8 Pharmaceutical Alternatives:

*Pharmaceutical Alternatives* means drug products that contain the identical therapeutic moiety, or its precursor, but not necessarily in the same amount or dosage form or as the same salt or ester. Each such drug product individually meets either the identical or its own respective compendial or other applicable standard of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times and/or dissolution rates.

### 3.7.1.9 Pharmaceutical Equivalent:

*Pharmaceutical Equivalent* means drug products that contain identical amounts of the identical active drug ingredient, i.e., the salt or ester of the same therapeutic moiety, in identical dosage forms, but not necessarily containing the same inactive ingredients, and

that meet the identical compendial or other applicable standard of identity, strength, quality, and purity, including potency and where applicable, content uniformity, disintegration times and/or dissolution rate.

## 3.7.2 Past Bioavailability Problems:

There are a number of examples of drugs products which have exhibited bioavailability problems in the past. These examples are all pre-1976 and as mentioned in the text were included in the earlier edition of the book with no further examples reported. This is an indication that more attention is now being given to formulation development during drug development.

### 3.7.2.1 Chlorpropamide:

*Chlorpropamide.* With three products tested the peak plasma concentration after one brand was less than 1/2 the peak after the other two products.

### 3.7.2.2 Digoxin:

*Digoxin.* The text reports a number of bioavailability problems with digoxin. One example is particularly interesting. Doctors in Israel noticed 15 cases of digoxin toxicity between Oct/Dec 1975 with almost no reports for the same period the previous year. It was found that the local manufacturer had changed the formulation to improve dissolution without telling the physicians. Urinary data suggested a two-fold increase in availability of the new formulation.

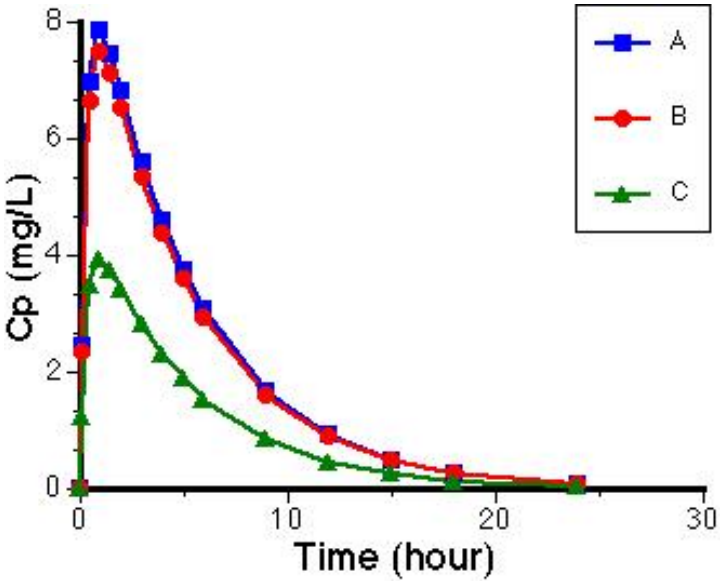



*Figure 3.7.3(1). Plot of Cp versus Time*

### 3.7.2.3 Phenytoin:

Again there are a number of examples in the text. One report described an incidence of phenytoin intoxication in Australia in 1968 and 1969. Apparently the tablet diluent was changed from calcium sulfate to lactose.

## 3.7.3 Reasons for Bioequivalence Requirements:

The FDA may decide to require bioavailability studies for a variety of reasons including:

- Results from clinical studies indicate that different drug products produce different therapeutics results.
- Results from bioavailability studies indicate that different products are not bioequivalent.
- Drug has a narrow therapeutic range.
- Low solubility and/or large dose.
- Absorption is considerably less than 100%

## 3.7.4 Bioavailability Study Characters:

With recently introduced products properly conducted bioavailability studies will have been performed before the product is allowed to be marketed. However products which were approved some time ago may not have been tested as thoroughly. It is therefore

helpful to be able to evaluate the testing which may have been undertaken. There are a number of situations where a pharmacist is required to evaluate bioavailability study testing. When selecting drug products for a prescription, product performance should be most important criteria. Once it is established that two or more products are equivalent, then the choice of brand can be made on the basis of economic factors, cost etc.

The evaluation of a drug product bioavailability study involves the consideration of various factors.

### 3.7.4.1 Drug:

The drug substance in each product must be the same. Bioavailability studies are conducted to compare two or more products containing the same chemical substance. We can't compare different chemical substances. The apparent volume of distribution and kel can be quite different for different drug substances, thus no interpretation of the results is possible. The first rule of bioavailability testing is that you compare the drug products with the same drug in each dosage from.

The only time that this rule is relaxed is in the case of pro-drug administration. A pro-drug is a compound which will form the drug of interest in the body. In this case it may be appropriate to compare the delivery of a dosage form containing the drug with another dosage form containing a pro-drug. This testing is generally conducted to evaluate the usefulness of the pro-drug, rather than a strict comparison of the drug products. Once the usefulness of the pro-drug is demonstrated comparisons between dosage forms all containing the pro-drug should be undertaken to evaluate the drug product performance.

### 3.7.4.2 Drug Product:

Usually the comparison is made between two (or more) similar products, containing exactly the same chemical substance. However, different dosage forms can be compared (when they contain the same drug). For example we could compare an IM dosage form with an IV one.

By calculating the AUC values we can determine the absolute bioavailability of the IM dosage form. In this case it appears to be close to 100%. The rate of absorption for the IM dose can be determined also, but of course no comparison is possible.

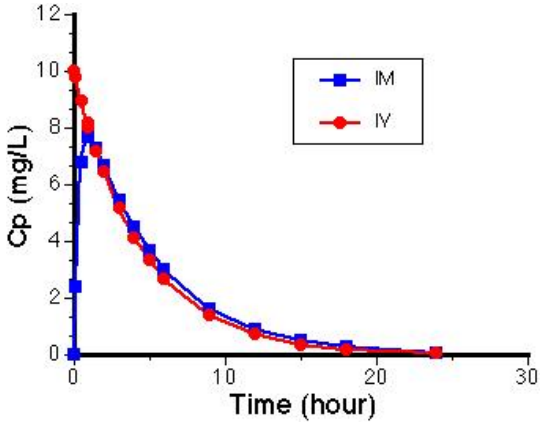



*Figure 3.7.4.2(1). Plot of Cp versus Time after IV and IM Administration.*

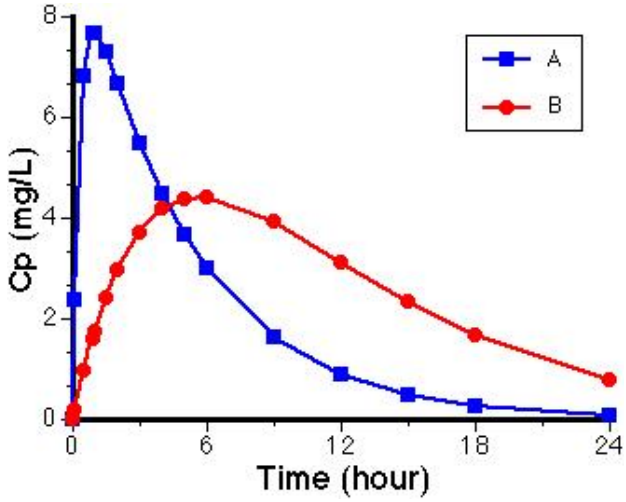



*Figure 3.7.4.2(2). Plot of Cp versus Time for A and B with B having Slower Absorption*

Alternately we could compare brand A tablet with brand B tablet or capsule. By comparing the AUC values and ka values we can make comparisons concerning both the extent and rate of absorption. In this case A appears to be faster than B but the extent of absorption doesn't appear to be all that different.

### 3.7.4.3 Subject:

A number of factors are of concern; health, age, weight, enzyme status, number.

### 3.7.4.4 Health:

Usually a study is designed so that each subject takes each product in turn. Thus the effect of the individual subject can be eliminated or reduced. Such a study design is called a

cross- over design. Even though each subject will act as their own control it is usually best to have subjects of similar kinetic characteristic so that major variations are not introduced. Thus healthy volunteers are often preferred by drug product evaluation studies. Informed consent should be obtained from each volunteer and some biochemical and medical examination will be used to confirm their medical state. For some drugs there may be special disease states which may cause the exclusion of some volunteers. For example, in one study we looked at propranolol products, and otherwise healthy volunteers with a past history of asthma were excluded from this study.

#### 3.7.4.5 Age:

As you will see later, age can have a significant effect on drug pharmacokinetics. Elderly patients and young children can have quite different kinetics compared with young adults. In the interest of a better matched group, subjects between the ages of 18 to 35 years are preferred. Kinetic changes usually aren't important until age greater than 60.

#### 3.7.4.6 Weight:

The apparent volume of distribution is usually proportional to weight in subjects of normal weight for height. However, in overweight (or underweight) subjects the V in L/kg maybe somewhat different. Again to better match the subjects, normal weights are preferred.

#### 3.7.4.7 Enzyme Status:

Smokers or subjects taking certain other drugs may have altered kinetics for the drug of interest. This can be caused by alteration of enzyme activity or by drug-drug interactions. These effects add complications to a study and an attempt is usually made to minimize these factors.

#### 3.7.4.8 Number:

The number of subjects included in the study should be sufficient to see any real (maybe 20% variation) differences in bioavailability. Usually 10 - 20 subjects are used in these studies. In clinical studies where the end-point is some clinical response, much larger numbers are required because of the greater variability in clinical response.

### 3.7.4.9 Assay:

The same assay method should be used for all phases of the study. It is not much use using one assay for product A samples and another assay for product B samples. This wouldn't be done in a single study; however, if you were trying to compare the results from one study with those from another, different assay methods may have been used. One assay method may pick up an interference which is not indicative of the drug concentration or the bioavailability. Also the assay method should be sensitive and specific.

### 3.7.4.10 Design:

*Design:* Usually a complete cross-over design is used. With this design each subject receives all products with a wash-out period between each dose administration.

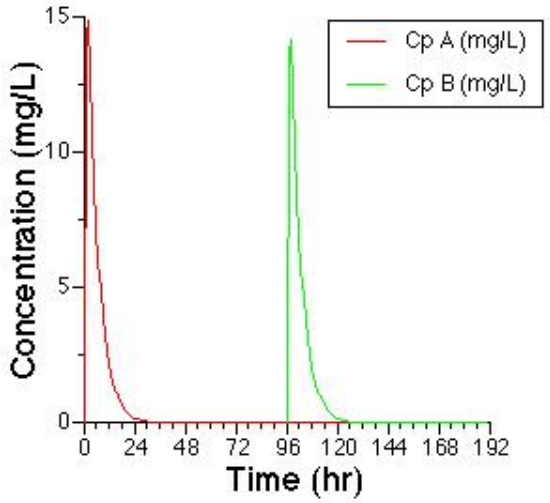



*Figure 3.7.4.10(1). Figure Showing Concentrations after two Separate Drug Administrations*

| | Week 1 | Week 2 | |
|---|---|---|---|
| Group 1 | A | B | |
| Group 2 | B | A | for two products |

*Table 3.7.4(1). Two Product Example*

| | Week 1 | Week 2 | Week 3 | |
|---|---|---|---|---|
| Group 1 | A | B | C | |
| Group 2 | B | C | A | |
| Group 3 | C | A | B | |
| Group 4 | A | C | B | |
| Group 5 | C | B | A | |
| Group 6 | B | A | C | for three products |

*Table 3.7.4(2). Three Product Examples*

When more than 3 or 4 products are involved it has been suggested that a different design is used whereby each subject will get maybe 3 or 4 products of a possible 8 to 12. This type of design, possibly an incomplete block design, usually requires more subjects to get the same information, but it does mean that each subject is not required to take as many doses. It is harder to recruit subjects for longer studies.

### 3.7.4.11 Data Analysis:

The rate of absorption can be characterized by the ka value and also the time of peak concentration. The extent of drug absorption is characterized by the F value or the peak concentration or total AUC values. Any differences in the average values of these parameters can then be analyzed statistically to determine the significance of the differences.

The 5 % confidence level is usually used as the criteria of acceptance. The analysis of variance is a technique for separating the effect of product, subject, and sequence. The significance of each of these factors can be tested.

| Source of Variation | d.f. | SS | MS | F | Significance Level |
|---|---|---|---|---|---|
| Total | 35 | 44.6 | - | - | - |
| Subject | 11 | 28.3 | 2.58 | 10.1 | $p < 0.001$ |
| Week | 2 | 0.14 | 0.068 | 0.27 | n.s. |
| Treatment | 2 | 11.0 | 5.55 | 21.8 | $P < 0.001$ |
| Residual | 20 | 5.09 | 0.255 | - | - |

*Table 3.7.4(3). Analysis of Variance Table for Three-Way Cross-Over Study*

In this example two effects are significant. There appears to be a significant effect due to treatment and subject. This would indicate that the subjects are significantly different from each other and that the treatments are significantly different in terms of the parameter measured. It is quite common that Cp or AUC values are significantly different for different subjects, because of their different weights or size. The different treatments would appear to be bio-inequivalent.

## 3.8 Physiological Factors Affecting Oral Absorption:

Looking briefly at the overall picture of drug absorption, distribution, and elimination.

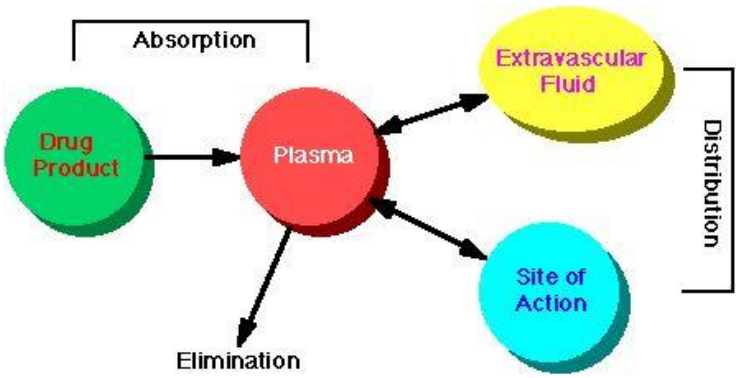



*Figure 3.8(1). Scheme of ADME processes*

The ultimate goal is to have the drug reach the site of action in a concentration which produces a pharmacological effect. No matter how the drug is given (other than I.V.) it must pass through a number of biological membranes before it reaches the site of action.

### 3.8.1 Membrane Physiology

#### 3.8.1.1 Membrane Structure:

In 1900 Overton performed some simple but classic experiments related to membrane structure. By measuring the permeability of various types of compounds across the membranes of a frog muscle he found that lipid molecules could readily cross this membrane, larger lipid insoluble molecules couldn't and small polar compounds could slowly cross the membrane.

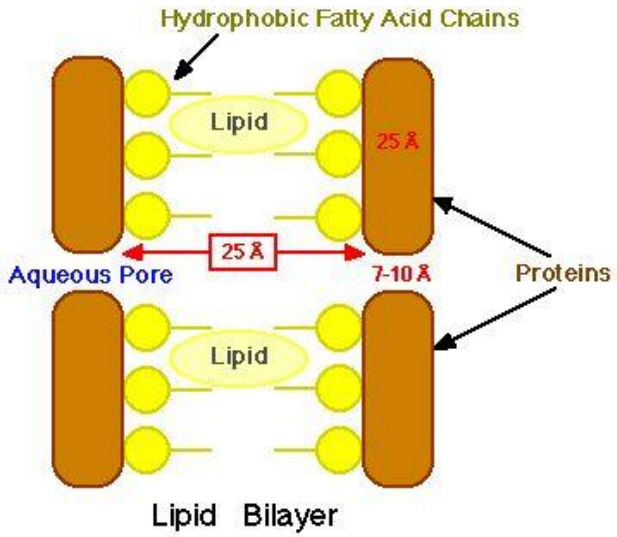



*Figure 3.8.1.1(1). The Davson-Danielli Model*

These results suggest that the biologic membrane is mainly lipid in nature but contains small aqueous channels or pores. Other experiments involving surface tension measurements have suggested that there is also a layer of protein on the membrane. These results and others have been incorporated into a general model for the biological membrane. This is the Davson-Danielli model.

The membrane then acts as a lipid barrier with small holes throughout.

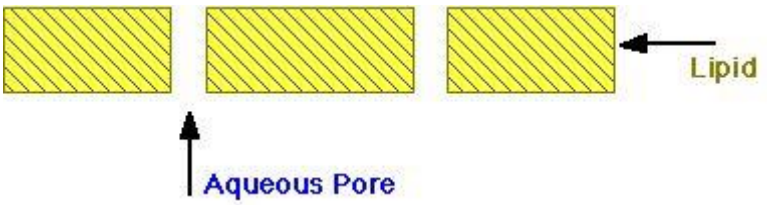



*Figure 3.8.1.1(2). Simplified Model of Membrane*

This is the general structure. Membranes in different parts of the body have somewhat different characteristics which influence drug action and distribution. In particular, pore size and pore distribution is not uniform between different parts of the body. Various examples of some membrane types are as below:

Blood-brain Barrier:

The membranes between the blood and brain have effectively no pores. This will prevent many polar materials (often toxic materials) from entering the brain. However,

smaller lipid materials or lipid soluble materials, such as diethyl ether, halothane, can easily enter the brain. These compounds are used as general anesthetics.

Renal Tubules:

In the kidney there are a number of regions important for drug elimination. In the tubules drugs may be reabsorbed. However, because the membranes are relatively non-porous, only lipid compounds or non-ionized species (dependent of pH and pKa) are reabsorbed.

Blood Capillaries and Renal Glomerular Membranes:

These membranes are quite porous allowing non-polar and polar molecules (up to a fairly large size, just below that of albumin, M.Wt 69,000) to pass through. This is especially useful in the kidney since it allows excretion of polar (drug and waste compounds) substances.

### 3.8.1.2 Transport across the Membranes

### Carrier mediated:

(1) Active

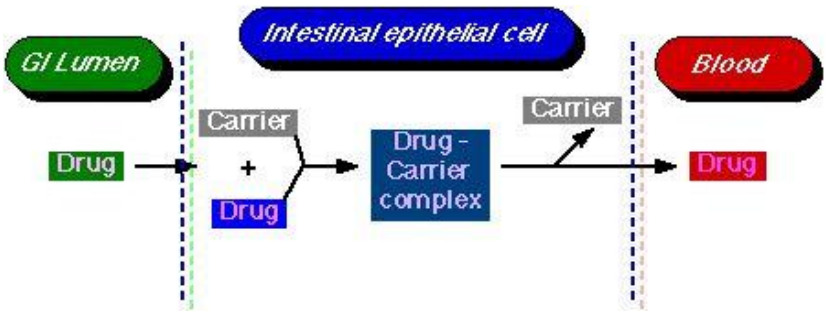



*Figure 3.8.1.2(1). Carrier-Mediated Transport Process*

The body has a number of specialized mechanisms for transporting particular compounds; for example, glucose and amino acids. Sometimes drugs can participate in this process. Active transport requires a carrier molecule and a form of energy.

- the process can be saturated
- transport can proceed against a concentration gradient
- competitive inhibition is possible

(2) Facilitated

A drug carrier is required but no energy is necessary. e.g. vitamin B12 transport

- saturable if not enough carrier
- no transport against a concentration gradient, downhill but faster

(2) Passive:

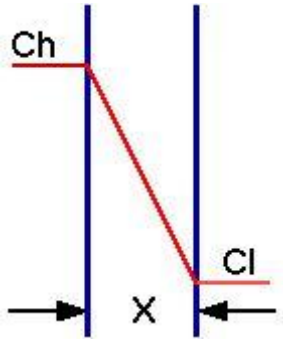



*Figure 3.8.1.2(2). Diagram of Passive Transport with a Concentration Gradient*

Most drugs cross biologic membranes by passive diffusion. Diffusion occurs when the drug concentration on one side of the membrane is higher than that on the other side. Drug diffuses across the membrane in an attempt to equalize the drug concentration on both sides of the membrane.

If the drug partitions into the lipid membrane a concentration gradient can be established. The rate of transport of drug across the membrane can be described by Fick's first law of diffusion:-

Rate of diffusion = $\frac{dM}{dt} = -\frac{D \cdot A \cdot (Ch - Cl)}{x}$

Equation XI-1 Rate of Diffusion:

The parameters of this equation are:-

D: diffusion coefficient. This parameter is related to the size and lipid solubility of the drug and the viscosity of the diffusion medium, the membrane. As lipid solubility increases or molecular size decreases then D increases and thus dM/dt also increases.

A: surface area. As the surface area increases the rate of diffusion also increase. The surface of the intestinal lining (with villae and microvillae) is much larger than the stomach. This is one reason absorption is generally faster from the intestine compared with absorption from the stomach.

x: membrane thickness. The smaller the membrane thickness the quicker the diffusion process. As one example, the membrane in the lung is quite thin thus inhalation absorption can be quite rapid.

(Ch -Cl): concentration difference. Since V, the apparent volume of distribution, is at least four liters and often much higher the drug concentration in blood or plasma will be quite low compared with the concentration in the GI tract. It is this concentration gradient which allows the rapid complete absorption of many drug substances.

Normally Cl << Ch then:-

$$\frac{dM}{dt} = -\frac{D \cdot A \cdot Ch}{x} = \frac{D \cdot A \cdot Xg}{x \cdot Vg}$$

constant ka

Thus the absorption of many drugs from the G-I tract can often appear to be first-order.

(3) Pinocytosis

For example Vitamin A, D, E, and K.

(4) Ion pair transport

For example quaternary ammonium compounds

## 3.8.2 Gastrointestinal Physiology:

### 3.8.2.1 Characteristics of G- I physiology:

| | pH | Membrane | Blood Supply | Surface Area | Transit Time | By-pass liver |
|---|---|---|---|---|---|---|
| BUCCAL | approx 7 | thin | Good, fast absorption with low dose | small | Short unless controlled | yes |
| ESOPHAGUS | 5 – 6 | Very thick, no absorption | - | small | short | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| STOMACH | 1 - 3 decomposition, weak acid unionized | normal | Good | small | 30 - 40 minutes, reduced absorption | no |
| DUODENUM | 6 - 6.5 bile duct, surfactant properties | Normal | Good | very large | very short (6" long), window effect | no |
| SMALL INTESTINE | 7 – 8 | Normal | Good | very large 10 - 14 ft, 80 $cm^2$ /cm | about 3 hours | no |
| LARGE INTESTINE | 5.5 – 7 | - | Good | not very large 4 - 5 ft | long, up to 24 hr | lower colon, rectum yes |

*Table 3.8.2.1(1). GI Physiology and Drug Absorption*

### 3.8.2.2 Gastric Emptying and Motility:

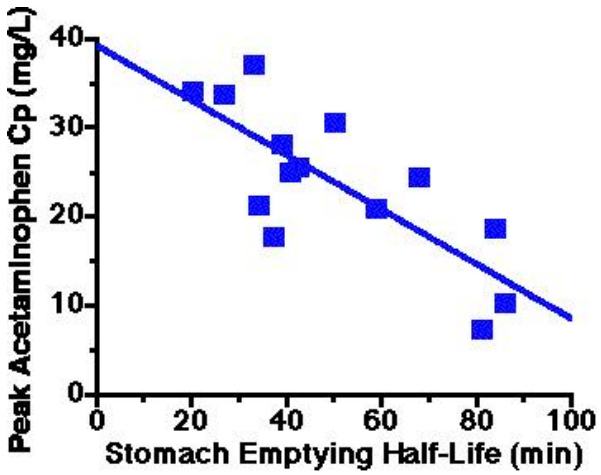



*Figure 3.8.2.2(1). Showing Dependence of Peak Acetaminophen Plasma Concentration as a Function of Stomach Emptying Half-life*

Generally drugs are better absorbed in the small intestine (because of the larger surface area) than in the stomach, therefore increasing stomach emptying will increase drug absorption. For example, a good correlation has been shown between stomach emptying time and peak plasma concentration for acetaminophen. The quicker the stomach emptying the higher the plasma concentration.

| | pH | Membrane | Blood Supply | Surface Area | Transit Time | By-pass liver |
|---|---|---|---|---|---|---|
| BUCCAL | approx 6 | thin | Good, fast absorption with low dose | small | Short unless controlled | yes |
| ESOPHAGUS | 6 | Very thick, no absorption | - | small | short | - |
| STOMACH | 1 - 3 decomposition, weak acid unionized | normal | good | small | 30 - 40 minutes, reduced absorption | no |
| DUODENUM | 5 - 7 bile duct, surfactant properties | normal | good | very large | very short (6" long), window effect | no |
| SMALL INTESTINE | 6 -7 | normal | good | V large 10 - 14 ft, 80 $cm^2$. | about 3 hours | no |
| LARGE INTESTINE | 6.8 – 7 | - | good | not very large 4 - 5 ft | long, up to 24 hr | lower colon, rectum yes |

*Table 3.8.2.2(2). Factors Affecting Gastric Emptying*

### 3.8.2.3 Influence of Food:

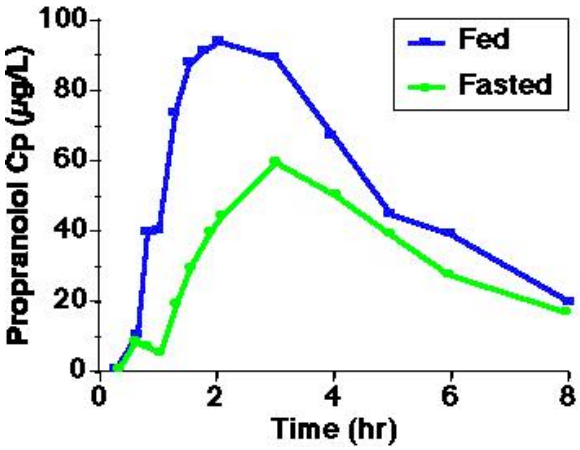



*Figure 3.8.2.3(1). Showing the Effect of Fasting versus Fed on Propranolol Concentrations*

Food can affect the rate of gastric emptying. For example fatty food can slow gastric emptying and retard drug absorption. Generally the extent of absorption is not greatly reduced. Occasionally absorption may be improved. Griseofulvin absorption is improved by the presence of fatty food. Apparently the poorly soluble griseofulvin is dissolved in the fat and then more readily absorbed.

Propranolol plasma concentrations are larger after food than in fasted subjects. This may be an interaction with components of the food.

### 3.8.2.4 Other Factors:

- Intestinal Motility and Transit Time
- Food Retards transit

## 3.9 Physical-Chemical Factors affecting Oral Absorption:

There are two major headings, which can be used to discuss this material as it affect oral drug absorption. These are i) the pH -partition theory and ii) the dissolution of drugs.

### 3.9.1 pH – Partition:

For a drug to cross a membrane barrier it must normally be soluble in the lipid material of the membrane to get into membrane, also it has to be soluble in the aqueous

phase as well to get out of the membrane. Most drugs have polar and non-polar characteristics or are weak acids or bases. For drugs which are weak acids or bases the pKa of the drug and the pH of the GI tract fluid and the pH of the blood stream will control the solubility of the drug and thereby the rate of absorption through the membranes, lining the GI tract.

Brodie et al. (in 1957) proposed the pH - partition theory to explain the influence of GI pH and drug pKa on the extent of drug transfer or drug absorption. Brodie reasoned that when a drug is ionized it will not be able to get through the lipid membrane, but only when it is non ionized and therefore has a higher lipid solubility. Brodie tested this theory by perfuming the stomach or intestine of rats, in situ, and injected the drug intravenously. He varied the concentration of drug in the GI tract until there was no net transfer of drug across the lining of the GI tract. He then determined the ratio D:-

$$D = \frac{\text{Total concentration in Blood}}{\text{Total concentration in G-I tract}}$$

$$\text{i.e. } D = \frac{[U]b + [I]b}{[U]g + [I]g}$$

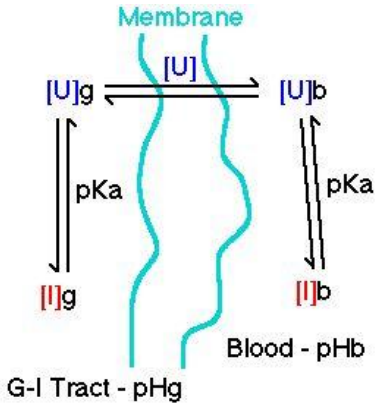



*Figure 3.9.1(1). Showing Transfer across Membrane*

These values were determined experimentally, but we should be able to calculate a theoretical value if we assume that only non ionized drug crosses the membrane and that net transfer stops when $[U]_b = [U]_g$

The ratio [U]/[I] is a function of the pH of the solution and the pKa of the drug; as described by the Henderson - Hasselbach equation

For weak acids:-

pKa - pH = $\log \frac{[U]}{[I]} = \log \frac{[HA]}{[A^-]}$

and for weak bases:-

pKa - pH = $\log \frac{[I]}{[U]} = \log \frac{[HB^+]}{[B]}$

Brodie found an excellent correlation between the calculated D value and the experimentally determined values.

Even though the D values refer to an equilibrium state a large D value will mean that more drug will move from the GI tract to the blood side of the membrane. The larger the D value, the larger the effective concentration gradient, and thus the faster the expected transfer or absorption rate.

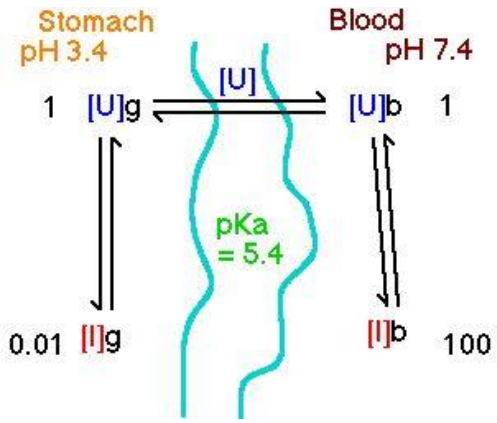



*Figure 3.9.1(2). Drug Distribution between Stomach and Blood*

Compare D for a weak acid (pKa = 5.4) from the stomach (pH 3.4) or intestine (pH 6.4), with blood pH = 7.4

Stomach

$[U]/[I] = 10^{pKa - pH} = 10^{5.4 - 3.4} = 10^2 = 100$

i.e. $[I] = 0.01 \times [U]$

Blood

$[U]/[I] = 10^{pKa - pH} = 10^{5.4 - 7.4} = 10^{-2} = 0.01$

i.e. $[I] = 100 \times [U]$

Therefore the calculated D value would be

$D = \frac{\text{Total in blood}}{\text{Total in stomach}} = \frac{1 + 100}{1 + 0.01} = \frac{101}{1.01} = 100$

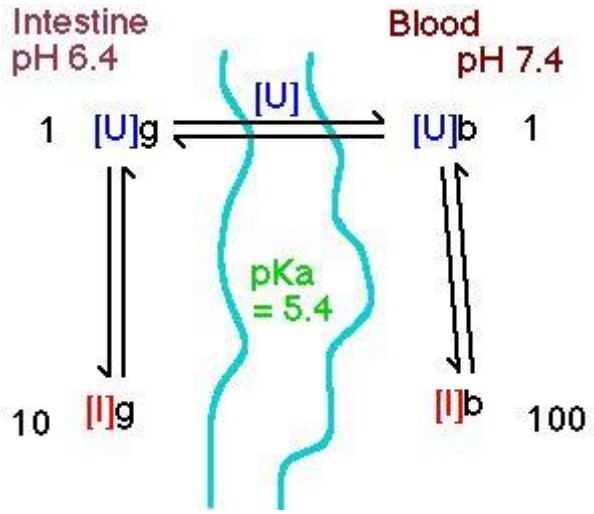



*Figure 3.9.1(3). Drug Distribution between Intestine and Blood*

By comparison in the intestine, pH = 6.4

The calculated D value is (100+1) / (10+1) = 9.2

From this example we could expect significant absorption of weak acids from the stomach compared with from the intestine. Remember however that the surface area of the intestine is much larger than the stomach. This approach can be used to compare a series of similar compounds with different pKa values.

We have applied the pH - partition theory to drug absorption, later we will use this theory to describe drug re-absorption in the kidney.

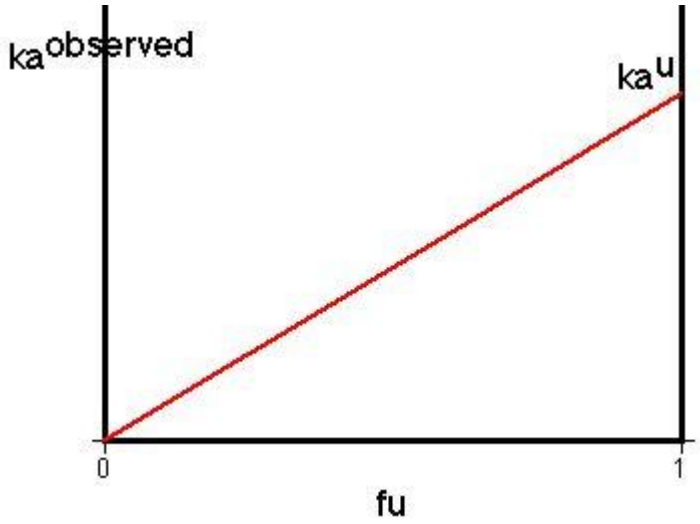



*Figure 3.9.1(4). Plot of ka versus fu*

With this theory it should be possible to predict that by changing the pH of the G-I tract that we would change the fraction non ionized and therefore the rate of absorption. This has some application in understanding drug absorption in overdose situations, but more readily used in relation to renal excretion.

Thus $ka_{observed}$ = ku * fu assuming that the ionized species is not absorbed.

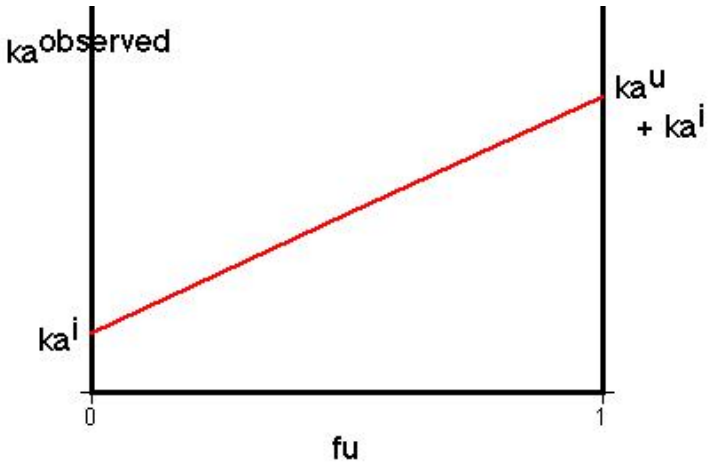



*Figure 3.9.1(5). Plot of ka Versus fu for Sulfaethidole*

For some drugs it has been found that the intercept is not zero in the above plot, suggesting that the ionic form is also absorbed. For example, results for sulfaethidole.

May be the ions are transported by a carrier which blocks the charge, a facilitated transport process.

## 3.9.2 Drug dissolution:

So far we have looked at the transfer of drugs in solution in the G-I tract, through a membrane, into solution in the blood. However, many drugs are given in solid dosage forms, and therefore must dissolve before absorption can take place.

*Dissolution* *Absorption*

SOLID ----------> SOLUTION ----------> BLOOD

*G-I Tract*

If absorption is slow relative to dissolution then all we are concerned with is absorption. However, if dissolution is the slow, rate determining step (the step controlling

the overall rate) then factors affecting dissolution will control the overall process. This is a more common problem with drugs which have a low solubility (below 1 g/100 ml) or which are given at a high dose, e.g. griseofulvin.

There are number of factors which affect drug dissolution and we can look at this process as diffusion controlled.

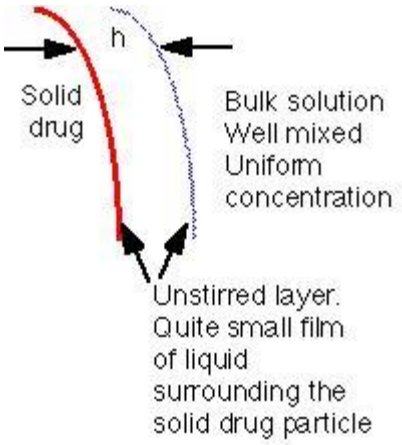



*Figure 3.9.2(1). Stagnant Layer*

First we need to consider that each particle of drug formulation is surrounded by a stagnant layer of solution.

After an initial period we will have a steady state set-up where drug is steadily dissolved at the solid-liquid interface and diffuses through the stagnant layer. If diffusion is the rate determining step we can use Fick's first law of diffusion to describe the overall process.

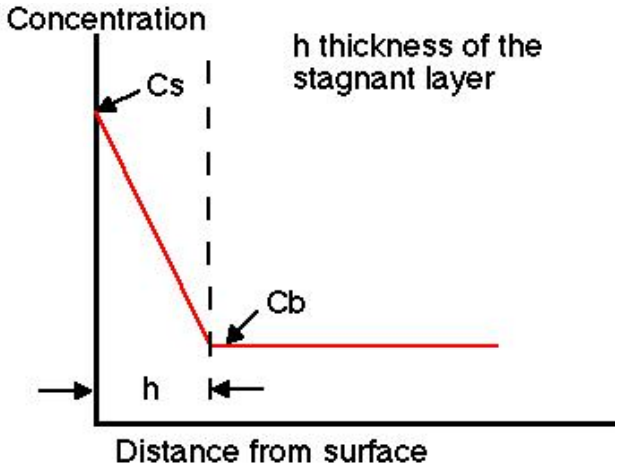



*Figure 3.9.2(2). Plot of Concentration Gradient*

If we could measure drug concentration at various distances from the surface of the solid we would see that a concentration gradient is developed.

### 3.9.2.1 Fick's First Law:

By Fick's first law of diffusion then:-

$$\text{Rate of solution} = \frac{D \cdot A \cdot (Cs - Cb)}{h}$$

where D is the diffusion coefficient, A the surface area, Cs the solubility of the drug, Cb the concentration of drug in the bulk solution, and h the thickness of the stagnant layer. If Cb is much greater than Cs then we have so-called "Sink Conditions" and the equation reduces to

$$\text{Rate of solution} = \frac{D \cdot A \cdot Cs}{h}$$

with each term in this equation contributing to the dissolution process.

### 3.9.2.2 Surface area, A:

The surface area per gram (or per dose) of a solid drug can be changed by altering the particle size. For example, a cube 1 cm on each side has a surface area of 6 $cm^2$. If this cube is broken into cubes with sides of 0.1 cm, the total surface area is 60 $cm^2$. Actually if we break up the particles by grinding we will have irregular shapes and even larger surface areas. Generally as A increases the dissolution rate will also increase. Improved bioavailability has been observed with griseofulvin, digoxin, etc.

Methods of particle size reduction include mortar and pestle, mechanical grinders, fluid energy mills, solid dispersions in readily soluble materials (PEG's).

### 3.9.2.3 Diffusion Layer Thickness, h:

This thickness is determined by the agitation in the bulk solution. In vivo we usually have very little control over this parameter. It is important though when we perform in vitro dissolution studies because we have to control the agitation rate so that we get similar results in vitro as we would in vivo.

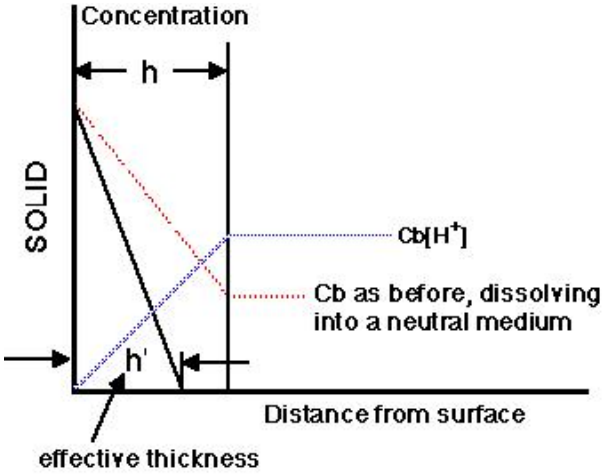



*Figure 3.9.2.3(1). Plot of Concentration versus Distance for Dissolution into a Reactive Medium*

The apparent thickness of the stagnant layer can be reduced when the drug dissolves into a reactive medium. For example, with a weakly basic drug in an acidic medium, the drug will react (ionize) with the diffusing proton ($H^+$) and this will result in an effective decrease in the thickness of the stagnant layer.

The effective thickness is now h' not h. Also the bulk concentration of the drug is effectively zero. For this reason weak bases will dissolve more quickly in the stomach.

### 3.9.2.4 Diffusion Coefficient, D:

The value of D depends on the size of the molecule and the viscosity of the dissolution medium. Increasing the viscosity will decrease the diffusion coefficient and thus the dissolution rate. This could be used to produce a sustained release effect by including a larger proportion of something like sucrose or acacia in a tablet formulation.

### 3.9.2.5 Drug Solubility, Cs:

Solubility is another determinant of dissolution rate. As Cs increases so does the dissolution rate. We can now look at ways of changing the solubility of a drug.

Salt form:

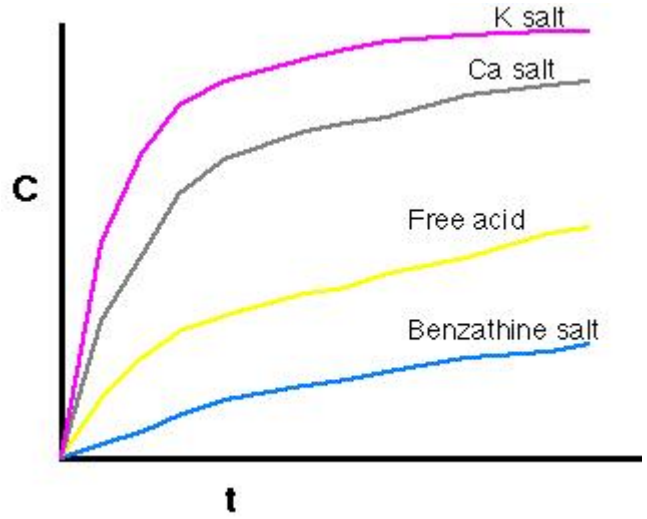



*Figure 3.9.2.5(1). Plot of Concentration versus Time*

Salts of weak acids and weak bases generally have much higher aqueous solubility than the free acid or base, therefore if the drug can be given as a salt the solubility can be increased and we should have improved dissolution. One example is Penicillin V. If we look at the dissolution profile of various salts.

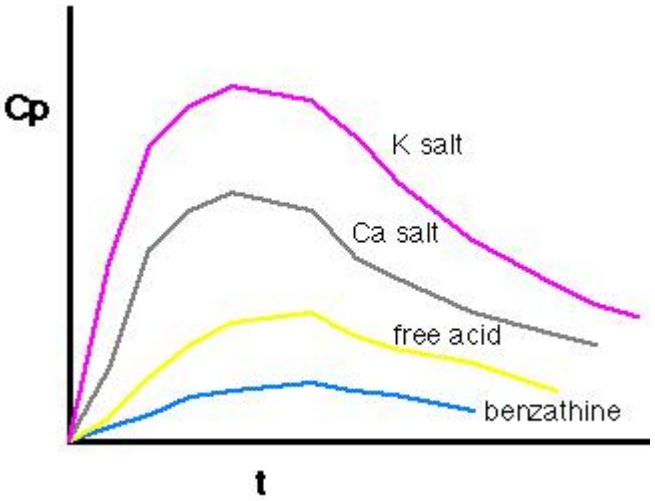



*Figure 3.9.2.5(2). Plot of Cp versus Time*

This can lead to quite different Cp versus time results after oral administration.

The t peak values are similar thus ka probably the same. C peak would show a good correlation with solubility. Maybe site limited (only that in solution by the time the drug

gets to the 'window' is absorbed). Use the potassium salt for better absorption orally. Use benzathine or procaine for IM depot use.

Crystal form:

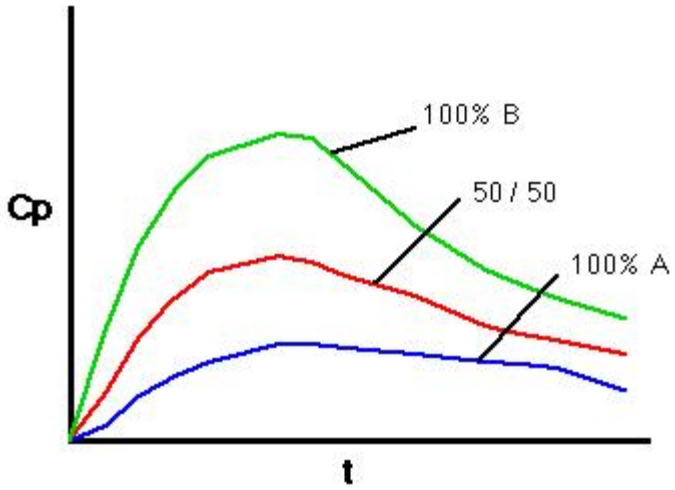



*Figure 3.9.2.5(3). Plot of Cp versus Time for Three Formulations of Chloramphenicol Palmitate*

Some drugs exist in a number of crystal forms or polymorphs. These different forms may well have different solubility properties and thus different dissolution characteristics. Chloramphenicol palmitate is one example which exists in at least two polymorphs. The B form is apparently more bioavailable*.

The recommendation might be that manufacturers should use polymorph B for maximum absorption. However, a method of controlling and determining crystal form would be necessary in the quality control process.

# 3.10 Formulation Factors:

The role of the drug formulation in the delivery of drug to the site of action should not be ignored. With any drug it is possible to alter its bioavailability considerably by formulation modification. With some drugs an even larger variation between a good formulation and a bad formulation has been observed. Since a drug must be in solution to be absorbed from the G-I tract, you may expect the bioavailability of a drug to decrease in the order solution > suspension > capsule > tablet > coated tablet.

This order may not always be followed but it is a useful guide. One example is the results for pentobarbital. Here the order was found to be aqueous solution > aqueous suspension = capsule > tablet of free acid form. This chapter will briefly discuss each of these formulation types particularly in regard to the relative bioavailability.

### 3.10.1 Solutions:

Drugs are commonly given in solution in cough/cold remedies and in medication for the young and elderly. In most cases absorption from an oral solution is rapid and complete, compared with administration in any other oral dosage form. The rate limiting step is often the rate of gastric emptying.

When an acidic drug is given in the form of a salt, it may precipitate in the stomach. However, this precipitate is usually finely divided and is readily redissolved and thus causes no absorption problems. There is the possibility with a poorly water soluble drug such as phenytoin that a well formulation suspension, of finely divided powder, may have a better bioavailability.

Some drugs which are poorly soluble in water may be dissolved in mixed water/alcohol or glycerol solvents. This is particularly useful for compounds with tight crystal structure, higher melting points that are not ionic. The crystal structure is broken by solution in the mixed solvent. An oily emulsion or soft gelatin capsules have been used for some compounds to produce improved bioavailability.

### 3.10.2 Suspensions:

A well formulated suspension is second only to a solution in terms of superior bioavailability. Absorption may well be dissolution limited; however a suspension of a finely divided powder will maximize the potential for rapid dissolution. A good correlation can be seen for particle size and absorption rate.

With very fine particle sizes the dispersibility of the powder becomes important. The addition of a surface active agent will improve dispersion of a suspension and may improve the absorption of very fine particle size suspensions otherwise caking may be a problem.

### 3.10.3 Capsules:

In theory a capsule dosage form should be quite efficient. The hard gelatin shell should disrupt rapidly and allow the contents to be mixed with the G-I tract contents. The capsule contents should not be subjected to high compression forces which would tend to reduce the effective surface area, thus a capsule should perform better than a tablet.

This is not always the case. If a drug is hydrophobic a dispersing agent should be added to the capsule formulation. These diluents will work to disperse the powder, minimize aggregation and maximize the surface area of the powder.

### 3.10.4 Tablets:

The tablet is the most commonly used oral dosage form. It is also quite complex in nature. The biggest problem is overcoming the reduction in effective surface area produced during the compression process. One may start with the drug in a very fine powder, but then proceeds to compress it into a single dosage unit.

#### 3.10.4.1 Ingredients:

Tablet ingredients include materials to break up the tablet formulation.

- Drug - may be poorly soluble, hydrophobic
- Lubricant - usually quite hydrophobic
- Granulating agent - tends to stick the ingredients together
- Filler - may interact with the drug, etc., should be water soluble
- Wetting agent - helps the penetration of water into the tablet
- Disintegration agent - helps to break the tablet apart

Coated tablets are used to mask an unpleasant taste, to protect the tablet ingredients during storage, or to improve the tablets appearance. Another barrier is placed between the solid drug and drug in solution. This barrier must break down quickly or it may hinder a drug's bioavailability.

### 3.10.5 Sustained Release Tablets:

Another form of coating is enteric coated tablets which are coated with a material which will dissolve in the intestine but remain intact in the stomach. Polymeric acid compounds have been used for this purpose with some success. This topic and the area of sustained release products has been discussed in more detail in other courses.

#### 3.10.5.1 Benefits:

- for short half-life drugs, sustained release can mean less frequent dosing and thus better compliance.
- reduce variations in plasma/blood levels for more consistent result.

#### 3.10.5.2 Problems:

- More complicated formulation may be more erratic in result. A sustained release product may contain a larger dose, i.e. the dose for two or three (or more) 'normal' dosing intervals. A failure of the controlled release mechanism may result in release of a large toxic dose.
- more expensive technology

#### 3.10.5.3 Types of Products:

- erosion tablets
- waxy matrix
    - matrix erodes or drug leaches from matrix
- coated pellets
    - different pellets (colors) have different release properties
- coated ion exchange
- osmotic pump
    - insoluble coat with small hole. Osmotic pressure pushes the drug out at a controlled rate.

#### 3.10.5.4 Results:

Reduced side effects

### 3.10.6 In-vitro Dosage Form Testing:

#### 3.10.6.1 Disintegration:

Disintegration time is the time to pass through a sieve while agitated in a specified fluid. Indicates the time to break down into small particles. Not necessarily solution. In the process of tablet manufacturer the drug is often formulated into a granular state (that is small but not fine) particles.

This is done as the granule often has better flow properties than the a fine powder and there is less de-mixing leading to better uniformity. The granules are then compressed to produce the tablet. The disintegration test may lead to an end point of tablet to granule only.

#### 3.10.6.2 Dissolution:

The time is takes for the drug to dissolve from the dosage form. Numerous factors affect dissolution. Thus the dissolution medium, agitation, temperature are carefully controlled. The dissolution medium maybe water, simulated gastric juice, or 0.1M HCl. The temperature is usually 37 degree C.

The apparatus and specifications may be found in the U.S.P. The U.S.P. methods are official however there is a wide variety of methods based on other apparatus. These are used because they may be faster, cheaper, easier, sensitive to a particular problem for a particular drug, or developed by a particular investigator.

Dissolution tests are used as quality control to measure variability between batches which maybe be reflected by in vivo performance. Thus the in vitro test may be a quick method of ensuring in vivo performance. Thus there has been considerable work aimed at defining the in vitro/in vivo correlation.

## 3.11 Multiple IV Bolus Dose Administration:

This will consider drug pharmacokinetics after multiple dose drug administration. But, first review the equations for single dose drug administration.

### 3.11.1 Single Dose Summary:

#### 3.11.1.1 IV Bolus:

IV Bolus

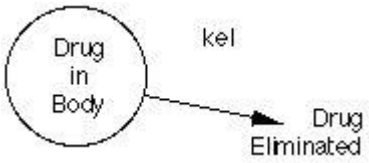



*Figure 3.11.1.1(1). Scheme for IV Bolus*

$$\frac{dCp}{dt} = -kel \cdot Cp$$

$$Cp = Cp^{0} \cdot e^{-kel \cdot t}$$

$$Cp = \frac{DOSE}{V} \cdot e^{-kel \cdot t}$$

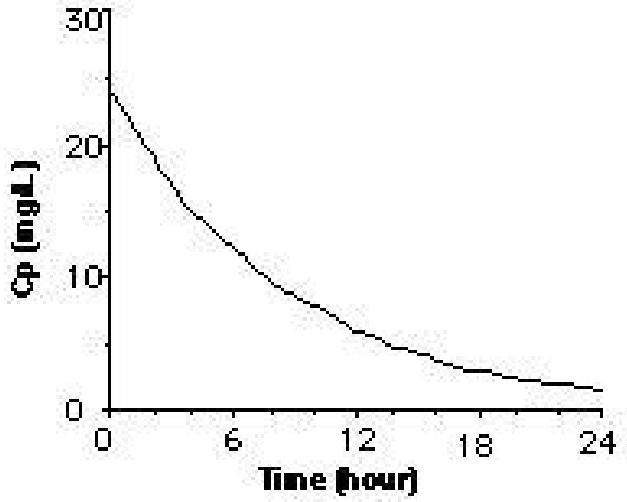



*Figure 3.11.1.1(2). Linear Plot of Cp versus Time*

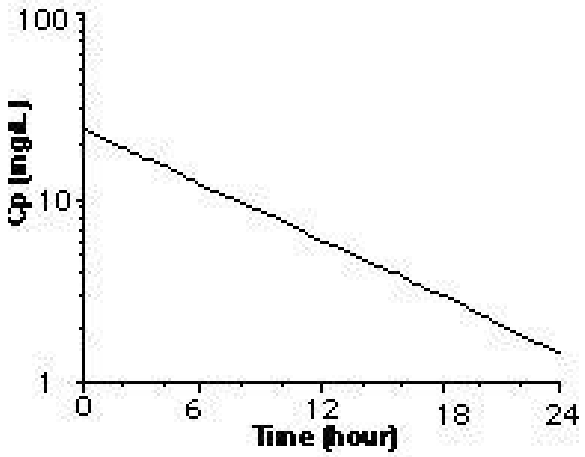



*Figure 3.11.1.1(3). Semi-log Plot of Cp versus Time*

### 3.11.1.2 IV infusion

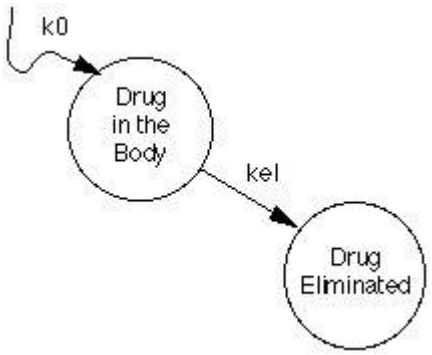



*Figure 3.11.1.2(1). Scheme for IV Infusion*

Differential Equations

- During:

$$\frac{dCp}{dt} = \frac{k0}{V} - kel \cdot Cp$$

Rate of Change of Cp

- After:

$$\frac{dCp}{dt} = -kel \cdot Cp$$

Cp versus Time

Integrated Equations:

- During

$$Cp = \frac{k0}{kel \cdot V} \cdot \left[ 1 - e^{-kel \cdot t} \right]$$

Cp versus Time During an IV Infusion

- At steady state

$$Cp^{ss} = \frac{k0}{kel \cdot V}$$

Cp at Steady State

- After

$$Cp = \frac{k0}{kel \cdot V} \cdot \left[ 1 - e^{-kel \cdot T} \right] \cdot e^{-kel \cdot (t-T)}$$

Cp versus Time after the Infusion

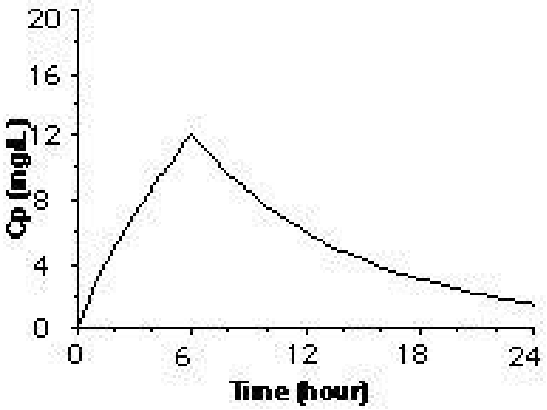



*Figure 3.11.1.2(2). Linear Plot of Cp versus Time*

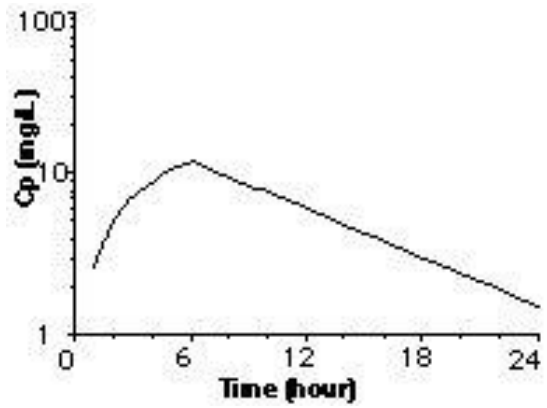



*Figure 3.11.1.2(3). Semi-log Plot of Cp versus Time*

### 3.11.1.3 Oral Administration:

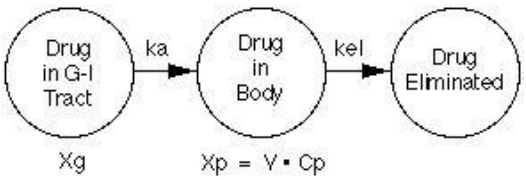



*Figure 3.11.1.3(1). Scheme for Oral Administration*

$$\frac{dCp}{dt} = \frac{ka \cdot Xg}{V} - kel \cdot Cp$$

Rate of Change of Cp

$$Cp = \frac{F \cdot DOSE \cdot ka}{V \cdot (ka - kel)} \cdot \left[ e^{-kel \cdot t} - e^{-ka \cdot t} \right]$$

Cp versus Time

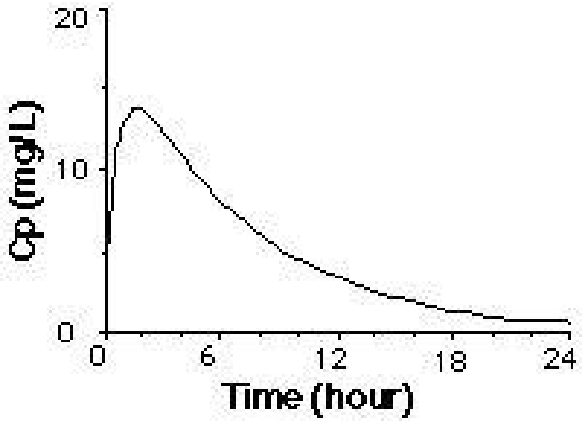



*Figure 3.11.1.3(2). Linear Plot of Cp versus Time*

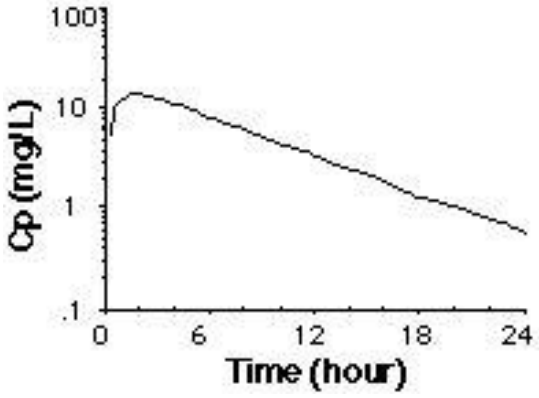



*Figure 3.11.1.3(3). Semi-log Plot of Cp versus Time*

With this refresher, drug pharmacokinetics after multiple dose administration may be easier to understand.

Aspirin given for a headache may be given as a single administration, whereas aspirin for arthritis will be given as a multiple dose. Antibiotics are usually given as a multiple dose regimen to produce and maintain effective plasma concentration. In fact, many drugs are given this way; anti-hypertensives, anti-epileptics etc.

Multiple dose administration is a very common method of drug administration. Up to this point we can calculate the drug concentration in plasma at any time after a single dose, we will continue now by looking at the equations for multiple dose administration.

## 3.11.2 Multiple IV Bolus:

### 3.11.2.1 Independent Doses:

After a single dose administration we assume that there is no drug in the body before the drug is administration and that no more is going to be administered. However, in the case of multiple dose administration we are expected to give second and subsequent doses before the drug is completely eliminated. Thus ACCUMULATION of the drug should be considered. On repeated drug administration the plasma concentration will be repeated for each dose interval giving a PLATEAU or STEADY STATE with the plasma concentration fluctuating between a minimum and maximum value.

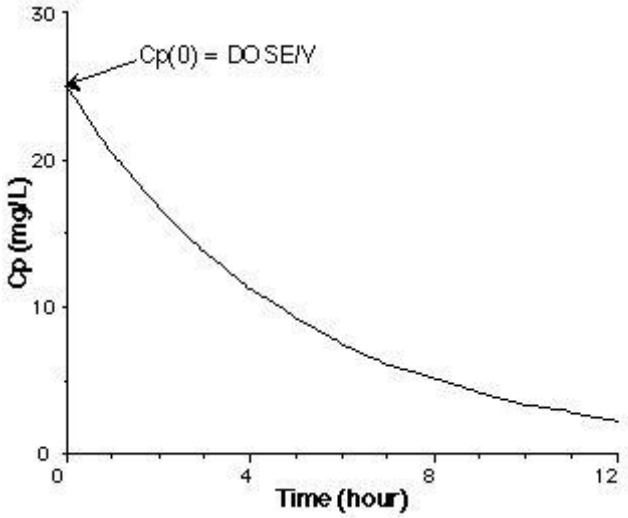



*Figure 3.11.2.1(1). Plot of Cp Versus Time Showing $Cp^0$*

We have already looked at the shape of the plasma concentration versus time curve following a single intravenous administration. If we assume instantaneous mixing we start off with an initial concentration $Cp^0$, given by $\frac{DOSE}{V}$ and then we have a fall in concentration with time controlled by the elimination rate constant.

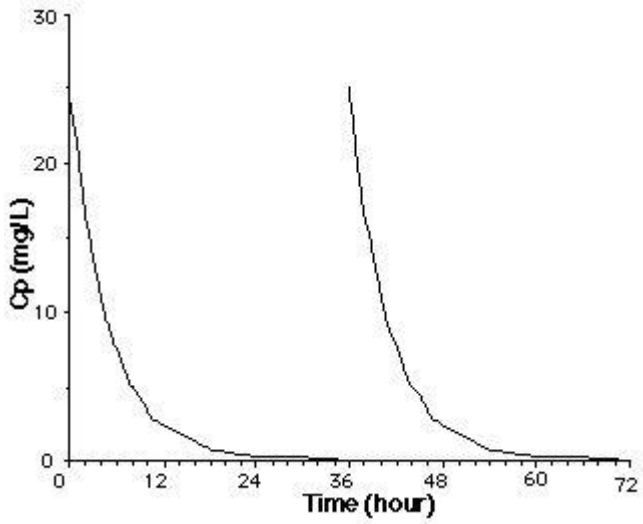



*Figure 3.11.2.1(2). Plot of Cp Versus Time after Two Separate Doses*

If the doses are given far enough apart then the concentration will have fallen to approximately zero before the next dose. There will then be no accumulation of drug in the body.

### 3.11.2.2 Accumulating Doses:

However if the second dose is given early enough so that not all of the first dose is eliminated then the drug will start to accumulate and we will get higher concentrations with the second and third dose. As an example we could consider a drug with a half-life of 6 hours. Giving a dose of 100 mg with an apparent volume of distribution of 25 liter the $Cp^0$ = 4 mg/liter.

After six hours the plasma concentration will fall to 2 mg/liter. If we give the same dose again the plasma concentration will increase by 4 mg/liter from 2 mg/liter to 6 mg/liter. Then after another half-life (6 hours) the plasma concentration will fall to 3 mg/liter. Again, another dose will increase the plasma concentration by 4 mg/liter to 7 mg/liter. After another half-life the plasma concentration will be 3.5 mg/liter. After repeated drug administration every six hours the plasma concentration will accumulate until it fluctuates between a maximum and minimum value of 8 mg/liter and 4 mg/liter.

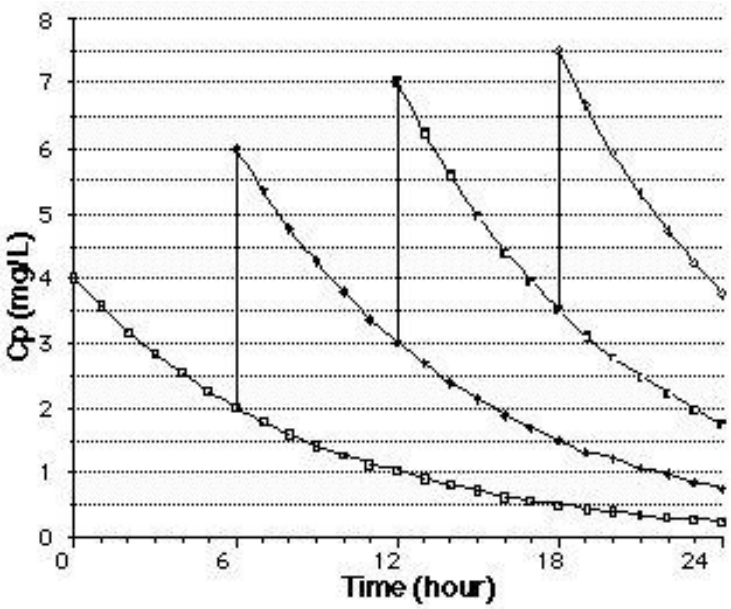



*Figure 3.11.2.2(1). Plot of Cp Versus Time Showing Doses Every Six Hours*

With each dose, drug accumulated until the amount of drug eliminated during each dosing interval was equal to the amount of the dose. In the first interval plasma concentrations fall from 4 to 2 mg/L. Continuing for a number of doses gives the following table.

| Start | | End | Concentration lost during dosage interval |
|---|---|---|---|
| 4 | --> | 2 mg/L | 2 mg/L |
| 6 | --> | 3 | 3 |
| 7 | --> | 3.5 | 3.5 |
| 7.5 | --> | 3.75 | 3.75 |
| | ... | | |
| 8 | --> | 4 | 4 <- which is the same as the concentration increase caused by each dose |

*Table 3.11.2.2(1). Concentration Lost During Dosage Interval*

There is a limit to drug accumulation because as the plasma concentration increased the amount of drug eliminated during the dosing interval will also increase as the rate of elimination is equal to the amount of the drug in the body multiplied by the rate constant for a first order elimination. (Compare this with the case of a continuous infusion).

So far we can see that if we give repeated doses before the body can eliminate the previous doses then we will get accumulation of the drug. We have also seen that when we have first order elimination this accumulation will not proceed indefinitely but will level off.

### 3.11.2.3 Development of General Equation:

We can now consider a general equation which could describe the plasma concentration at any time after multiple IV. bolus drug administration.

Concentration at the end of the first dosing interval

$$Cp_1^{\tau} = Cp_1^0 \cdot e^{-kel \cdot \tau}$$

Cp after the First Dose

where $Cp_{\text{dose number}}^{\text{time since last dose}}$

This gives the plasma concentrations at the end of first interval, where t is the dosing interval in hours.

At the start of the second interval

$$Cp_2^0 = Cp_1^{\tau} + Cp_1^0 = Cp_1^0 \cdot e^{-kel \cdot \tau} + Cp_1^0$$

then at the end of the second dose interval

$$Cp_2^{\tau} = \left[ Cp_1^0 \cdot e^{-kel \cdot \tau} + Cp_1^0 \right] \cdot e^{-kel \cdot \tau}$$

and so on.

It will help if we define the parameter

$$R = e^{-kel \cdot \tau}$$

which is the fraction of the initial plasma concentration remaining at the end of the dosing interval. Then

$$Cp_2^{\tau} = Cp_1^0 \cdot R + Cp_1^0 \cdot R^2$$

$$Cp_3^0 = Cp_1^0 + Cp_1^0 \cdot R + Cp_1^0 \cdot R^2$$

This is a geometric series with each term R times the preceding term.

Start

$$Cp_n^0 = Cp_1^0 + Cp_1^0 \cdot R + Cp_1^0 \cdot R^2 + \ldots + Cp_1^0 \cdot R^{n-1}$$

*Cp Immediately after the nth Doses*

End

$$Cp_n^{\tau} = Cp_1^0 \cdot R + Cp_1^0 \cdot R^2 + Cp_1^0 \cdot R^3 + \ldots + Cp_1^0 \cdot R^n$$

*Cp at the end of nth Dosing Interval*

These two sums can be simplified to give

Start

$$Cp_n^0 = Cp_1^0 \cdot \left[ \frac{1 - R^n}{1 - R} \right] = \frac{DOSE}{V} \cdot \left[ \frac{1 - e^{-n \cdot kel \cdot \tau}}{1 - e^{-kel \cdot \tau}} \right]$$

*Cp at the Beginning on the nth Dosing Interval*

and

End

$$Cp_n^{\tau} = Cp_1^0 \cdot \left[ \frac{1 - R^n}{1 - R} \right] \cdot R = \frac{DOSE}{V} \cdot \left[ \frac{1 - e^{-n \cdot kel \cdot \tau}}{1 - e^{-kel \cdot \tau}} \right] \cdot e^{-kel \cdot \tau}$$

*Cp at the End of the nth Dosing Interval*

using the first equation (Equation XIV-14) we can calculate the concentration in plasma at any time following uniform multiple IV bolus administration.

$$Cp_n^t = Cp_n^0 \cdot e^{-kel \cdot t}$$

where t = time since the last dose. Then

$$Cp_n^t = \frac{DOSE}{V} \cdot \left[ \frac{1 - e^{-n \cdot kel \cdot \tau}}{1 - e^{-kel \cdot \tau}} \right] \cdot e^{-kel \cdot t}$$

*Cp at any Time after n Doses*

### 3.11.2.4 Cpmax and Cpmin Equations:

More useful equations can be derived from this general equation. These are equations to calculate the maximum and minimum plasma concentration after many doses. That is as n ---> $^{\infty}$ and t = 0 or t = t. These are the limits of the PLATEAU CONCENTRATIONS.

$$Cp_{\infty}^0 = Cp_{max} = Cp_1^0 \cdot \left[ \frac{1}{1 - e^{-kel \cdot \tau}} \right] = Cp_1^0 \cdot \frac{1}{(1 - R)}$$

$$= \frac{DOSE}{V \cdot (1 - R)}$$

*Cp Immediately after Many Doses*

and

$$Cp_{\infty}^{\tau} = Cp_{min} = Cp_1^0 \cdot \left[ \frac{e^{-kel \cdot \tau}}{1 - e^{-kel \cdot \tau}} \right] \qquad = Cp_1^0 \cdot \frac{R}{(1 - R)}$$

$$= \frac{DOSE \cdot R}{V \cdot (1 - R)}$$

*Cp Immediately before Many Doses*

An example may be helpful: $t_{1/2}$ = 4 hr; IV dose 100 mg every 6 hours; V = 10 liter

then

$$Cp_1^0 = \frac{100}{10} = 10\ mg/L$$

What are the $Cp_{max}$ and $Cp_{min}$ values when the plateau values are reached

$$kel = \frac{0.693}{4} = 0.17\ hr^{-1}$$

$R = e^{-kel * \tau} = e^{-0.17 \times 6} = 0.35$

therefore

$$Cp_{max} = \frac{Cp_1^0}{(1 - R)} = \frac{10}{1 - 0.35} = 15.5\ mg/L$$

and

$$Cp_{min} = \frac{Cp_1^0 \cdot R}{(1 - R)} = Cp_{max} \cdot R = 15.5 \times 0.35 = 5.4\ mg/L$$

therefore the plasma concentration will fluctuate between 15.5 and 5.4 mg/liter during each dosing interval when the plateau is reached.

We can now calculate the plasma concentration at any time following multiple IV bolus administration and we can calculate the $Cp_{max}$ and $Cp_{min}$ values.

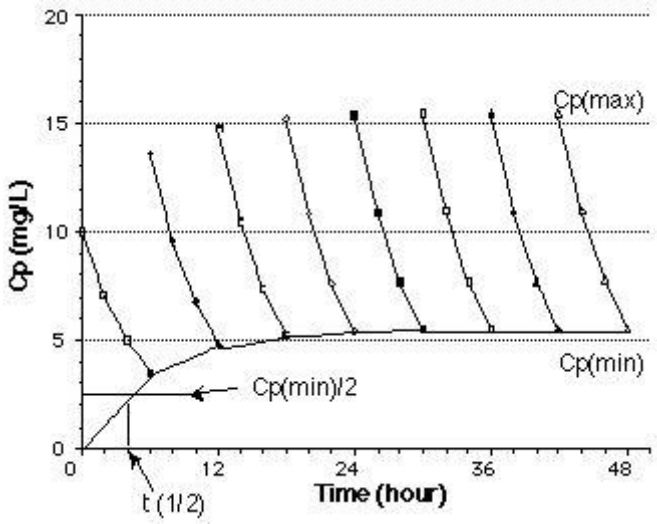



*Figure 3.11.2.4(1). Plot of Cp Versus Time showing Time to Approach 50% of Plateau during Multiple Dose Regimen*

It can be shown that the time to reach a certain fraction of the plateau concentration is dependent on the drug elimination half-life only, much the same as for the approach to steady state during an IV infusion. Thus we may have a problem with an excessive time required to reach the plateau. Therefore we may want to determine a suitable loading dose to achieve steady state rapidly.

In the previous example $Cp_{max}$ = 15.5 mg/liter

A suitable loading dose would be $Cp_{max}$ * V

155 mg as a bolus would give Cp = 15.5 mg/liter, followed by 100 mg every 6 hours to maintain the $Cp_{max}$ and $Cp_{min}$ values at 15.5 and 5.5 mg/liter respectively.

In general:-

The loading dose is $Cp_{max}$ * V

And since

$$Cp_{max} = \frac{\text{Maintenance DOSE}}{V \cdot (1 - R)}$$

(see Equation XIV-17, page XIV-11)

$$\text{Loading DOSE} = \frac{\text{Maintenance DOSE}}{(1 - R)}$$

*Loading Dose*

or

Maintenance DOSE = Loading DOSE * (1 - R)

We can try another example of calculating a suitable dosing regimen.

Consider V = 25 liter; kel = 0.15 $hr^{-1}$ for a particular drug and we need to keep the plasma concentration between 35 mg/liter (MTC) and 10 mg/liter (MEC).

What we need is the maintenance dose, the loading dose, and the dosing interval.

Since

$$\frac{Cp_{max}}{Cp_{min}} = \frac{DOSE}{V \cdot (1 - R)} \cdot \frac{V \cdot (1 - R)}{DOSE \cdot R} = \frac{1}{R}$$

therefore

$$R = \frac{Cp_{min}}{Cp_{max}} = \frac{10}{35} = 0.2857$$

Also

$R = e^{-kel * \tau} = 0.2857$

then

$- kel * \tau = -1.2528$ or

$\tau = 8.35$ hour; the dosing interval.

A dosing interval of 8 hours would be more reasonable. Thus with $\tau = 8$ hr and kel = 0.15 $hr^{-1}$

$R = e^{-kel * \tau} = e^{-8 \times 0.15} = 0.3012$

If we use $Cp_{max} = \frac{DOSE}{V \cdot (1 - R)}$

Maintenance dose = $Cp_{max} * V * (1 - R) = 35 \times 25 \times (1 - 0.3012) = 611$ mg

Again a more realistic dose would be 600 mg every 8 hours.

To check

$$Cp_{max} = \frac{DOSE}{V \cdot (1 - R)} = \frac{600}{25 \times (1 - 0.3012)} = 34.3 \text{ mg/L}$$

and

$Cp_{min} = Cp_{max} * R = 10.3$ mg/L

This regimen would be quite suitable as the maximum and minimum values are still within the limits suggested. All that remains is to calculate a suitable loading dose.

Loading dose = $Cp_{max} * V = 35 \times 25 = 875$ mg either 875, 850 or 800 mg

This answer can be expressed graphically.

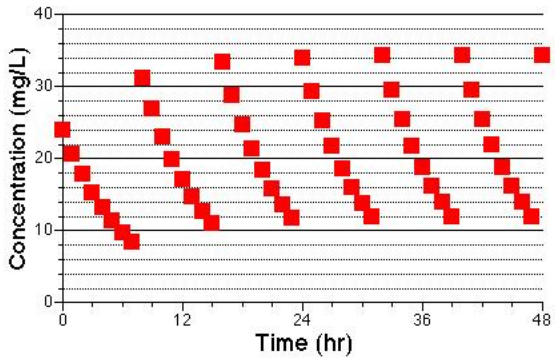



*Figure 3.11.2.4(2). Plasma Concentration after Multiple IV Bolus Doses*

# 3.12 Multiple Oral Dose Administration:

So far we have looked at multiple IV bolus administration. In an analogous fashion, equations can be developed which enable you to calculate the plasma concentration achieved following multiple oral administration. To start the plasma concentration achieved following a single oral dose can be given by:-

$$Cp = \frac{F \cdot DOSE \cdot ka}{V \cdot (ka - kel)} \cdot \left[ e^{-kel \cdot t} - e^{-ka \cdot t} \right]$$

*Cp after a Single Oral Dose*

This can be converted to an equation describing plasma concentration at any time following n equal doses with constant dosing interval t.

$$Cp = \frac{F \cdot Dose \cdot ka}{V \cdot (ka - kel)} \cdot \left[ \frac{1 - e^{-n \cdot kel \cdot \tau}}{1 - e^{-kel \cdot \tau}} \cdot e^{-kel \cdot t} - \frac{1 - e^{-n \cdot ka \cdot \tau}}{1 - e^{-ka \cdot \tau}} \cdot e^{-ka \cdot t} \right]$$

*General Equation*

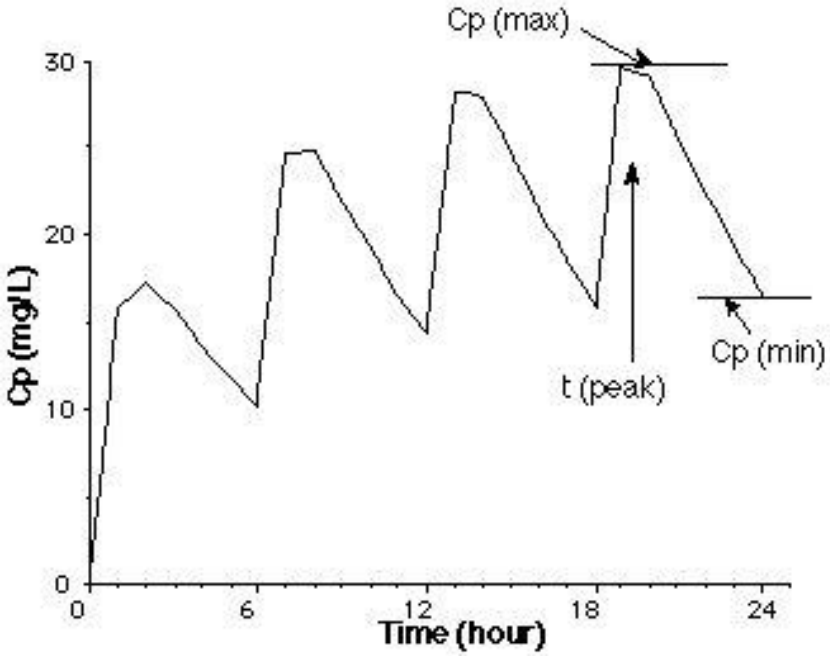



*Figure 3.12(1). Plot of Cp Versus Time for Multiple Oral Doses showing $Cp_{max}$ and $Cp_{min}$*

The plasma concentration versus time curve described by this equation is similar to the IV curve in that there is accumulation of the drug in the body to some plateau level and the plasma concentrations fluctuate between a minimum and a maximum value.

The Cpmax value can be calculated at the time t = $t_{peak}$ after many doses (n -> $^{\infty}$) but it is complicated by the need to determine the value for $t_{peak}$.

## 3.12.1 Cpmin Equation:

However Cpmin can be more easily determined at t = 0 or t = t. Thus at t = 0 and n -> $^{\infty}$.

$$Cp_{min} = \frac{F \cdot DOSE \cdot ka}{V \cdot (ka - kel)} \cdot \left[ \frac{1}{1 - e^{-kel \cdot \tau}} - \frac{1}{1 - e^{-ka \cdot \tau}} \right]$$

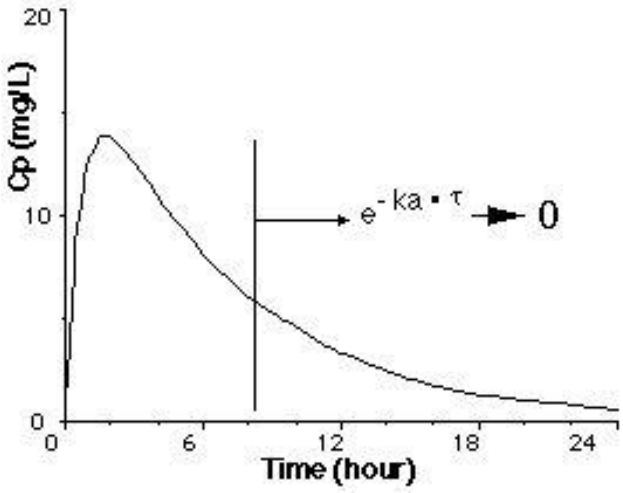



*Figure 3.12.1(1). Plot Cp Versus Time after a Single Dose showing Possible Time of Second Dose*

This can be further simplified if we assume that the subsequent doses are given after the plasma concentration has peaked and $e^{-ka * t}$ is close to zero. That is the next dose is given after the absorption phase is complete.

Cpmin then becomes:-

$$Cp_{min} = \frac{F \cdot DOSE \cdot ka}{V \cdot (ka - kel)} \cdot \left[ \frac{e^{-kel \cdot \tau}}{1 - e^{-kel \cdot \tau}} \right]$$

*Cp Equation Simplified*

$$Cp_{min} = A \cdot \left[ \frac{R}{1 - R} \right]$$

*Cp Equation Simplified Again*

The relationship between loading dose and maintenance dose and thus drug accumulation during multiple dose administration can be studied by looking at the ratio between the minimum concentration at steady state and the concentration one dosing interval (t) after the first dose.

$$\frac{Cp_{min}}{Cp_1^{\tau}} = \frac{\frac{F \cdot DOSE \cdot ka}{V \cdot (ka - kel)} \cdot \left[ \frac{e^{-kel \cdot \tau}}{1 - e^{-kel \cdot \tau}} \right]}{\frac{F \cdot DOSE \cdot ka}{V \cdot (ka - kel)} \cdot e^{-kel \cdot \tau}}$$

$$\frac{Cp_{min}}{Cp_1^{\tau}} = \frac{A \cdot \left[ \frac{R}{1 - R} \right]}{A \cdot R}$$

Which can be simplified to give:-

$$\frac{Cp_{min}}{Cp_1^{\tau}} = \frac{1}{1 - e^{-kel \cdot \tau}} = \frac{1}{(1 - R)}$$

*Ratio Between Cp after First and Last Dose*

This turns out to be the same equation as for the IV bolus. Therefore we can calculate a loading dose just as we did for an IV multiple dose regimen.

$$\text{Loading Dose} = \frac{\text{Maintenance Dose}}{(1 - R)}$$

*Loading Dose Equation*

This equation holds if each dose is given after the absorption phase of the previous dose is complete. We can further simplify Equation XV-4, if we assume that ka >> kel

then (ka - kel) approximatley equal to ka and thus $\frac{ka}{(ka - kel)}$ approximately = 1.

$$Cp_{min} = \frac{F \cdot DOSE}{V} \cdot \left[ \frac{e^{-kel \cdot \tau}}{1 - e^{-kel \cdot \tau}} \right]$$

*Cp after Many Oral Doses (Uniform Dose and Interval)*

It is an even more extreme simplification. However, it can be very useful if we don't know what the ka value is but can assume that absorption is reasonably fast. Equation 71 will tend to give concentrations that are lower than those obtained with the full equation. Thus any estimated fluctuation between Cpmin and Cpmax will be overestimated using the simplified equation.

## 3.12.2 Average Cp Equation:

Another very useful concentration term for the calculation of oral dosing regimens is the average plasma concentration, $\overline{Cp}$, during the dosing interval at steady state.

This term is defined as the area under the plasma concentration versus time curve during the dosing interval at steady state divided by the dosing interval.

$$\overline{Cp} = \frac{\int_0^{\tau} Cp \cdot dt}{\tau}$$

*Average Cp for a Dosing Interval at Steady State*

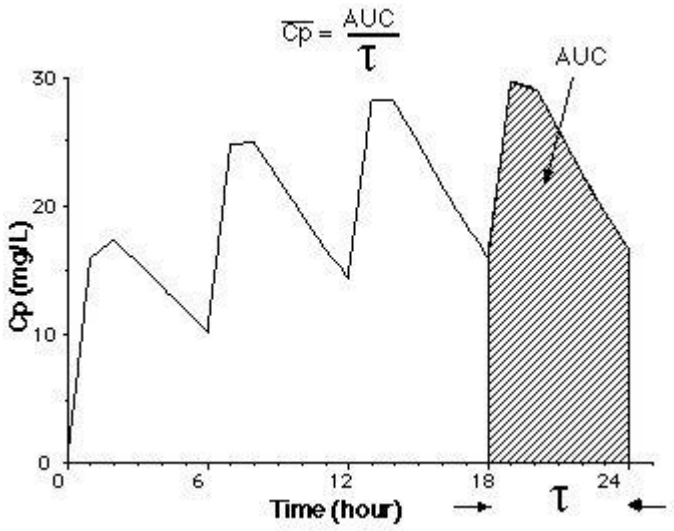



*Figure 3.12.2(1). Plot of Cp versus Time after Multiple Oral Administration showing AUC at Steady State*

By integrating the equation for plasma concentration at the plateau, between t = 0 and t = t gives:-

$$\overline{Cp} = \frac{F \cdot DOSE}{V \cdot kel \cdot \tau}$$

*Average Cp for a Dosing Interval at Steady State*

An interesting result of this equation is that we get the same average plasma concentration whether the dose is given as a single dose every t dosing interval or is subdivided into shorter dosing intervals.

For example 300 mg every 12 hours will give the same average plasma concentration as 100 mg every 4 hours. Of course, the difference between the maximum and minimum plasma concentration will be larger in the case of the less frequent dosing.

For example F = 1.0; V = 30 liter; $t_{1/2}$ = 6 hours or kel = 0.693/6 = 0.116 $hr^{-1}$.

We can now calculate the dose given every 12 hours required to achieve an average plasma concentration of 15 mg/L.

$$\text{Since} \quad \overline{Cp} = \frac{F \cdot DOSE}{V \cdot kel \cdot \tau}$$

$$DOSE = \frac{\overline{Cp} \cdot V \cdot kel \cdot \tau}{F} = \frac{15 \times 30 \times 0.116 \times 12}{1.0} = 624 \text{ mg}$$

We could now calculate the loading dose

$R = e^{-kel * [[tau]]} = e^{-0.116 \times 12} = 0.25$

$$\text{Loading DOSE} = \frac{\text{Maintenance DOSE}}{1 - R} = \frac{624}{1 - 0.25} = 832 \text{ mg}$$

To get some idea of the fluctuations in plasma concentration we could calculate the Cpmin value. Assuming that ka >> kel and that $e^{-ka * t}$ --> 0, using Equation XV-8.

$$Cp_{min} = \frac{F \cdot DOSE}{V} \cdot \left[ \frac{e^{-kel \cdot \tau}}{1 - e^{-kel \cdot \tau}} \right]$$

$$Cp_{min} = \frac{1.0 \times 624}{30} \times \left[ \frac{0.25}{1 - 0.25} \right] = 6.93 \text{ mg/L}$$

Therefore the plasma concentration would probably fluctuate between 7 and 23 mg/L (very approximate) with an average concentration of about 15 mg/L. [23 = 15 + (15-7), i.e. high = average + (average - low), very approximate!].

As an alternative we could give half the dose, 312 mg, every 6 hours give:-

$$Cp_{min} = \frac{F \cdot DOSE}{V} \cdot \left[ \frac{e^{-kel \cdot \tau}}{1 - e^{-kel \cdot \tau}} \right]$$

$$Cp_{min} = \frac{1 \times 312}{30} \times \left[ \frac{0.5}{1 - 0.5} \right] = 10.4 \text{ mg/L}$$

The $\overline{Cp}$ would be the same

$$\overline{Cp} = \frac{F \cdot DOSE}{V \cdot kel \cdot \tau} = \frac{1 \times 312}{30 \times 0.116 \times 6} = 15 \text{ mg/L}$$

Thus the plasma concentration would fluctuate between about 10.4 to 20 with an average of 15 mg/L.

## 3.12.3 Non-uniform Dosing Intervals:

Applies when all disposition processes are linear. That is, distribution, metabolism, and excretion (DME) processes are linear or first order. Thus, concentrations after multiple doses can be calculated by adding together the concentrations from each dose.

For example, calculate drug concentration at 24 hours after the first dose of 200 mg. The second dose of 300 mg was given at 6 hours and the third dose of 100 mg at 18 hours.

$$Cp = Cp^1 + Cp^2 + Cp^3 =$$

Graphically, this will look like the results in the figure below:

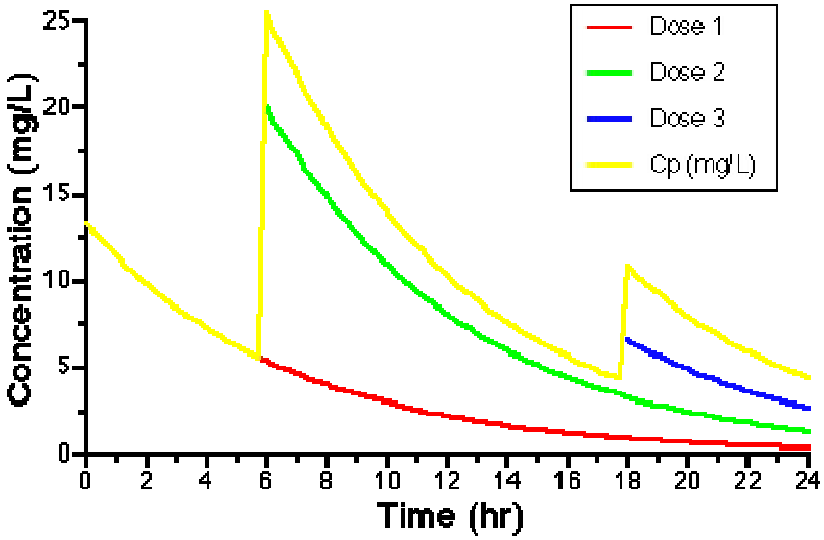



*Figure 3.12.3(1). Drug Concentration after Three IV Bolus Doses*

The calculations we have looked at consider that the dosing intervals are quite uniform, however, commonly this ideal situation is not adhered to completely.

Dosing three times a day may be interpreted as with meals, the plasma concentration may then look like the plot in Figure. The ratio between Cpmax and Cpmin is seven fold (8.2/1.1 = 7.45) in this example.

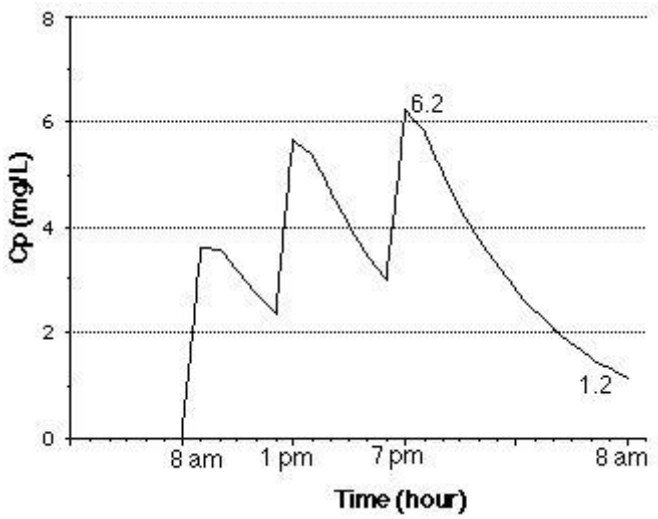



*Figure 3.12.3(3). Cp versus Time during Dosing at 8 am, 1 pm, and 7 pm*

However this regimen may be acceptable if

1) the drug has a wide therapeutic index

2) there is no therapeutic disadvantage to low overnight plasma concentrations, e.g., analgesic of patient stays asleep.

## 3.13 Non-Linear Pharmacokinetic Models:

All of the rate processes discussed so far in this course, except for the infusion process, follow first order kinetics. In particular the elimination process has been assumed to follow first order kinetics. However occasionally it is observed that the elimination of a drug appears to be zero order at high concentrations and first order at low concentrations. That is 'concentration' or 'dose' dependent kinetics are observed. That is at high doses, which produce higher plasma concentrations, zero order kinetics are observed, whereas at lower doses the kinetics are linear, that is first order.

This occurs especially with drugs which are extensively metabolized. A typical characteristic of enzymatic reactions and active transport is a limitation on the capacity of the process. There is only so much enzyme present in the liver, and therefore there is a maximum rate at which metabolism can occur. A further limitation in the rate of metabolism can be the limited availability of a co-substance or co-factor required in the enzymatic process. This might be a limit in the amount of glucuronide or glycine, for example.

Most of our knowledge of enzyme kinetics is derived from *in vitro* studies where substrate, enzyme, and co-factor concentrations are carefully controlled. Many factors are involved *in vivo* so that each cannot be easily isolated in detail. However, the basic principles of enzyme kinetics have application in pharmacokinetics.

Dose dependent pharmacokinetics can often be described by Michaelis-Menten kinetics with the RATE of elimination approaching some maximum rate, Vm.

$$\text{The rate of metabolism} = \frac{Vm \cdot Cp}{Km + Cp}$$

with Km a Michaelis-Menten constant. Km is the concentration at which the rate of metabolism is 1/2 the maximum rate, Vm

## 3.13.1 Scheme:

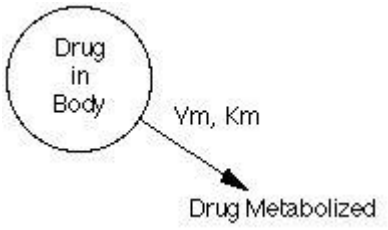



*Figure 3.13.1(1). Scheme for One Compartment Model with Michaelis-Menten Elimination*

We can use above figure, when M-M kinetics is included in a one compartment pharmacokinetic model as the only the route of elimination.

## 3.13.2 Differential equation:

Thus:-

$$\frac{dCp}{dt} = - \frac{Vm \cdot Cp}{Km + Cp}$$

It is not possible to integrate this equation but by looking at low and high concentrations we can get some idea of the plasma concentration versus time curve.

### 3.13.2.1 Low Cp approximation to first order:

At low concentrations, where Km > Cp, Km + Cp is approximately equal to Km

$$\text{therefore } \frac{dCp}{dt} = - \frac{Vm}{Km} \cdot Cp$$

where the Vm/Km is a constant term and the whole equation now looks like that for first order elimination, with Vm/Km a first order elimination rate constant.

Therefore at low plasma concentrations we would expect first order kinetics. Remember, this is the usual situation for most drugs. That is Km is usually larger than the plasma concentrations that are achieved.

### 3.13.2.2 High Cp approximation to Zero Order:

For some drugs, higher concentrations are achieved, that is Cp > Km, then Km + Cp is approximately equal to Cp.

$$\text{therefore } \frac{dCp}{dt} = -\frac{Vm \cdot Cp}{Cp} = -Vm$$

and we now have zero order elimination of drug. At high plasma concentrations we have zero order or concentration independent kinetics.

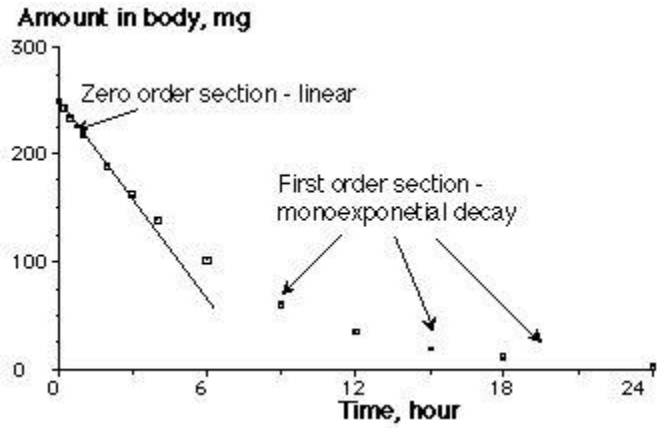



*Figure 3.13.2.2(1). Linear Plot of Cp Versus Time Showing High Cp and Low Cp - Zero Order and First Order Elimination*

From the plot.

High Cp, in the zero order part, the slope is fairly constant but steeper, that is, the rate of elimination is faster than at lower concentrations.

At higher concentrations the slope = -Vm. At lower concentrations we see an exponential decline in plasma concentration such as with first order elimination.

On semi-log graph paper we can see that in the zero order region the slope is more shallow, thus the rate constant is lower. The straight line at lower concentrations is indicative of first order kinetics.

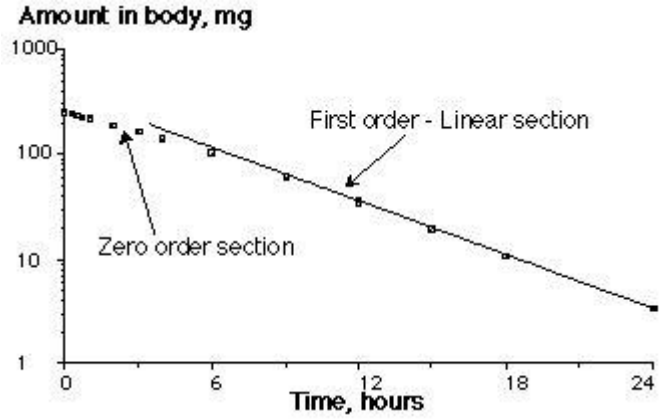



*Figure 3.13.2.2(2) Semi-Log Plot of Cp Versus Time Showing High Cp and Low Cp*

The presence of saturation kinetics can be quite important when high doses of certain drugs are given, or in case of over-dose. In the case of high dose administration the effective elimination rate constant is reduced and the drug will accumulate excessively if saturation kinetics is not understood.

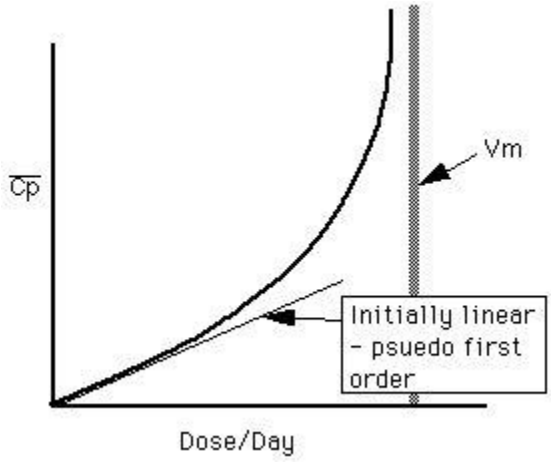



*Figure 3.13.2.2(3). Linear Plot of $\overline{Cp}$ Versus Dose Per Day*

Phenytoin is an example of a drug which commonly has a Km value within or below the therapeutic range. The average Km value about 4 mg/L. The normally effective plasma concentrations for phenytoin are between 10 and 20 mg/L. Therefore it is quite possible for patients to be overdosed due to drug accumulation. At low concentration the apparent half-life is about 12 hours, whereas at higher concentration it may well be much greater than 24 hours. Dosing every 12 hours, the normal half-life, can rapidly lead to dangerous accumulation.

At concentrations above 20 mg/L elimination maybe very slow in some patients. Dropping for example from 25 to 23 mg/L in 24 hours, whereas normally you would expect it to drop from 25 -> 12.5 -> 6 mg/L in 24 hours. Typical Vm values are 300 to 700 mg/day. These are the maximum amounts of drug which can be eliminated by these patients per day. Giving doses approaching these values or higher would cause dangerous accumulation of drug. Figure 90 is a plot of $\overline{Cp}$ versus dose for example.

## 3.13.3 Parallel Pathway:

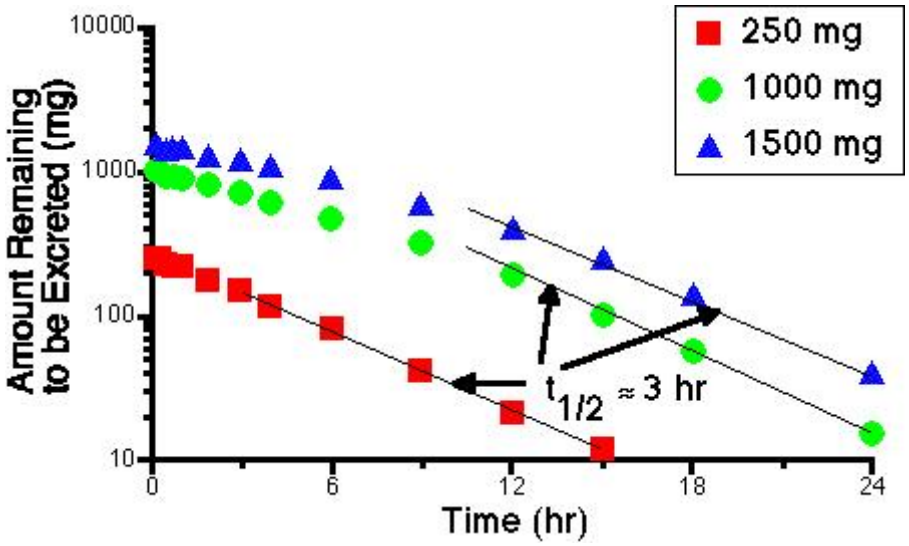



*Figure 3.13.3(1). Plot of Salicylate Amount in the Body Versus Time. Similar $t_{1/2}$ at Lower Concentrations Only*

Another drug with saturable elimination kinetics is aspirin or maybe more correctly salicylate. In the case of aspirin or salicylate poisoning the elimination maybe much slower than expected because of Michaelis-Menten kinetics.

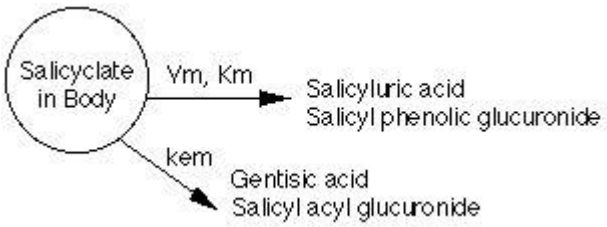



*Figure 3.13.3(2). Scheme for Aspirin/Salicylate Elimination*

In the case of aspirin we have parallel first order process with the Michaelis-Menten kinetics. Therefore as dose increases proportionally more drug would be removed by the first order processes rather than the saturable one. This is shown in the figure below:

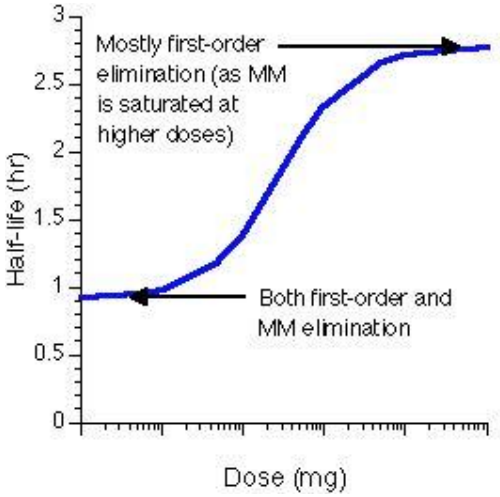



*Figure 3.13.3(3). Plot of Apparent $t_{1/2}$ Versus log(DOSE)*

The phenomena of non-linear pharmacokinetics is of great importance in multiple dose therapy in which more significant changes in the plateau levels are produced by the accumulation of drug in the body than can be expected in single dose studies. This accumulation will result in toxic responses especially when the therapeutic index of the drug is low.

## 3.13.4 Dosing Approaches:

### 3.13.4.1 First Dose:

One approach is to use the population values for phenytoin. With this method we would use the population values of Vm = 7 mg/kg/day and Km = 5 mg/L. Aiming at 15 mg/L for $\overline{Cp}$ with a patient weight of 80 kg, the equation

$$D.R. = \frac{Vm \cdot \overline{Cp}}{Km + \overline{Cp}} \text{ can be used}$$

$$D.R. = \frac{Vm \cdot \overline{Cp}}{Km + \overline{Cp}} = \frac{7 \times 80 \times 15}{(5 + 15)} = 420 \text{ mg/day}$$

Probably better to start out low since toxicity is more probable above 20 mg/L.

### 3.13.4.2 Second Dosing Regimen:

That is after giving a continuous dose regimen to steady state, measure plasma concentration and adjust dose. For example if after 420 mg/day, $\overline{Cp}$ is 20 mg/L then a downward adjustment would be necessary. If we assume that the Km is close to the average value of 5 mg/L we can estimate Vm from the equation above

$$Vm = D.R. + \frac{D.R. \cdot Km}{\overline{Cp}}$$

$$= 420 + \frac{420 \times 5}{20} = 420 + 105 = 525 \text{ mg/day}$$

thus a new dose rate can be calculated

$$D.R. = \frac{Vm \cdot \overline{Cp}}{Km + \overline{Cp}} = \frac{525 \times 15}{5 + 15} = 394 \text{ mg/day}$$

approximately 400 mg/day. Note: A reduction in dose of 20 mg/day (5 %) is calculated to give a 5 mg/L change (25 %) in $\overline{Cp}$. Another approach can be describe using the 'graph' (nomogram) shown below.

Line A represents $Cp^{ss}$ = 8 mg/L on 300 mg/day (70 kg = 4.3 mg/kg/day). Line B was drawn to achieve a new $Cp^{ss}$ = 15 mg/L with a dose of 5.2 mg/kg/day (= 364 mg/day)

The graph is used by plotting the line described by the current $Cp^{ss}$ and R on the graph, marking a point in the middle of the contour. From that point draw a line to the desired $Cp^{ss}$, the value on the vertical axis gives the required dose rate, R.

### 3.13.4.3 Third Dosing Regimen:

If we already have two plasma concentrations after two dose rates we can solve the equation

$$D.R._1 = \frac{Vm \cdot \overline{Cp_1}}{Km + \overline{Cp_1}}$$

using simultaneous equations.

With $\overline{Cp_1}$ = 8.0 mg/L and $\overline{Cp_2}$ = 27.0 mg/L for R1 = 225 mg/day and R2 = 300 mg/day

$$225 = \frac{Vm \cdot 8}{Km + 8} \text{ and } 300 = \frac{Vm \cdot 27}{Km + 27}$$

225 * Km + 225 * 8 = 8 * Vm (1)

and

300 * Km + 300 * 27 = 27 * Vm (2)

or multiplying (1) x 300

300 * 225 * Km + 300 * 225 * 8 = 300 * 8 * Vm (3)

and multiplying (2) x 225

300 * 225 * Km + 300 * 225 * 27 = 225 * 27 * Vm (4)

subtracting (4) - (3)

300 * 225 * (27 - 8) = (225 * 27 - 300 * 8) * Vm

$$Vm = \frac{1282500}{3675} = 349\ mg/day$$

and

$$Km = \frac{8 \cdot Vm - 225 \cdot 8}{225} = \frac{992}{225} = 4.4\ mg/L$$

With these Vm and Km values we can now calculate the next dosing regimen to try.

## 3.14 Routes of Excretion:

The major routes included will be renal, biliary, pulmonary, and salivary.

### 3.14.1 Renal Excretion:

The major organ for the excretion of drugs is the KIDNEY. The functional unit of the kidney is the nephron in which there are three major processes to consider:-

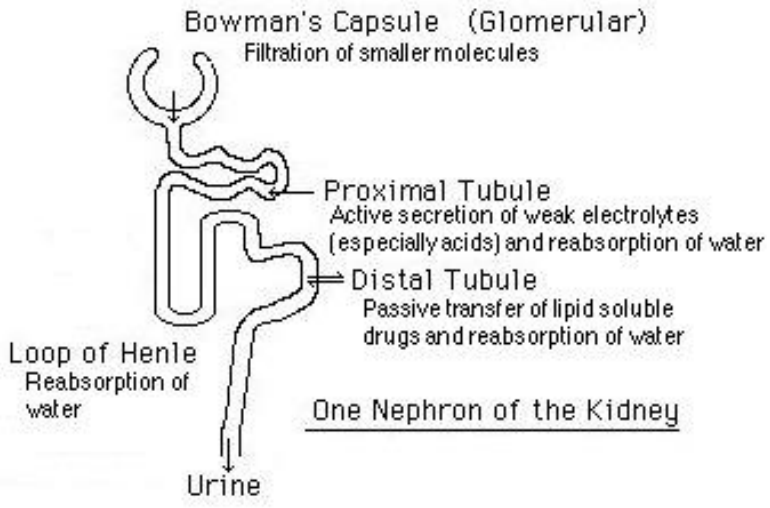



*Figure 3.14.1(1). One Nephron of the Kidney*

### 3.14.1.1 Glomerular Filtration:

In the glomerular all molecules of low molecular weight are filtered out of the blood. Most drugs are readily filtered from the blood unless they are tightly bound to large molecules such as plasma protein or have been incorporated into red blood cells. The glomerular filtration rate varies from individual to individual but in healthy individuals the normal range is 110 to 130 ml/min. About 10% of the blood which enters the glomerular is filtered. This filtration rate is often measured by determining the renal clearance of inulin. Inulin is readily filtered in the glomerular, and is not subject to tubular secretion or re-absorption. Thus inulin clearance is equal to the glomerular filtration rate.

Again, most drugs are filtered from blood in the glomerular, the overall renal excretion however is controlled by what happens in the tubules. More than 90% of the filtrate is reabsorbed. 120 ml/min is 173 L/day. Normal urine output as you may realize is much less than this, about 1 to 2 liter per day.

### 3.14.1.2 Tubular Secretion:

In the proximal tubule there is re-absorption of water and active secretion of some weak electrolyte but especially weak acids. As this process is an active secretion it requires a carrier and a supply of energy. This may be a significant pathway for some compounds such as penicillins. Because tubular secretion is an active process there may be competitive inhibition of the secretion of one compound by another. A common example of these phenomena is the inhibition of penicillin excretion by competition with probenecid. When penicillin was first used it was expensive and in short supply, thus probenecid was used to reduce the excretion of the penicillin and thereby prolong penicillin plasma concentrations (PDR). Since then it has been shown that probenecid also alters the distribution of penicillins to various tissues causing more drug to distribute out of plasma, causing even less to be eliminated.

Drugs or compounds which are extensively secreted, such as *p*-aminohippuric acid (PAH), may have clearance values approaching the renal plasma flow rate of 425 to 650 ml/min, and are used clinically to measure this physiological parameter.

### 3.14.1.3 Tubular Re-absorption:

In the distal tubule there is passive excretion and re-absorption of lipid soluble drugs. Drugs which are present in the glomerular filtrate can be reabsorbed in the tubules.

The membrane is readily permeable to lipids so filtered lipid soluble substances are extensively reabsorbed. A reason for this is that much of the water, in the filtrate, has been reabsorbed and therefore the concentration gradient is now in the direction of re-absorption. Thus if a drug is non-ionized or in the unionized form it maybe readily reabsorbed.

Many drugs are either weak bases or acids and therefore the pH of the filtrate can greatly influence the extent of tubular re-absorption for many drugs. When urine is acidic weak acid drugs tend to be reabsorbed. Alternatively when urine is more alkaline, weak bases are more extensively reabsorbed. These changes can be quite significant as urine pH can vary from 4.5 to 8.0 depending on the diet (e.g. meat can cause a more acidic urine) or drugs (which can increase or decrease urine pH).

In the case of a drug overdose it is possible to increase the excretion of some drugs by suitable adjustment of urine pH. For example, in the case of pentobarbital ( a weak acid) overdose it may be possible to increase drug excretion by making the urine more alkaline with sodium bicarbonate injection. This method is quite effective if the drug is extensively excreted as the unchanged drug (i.e. fe -> 1). If the drug is extensively metabolized then alteration of kidney excretion will not alter the overall drug metabolism all that much.

The effect of pH change on tubular re-absorption can be predicted by consideration of drug pKa according to the Henderson-Hesselbalch equation.

### 3.14.1.4 Renal Clearance:

One method of quantitatively describing the renal excretion of drugs is by means of the renal clearance value for the drug. Remember that renal clearance can be calculated as part of the total body clearance for a particular drug. Renal clearance can be used to investigate the mechanism of drug excretion. If the drug is filtered but not secreted or reabsorbed the renal clearance will be about 120 ml/min in normal subjects. If the renal clearance is less than 120 ml/min then we can assume that at least two processes are in operation, glomerular filtration and tubular re-absorption. If the renal clearance is greater than 120 ml/min then tubular secretion must be contributing to the elimination process. It is also possible that all three processes are occurring simultaneously.

Renal clearance is then:-

$$CL_{renal} = \frac{\text{filtration rate} + \text{secretion rate} - \text{reabsorption rate}}{Cp}$$

Renal clearance values can range from 0 ml/min, the normal value for glucose which is usually completely reabsorbed to a value equal to the renal plasma flow of about 650 ml/min for compounds like *p*-aminohippuric acid.

We can calculate renal clearance using the pharmacokinetic parameters ke and V. Thus $CL_{renal}$ = ke * V. We can also calculate renal clearance by measuring the total amount of drug excreted over some time interval and dividing by the plasma concentration measured at the midpoint of the time interval.

$$\text{Renal Clearance} = \frac{\text{Rate of Excretion}}{\text{Plasma Concentration}}$$

$$\text{Renal Clearance} = CL_{renal} = \frac{\Delta U / \Delta t}{Cp_{midpoint}}$$

To continue we can briefly look at some other routes of drug excretion. We will then return to the topic of renal excretion by considering drug dosage adjustments in patients with reduced renal function.

## 3.14.2 Hemodialysis:

Hemodialysis or `artificial kidney' therapy is used in renal failure to remove toxic waste material normally removed by the kidneys, from the patient's blood. In the procedure blood is diverted externally and allowed to flow across a semi-permeable membrane that is bathed with an aqueous isotonic solution. Nitrogenous waste products and some drugs will diffuse from the blood, thus these compounds will be eliminated. Therefore in patients with kidney failure, hemodialysis will be an important route of drug elimination.

This technique is particularly important with drugs which:

1) have good water solubility;

2) are not tightly bound to plasma protein;

3) are smaller (less than 500) molecular weight; and

4) have a small apparent volume of distribution.

Conversely drugs which are tightly bound or extensively stored or distributed into tissues are only poorly removed by this route, or process.

## 3.14.3 Biliary Excretion:

The liver secretes 0.25 to 1 liter of bile each day. Some drugs and their metabolites are excreted by the liver into bile. Anions, cations, and non-ionized molecules containing both polar and lipophilic groups are excreted into the bile provided that the molecular

weight is greater than about 300. Molecular weights around 500 appears optimal for biliary excretion in humans. Lower molecular weight compounds are reabsorbed before being excreted from the bile duct. Conjugates, glucuronides (drug metabolites) are often of sufficient molecular weight for biliary excretion. This can lead to biliary recycling. Indomethacin is one compound which undergoes this form of recycling.

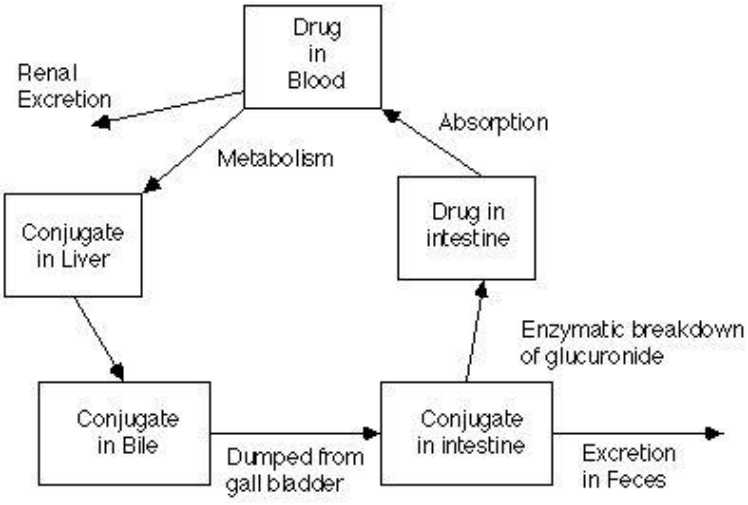



*Figure 3.14.3(1). Enteroheptic Recycling*

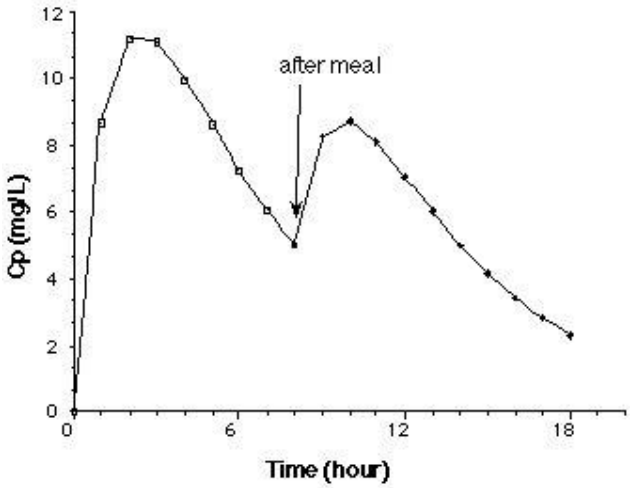



*Figure 3.14.3(2). Cp versus Time showing a Second Peak*

Other compounds extensively excreted in bile include cromoglycate (unchanged drug), morphine, and chloramphenicol (as glucuronide). At least part of the biliary secretion is active since bile/plasma concentrations maybe as high as 50/1. There can also be competition between compounds.

The efficiency of this biliary excretion system can be assessed by use of a test substance, such as Bromsulphalein.

### 3.14.4 Pulmonary Excretion:

The lung is the major organ of excretion for gaseous and volatile substances. The breathalyzer test is based on a quantitative pulmonary excretion of ethanol. Most of the gaseous anesthetics are extensively eliminated in expired air.

### 3.14.5 Salivary Excretion:

This is not really a method of drug excretion as the drug will usually be swallowed and reabsorbed, thus a form of 'salivary recycling'. Drug excretion into saliva appears to be dependent on pH partition and protein binding. This mechanism appears attractive in terms of drug monitoring, that is determining drug concentration to assist in drug dosage adjustment. For some drugs, the saliva/free plasma ratio is fairly constant. Therefore drug concentrations in saliva could be a good indication of drug concentration in plasma. For some drugs localized side effects maybe due to salivary excretion of the drug.

### 3.14.6 Renal Disease Considerations:

#### 3.14.6.1 Dose Adjustment:

Getting back to the renal excretion of drugs. If a drug is extensively excreted unchanged into urine, alteration of renal function will alter the drug elimination rate. Fortunately creatinine clearance can be used as a measure of renal function. For most drugs which are excreted extensively as unchanged drug it has been found that there is a good correlation between creatinine clearance and drug clearance or observed elimination rate (since V is usually unchanged).

Creatinine Clearance:

Creatinine is produced in the body by muscle metabolism from creatine phosphate. Creatinine production is dependent on the age, weight, and sex of the patient. Elimination of creatinine is mainly by glomerular filtration with a small percentage by active secretion. With the patient in stable condition the production is like a continuous infusion to steady state with the infusion rate controlled by muscle metabolism and the elimination controlled by renal function. Thus as renal function is reduced serum creatinine concentrations

increases. Other compounds such as inulin are also used for GFR measurement. Although inulin GFR values are probably more accurate they involve administration of inulin and careful collection of urine for inulin determination. The major advantage of creatinine is that its formation is endogenous. Determination of creatinine clearance consists of collection of total urine and a plasma/serum determination at the mid-point time.

$$\text{Creatinine Clearance} = \frac{\text{rate of excretion into urine}}{\text{serum concentration}}$$

with serum creatinine expressed as mg/100 ml and creatinine clearance as ml/min. Normal inulin clearance values are 124 ml/min for men and 109 ml/min for women. Because of some small renal secretion of creatinine, normal values of creatinine clearance are slightly higher than GFR measured with inulin. Thus, normal creatinine clearance values are about 120 to 130 ml/min.

Various investigators have developed equations which allow calculation of creatinine clearance using serum creatinine values. Thus a single serum level can used. For example, the equation of Cockcroft and Gault.

Males:

$$CL_{Cr} = \frac{[\,140 - \text{age (yr)}\,] \cdot \text{body weight (kg)}}{72 \cdot Cs_{Cr}}$$

Females: Use 85% of the value calculated for males. Lean body weight can be used in this equation.

Estimation of kel in a Patient:

The relationship between creatinine clearance and overall drug elimination can be easily seen by looking at plots of kel observed versus creatinine clearance.

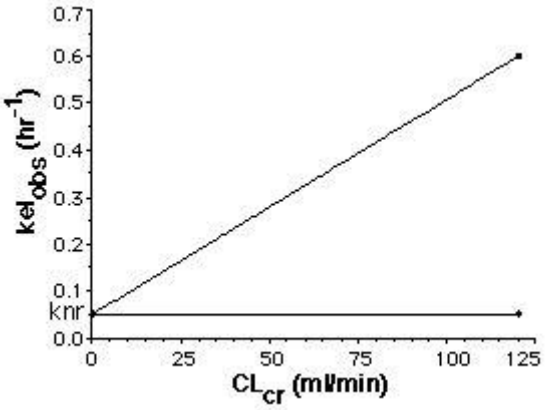



*Figure 3.14.6.1(1). Plot of kel versus CLCR (Dettli Plot)*

These are often called Dettli plots. Above figure shows the situation with considerable excretion as unchanged drug. i.e. fe 0.5 <--> 0.9

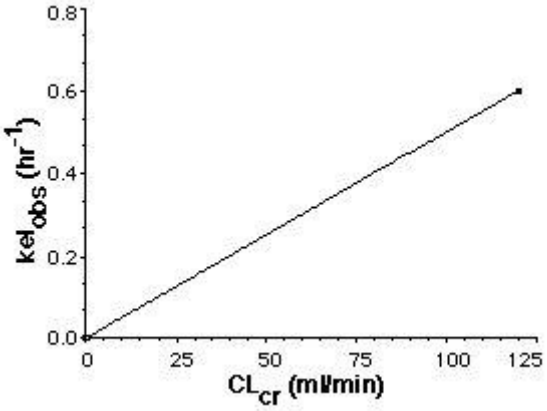



*Figure 3.14.6.1(2). Dettli Plot (fe = 1)*

In above figure drug is excreted entirely as unchanged drug. i.e. fe = 1

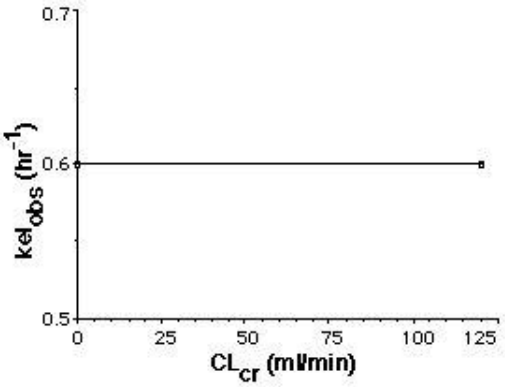



*Figure 3.14.6.1(3). Dettli Plot (fe = 0)*

In above drug is excretion only as metabolized drug. i.e. fe = 0. If we can determine the relationship between CLcr and drug clearance or kel from a number of patients we can then determine the creatinine clearance in a new patient and estimate the elimination rate constant for the drug of interest in this patient. We can therefore calculate an optimum dose and dosing interval.

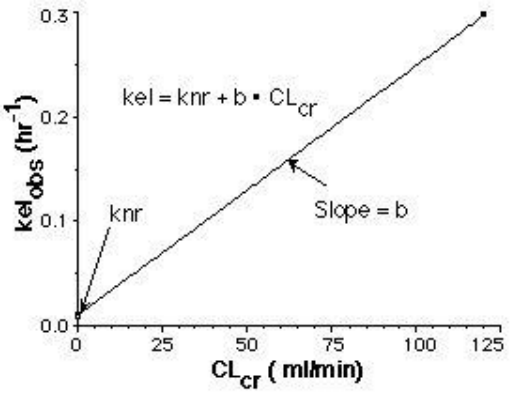



*Figure 3.14.6.1(4). Dettli Plot Showing kel Observed Versus $CL_{CR}$*

The question now arises, how do we calculate kel for a particular drug and patient? For this we need to rely on data previously obtained and published in literature. With this information we can construct a plot of kel versus CLcr. This plot maybe built into a computer program or nomogram.

This is the plot shown before. In the references given earlier there is information useful for calculating kel in patients with impaired renal function.

| For example | knr | B |
|---|---|---|
| Kanamycin | 0.01 | 0.0024 |
| Sulfadiazine | 0.03 | 0.0005 |
| Tetracycline | 0.008 | 0.00072 |

*Table 3.14.6.1(1). Calculating kel in patients with impaired renal function*

As an example these data could be used to calculate the kel for a patient with a CLcr of 10 ml/min compared with normal renal function of 120 ml/min

For kanamycin

kelpatient = knr + b * CLcr = 0.01 + 0.0024 x 10 = 0.01 + 0.024 = 0.034 $hr^{-1}$

cf: kel = 0.01 + 0.0024 x 120 = 0.298 $hr^{-1}$

For sulfadiazine

kelpatient = 0.03 + 0.0005 x 10 = 0.03 + 0.005 = 0.035 $hr^{-1}$

cf: kel = 0.03 + 0.0005 x 120 = 0.09 $hr^{-1}$

For tetracycline

kelpatient = 0.008 + 0.00072 x 10 = 0.008 + 0.0072 = 0.0152 $hr^{-1}$

cf: kel = 0.008 + 0.00072 x 120 = 0.0944 $hr^{-1}$

### 3.14.6.2 Average Cp calculations:

For example consider the drug kanamycin. A patient of 70 kg with normal kidney function may receive 250 mg IM every six hours (about 3 half-lives; $t_{1/2}$ = 2.3 hours). If F = 1.0 and V = 13.3 liter, kel = 0.693/2.3 = 0.30 $hr^{-1}$.

Then

$$\overline{Cp} = \frac{F \cdot DOSE}{V \cdot kel \cdot \tau} = \frac{1 \times 250}{13.3 \times 0.3 \times 6} = 10.4\ mg/L$$

If ka >> kel then

$$Cpmin = \frac{F \cdot DOSE}{V} \cdot \left[ \frac{R}{1 - R} \right]$$

$R = e^{-0.3 * 6} = 0.165$

Cpmin = 3.7 mg/L

These are the results you should expect in a patient with a normal creatinine clearance value. However in a patient with a creatinine clearance of only 10 ml/min the elimination rate constant will be quite different and if the same dosage regimen were used quite different plasma concentrations would be achieved.

The elimination rate constant for this patient would be 0.034 $hr^{-1}$ ($t_{1/2}$ = 20 hr). Using the same dosing regimen:

$$\overline{Cp} = \frac{F \cdot DOSE}{V \cdot kel \cdot \tau} = \frac{1 \times 250}{13.3 \times 0.034 \times 6} = 92\ mg/L$$

This average plasma concentration is well above the 35 mg/L which should be avoided (in the PDR 89 p740). Clearly some dosage adjustment should be made to the dosage regimen.

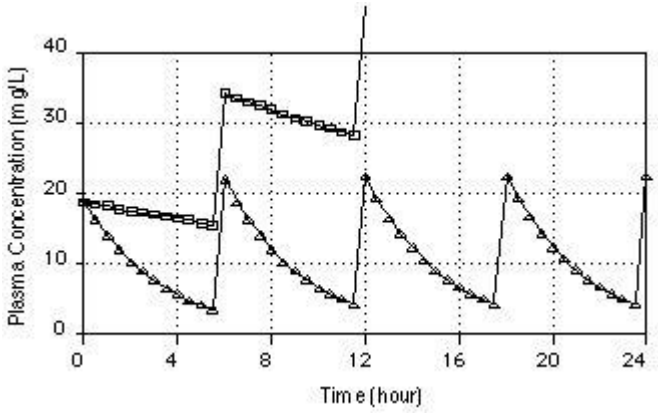



*Figure 3.14.6.2(1). Linear Plot of Cp versus Time*

We should consider

a) changing the dose

b) changing the dosing interval

c) changing both the dose and the dosing interval.

We can make these alterations easily using the $\overline{Cp}$ equation

$$\overline{Cp} = \frac{F \cdot DOSE}{V \cdot kel \cdot \tau}$$

From this we can see that decreasing the dose or increasing the dosing interval will have the desired response.

Altered Dose:

Assuming that a Cp of 10.4 mg/L (the value obtained in the normal patient on a normal dosage regimen) is satisfactory we can calculate a dose to achieve this value by:-

$$DOSE = \frac{\overline{Cp} \cdot V \cdot kel \cdot \tau}{F} = \frac{10.4 \times 13.3 \times 0.034 \times 6}{1} = 28.2 \text{ mg (instead of 250 mg)}$$

Assuming ka >> kel, R = 0.815 and Cpmin = 9.3 mg/L

Thus this new dosing regimen of 28 mg every 6 hours should work

Altered Dose Interval:

$$\tau = \frac{DOSE \cdot F}{\overline{Cp} \cdot V \cdot kel} = \frac{250 \times 1}{10.4 \times 13.3 \times 0.034} = 53 \text{ hours (instead of 6 hours)}$$

Therefore giving 250 mg every 53 hours should achieve a satisfactory plasma concentration profile.

R = 0.165; Cpmin = 3.7 mg/L

We would expect greater fluctuations with this method and dosing every 53 hours is not all that convenient. Every 6 hours is not all that great either if a longer dosing interval would work. We might consider dosing every 24 hours.

Altered Dose and Interval:

Using t = 24 hours

$$DOSE = \frac{\overline{Cp} \cdot V \cdot kel \cdot \tau}{F} = \frac{10.4 \times 13.3 \times 0.034 \times 24}{1}$$ = 113 mg every 24 hours (maybe 100 mg every 24 hours)

R = 0.442; Cpmin = 6.7 mg/L.

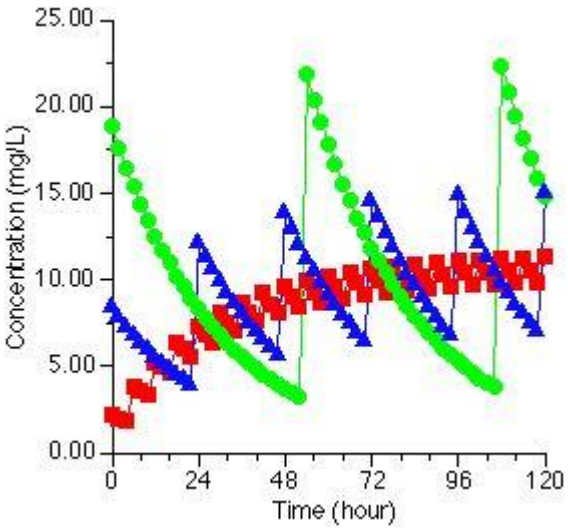



*Figure 3.14.6.2(2). Plot of Cp versus Time*

The lines in Figure were calculated to achieve a $\overline{Cp}$ of 10.4 mg/L using 28 mg q6h, 250 mg q54h, pr 113 mg q24h.

### 3.14.6.3 Cpmax/Cpmin Calculations:

This is another approach, the steps to be taken include

*a. Define Cpmin/Cpmax.:*

From information on the drug with reference to the patient's clinical requirements. For example the normal upper limit for gentamicin peak concentrations might be 6 mg/L, however in case of life-threatening infection higher levels may be approached. Initial

calculation might be based on a peak of 6 mg/L and a trough below 1 mg/L. (Use 1 mg/L as the trough and extend the interval when making the adjustment in $\tau$.

*b. Determine CLcr:*

Probably from serum creatinine levels using the Cockcroft-Gault equation.

*c. Determine kel:*

Using the equation kel = km + b * CLCr with km and b values from the literature

*d. Calculate Tau:*

Since $\frac{Cp_{min}}{Cp_{max}} = R = e^{-kel \cdot \tau}$ and we know Cpmin, Cpmax, and kel we can calculate tau, $\tau$. Typically this will be some uneven time value.

*e. Round Tau:*

A more usual dosing interval should now be chosen. For example a tau of 7.8 or 6.7 hour could be rounded to 8 hours, thus dosing three times a day.

*f. Recalculate R:*

A new value of tau results in a new value of R.

*g. Calculate Maintenance Dose:*

The maintenance dose can be calculated from the minimum or the maximum plasma concentration. Thus

Maintenance dose = Cpmax * V * (1 - R) OR = Cpmin * V * (1 - R)/R

*h. Calculate Loading DOSE:*

The loading dose can be calculated directly (for an iv bolus) by equating $Cp^0$ and the Cpmax value. Thus, Loading dose = Cpmax * V

*Example:* A 75 kg, 65 year old male patient, serum creatinine concentration of 2.3 mg/100 ml, is to be given gentamicin iv to achieve a peak plasma concentration of 6 mg/L and trough concentration below 1 mg/L. The apparent volume of distribution is reported to be 0.28 L/kg. From table 16-3 (Niazi), km and b values are 0.02 and 0.0028, respectively.

a. Cpmax = 6 mg/L and Cpmin = 1 mg/L

b. CLcr = $\frac{[140 - 65] \times 75}{72 \times 2.3} = 34$ ml/min

c. kel = km + b * CLcr = 0.02 + 0.0028 x 34 = 0.115 $hr^{-1}$

d. R = $\frac{1}{6} = 0.1667 = e^{-0.115 \times \tau}$

ln (0.1667) = -1.792 = -0.115 x $\tau$

$\tau$ = 15.6 hour

e. Since a longer dosing interval is needed to keep the trough level below 1 mg/L use a tau value of 18 hours.

f. New R value. $R = e^{-0.115 \times 18} = 0.1262$

g. Calculate maintenance dose using Cpmax = 6 mg/L as reference point. Thus

Maintenance dose = Cpmax * V * (1 - R) = 6 x 75 x 0.28 x (1 - 0.1262) = 110 mg

Thus use 100 mg iv every 18 hours

$$Cp_{max} = \frac{DOSE}{V \cdot (1 - R)} = \frac{100}{75 \times 0.28 \times (1 - 0.1262)} = 5.45 \text{ mg/L}$$

Cpmin = Cpmax * R = 5.45 x 0.1262 = 0.69 mg/L

h. The loading dose can be calculated as:-

Loading dose = Cpmax * V = 6 x 75 x 0.28 = 126 mg. Using 125 mg would give a

Cpmax = $\frac{125}{75 \times 0.28}$ = 5.95 mg/L.

Thus a loading dose of 125 mg followed by 100 mg every 18 hours should be satisfactory.

*Comparison with PDR recommendation.*

Usual dose for 75 kg patient is 75 mg q8h. With serum creatinine 2.3 mg/100 ml give 40 percent of 75 mg q8h. That is 30 mg q8h (R = 0.399) giving 2.38 and 0.95 for Cpmax and Cpmin, respectively.

## 3.15 Metabolism:

The body has another way of deactivating drugs in the body. This method of elimination is metabolism or biotransformation. Metabolic processes, in general, have the overall effect of converting drug molecules into more polar compounds. Again, in general, the effect of this should be to decrease tubular re-absorption in the kidney and thus increase drug elimination.

Generally, it also means an immediate loss of pharmacological activity because transport into the site of action is hindered (less lipid soluble) or the molecule no longer fits into the receptor site. There are exceptions however, and a number of `new' drugs have been discovered as active metabolites.

Metabolism takes place by enzymatic catalysis. Most metabolism occurs in the liver although other sites have been described, such as intestinal wall, kidney, skin, blood.

## 3.15.1 Metabolic Reactions:

There are four main patterns of drug metabolism.

These are:

1) oxidation

2) reduction

3) hydrolysis

4) conjugation

The first three are often lumped together as phase I reactions, while the fourth process, conjugation, is called phase II metabolism. A common scheme in the overall metabolism of drugs is that metabolites are metabolized. In particular a drug maybe oxidized, reduced or hydrolyzed and then another group may be added in a conjugation step.A common cause of capacity limited metabolism is a limit in the amount of the conjugate added in the conjugation step.

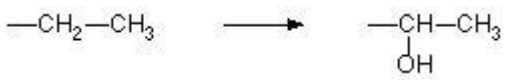
### 3.15.1.1 Phase I:

*Oxidation:*

Oxidation is the addition of oxygen and/or the removal of hydrogen. Most oxidation steps occur in the endoplasmic reticulum.

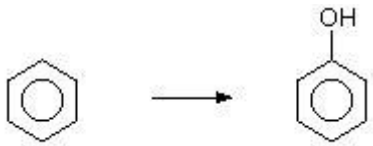
Common reactions include :-

*Alkyl group ----> alcohol*




for example phenobarbitone

*Aromatic ring ----> phenol*



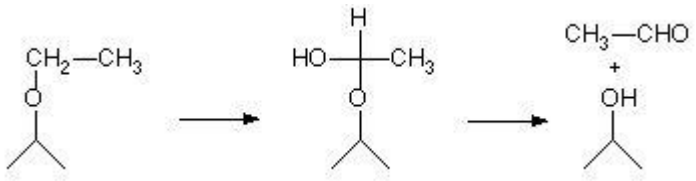

for example phenytoin

*Oxidation at S or N*

*sulfoxide*

for example chlorpromazine

*in two steps oxidative dealkylation is possible*



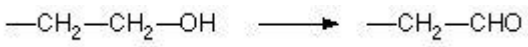

for example phenacetin

Outside the microsomes - in liver and brain

Monoamineoxidaze


for example 5-hydroxytryptamine

Alcohol dehydrogenase - in liver, kidney, lung




*Reduction:*

Add a hydrogen or remove oxygen

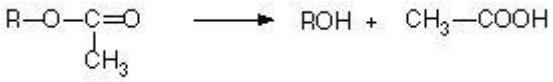
azo (-N=N-) or nitro groups ($-NO_2$) -----> amines ($-NH_2$)

for example nitrazepam

*Hydrolysis:*

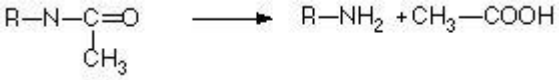
Addition of water with breakdown of molecule. In blood plasma (esterases) and liver

Esters ---> alcohol and acid




for example aspirin to salicylic acid

Amides to amine and acid




for example procainamide

### 3.15.1.2 Phase II:

*Conjugation:*

Conjugation reactions involve the addition of molecules naturally present in the body to the drug molecule. The drug may have undergone a phase I reaction.

*Glucuronidation:*

This is the main conjugation reaction in the body. This occurs in the liver. Natural substrates are bilirubin and thyroxine. Aliphatic alcohols and phenols are commonly conjugated with glucuronide. Thus hydroxylated metabolites can also be conjugated. for example morphine

*Acylation:*

Acylation, especially acetylation with the acetyl group, e.g. sulfonamides

Glycine:

Glycine addition ($NH_2CH_2COOH$) for example nicotinic acid

*Sulfate:*

Sulfate ($-SO_4$) for example morphine, paracetamol

In most cases the metabolite is formed by production of a more polar group, for example C-H -> C-OH, or addition of a polar group, for example acetyl ($CH_3COO-$). Generally the resultant metabolite is more water soluble, and certainly less lipid soluble. Less drug is reabsorbed from the kidney.

Occasionally the metabolite is less water soluble. A significant example is the acetyl metabolite of some of the sulfonamides. Some of the earlier sulfonamides are acetylated to relatively insoluble metabolites which precipitated in urine, crystalluria. The earlier answer this was the triple sulfa combination, now the more commonly used sulfonamides have different elimination and solubility properties and exhibit less problems.

In most cases the metabolites are inactive, however, occasionally the metabolite is also active, even to the extent that the metabolite may be the preferred compound to be administered. The original drug may take on the role of a pro-drug. For example:-

amitriptyline ---> nortriptyline

codeine ---> morphine

primidone ---> phenobarbital

Drug metabolism can be quantitatively altered by drug interactions. This alteration can be an increase by induction of enzyme activity or a reduction by competitive inhibition.

## 3.15.2 Induction:

A large number of drugs can cause an increase over time in liver enzyme activity. This in turn can increase the metabolic rate of the same or other drugs. Phenobarbitone will induce the metabolism of itself, phenytoin, warfarin, etc. Cigarette smoking can cause increased elimination of theophylline and other compounds. Dosing rates may need to be increased to maintain effective plasma concentrations.

## 3.15.3 Inhibition:

Alternately some drugs can inhibit the metabolism of other drugs. Drug metabolism being an enzymatic process can be subjected to competitive inhibition. For example, warfarin inhibits tolbutamide elimination which can lead to the accumulation of drug and may require a downward adjustment of dose.

## 3.15.4 Hepatic Clearance:

The systemic clearance, CL, is a measure of the efficiency with which a drug is irreversibly removed from the body. Under first order conditions, clearance can be calculated as:-

Single dose

$$CL = \frac{F \cdot DOSE}{AUC} = \frac{0.693 \cdot V_{area}}{t_{1/2}}$$

*Clearance after a Single Dose*

During multiple dose regimens, clearance can be calculated under steady state conditions as:-

$$CL = \frac{k0}{Cp^{ss}} = \frac{F \cdot DOSE}{\overline{Cp} \cdot \tau}$$

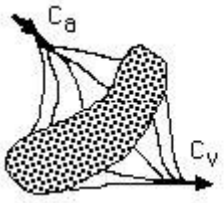
*Clearance at Steady State*

where $Cp^{ss}$ is the steady state plasma concentration and Cp is the average plasma concentration during the dosing interval $\tau$.

### 3.15.4.1 Venous Equilibration Model Equation:

The above equations apply to the overall clearance of drug from the body. We can also consider the organ clearance as it may be measured in an isolated organ system. Here

we would have for example an isolated liver, perfused with blood containing the drug of interest. By measuring the drug concentration in the blood entering and leaving the organ at steady state, the organ clearance can be measured for the drug.




$$\text{Organ Clearance} = \frac{Q.(C_a - C_v)}{C_a}$$

$$= Q.E$$

*Figure 3.15.4.1(1). Blood Flow through the Liver*

where Q is the blood flow rate to the organ, Ca is the concentration of drug in the blood entering the organ, and Cv is the concentration of drug in the blood leaving the organ. The term E is the steady state extraction ratio. High E values mean high clearance by the liver and thus extensive metabolism.

The sum of the individual organ clearance values are equal to the systemic clearance, CL. For a drug which is eliminated entirely via the liver, the hepatic clearance is equal to the systemic or total body clearance. From the equation above we can see that the organ clearance is a function of the liver blood flow and the extraction ratio of the drug. The liver blood flow is a physiological parameter which may be altered in disease states. The extraction ratio, we shall see shortly is a parameter dependent not only of the condition of the liver but also the drug.

Both the hepatic clearance and the extraction ratio are empirical parameters which can be used as measures of the efficiency of the elimination process. They are dependent on three independent variables:-

i) total hepatic blood flow (Q),

ii) fraction unbound (fu) or the extent of drug binding to blood constituents. This may be saturable with high dose, polar compounds, and

iii) the free intrinsic clearance (CLint) or the rate-limiting step in drug uptake from blood, intracellular transport, metabolism, and where necessary biliary secretion. The free intrinsic clearance may be thought of as the clearance of drug from liver plasma water, devoid of the influence of blood flow or binding. Since a major part of this parameter is metabolism which is typically enzyme mediated this parameter may be saturated at higher doses, for some drugs.

The equation describing hepatic clearance in terms of these parameters using the venous equilibration model can be defined as:

$$CL = Q \cdot \frac{fu \cdot CL_{int}}{(Q + fu \cdot CL_{int})} = \frac{Q \cdot CL_{int}^{total}}{Q + CL_{int}^{total}}$$

*Clearance*

$$\text{with } E = \frac{fu \cdot CL_{int}}{Q + fu \cdot CL_{int}}$$

$CL = Q * E$

With this equation it is possible to look at the influence of free intrinsic clearance, drug binding, and liver blood flow on the overall hepatic clearance of a drug. Drugs can be classified into three types depending on the intrinsic clearance and binding. Flow limited, capacity limited, and others.

### 3.15.4.2 Flow Limited Drugs:

High $fu * CL_{int}$ ( $= \mathbf{CL_{int}^{total}}$ ) value:

For drugs with high total intrinsic clearance the extraction ratio, E, approaches 100%, the hepatic clearance approximates and is dependent of hepatic blood flow. Hepatic clearance is said to be FLOW LIMITED. Also, we can note that the hepatic clearance is not dependent on moderate changes in free intrinsic clearance or binding to blood constituents.

$$CL = Q \cdot \frac{fu \cdot CL_{int}}{fu \cdot CL_{int}} = Q$$

Examples include:- lidocaine, propranolol, morphine.

### 3.15.4.3 Capacity Limited Drugs:

Very Low Total Intrinsic Clearance:

With drugs having very low intrinsic clearance, hepatic extraction is inefficient and hepatic clearance becomes independent of hepatic blood flow. Now changes in free intrinsic clearance and/or binding to blood constituents becomes very important in determination of the overall hepatic clearance. Hepatic clearance is said to be CAPACITY LIMITED as the intrinsic capacity of the liver controls the drug clearance.

$$CL = Q \cdot \frac{fu \cdot CL_{int}}{Q} = fu \cdot CL_{int}$$

Examples include: phenytoin, warfarin, and quinidine. For such drugs it is possible that liver disease will cause a decrease in $CL_{int}$ but also an increase in fu. In this case the overall hepatic clearance doesn't reflect just the hepatic metabolic activity but also the drug binding. This is illustrated with tolbutamide. In patients with hepatitis there is an increase in fu but no change in $CL_{int}$. As a result CL is increased and the elimination half-life decreases. The change in elimination half-life reflects changes in binding and not changes in drug metabolizing activity.

#### 3.15.4.4 Other Drugs:

Between these two extremes. Capacity-limited but binding-insensitive drugs: The three parameters; Q, fu, and $CL_{int}$ are important determinants of drug elimination.
Examples include:- theophylline, antipyrine

### 3.15.5 Systemic Availability:

Even if we can assume that a drug is completely absorbed across the G-I tract, a proportion of the dose may be eliminated by the liver before reaching the systemic circulation because of the anatomical arrangement of the portal circulation. This pre-systemic or first-pass elimination can be determined from the extraction ratio, E, such that the fraction of the dose that is available to the central circulation is 1-E. This 1-E value becomes the maximum availability possible before allowing for reduced product performance.

For drugs which are extensively metabolized, first pass metabolism can be quite important. It means that higher doses must be given orally compared with parenteral administration.

for example morphine p.o. 30 mg cf. IV. 5 mg

lidocaine not active p.o.

In liver disease there is potential for changing the systemic availability of high extraction drugs and thereby affecting steady state concentrations.

If liver disease causes a modest reduction in the extraction ratio, from for example 0.95 to 0.9, the fraction of the orally administered drug reaching the systemic circulation (1-E) will be doubled. One of the consequences of the pathogenesis of chronic liver disease is the development of porta-systemic shunts that may carry drug absorbed from the G-I tract through the mesenteric veins directly into the systemic circulation. Thus in a disease where

biochemical hepatic function is relatively well maintained (e.g., schistosomiasis), oral treatment with high clearance drugs such as morphine or propranolol can lead to high blood levels and an increase in adverse drug effects. For example, 30 mg morphine orally may act like 30 mg IV. and lead to over dosage with respiratory depression.

## 3.16 Drug Distribution:

Drug distribution means the reversible transfer of drug from one location to another within the body. Once a drug has entered the vascular system it becomes distributed throughout the various tissues and body fluids in a pattern that reflects the physiochemical nature of the drug and the ease with which it penetrates different membranes. The ONE COMPARTMENT model assumes rapid distribution but it does not preclude extensive distribution into various tissues.

### 3.16.1 Drug Distribution Patterns:

Distribution can be thought of as following one of four types of patterns.

1) The drug may remain largely within the vascular system. Plasma substitutes such as dextran are an example of this type, but drugs which are strongly bound to plasma protein may also approach this pattern.

2) Some low molecular weight water soluble compounds such as ethanol and a few sulfonamides become uniformly distributed throughout the body water.

3) A few drugs are concentrated specifically in one or more tissues that may or may not be the site of action. Iodine is concentrated by the thyroid gland. The antimalarial drug chloroquine may be present in the liver at concentrations 1000 times those present in plasma. Tetracycline is almost irreversibly bound to bone and developing teeth. Consequently tetracyclines should only be given to young children or infants in extreme conditions as it can cause discoloration and mottling of the developing second set of teeth. Another type of specific concentration may occur with highly lipid soluble compounds which distribute into fat tissue.

4) Most drugs exhibit a non-uniform distribution in the body with variations that are largely determined by the ability to pass through membranes and their lipid/water solubility. The highest concentrations are often present in the kidney, liver, and intestine usually reflecting the amount of drug being excreted.

Pattern 4 is the most common being a combination of patterns 1, 2 and 3.

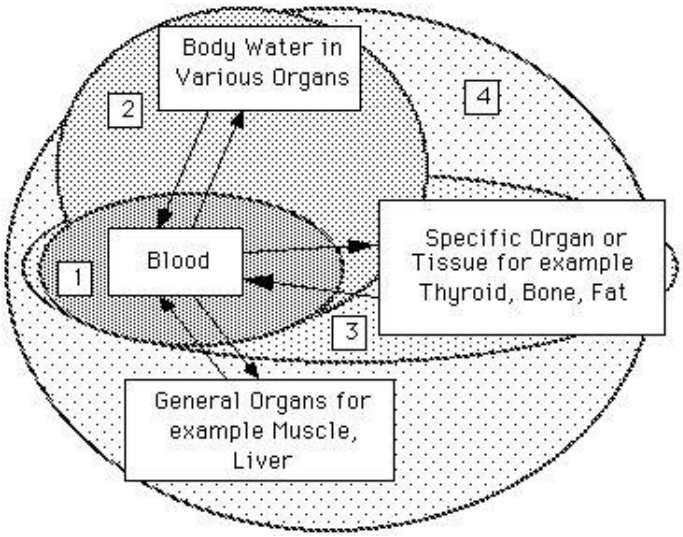



*Figure 3.16.1(1). Representing Various Volumes Distribution Patterns*

| Drug | Liters/Kg | Liter/70 Kg |
|---|---|---|
| Chloroquine | 94 - 250 | 6600 - 17500 |
| Nortriptyline | 21 | 1500 |
| Digoxin | 7 | 500 |
| Lidocaine | 1.7 | 120 |
| Theophylline | 0.5 | 35 |
| Tolbutamide | 0.11 | 8 |

*Table 3.16.1(1). Apparent Volumes of Distribution*

A useful indicator of the type of pattern that characterizes a particular drug is the apparent volume of distribution.

A value of V in the region of 3-5 liter (in an adult) would be compatible with pattern 1. This is approximately the volume of plasma. Pattern two would be expected to produce a V value of 30 to 50 liter, corresponding to total body water. Agents or drugs exhibiting pattern 3 would exhibit very large values of V if the drug concentration effect was acting on most of the dose. Chloroquine has a V value of approximately 17,000 liter. Drugs following pattern 4 may have a V value within a wide range of values. These patterns of variation have been used to determine body fluid volumes.

| Fluid substances | Volume (liter) | Test |
|---|---|---|
| Extracellular Fluid | 13-16 | Inulin, Na23, $Br^-$, $I^-$ |
| Plasma | 3-4 | Evans blue, $I^{131}$ albumin, dextrans |
| Interstitial fluids | 10-13 | |
| Intracellular fluids | 25-28 | |
| Total body water | 40-46 | Antipyrine, $D_2O$, ethanol |

*Table 3.16.1(2). Volumes Measured by Various Test Materials*

## 3.16.2 Factors affecting Drug Distribution

### 3.16.2.1 Rate of Distribution:

| Rate of distribution - | Membrane permeability |
|---|---|
| | Blood perfusion |
| Extent of Distribution - | Lipid Solubility |
| | pH - pKa |
| | Plasma protein binding |
| | Intracellular binding |

*Table 3.16.2.1(1). Rate of distribution*

Membrane Permeability:

We have already covered some material about membrane permeability. The capillaries are typically lined with endothelium whose cells overlap, though to a lesser degree than epithelial cells. Also, the junctions between cells are discontinuous. Capillary walls are quite permeable. Lipid soluble drugs pass through very rapidly. Water soluble compounds penetrate more slowly at a rate more dependent on their size. Low molecular weight drugs pass through by simple diffusion. For compounds with molecular diameter above 100 Å transfer is slow.

For drugs which can be ionized the drug's pKa and the pH of the blood will have a large effect on the transfer rate across the capillary membrane.

There are two deviations to the typical capillary structure which result in variation from normal drug tissue permeability.

i) Permeability is greatly increased in the renal capillaries by pores in the membrane of the endothelial cells, and in specialized hepatic capillaries, known as sinusoids which may lack a complete lining. This results in more extension distribution of many drugs out of the capillary bed.

ii) On the other hand brain capillaries seem to have impermeable walls restricting the transfer of molecules from blood to brain tissue. Lipid soluble compounds can be readily transferred but the transfer of polar substances is severely restricted. This is the basis of the "blood- brain" barrier.

Membrane permeability tends to restrict the transfer and distribution of drugs once they are delivered to the tissue. The other major factor which determines the rate of drug distribution is blood perfusion.

Blood Perfusion Rate:

The rate at which blood perfuses to different organs varies widely.

| Organ | Perfusion Rate (ml/min/ml of tissue) | % of cardiac output |
|---|---|---|
| Bone | 0.02 | 5 |
| Brain | 0.5 | 14 |
| Fat | 0.03 | 4 |
| Heart | 0.6 | 4 |
| Kidneys | 4.0 | 22 |
| Liver | 0.8 | 27 |
| Muscle | 0.025 | 15 |
| Skin | 0.024 | 6 |

*Table 3.16.2.1(2). Blood Perfusion Rate*

Total blood flow is greatest to brain, kidneys, liver, and muscle with highest perfusion rates to brain, kidney, liver, and heart. It would be expected that total drug concentration would rise most rapidly in these organs. Certain organs such as the adrenals (1.2/0.2%) and thyroid (2.4/1%) also have large perfusion rates.

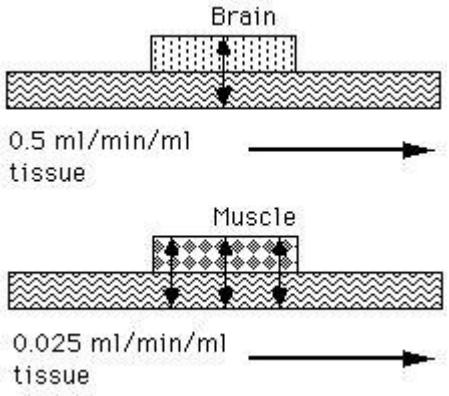



*Figure 3.16.2.1(1). Comparison between Drug transfer to Brain and Muscle*

As an example; thiopental gets into the brain faster than muscle, whereas, penicillin gets into muscle more quickly than it gets into brain.

i) Thiopental is only partly ionized and passes into the brain or muscle easily. Perfusion limits the transport. Since brain has a higher perfusion rate the thiopental can transfer in and out more quickly.

ii) Penicillin is quite polar and is thus slowly permeable. Permeability limited transfer is faster in muscle as muscle capillaries are less restrictive. Thus transfer of penicillin is faster in muscle than brain.

In brain, perfusion or membrane permeability limits drug transport or distribution. Thiopental diffuses readily, thus perfusion limits its distribution. Since perfusion is higher to the brain than to muscle, transport to the brain is faster. Penicillin less readily diffuses thus it is diffusion which limits penicillin distribution. Muscle diffusion is easier thus distribution into muscle is faster for penicillin than distribution into brain.

### 3.16.2.2 Extent of Distribution:

We can now consider factors which alter the extent of drug distribution

Plasma Protein Binding:

Extensive plasma protein binding will cause more drugs to stay in the central blood compartment. Therefore drugs which bind strongly to plasma protein tend to have lower volumes of distribution.

Proteins Involved:

Although drugs are bound to many macromolecules, binding to plasma protein is the most common. Of these plasma proteins, albumin, which comprises 50 % of the total proteins binds the widest range of drugs. Acidic drugs commonly bind to albumin, while basic drugs often bind to alpha$_1$-acid glycoproteins and lipoproteins. Many endogenous substances, steroids, vitamins, and metal ions are bound to globulins.

| Drugs | Binding Sites for Acidic Agents |
|---|---|
| Bilirubin, Bile acids, Fatty Acids,Vitamin C, Salicylates, Sulfonamides,Barbiturates, Phenylbutazone,Penicillins, Tetracyclines, Probenecid | Albumins |
| | Binding Sites for Basic Agents |
| Adenisine, Quinacrine, Quinine,Streptomycin, Chloramphenicol,Digitoxin, Ouabain, Coumarin | Globulins, alpha$_1$, alpha$_2$, beta$_1$, beta$_2$, gamma |

*Table 3.16.2.2(1). Proteins with Potential Binding Sites for Various Drugs*

Forces involved:

Groups on the protein molecules that are responsible for electrostatic interactions with drugs include:

the $-NH_3^+$ of lysine and N- terminal amino acids,

the $-NH_2^+-$ of histidine, the - S$^-$ of cysteine, and

the - COO$^-$ of aspartic and glutamic acid residues.

In order to achieve reasonably stable complexes, however, it is likely that in most cases the initial electrostatic attraction is reinforced at close range by van der Waal's forces (dipole-dipole; dipole-induced dipole; induced dipole-induced dipole) and hydrogen bonding. This is suggested by the frequently crucial role of protein configuration in the binding phenomenon. Agents which denature protein may cause the release of bound drug. Often there may be competition between drugs, in which agents that are bound very tightly, such as coumarin anticoagulants, are able to displace less tightly bound compounds from their binding sites.

| Drug | Percent Unbound (100 * fu) |
|---|---|
| Caffeine | 90 |
| Digoxin | 77 |
| Gentamicin | 50 |
| Theophylline | 85 |
| Phenytoin | 13 |
| Diazepam | 4 |
| Warfarin | 0.8 |
| Phenylbutazone | 5 |
| Dicumarol | 3 |

*Table 3.16.2.2(2). Percent Unbound for Selected Drugs*

Slight changes in the binding of highly bound drugs can result in significant changes in clinical response or cause a toxic response. Since it is the free drug in plasma which equilibrates with the site of pharmacological or toxic response, a slight change in the extent of binding, such as 99 to 98 % bound, which can result in an almost 100 % change in free concentration, can cause very significant alteration in response. For a large number of drugs, including warfarin and phenytoin, drug response will be dependent on free drug concentration. Alteration of free concentration by drug interaction or disease state can alter the intensity of action of these drugs. Examples include phenylbutazone and salicylates displacing tolbutamide to give an increased effect, hypoglycemia.

As you can see from Table XVIII-5, the extent of protein binding can vary considerably from one drug to another.

Protein Binding Determination:

*Spectral changes:* Most drugs have distinct UV spectra because of the conjugated chromophores in the molecule. When a drug interacts with a protein the UV or visible spectrum may be changed because of alterations in the electronic configuration. These alterations can be quantitated and used to determine the extent of binding. Changes in fluorescence spectra can be used in the same way. Spectra for warfarin.

*Gel filtration:* This involves the use of porous gels that are molecular sieves. They separate components on the basis of size. Low molecular weight drugs are held on the gel whereas bound drug and protein are washed through.

*Equilibrium dialysis:*

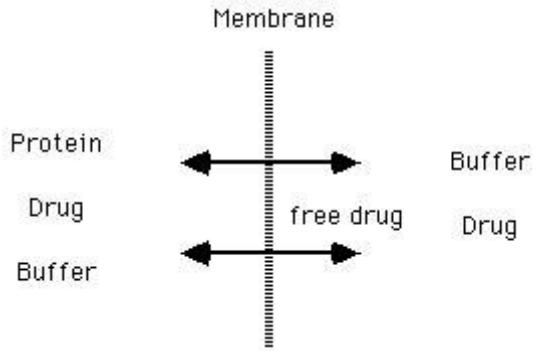



*Figure 3.16.2.2(1). Equilibrium Across a Semi-permeable Membrane*

The protein solution (e.g. plasma) containing drug and a buffer solution are placed on opposite sides of a dialysis membrane.

After a sufficient time (maybe 12- 24 hours), free drug concentration will be the same on either side of the membrane. Protein binding can be determined by measuring the concentration of drug on either side of the membrane. On left the concentration will involve free and bound drug, whereas on the right there is no binding and the concentration will equal to the free drug concentration.

*Ultrafiltration:*

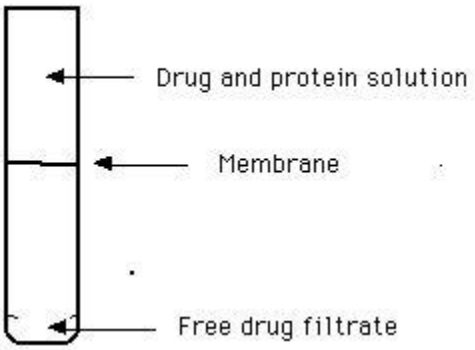



*Figure 3.16.2.2(2). Ultrafiltration as a Method of Measuring Protein Binding*

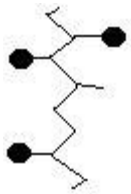
A quicker method of separating free and bound drug is the ultrafiltration method. Drug and protein solution are placed in a filter membrane and liquid containing free drug is forced through the membrane by centrifugation.

### Protein binding equilibria

With one type of binding site, protein binding can be described mathematically by the equation:

$$[D] + [nP - rP] \underset{Kd}{\overset{Ka}{\rightleftharpoons}} [DP]$$

With [D] free drug concentration, [P] total protein concentration with 'n' binding sites per molecule, thus [nP] is the total concentration of protein binding sites and [rP] = [DP] is the concentration of bound drug or bound protein with r drug molecules bound per protein molecule. Typically there may be 1 - 4 binding sites per protein molecule.




Ka = association constant $= \frac{[\text{Concentration Bound}]}{[D] \cdot [\text{Protein Free}]} = \frac{[rP]}{[D] \cdot [nP - rP]}$

where $r = \frac{[DP]}{[P]_{total}} = \frac{[\text{Drug Bound}]}{[\text{Total Protein}]}$

*Plots:*

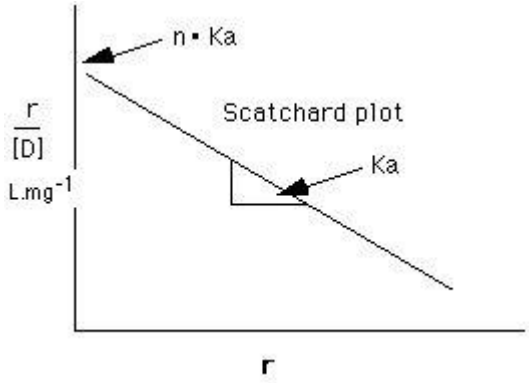



*Figure 3.16.2.2(3). Plot of r/[D] Versus r*

This can be rearranged to give $\frac{r}{[D]} = n \cdot Ka - Ka \cdot r$, thus plotting r/[D] versus r should give a straight line. This is called a Scatchard plot.

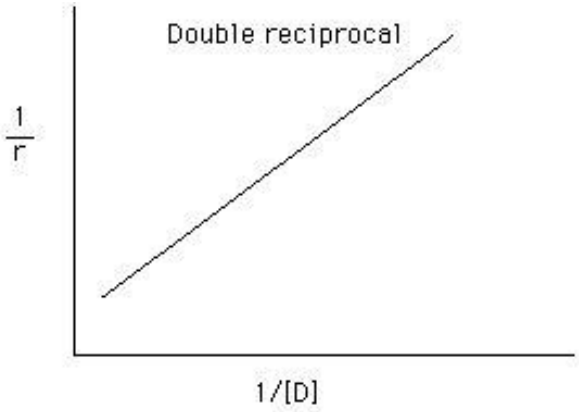



*Figure 3.16.2.2(4). Plot of 1/r Versus 1/[D]*

Alternate rearrangement gives

$\frac{1}{r} = \frac{1}{(n \cdot Ka \cdot [D])} + \frac{1}{n}$, thus a plot of 1/r versus 1/[D] should also give a straight line. This the double reciprocal plot.

With one type of binding site these plots produce straight lines which can be used to determine Ka and n values. With more than one type of binding site, these plots are curved.

Tissue localization of drugs:

In addition to plasma protein binding, drugs may bind to intracellular molecules. Certain of these may be actual drug receptors, and the interaction that occurs may represent the molecular basis of the pharmacological action.

The affinity of a tissue for a drug may be for any of several reasons, including binding to tissue proteins (such as albumin) or to nucleic acids, or in the case of adipose tissue, dissolution in the lipid material.

The concentration of chloroquine in the liver is due to the binding of the drug to DNA. Barbiturates distribute extensively into adipose tissue, primarily because of their high lipid solubility. Tetracyclines bind to bone thus should be avoided in young children or discoloration of permanent teeth may occur.

Unlike plasma binding, tissue binding of a drug cannot be measured directly as handling of the tissue results in disruption of the binding. This doesn't mean that tissue binding and changes in tissue binding are not important.

### 3.16.3 Weight Considerations:

The apparent volume of distribution will often be proportional to the total body weight of a patient. In fact many V values found in the literature will be given as so many liter per kilogram total body weight. The assumption made is that the body composition is unchanged on a percentage basis, thus distribution will be identical no matter what the patient weighs. This works within some limits.

For example body composition of the very young and the very old may be quite different from `normal', that is the average subject in whom the parameter values may have been originally determined.

For example the apparent volume of distribution of antipyrine is 0.62 l/kg in normal weight subjects but 0.46 l/kg in obese patients. Other drugs such as digoxin and gentamicin are also quite polar and tend to distribute into water rather than adipose tissue.

### 3.16.4 Protein Binding Interactions:

The role of protein binding in drug interactions can be quite involved. Although drugs may well displace each other from common binding sites, the clinical (and pharmacokinetic) importance of these interactions may require considerable investigation.

For these effects to be important one drug must be extensively protein bound, while the displacer must have a high affinity for the same binding site.

Therapeutically the major criteria are the free drug concentration. One result of a drug interaction is to tend to produce an increase in free drug concentration, however, that will cause an increase in elimination and thus an overall reduction in total drug concentration, potentially maintaining the free concentrations unchanged.

## 3.17 Multi-Compartment Pharmacokinetic Models:

So far we have talked about the pharmacokinetics of drugs in terms of a one compartment model. We have assumed that the drug, once administered is mixed instantaneously in the blood and that the drug distributes throughout the body rapidly reaching equilibrium throughout the tissue into which the drug enters. We have in essence considered that the body acts as a well mixed container.

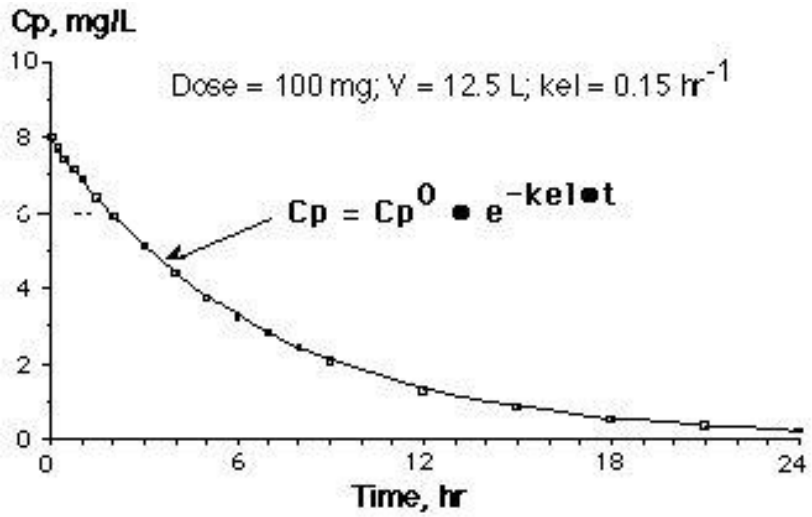



*Figure 3.17(1). Linear Plot of Cp Versus Time for a One-Compartment - IV Bolus*

With first order drug elimination we found that the plasma concentration will fall monoexponentially with time following IV bolus administration.

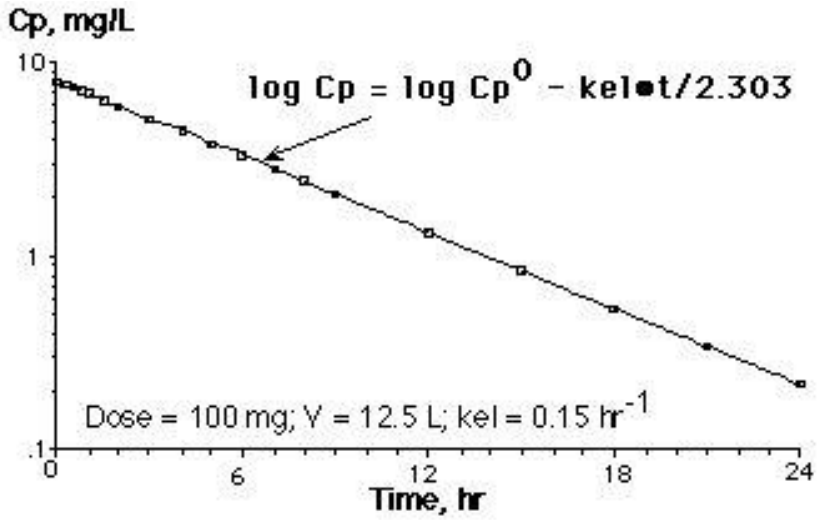



*Figure 3.17(2). Semi-Log Plot of Cp Versus Time*

And the log of the plasma concentration will fall as a straight line.

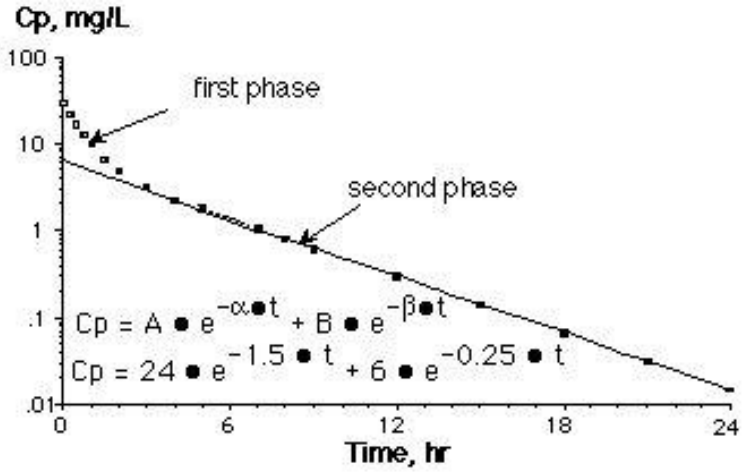



*Figure 3.17(3). Semi-Log Plot of Cp Versus Time. Two-Compartment - IV Bolus.*

Commonly we find with real data, especially if we have a number of early data points, that the log Cp versus time plot is not a straight line. We see an initial early deviation from the straight line, followed by a log-linear phase. The initial phase is a more rapid drop in plasma concentration before settling into the log-linear fall in plasma concentration.

This suggests that the body is not behaving as a single well mixed compartment. There appears, mathematically, to be distribution between two (or more) compartments. That is we don't have instantaneous equilibrium between the drug in all the various tissues of the body. In the next approximation we can consider that the body is behaving as two distinct compartments. These compartments can be called the central compartment and the

peripheral compartment. Exact anatomical assignment to these compartments is not always possible. However, generally the rapidly perfused tissues often belong in the central compartment.

## 3.17.1 Intravenous administration

### 3.17.1.1 Scheme or Diagram:

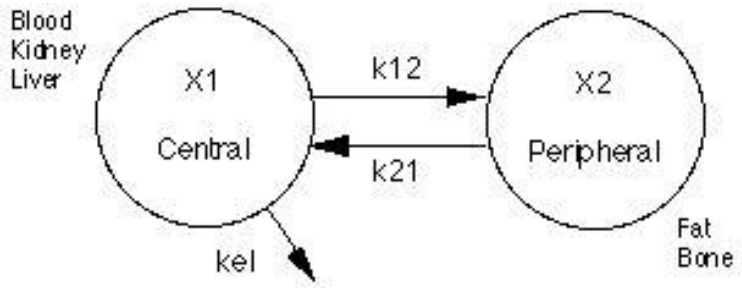



*Figure 3.17.1.1(1). Two Compartment Pharmacokinetic Model*

### 3.17.1.2 Differential Equation:

The differential equation for drug in the central compartment following intravenous bolus administration is:-

$$\frac{dX1}{dt} = -kel \cdot X1 - k12 \cdot X1 + k21 \cdot X2$$

The kel * X1 term describes elimination of the drug from the central compartment, while the k12 * X1 and k21 * X2 terms describe the distribution of drug between the central and peripheral compartments.

### 3.17.1.3 Integrated equation

Integration of this equation (using Laplace transforms) leads to a biexponential equation for plasma concentration as a function of time.

Thus,

$Cp = A * e^{-\alpha * t} + B * e^{-\beta * t}$ with $\alpha > \beta$.

The A, B, $\alpha$, and $\beta$ terms were derived from the microconstants during the integration process. They are functions of the microconstant k12, k21, kel and V1

$\alpha + \beta = kel + k12 + k21$

$\alpha * \beta = kel * k21$

$$\alpha, \beta = \frac{(\alpha + \beta) \pm \sqrt{(\alpha + \beta)^2 - 4 \cdot \alpha \cdot \beta}}{2}$$

$$A = \frac{DOSE \cdot (\alpha - k21)}{V_1 \cdot (\alpha - \beta)}$$

$$B = \frac{DOSE \cdot (k21 - \beta)}{V_1 \cdot (\alpha - \beta)}$$

### 3.17.1.4 Parameter Determination:

Method of Residuals:

Values for all these parameters can be determined by first calculating A, B, $\alpha$, and $\beta$. For this we can use the method of residuals (in a similar fashion to determining ka and kel for the one compartment model after oral administration). By definition $\alpha$ is greater than $\beta$ then as t --> $\infty$, $e^{-\alpha * t}$ --> 0 faster than $e^{-\beta * t}$. Therefore if the ratio $\alpha$ / $\beta$ is large enough (greater than 5) the terminal data points will fall on the line

$Cp^{late} = B * e^{-\beta * t}$

This equation is similar to the equation for the late plasma concentration values after oral administration with a one compartment model. This line will be linear if plotted on semi-log graph paper.

From the slope of this line a value of $\beta$ can be determined. The $t_{1/2}$ calculated as 0.693/ $\beta$ is often called the biological half-life or terminal half-life. It is the half-life describing the terminal elimination of the drug from plasma. [For the one compartment model the biological half-life is equal to 0.693/kel].

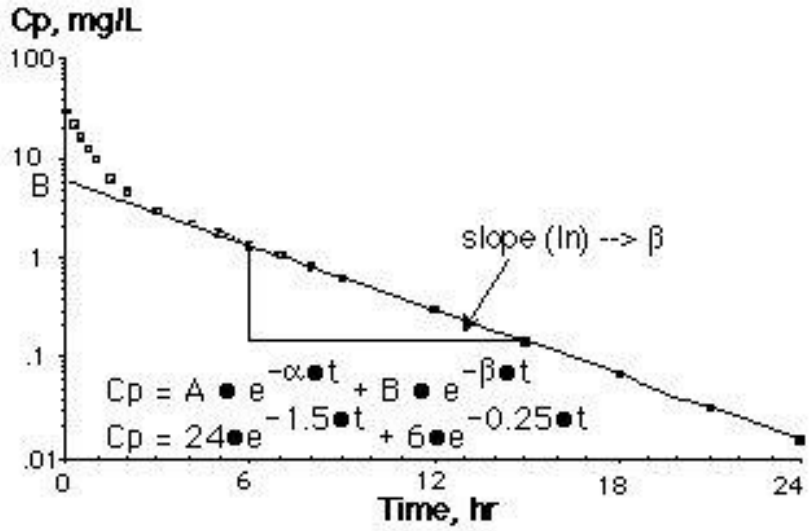



*Figure 3.17.1.4(1). Semi-Log Plot of Cp Versus Time Showing $Cp^{late}$ Extrapolated Back to B*

The difference between the $Cp^{late}$ values at early times and the actual data at early times is again termed the 'residual'

Residual = Cp - $Cp^{late}$ = A * $e^{-\alpha *t}$

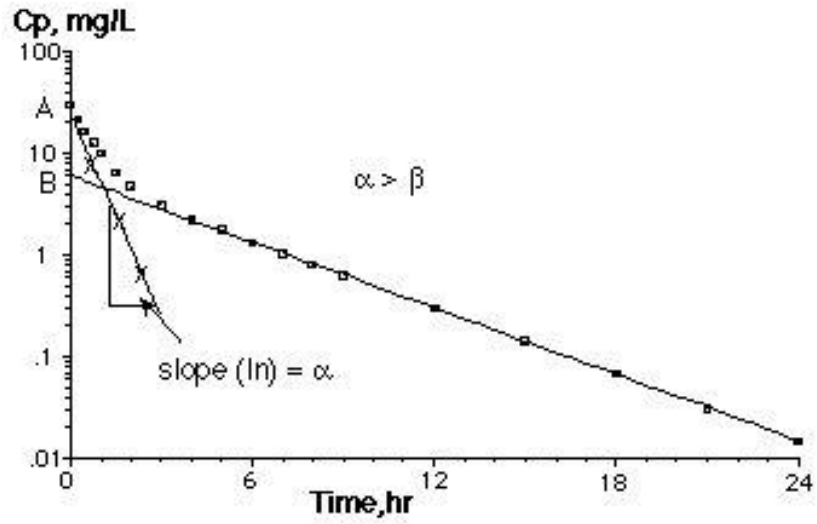



*Figure 3.17.1.4(2). Semi-Log Plot of Cp Versus Time Showing Residual Line and β Line*

From the slope of the residual line the value of α can be calculated with the A read off the concentration axis. With A, B, α, and βcalculated we can calculate the microconstants given the formulas.

$$k21 = \frac{A \cdot \beta + B \cdot \alpha}{A + B}\ ;\ kel = \frac{\alpha \cdot \beta}{k21}\ ;\ k12 = \alpha + \beta - k21 - kel$$

### 3.17.1.5 Effect of k12 and k21:

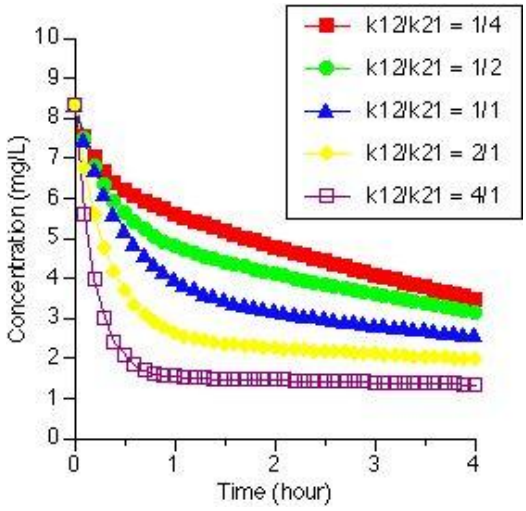



*Figure 3.17.1.5(1). Plot of Cp versus Time Showing the Effect of Different k12/21 Ratio Values*

From the k12 and k21 values we can assess the extent of distribution of drug into the peripheral compartment. The higher the ratio k12/k21 the greater the distribution of drug into the peripheral compartment. The larger the individual values of k12 and k21 the faster is the transfer between the central and peripheral compartments and the more the body behaves as a single compartment.

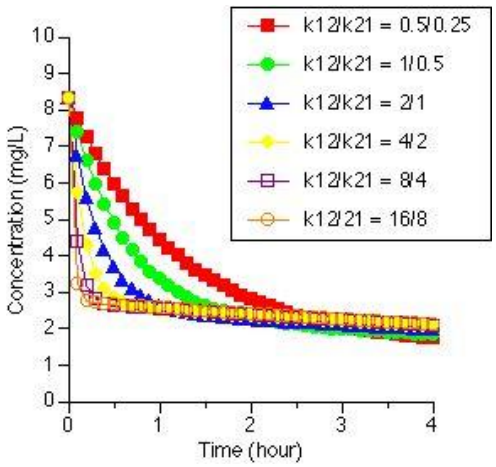



*Figure 3.17.1.5(2). Plot of Cp versus Time Showing the Effect of the Magnitude of k12 and k21*

As the ratio increases the distribution phase is more pronounced. Conversely with the ratio 1/4 there is very little distribution phase. Also note that the $\beta$value or the slope of the terminal phase is changing even though the kel is fixed at 0.2 $hr^{-1}$.

With faster and faster distribution the initial drop in plasma concentration becomes quite rapid. If you were sampling every 30 minutes, the initial phase would be missed. The data would look just like a one compartment model. Redrawing the slow plot with k12/k21 (0.5/0.25) over 24 hours and gives a plot that is definitely still biexponential.

### 3.17.1.6 Apparent Volumes of Distribution:

The concentration of drug in the body is determined not only by the rate constant values but also by the apparent volume of distribution. In the case of the two compartment model a number of volume terms can been defined.

$V_1$:

The apparent volume of the central compartment, $V_1$ or $V_c$, can be calculated as:-

$$V_1 = \frac{DOSE}{A + B} = \frac{DOSE}{Cp^0} \text{ (since } A + B = Cp^0)$$

This parameter is important because it allows the calculation of the highest plasma concentration or $Cp^0$ after an IV bolus administration. This concentration may result in transient toxicity. $V_1$ can also be used in dose calculations.

$V_{area}$ ( = $V_ß$):

$V_{area}$ or $V_ß$ is defined as:-

$$V_{area} = \frac{DOSE}{\beta \cdot AUC} = \frac{V_1 \cdot kel}{\beta} = \frac{Clearance}{\beta} = V_ß$$

Because of the relationship with clearance and $\beta$and with $V_1$ and kel this parameter is quite useful in dosing calculations. This parameter can be readily calculated via AUC and $\beta$values from the 'raw' data and is therefore commonly quoted.

$V_{extrap}$:

$V_{extrap}$ calculated as:-

$$V_{extrap} = \frac{DOSE}{B}$$

This is the volume value calculated if the distribution phase is ignored. Generally not very useful. However, you may see it used. You will be using this if the distribution phase is ignored. (That is, it is the V calculated using a one compartment model with a two compartment drug).

*$V_{ss}$*

$V_{ss}$, V steady state defined as:-

$$V_{ss} = V_1 \cdot \frac{k12 + k21}{k21}$$

This term relates the total amount of drug in the body at 'steady state' with the concentration in plasma or blood

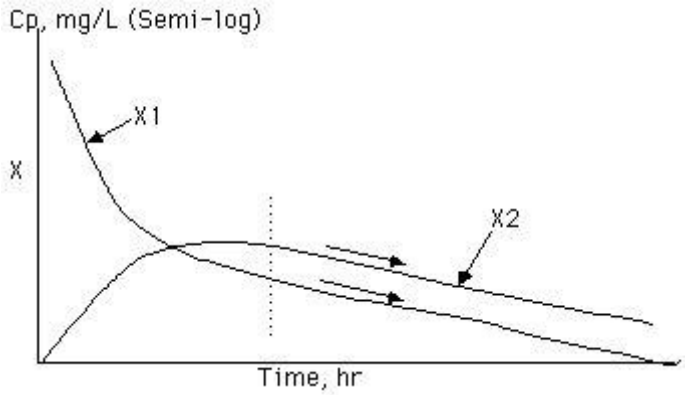



*Figure 3.17.1.6(1). Plot of X1 (Plasma) and X2 (Tissue) Compartment Concentrations, Showing ÔSteady StateÕ with Both Lines Parallel*

The relationship between volume terms is that:

$V_{extrap} > V_{area} > V_{ss} > V_1$ and for a one compartment model the values for all these parameters are equal.

The first two columns are the time and plasma concentration which may be collected after IV bolus administration of 500 mg of drug. At longer times, after 4 hours, out to 12 hours the data appears to follow a straight line on semi- log graph paper. Since $\alpha > \beta$ this terminal line is described by $B * e^{-\beta * t}$.

As an example we can look at the data in the table below.

| Time (hr) | Concentration (mg/L) | Cplate (mg/L) | Residual (mg/L) |
|---|---|---|---|
| 0.5 | 20.6 | 8.8 | 11.8 |
| 1 | 13.4 | 7.8 | 5.6 |
| 2 | 7.3 | 6.1 | 1.2 |
| 3 | 5.0 | 4.7 | 0.3 |
| 4 | 3.7 | 3.7 | - |
| 6 | 2.2 | | |
| 8 | 1.4 | | |
| 10 | 0.82 | | |
| 12 | 0.50 | | |

*Table 3.17.1.6(1). Two Compartment Pharmacokinetics*

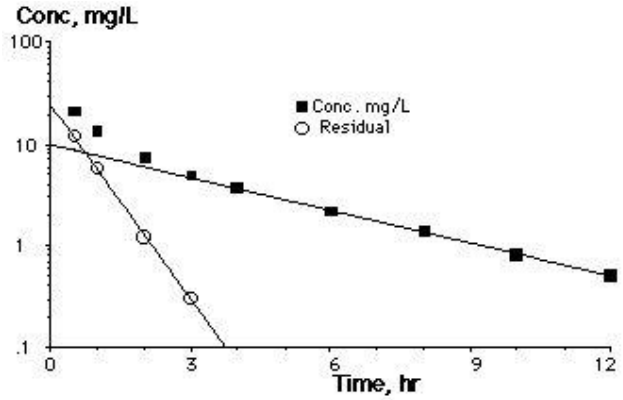



*Figure 3.17.1.6(2). Plot of Cp versus Time Illustrating the Method of Residuals*

Following it back to t = 0 gives B = 10 mg/L. From the slope of the line [ $\beta = 0.25\ hr^{-1}$. $Cp^{late}$ values at early times are shown in column 3 and the residual in column 4. The residual values are plotted (o) also giving a value of A = 25 mg/L and $\alpha = 1.51\ hr^{-1}$

Note that $\alpha / \beta = 6$, thus these values should be fairly accurate.

B = 10 mg/L, $\beta = (\ln 10 - \ln 0.5)/12 = 2.996/12 = 0.25\ hr^{-1}$

A = 25 mg/L, $\alpha = (\ln 25 - \ln 0.27)/3 = 4.528/3 = 1.51\ hr^{-1}$

Therefore $Cp = 25 * e^{-1.51 * t} + 10 * e^{-0.25 * t}$

We can now calculate the microconstants.

$$k21 = \frac{A \cdot \beta + B \cdot \alpha}{A + B} = \frac{25 \times 0.25 + 10 \times 1.51}{25 + 10} = 0.61\ hr\text{-}1$$

$$kel = \frac{\alpha \cdot \beta}{k21} = \frac{1.51 \cdot 0.25}{0.61} = 0.62\ hr^{-1}$$

$k12 = \alpha + \beta - k21 - kel$

$= 1.51 + 0.25 - 0.61 - 0.62$

$= 0.53\ hr^{-1}$

$$V_1 = \frac{DOSE}{A + B} = \frac{500}{35} = 14.3\ L$$

The AUC by the trapezoidal rule + $Cp^{last}/\beta = 56.3 + 2.0 = 58.3\ mg.hr.L^{-1}$, [Note the use of $\beta$] thus

$$V_{area} = \frac{DOSE}{\beta \cdot AUC} = \frac{500}{0.25 \times 58.3} = 34.3\ L$$

$$V_{extrap} = \frac{DOSE}{B} = \frac{500}{10} = 50\ L$$

$$V_{ss} = V_1 \cdot \frac{k21 + k12}{k21} = 14.3 \times \frac{0.61 + 0.53}{0.61} = 26.7\ L$$

Notice that $V_{extrap} > V_{area} > V_{ss} > V_1$ [50 > 34.3 > 26.7 > 14.3]

### 3.17.1.7 Dosage Calculations:

Dosage calculations are complicated by the extra terms in the equations however some calculations are still reasonably straightforward. The dose required for a particular initial plasma concentration can be calculated if $V_1$ is known.

Thus: $DOSE = V_1 * Cp^0$(required)

To achieve an initial Cp of 20 mg/L given $V_1$ = 30 liter would require a DOSE = 20 * 30 = 600 mg.

Alternately if a dose of 500 mg is given and the $V_1$ value is 16 L, the expected $Cp^0$ can be calculated.

$Cp^0 = 500/16 = 31.3\ mg/L$

If the A, B, $\alpha$, and $\beta$ values are known or calculated, then the plasma concentration at any time after a single IV dose can be calculated.

The plasma concentration achieved after a continuous IV infusion is given by the same equation described for the one compartment model, i.e.:-

$k0 = Cp^{ss} * clearance = Cp^{ss} * V_1 * kel = Cp^{ss} * V_{area} * \beta$

If a plasma concentration of 30 mg/L is required and $V_1$ = 15 L and kel is 0.2 $hr^{-1}$ then the required infusion rate can be readily determined. k0 = 30 x 15 x 0.2 = 90 mg/hr

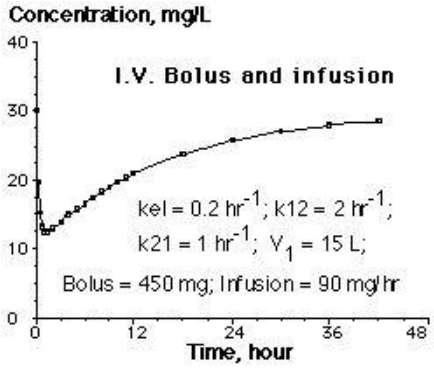



*Figure 3.17.1.7(1). Linear Plot of Cp Versus Time With IV Bolus and Infusion to Give 30 mg/L*

Since the time to reach the steady state concentration is controlled by the $\beta$value this could mean a slow approach to the desired value, thus an IV bolus loading dose may be useful. Unfortunately this calculation is not straight forward as you found in the lab experiment.

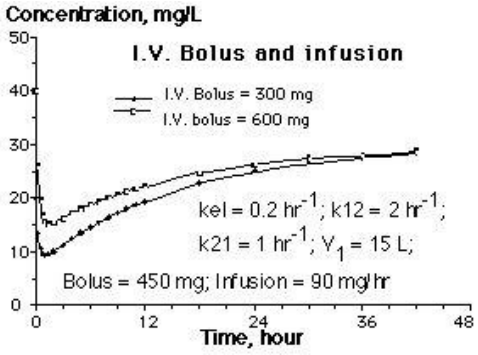



*Figure 3.17.1.7(2). Linear Plot with Higher and Lower Bolus Dose*

With $V_1$ = 15 L, kel = 0.2 $hr^{-1}$, and required Cp = 30 mg/L

Bolus DOSE = 15 x 30 = 450 mg and

Infusion Rate = k0 = 30 x 15 x 0.2 = 90 mg/hr

As you can see this gives quite a dip in the Cp versus time curve. With Bolus DOSEs, either 600 or 300 mg the curves may or may not be better depending on the therapeutic range of the drug.

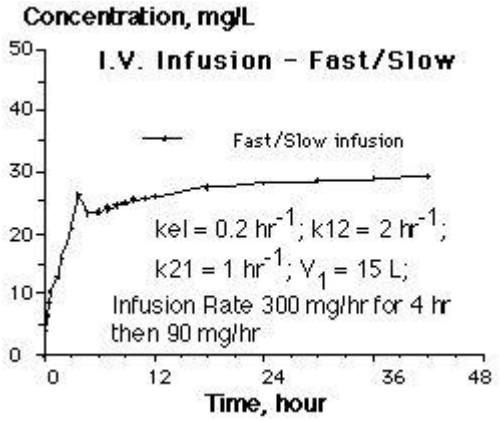



*Figure 3.17.1.7(3). Linear Plot of Cp Versus Time With Fast and Slow Infusion*

Another alternative is to give a fast infusion followed by the maintenance infusion. Here 1200 mg was given over 4 hours (at 300 mg/hr) before switching to the slower 90 mg/hr maintenance rate.

## 3.17.2 Oral Administration:

Following oral administration of a drug with two compartment characteristics, Cp is described by an equation with three exponential terms.

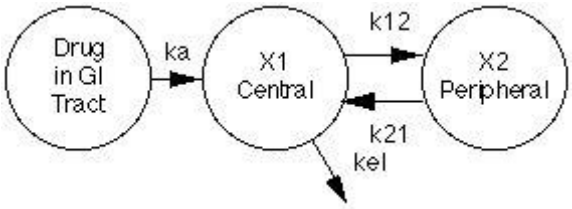



*Figure 3.17.2(1). Scheme for Oral Two-Compartment Pharmacokinetic Model*

The model is shown in above figure:

$$\frac{dX1}{dt} = ka \cdot Xg + k21 \cdot X2 - (k12+kel) \cdot X1$$

*Differential equation*

$Cp = A.e^{-alpha.t} + B.e^{-beta.t} + C.e^{-ka.t}$

where $A + B + C = 0$

*Integrated Equation*

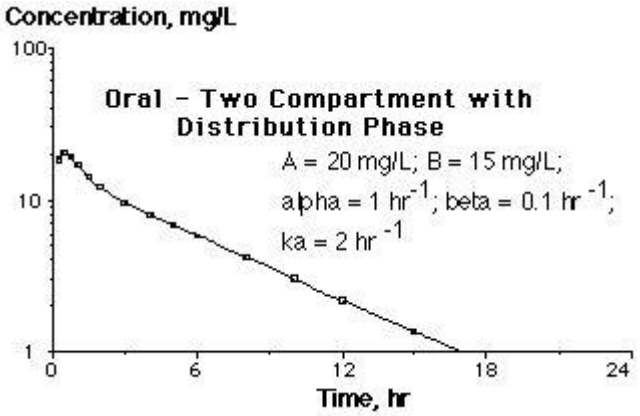



*Figure 3.17.2(2). Semi-Log Plot Showing Pronounced Distribution*

Bioavailability calculations are the same as for the one compartment model, i.e., by comparison of AUC or $U^{\infty}$. These apply for any linear system. Also if $\alpha$, $\beta$, and ka are sufficiently separated the method of residuals can be applied to determine the three values.

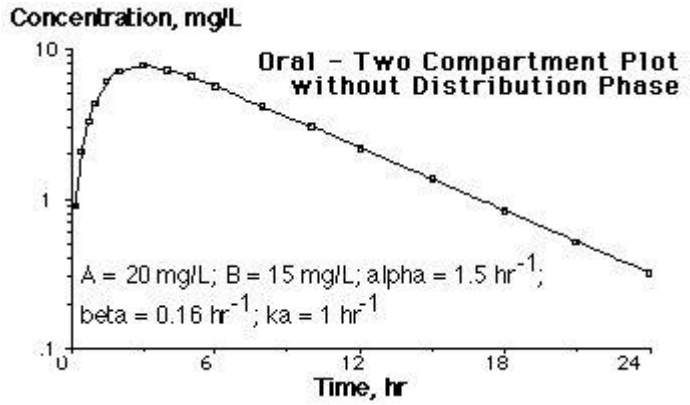



*Figure 3.17.2(3). Semi-Log Plot Without Distribution Phase Evident*

The $\overline{Cp}$ equation can also be used to calculate appropriate dosing regimens. For example if an average plasma concentration of 20 mg/L is required and V1 = 15 L, kel = 0.15 $hr^{-1}$, F = 0.9 and a dosing interval of 12 hours is to be used then the required dose is:-

$$DOSE = \frac{20 \times 15 \times 0.15 \times 12}{0.9} = 600 \text{ mg every 12 hours}$$

$$\text{Since } \overline{Cp} = \frac{F \cdot DOSE}{Clearance \cdot \tau}$$

$$= \frac{F \cdot DOSE}{kel \cdot V_1 \cdot \tau}$$

$$= \frac{F \cdot DOSE}{\beta \cdot V_\beta \cdot \tau}$$

### 3.17.3 Non Compartmental Analysis:

| Data Obtained After 100 mg iv | | | | |
|---|---|---|---|---|
| Time (hr) | Cp (mg/L) | Cp * t (mg.hr/L) | AUC | AUMC |
| 0 | 8 | 0 | 0 | 0 |
| 1 | 7.09 | 7.09 | 7.54 | 3.54 |
| 2 | 6.29 | 12.58 | 14.24 | 13.38 |
| 3 | 5.58 | 16.74 | 20.17 | 28.05 |
| 4 | 4.95 | 19.80 | 25.44 | 46.32 |
| 6 | 3.89 | 23.36 | 34.28 | 89.49 |
| 9 | 2.71 | 24.45 | 44.20 | 161.21 |
| 12 | 1.89 | 22.74 | 51.12 | 232.00 |
| 18 | 0.92 | 16.60 | 59.57 | 350.06 |
| 24 | 0.44 | 10.77 | 63.69 | 432.21 |
| ∞ | | | 67.43 | 553.21 |

*Table 3.17.3(1). Typical Cp versus Time Data after IV Bolus Administration*

Non compartmental methods can be used to determine certain pharmacokinetic parameters without deciding on a particular compartmental model. The basic calculations are based on the area under the plasma concentration versus times curve (zero moment) and the first moment curve (AUMC). The AUC can be calculated as before using by the trapezoidal rule.

The first moment is calculated as concentration times time (Cp * t). The AUMC is the area under the concentration times time versus time curve. Maybe best covered with an example. Consider a drug given both by iv and oral administration. Both the AUC and AUMC were calculated using the trapezoidal rule without making any assumption concerning the number of compartments. The final segment of the AUC curve is calculated as Cp(last)/k, where k is the last exponential (the slowest). The last segment for the AUMC curve is:

$$\frac{Cp(last) \cdot t(last)}{k} + \frac{Cp(last)}{k^2}$$

From the AUC and AUMC values we can calculate the mean residence time, MRT. This is the average time that the drug stays in the body (or plasma as measured here). It can be related to the average elimination rate constant as 1/MRT. The values from the above data are MRT = 553.21/67.43 = 8.2 hr and k = 1/8.2 = 0.122 $hr^{-1}$. Remember we can also calculate the clearance, CL = Dose/AUC = 100/67.43 = 1.48 $L.hr^{-1}$. Finally a steady state volume can be calculated as CL * MRT = 1.48 x 8.2 = 12.2 L.

$$MRT = \frac{AUMC}{AUC}$$

$$\bar{k} = \frac{1}{MRT}$$

$$CL = \frac{Dose}{AUC}$$

$$V_{ss} = CL \cdot MRT$$

| Data Obtained After 250 mg po | | | | |
|---|---|---|---|---|
| Time (hr) | Cp (mg/L) | Cp * t (mg.hr/L) | AUC | AUMC |
| 0 | 0 | 0 | 0 | 0 |
| 1 | 12.18 | 12.18 | 6.09 | 6.09 |
| 2 | 14.12 | 28.25 | 19.24 | 26.30 |
| 3 | 13.43 | 40.30 | 33.02 | 60.58 |
| 4 | 12.16 | 48.64 | 45.82 | 105.06 |
| 6 | 9.64 | 57.86 | 67.63 | 211.57 |
| 9 | 6.73 | 60.60 | 92.19 | 389.28 |
| 12 | 4.69 | 56.38 | 109.34 | 564.76 |
| 18 | 2.28 | 41.16 | 130.30 | 857.40 |
| 24 | 1.11 | 26.71 | 140.50 | 1061.04 |
| ∞ | | | 149.78 | 1360.98 |

*Table 3.17.3(2). Typical Cp versus Time Data after Oral Administration*

The following data were calculated after a 250 mg oral dose of the same drug. From these data a MRT was calculated as 1360.98/149.78 = 9.08 hr. We can subtract from this MRT(PO) the MRT(iv) to get an idea of the absorption process, the mean absorption time (MAT). That is MAT = MRT(PO) - MRT(iv) = 9.08 - 8.20 = 0.88 hr. From this we can calculate an average absorption rate constant = 1/MAT = 1/0.88 = 1.14 $hr^{-1}$. Of course we can calculate the bioavailability of the oral dosage form using the dose adjusted AUC ratio. Thus F = (149.78/67.43) x (100/250) = 0.89.

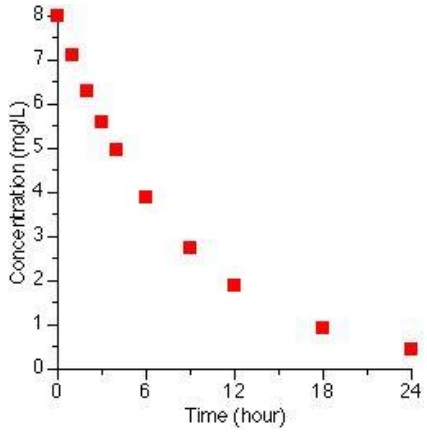



*Figure 3.17.3(1). Plot of Cp versus Time (IV)*

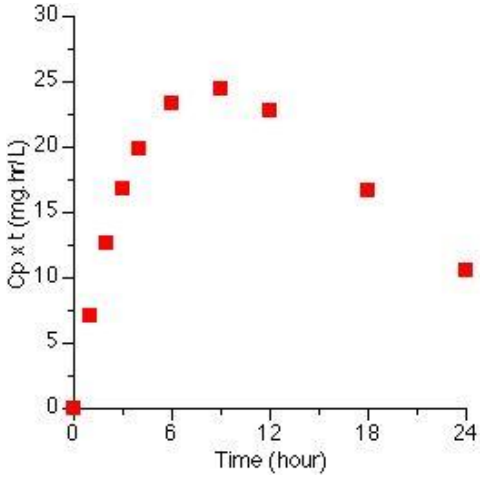



*Figure 3.17.3(2). Plot of Cp x Time versus Time (IV)*

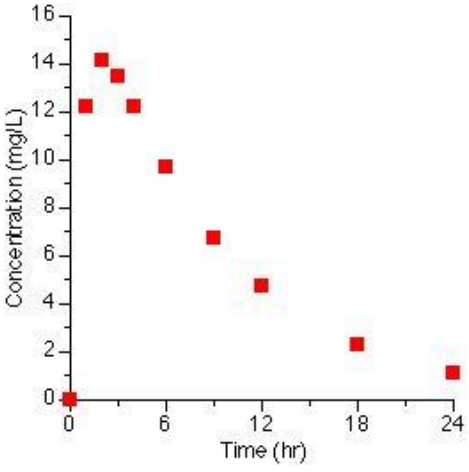



*Figure 3.17.3(3). Plot of Cp versus Time (PO)*

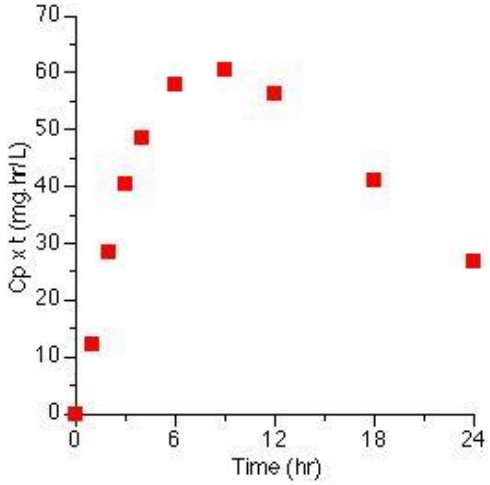



*Figure 3.17.3(4). Plot of Cp x Time versus Time (PO)*

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# Chapter 4: DRUG DISSOLUTION PARAMETERS COMPUTATIONS

## 4.1. Introduction:

Dissolution, or Solvation, in chemistry, the process of dissolving a solid substance into a solvent to yield a solution. Drug dissolution testing is an approach to evaluate drug release characteristics of a product (tablets/capsules) in vitro. The technique is very well established and extensively used at every stage of product manufacturing. [1-2].

Dissolution testing of pharmaceutical dosage forms is considered to be one of the most important quality control tests in the pharmaceutical industry for the characterization of drug substance and solid dosage forms. It is also a useful tool in correlation of formulation / process variables to the rate and extent of release of the drug from the dosage form. The optimization of formulation parameters such as particle size, amount and type of surfactant, disintegrates and lubricants incorporated, compressional forces applied to tablets, coatings employed to extend release, and other factors to produce desired release characteristics for a drug product are conveniently monitored by dissolution techniques. Dissolution has been accepted as an important quality control test for oral solid dosage forms for over 20 years. Though not often validated by *in vivo* studies, the results of a well-designed dissolution test generally offer a sense of assurance for product quality that is needed for proper *in vivo* performance. [3-5]

Drug dissolution (or release) testing is an analytical technique used to assess release profiles of drugs in pharmaceutical products, generally solid oral products such as tablets and capsules. This test gains its significance from the fact that if a drug from a product is to produce its effect; it must be released from the product and should generally be dissolved in the fluids of the gastrointestinal (GI) tract. Thus, a drug dissolution test may be considered as an indicator of potential drug release and absorption characteristics of a product in humans as well as in animals. Therefore, a dissolution test is often considered a surrogate for the assessment of availability of drugs in the body, generally termed bioavailability. This

link of dissolution (in vitro) to drug release in the body (in vivo) as commonly determined by bioavailability assessment is formally referred to as in vitro–in vivo correlation (IVIVC).This concept of IVIVC, in a quantitative and/or qualitative format, provides the basis for the assessment of quality of the products. Thus, the dissolution test is not only a procedure for product development but is also extensively used as a quality control technique because of this in vitro–in vivo association. [5-8]

## 4.1.1 Dissolution Profiles:

The ideal immediate release profile goes through 5 main stages. While many of these actions are occurring through out the profile, these named are the primary factor affecting the shape of the profile. It is a term used to describe the diminishing light of a star as a celestial body slowly blocks it from view. The fifth term is a borrowed medical term used to describe the complete blockage of an artery, no longer able to deliver any more blood. [9-11]

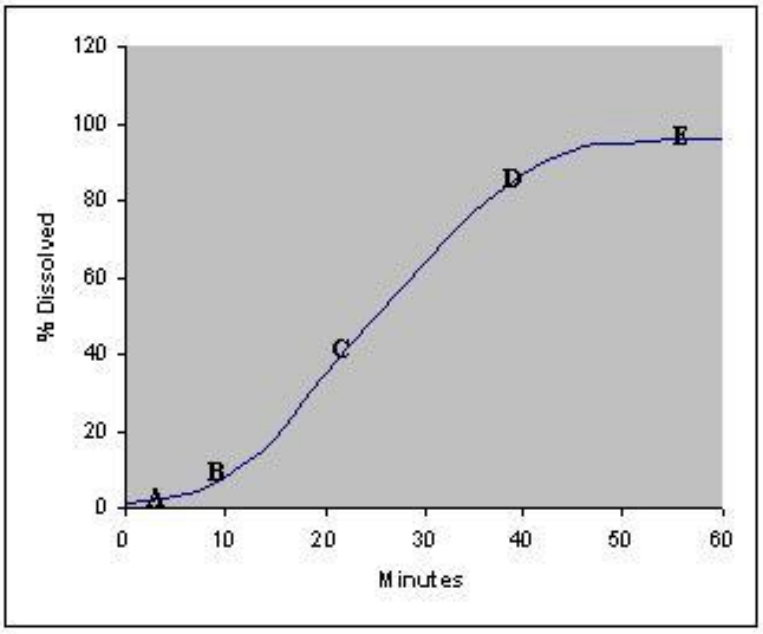


*Figure 4.1.1(1): Plot of Concentration and Time*

A: Disintegration

B: Deaggegation

C: Release

D: Occultation

E: Occlusion

### 4.1.2 Dissolution Profiling:

Dissolution profile of two products (12 units each) of the test and reference products. To use the mean dissolution values from both curves, the percent coefficient of variation at the earlier time points should not be more than 20% and at other time points should not be more than 10%. Only one measurement should be considered after 85% dissolution of both of the products. The dissolution measurements of the test and reference batches should be made under exactly the same conditions. [12-13].

### 4.1.3 Dissolution and Particle Size:

In order for a drug to have its effect after oral administration it must go into solution and then diffuse through the gut wall into the body. The first step in that process is the disintegration of the dosage form followed by dissolution of the active ingredient. Dissolution of a pure substance follows the Noyes Whitney Equation.

—

Where, — is the rate of dissolution,

k is the dissolution rate constant,

S is the surface area of the dissolving solid,

Cs is the saturation concentration of drug in the diffusion layer and

Ct is the concentration of drug in dissolution media (or the bulk).

One way to increase dissolution rate of poorly soluble drugs is to increase the surface available for dissolution. This is done by reducing particle size or by dividing the dosage form into two smaller tablets or capsules, with a larger combined surface area. Other ways include increasing the disintegration rate and deaggregation. [14]

A wide range of principles and methods is available for the purpose of enhancing dissolution and/or dissolution rate of 'low-solubility' substances, for example: selection of salt form for weak acids and bases, reduction of particle size and, thereby, increased specific surface area, use of surfactants for increased wettability, complex formation with excipients, e.g. hydrophilic polymers or cyclodextrins, preparation of solid dispersions, change of crystal form by precipitation with hydrophilic polymers, lipophilic formulations, i.e. emulsions, microemulsions etc. [14-15].

To reflect drug absorption behavior in vivo or, more accurately drug release in vivo, drug dissolution tests are conducted in vitro, mimicking the physiological environment of

the GI tract. The GI tract environment is represented by mild stirring of drug products in aqueous-based solutions, such as 0.1 N HCl or buffers having pH values in the range of 4–7.5. The cumulative percentage of a drug dissolved at a number of time points is determined and may be reported as a plot of % drug dissolved versus sampling times. The resulting graph is commonly referred to as a "dissolution profile" and provides a means of comparison with in vivo drug release to establish absorption characteristics of drugs from products in humans. [16].

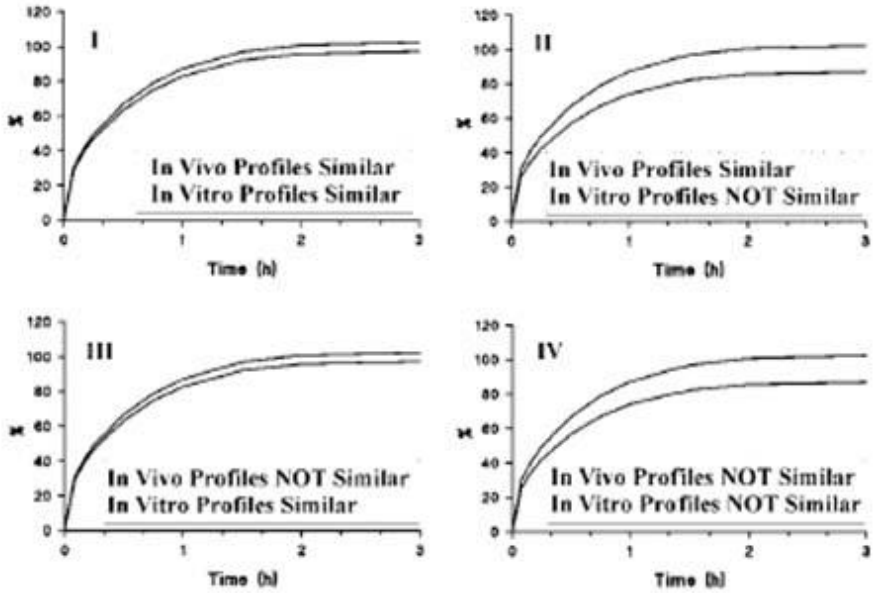


*Figure 4.1.3(1):Possible testing outcome scenarios, as drug dissolution (release) profiles, of products with in vivo similar (bioequivalent) and dissimilar (bioinequivalent) release characteristics.*

Further, because of the above-mentioned in vitro–in vivo association, it is generally considered that if a dissolution profile of a test product matches that of a reference product, then the test product should behave similarly to the reference product in vivo (i.e., both will have similar bioavailability and will be considered as bioequivalent). Conversely, if the profile of the test product is different from that of the reference product, then the test product might behave differently in humans. This practice of describing dissolution results or profiles is commonly referred to as providing discriminating dissolution profiles, and the test as a discriminatory test. That is, a dissolution test is expected to discriminate whether dissimilar products are from different manufacturing batches of the same product or from

different products such as generics. It is most important to note that the terminology of "discrimination" should be related to and based on similarity or dissimilarity of in vitro results to in vivo results only. It is further important to note that a test should only be considered discriminatory if dissolution profiles obtained are dissimilar for dissimilar in vivo profiles (i.e., products should be bioinequivalent). [17-19].

If different dissolution profiles are obtained for products with the same or different formulations or manufacturing attributes but with similar in vivo characteristics, they may not be considered as discriminating profiles, and the test that produces such profiles should not be considered as a discriminatory test. The differences in profiles for products having similar release characteristics in vivo (i.e., for bioequivalent products) should be considered as an expected and acceptable variation in dissolution results from acceptable products without any negative therapeutic consequences. Such differences in dissolution profiles should form the basis of setting tolerances for quality control purposes for acceptable products and not for establishing discrimination or differences. Dissolution is the process by which a solid or liquid enters its aqueous phase (solution) [20-21].

## 4.2 Mechanism of Drug Dissolution:

So far we have looked at the transfer of drugs in solution in the G-I tract, through a membrane, into solution in the blood. However, many drugs are given in solid dosage forms, and therefore must dissolve before absorption can take place. [22].


*Figure 4.2(1) Dissolution and Absorption*

If absorption is slow relative to dissolution then all we are concerned with is absorption. However, if dissolution is the slow, rate determining step (the step controlling the overall rate) then factors affecting dissolution will control the overall process. This is a more common problem with drugs which have a low solubility (below 1 g/100 ml) or which are given at a high dose, e.g. griseofulvin.

There are number of factors which affect drug dissolution. One model that is commonly used is to consider this process to be diffusion controlled through a stagnant layer surrounding each solid particle.

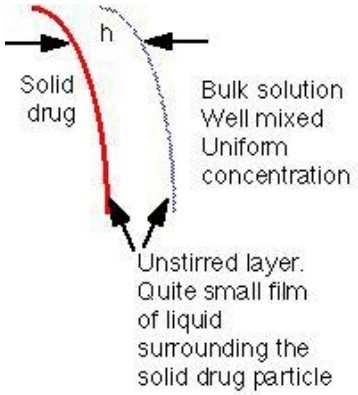


*Figure 4.2(2) Diagram Representing Diffusion through the Stagnant Layer*

A physical model is shown in Figure 4.2(1).

First we need to consider that each particle of drug formulation is surrounded by a stagnant layer of solution.

After an initial period we will have a steady state set-up where drug is steadily dissolved at the solid-liquid interface and diffuses through the stagnant layer. If diffusion is the rate determining step we can use Fick's first law of diffusion to describe the overall process.

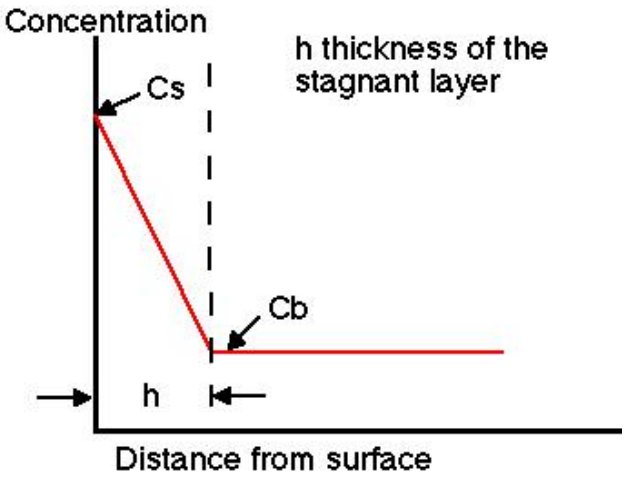


*Figure 4.2(3) Plot of Concentration Gradient*

If we could measure drug concentration at various distances from the surface of the solid we would see that a concentration gradient is developed. [23-25].

## 4.2.1 Fick's First Law:

By Fick's first law of diffusion applied to dissolution:

$$\text{Rate of Solution} = \frac{D \bullet A \bullet (Cs - Cb)}{h}$$

where D is the diffusion coefficient, A the surface area, Cs the solubility of the drug, Cb the concentration of drug in the bulk solution, and h the thickness of the stagnant layer. If Cb is much smaller than Cs then we have so-called "Sink Conditions" and the equation reduces to

$$\text{Rate of Solution} = \frac{D \bullet A \bullet Cs}{h}$$

with each term in this equation contributing to the dissolution process.

### 4.2.1.1 Surface area, A:

The surface area per gram (or per dose) of a solid drug can be changed by altering the particle size. For example, a cube 1 cm on each side has a surface area of 6 $cm^2$. If this cube is broken into cubes with sides of 0.1 cm, the total surface area is 60 $cm^2$. Actually if we break up the particles by grinding we will have irregular shapes and even larger surface areas. Generally as A increases the dissolution rate will also increase. Improved bioavailability has been observed with griseofulvin, digoxin, etc.

Methods of particle size reduction include mortar and pestle, mechanical grinders, fluid energy mills, solid dispersions in readily soluble materials (PEG's).

### 4.2.1.2 Diffusion layer thickness, h

This thickness is determined by the agitation in the bulk solution. In vivo we usually have very little control over this parameter. It is important though when we perform in vitro dissolution studies because we have to control the agitation rate so that we get similar results in vitro as we would in vivo.

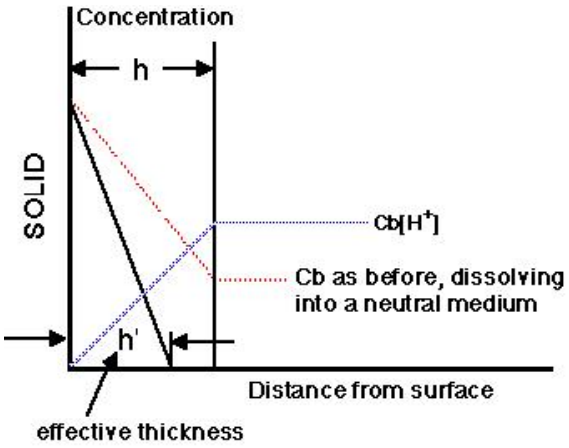


*Figure 4.2.1.2(1) Plot of Concentration versus Distance for Dissolution into a Reactive Medium*

The apparent thickness of the stagnant layer can be reduced when the drug dissolves into a reactive medium. For example, with a weakly basic drug in an acidic medium, the drug will react (ionize) with the diffusing proton ($H^+$) and this will result in an effective decrease in the thickness of the stagnant layer.

The effective thickness is now h' not h. Also the bulk concentration of the drug is effectively zero. For this reason weak bases will dissolve more quickly in the stomach.

### 4.2.1.3 Diffusion Coefficient, D

The value of D depends on the size of the molecule and the viscosity of the dissolution medium. Increasing the viscosity will decrease the diffusion coefficient and thus the dissolution rate. This could be used to produce a sustained release effect by including a larger proportion of something like sucrose or acacia in a tablet formulation.

### 4.2.1.4 Drug Solubility, Cs

Solubility is another determinant of dissolution rate. As Cs increases so does the dissolution rate. We can now look at ways of changing the solubility of a drug.

Salt Form:

If we look at the dissolution profile of various salts.

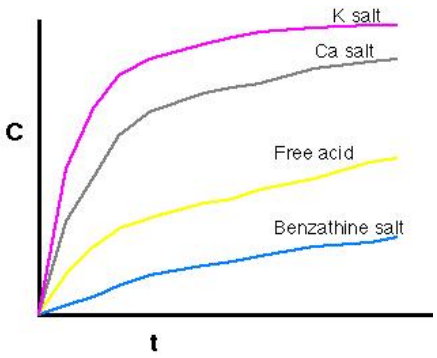


*Figure 4.2.1.4(1) Plot of Dissolved Drug Concentration versus Time*

Salts of weak acids and weak bases generally have much higher aqueous solubility than the free acid or base, therefore if the drug can be given as a salt the solubility can be increased and we should have improved dissolution. One example is Penicillin V.

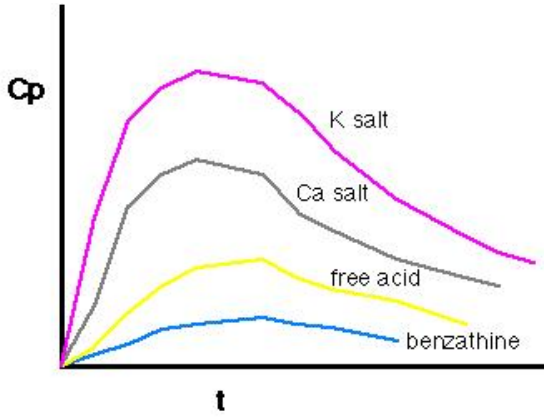


*Figure 4.2.1.4(2) Plot of Cp versus Time*

Crystal Form:

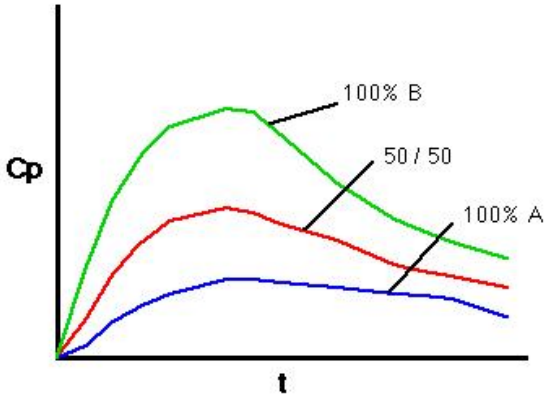


*Figure 4.2.1.4(3) Plot of Cp versus Time for Three Formulations of Chloramphenicol Palmitate*

This can lead to quite different Cp versus time results after oral administration. The $t_{peak}$ values are similar thus ka probably the same. $C_{peak}$ would show a good correlation with solubility. Maybe site limited (only that in solution by the time the drug gets to the 'window' is absorbed). Use the potassium salt for better absorption orally. These results might support the use benzathine or procaine for IM depot use.

Some drugs exist in a number of crystal forms or polymorphs. These different forms may well have different solubility properties and thus different dissolution characteristics. Chloramphenicol palmitate is one example which exists in at least two polymorphs. The B form is apparently more bioavailable.

The recommendation might be that manufacturers should use polymorph B for maximum solubility and absorption. However, a method of controlling and determining crystal form would be necessary in the quality control process. Shelf-life could be a problem as the more soluble (less stable) form may transform into the less soluble form. In time the suspension may be much less soluble with variable absorption. [26-27]

## 4.3 Dissolution Testing Parameters:

Dissolution tests in vitro measures the rate and extent of dissolution of a drug in aqueous medium from a dosage form. Extensive research work on in vitro dissolution and in vivo availability of drugs revealed the fact that in vitro dissolution test can be used to predict the in-vivo performance of the dosage form. There are number of parameters that must be considered when performing dissolution test. [28-31].

### 4.3.1 Apparatus:

The most commonly employed dissolution test methods are (1) the basket method (Apparatus 1) and (2) the paddle method (Apparatus 2). The basket and the paddle methods are simple, robust, well standardized, and used worldwide. These methods are flexible enough to allow dissolution testing for a variety of drug products. For this reason, the official in vitro dissolution methods described in *U.S. Pharmacopeia* (USP), Apparatus 1 and Apparatus 2 should be used unless shown to be unsatisfactory. The in vitro dissolution procedures, such as the reciprocating cylinder (Apparatus 3) and a flow-through cell system (Apparatus 4) described in

the USP, may be considered if needed. These methodologies or other alternatives/ modifications should be considered on the basis of their proven superiority for a particular product. Because of the diversity of biological and formulation variables and the evolving nature of understanding in this area, different experimental modifications may need to be carried out to obtain a suitable in vivo correlation with in vitro release data. Dissolution methodologies and apparatus described in the USP can generally be used either with manual sampling or with automated procedures. [32-33].

### 4.3.2 Dissolution Medium:

Dissolution testing should be carried out under physiological conditions, if possible. This allows interpretation of dissolution data with regard to in vivo performance of the product. However, strict adherence to the gastrointestinal environment need not be used in routine dissolution testing. The testing conditions should be based on physicochemical characteristics of the drug substance and the environmental conditions the dosage form might be exposed to after oral administration. [34].

The volume of the dissolution medium is generally 500, 900, or 1000 mL.

- Generally as dissolution media following are used:
- 0.1N HCL
- 0.01N HCL
- 0.06N HCL
- 0.05M Potassium Phosphate pH 6.8
- 0.05M Potassium Phosphate pH 7.2
- 0.05M Potassium Phosphate pH 7.4
- 0.05M Acetate Buffer pH
- 4.5 Simulated Gastric Fluid

Sink conditions are desirable but not mandatory. An aqueous medium with pH range 1.2 to 6.8 (ionic strength of buffers the same as in USP) should be used. To simulate intestinal fluid (SIF), a dissolution medium of pH 6.8 should be employed. A higher pH should be justified on a case-by-case basis and, in general, should not

exceed pH 8.0. To simulate gastric fluid (SGF), a dissolution medium of pH 1.2 should be employed without enzymes. The need for enzymes in SGF and SIF should be evaluated on a case-by-case basis and should be justified.

Use of water as a dissolution medium also is discouraged because test conditions such as pH and surface tension can vary depending on the source of water and may change during the dissolution test itself, due to the influence of the active and inactive ingredients. For water insoluble or sparingly water soluble drug products, use of a surfactant such as sodium lauryl sulfate is recommended. The need for and the amount of the surfactant should be justified. [35-37].

All dissolution tests for IR dosage forms should be conducted at 37±0.5°C. The basket and paddle method can be used for performing dissolution tests under multimedia conditions (e.g., the initial dissolution test can be carried out at pH 1.2, and, after a suitable time interval, a small amount of buffer can be added to raise pH to 6.8). Alternatively, if addition of an enzyme is desired, it can be added after initial studies (without enzymes).

Use of Apparatus 3 allows easy change of the medium. Apparatus 4 can also be adopted for a change in dissolution medium during the dissolution run. Certain drug products and formulations are sensitive to dissolved air in the dissolution medium and will need deaeration. In general, capsule dosage forms tend to float during dissolution testing with the paddle method. [38].

The apparatus suitability tests should be carried out with a performance standard (i.e., calibrators) at least twice a year and after any significant equipment change or movement. However, a change from basket to paddle or vice versa may need recalibration. The equipment and dissolution methodology should include the product related operating instructions such as deaeration of the dissolution medium and use of a wire helix for capsules. Validation of automated procedures compared to the manual procedures should be well documented. [39].

### 4.3.3 Types of Dosage Form: [40]

Dissolution testing is a useful tool for evaluation and testing of various dosage forms. Dosage forms are the means by which drug molecules are delivered to sites of action

within the body. The different forms in which drugs may be supplied to a patient are described briefly:

#### 4.3.3.1 Implants:

These are sterile disks or cylinders introduced surgically into body tissues and designed to release one or more medicaments over an extended period of time.

#### 4.3.3.2 Insufflations:

These are medicated powders designed to be blown into the ear, nose, throat or body cavities by means of a device known as an *insufflator*. Bulk insufflation has largely disappeared and has been replaced by individual doses of powdered drugs supplied in hard capsules and inhaled from a device which breaks the capsule and allows the patient to inhale the powder. This type of insufflation is used mainly for drug delivery into the respiratory tract by inhalation.

#### 4.3.3.3 Irrigation Solutions:

These are sterile, pyrogen-free solutions usually intended for irrigation of body cavities, operation cavities, wounds or the urogenital system.

#### 4.3.3.4 Linctuses:

Linctuses are viscous, liquid oral preparations that are usually prescribed for the relief of cough. They usually contain a high proportion of syrup and glycerol which have a demulcent effect on the membranes of the throat. The dose volume is small (5ml) and, to prolong the demulcent action, they should be taken undiluted.

#### 4.3.3.5 Liniments:

Liniments are fluid, semi-fluid or, occasionally, semi-solid preparations intended for application to the skin. They may be alcoholic or oily solutions or emulsions. Most are massaged into the skin but some are applied on a warm dressing or with a brush (analgesic and soothing types). Liniments should not be applied to broken skin.

#### 4.3.3.6 Lozenges:

Lozenges are solid preparations consisting of sugar and gum, the latter giving strength and cohesiveness to the lozenge and facilitating slow release of the medicament. They are used to medicate the mouth and throat and for the slow administration of indigestion or cough remedies.

#### 4.3.3.7 Lotions:

These are fluid preparations for external application without friction. They are either dabbed on the skin or applied on a suitable dressing and covered with a waterproof dressing to reduce evaporation.

#### 4.3.3.8 Mixtures:

Mixtures are liquid oral preparations consisting of one or more medicaments dissolved or suspended in an aqueous vehicle. Official mixtures are not usually formulated for a long shelf-life.

#### 4.3.3.9 Mouthwashes:

These are similar to gargles but are used for oral hygiene and to treat infections of the mouth.

#### 4.3.3.10 Ointments:

Ointments are semi-solid, greasy preparations for application to the skin, rectum or nasal mucosa. The base is usually anhydrous and immiscible with skin secretions. Ointments may be used as emollients or to apply suspended or dissolved medicaments to the skin. Ointments intended for application to large open wounds should be sterile.

#### 4.3.3.11 Oral Emulsions:

The term 'oral emulsion' as an oral dosage form may be defined as 'a fine dispersion of droplets of an oily liquid in an aqueous liquid which forms the continuous phase'. Drugs may be dissolved in either of the phases or suspended in the emulsion.

#### 4.3.3.12 Nasal Drops and Sprays:

Drugs in solution may be instilled into the nose from a dropper or from a plastic squeeze bottle. The drug may have a local effect, e.g. antihistamine, vasoconstrictor, decongestant. Alternatively the drug may be absorbed through the nasal mucosa to exert a systemic effect, e.g. the peptide hormones oxytocin and vasopressin. The use of oily nasal drops should be avoided because of possible damage to the cilia of the nasal mucosa. Prolonged use of nasal vasoconstrictors may result in rebound vasodilatation and further nasal congestion.

#### 4.3.3.13 Oral Liquids:

Oral Liquids are homogeneous preparations containing one or more active ingredients dissolved or suspended in a suitable vehicle. Elixirs, linctuses, mixtures, oral drops, oral emulsions, oral solutions and oral suspensions are included in the general category of oral liquids.

#### 4.3.3.14 Parenteral Preparations (Injectable Preparations):

These are sterile dosage forms containing one or more medicaments and designed for parenteral administration.

*Injections* are sterile solutions, suspensions or emulsions in a suitable aqueous or non-aqueous vehicle and are usually classified according to their route of administration.

*Powders for injections* are sterile solid substances to be dissolved or suspended by adding a prescribed volume of the appropriate sterile fluid. The solution or suspension is usually prepared immediately prior to use to avoid deterioration of the product on storage. *Intravenous infusions* are sterile aqueous solutions or emulsions, free from pyrogens and usually made isotonic with blood. they do not contain added antimicrobial preservatives or buffering agents and are designed for intravenous administration in volumes usually greater than 10-15 ml.

#### 4.3.3.15 Pastes:

Pastes are semi-solid preparations for external application that differ from similar ointments and gels in that they contain a high proportion of finely powdered medicaments. The base may be anhydrous (liquid or soft paraffin) or water soluble (glycerol or a

mucilage). Their stiffness makes them useful protective coatings. Pastes intended for application to large open wounds should be sterile.

#### 4.3.3.16 Paints:

Paints are liquids for application to the skin or mucous membranes. Skin paints often have a volatile solvent that evaporates quickly to leave a dry resinous film of medicament. Throat paints are more viscous due to a high content of glycerol, designed to prolong contact of the medicament with the affected site.

#### 4.3.3.17 Pills:

Pills are oral dosage forms which consist of spherical masses prepared from one or more medicaments incorporated with inert excipients. Pills are now rarely used. The term 'pill' is used colloquially (yet incorrectly) as a synonym for oral contraceptive tablets which are actually prepared by compression.

#### 4.3.3.18 Poultices:

Poultices are paste-like preparations used externally to reduce pain and inflammation because they retain heat well. After heating, the preparation is spread thickly on a dressing and applied as hot as the patient can bear, to the affected area.

#### 4.3.3.19 Powders (Oral):

There are two kinds of powder intended for internal use.

*Bulk Powders* usually contain non-potent medicaments such as antacids since the patient measures a dose by volume using a 5ml medicine spoon. The powder is then usually dispersed in water or, in the case of effervescent powders, dissolved before taking.

*Divided Powders* are packaged individually - each dose is seperately wrapped in paper or sealed into a sachet.

### 4.3.4 Dissolution Rate:

Dissolution rate Measurement of the intrinsic dissolution rate of materials to aid the selection of the best form of the API for progression. Examples of test materials include: Salts, Polymorphs, Amorphous, Hydrates and Formulations. [41].

Dissolution rate of a material is a much better parameter for correlation with the likely in-vivo performance of an oral formulation compared to thermodynamic solubility.

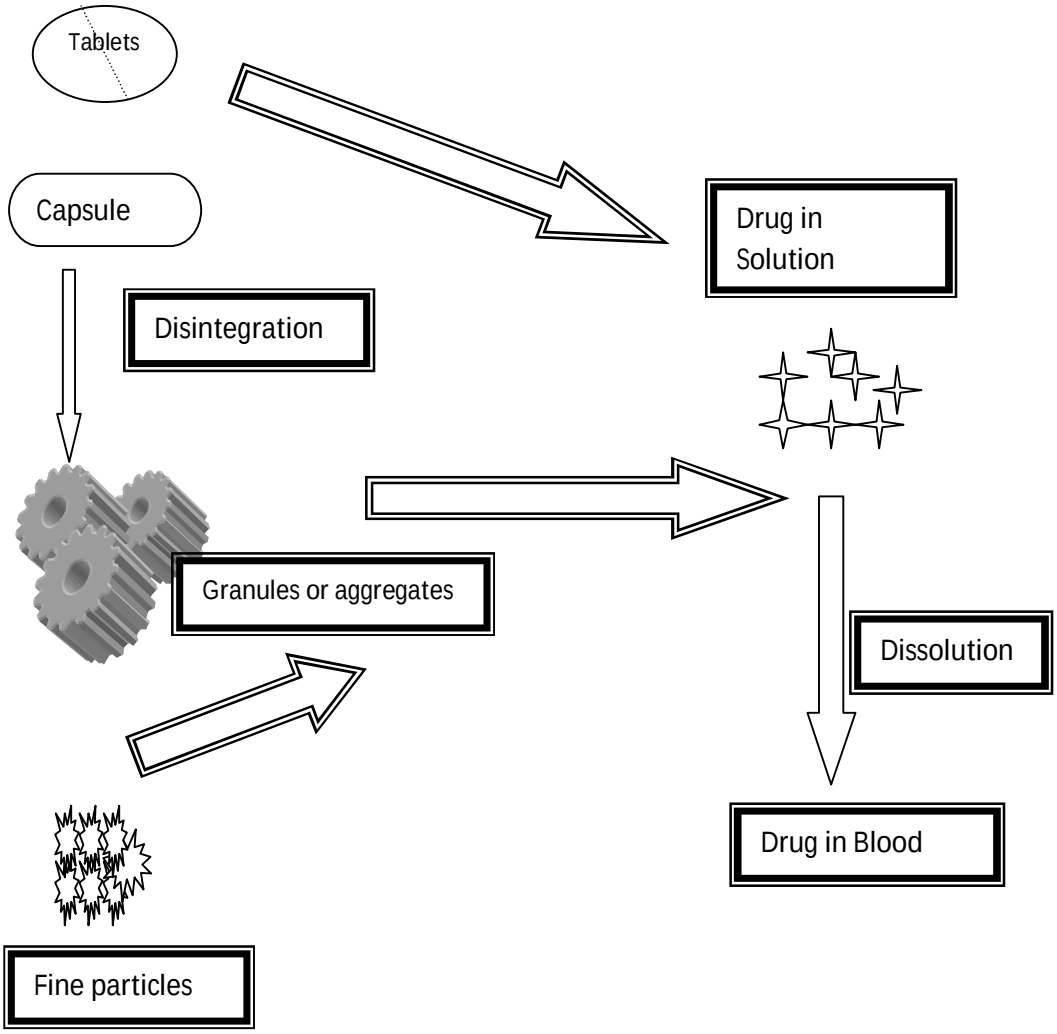


*Figure 4.3.4(1) Dissolution Rate*

## 4.3.5 Solubility:

Solubility is a characteristic physical property referring to the ability for a given substance, the solute, to dissolve in a solvent. It is measured in terms of the maximum amount of solute dissolved in a solvent at equilibrium. The resulting solution is called a saturated solution. Certain liquids are soluble in all proportions with a given solvent, such as ethanol in water. This property is known as miscibility. Also, the equilibrium solubility

can be exceeded under various conditions to give a so-called supersaturated solution, which is metastable.

A supersaturated solution will often lose the excess solute immediately if a suitable site on which to do so, such as a scratched beaker wall, a gas bubble, or a crystal of solute, is introduced into the solution.

- Very Soluble < 1
- Freely Soluble between 1 and 10
- Soluble between 10 and 30
- Sparingly Soluble between 30 to 100
- Slightly Soluble between 100 to 1000
- Very Slightly Soluble between 1000 to 10000
- Practically insoluble > 10000

### 4.3.6 Type of Dissolution Apparatus:

Varying the test apparatus and conditions is the first step in defining an *in vitro* method that is sufficiently discriminatory for evaluating bioavailability and/or control manufacturing variables. This is especially true for modified-release oral solid dosage forms. For immediate-release oral solid dosage forms such as tablets, chewable tablets, and hard and soft gelatin capsules, however, needless proliferation of methods that often do not have any distinct advantages over simple United States Pharmacopeia (USP) methods is not desirable. In test medium selection, physiological relevance should always be the primary consideration.

The most commonly used USP basket (Method I) and paddle (Method II) methods first appeared in USP XIII in early 1970. These methods are referred to as closed system methods in which a fixed volume of dissolution medium is used. In practice, a rotating basket or paddle provides a steady stirring motion in a large vessel with 500â€“ 1000 ml fluid that is immersed in a temperature-controlled water bath. The devices are very simple, robust, and can be easily standardized. The descriptions for apparatus specifications are detailed in chapter 711 of USP XXIII. When properly calibrated, USP methods I and II have been the methods of choice for dissolution testing of immediate release oral solid dosage forms. [42].

### 4.3.7 Stirrer Type:

Dissolution Testers for USP method 1 (Basket), 2 (Paddle), 5 (Paddle over Disc) and 6 (Rotating Cylinder). A stirrer is a type of laboratory equipment consisting of a rotating magnet or stationary electomagnets creating a rotating magnetic field. The stirrer is used to cause a stir bar, immersed in a liquid to be stirred, to spin very quickly, stirring it. Often, the stirrer can provide heating.

Stirrers are often used in laboratories, especially in the field of chemistry. They are preferred over gear-driven motorized stirrers in chemical research because they are quieter, more efficient, and have no moving parts to break or wear out (other than the simple bar magnet itself). Due to the small size, the stirring bar is more easily cleaned and sterilized than other stirring devices. [43].

### 4.3.8 Dissolution Media:

The nature and quantity of the dissolution medium will also affect the dissolution test. Dissolution medium selected for the study must be able to show the in vitro and in vivo correlation. [44].

According to the nature of the drug product and the location in the body where the drug is expected to dissolve, which medium is best is a matter of considerable controversy.

Various investigators have used dissolution media are as following:

- 0.1N Hydrochloric Acid
- Phosphate Buffer (pH 4)
- Phosphate Buffer (pH 6.8)
- Phosphate Buffer (pH 7)
- Phosphate Buffer (pH 7.4)
- Phosphate Buffer (pH 8)

### 4.3.9 Volume of Dissolution Media:

In selecting the volume of dissolution medium, the solubility as well as the amount of drug in the dosage form must be considered. Usually a volume of dissolution medium larger then the amount of solvent needed to completely dissolved the drug is used in such test. Generally used the volume is 250 ml, 500 ml, 900 ml and 1000 ml. [45].

## 4.3.10 Temperature (Degree Centigrade):

The temperature of dissolution medium must also be controlled and variation in temperature must be avoided. Most of dissolution tests are performed at $37^0$ c, the body temperature. [46].

## 4.3.11 Resolution per Min (rpm):

The rotational speed is selected and maintained according to the monograph specified in the individual monograph. For many drugs dissolution rates can be higher with the paddle method so according to it resolution of basket and paddle method is preferred.

## 4.3.12 Assay Method:

### 4.3.12.1 UV:

A wide range of spectrophotometers is now available for dissolution applications. The choice of spectrophotometer will depend to a large extent on cost and the degree of sophistication required i.e., single beam, double beam, etc. All of UV-VIS on-line systems are based on the â€˜continuous flowâ€™ method which is understandably the most popular approach to automated Tablet Dissolution Testing. Such systems are simple, clean, easy to set up and maintain. UV visible spectrometers measures the absorbance at the wavelength and it helps to find in vitro release of drug.

### 4.3.12.2 HPLC:

It is known as high performance liquid chromatography. It is based on methods of separation pharmaceutical companies have automated testing options when working with solid dosage forms. Dissolution testing using HPLC offers high-quality results. (Chromatography). (high-performance liquid chromatography)

### 4.3.12.3 IR:

Infrared Spectroscopy involving the determination of dissolution profiles in pharmaceutical tablets is IR Fourier transform infrared (FT-IR) imaging was used to study dissolution of VARIOUS dosage forms. Infra red spectroscopy to measure the percentage drug dissolution from a dosage forms. [47-48].

## 4.3.13 Transitional Diffusion Layer Thickness (Microns):

Good simulation of powder dissolution data has been achieved by assuming that the hydrodynamic diffusion layer thickness is equal to the drug particle radius for particles below a certain radius and equal to a constant value for all larger particles. As a default, this value is 30 µm on the interface. Given all other parameters constant, changing this value allows for different dissolution rates due to changes in the stirring rate. Smaller values for the transitional diffusion layer thickness will result in a faster rate of dissolution.

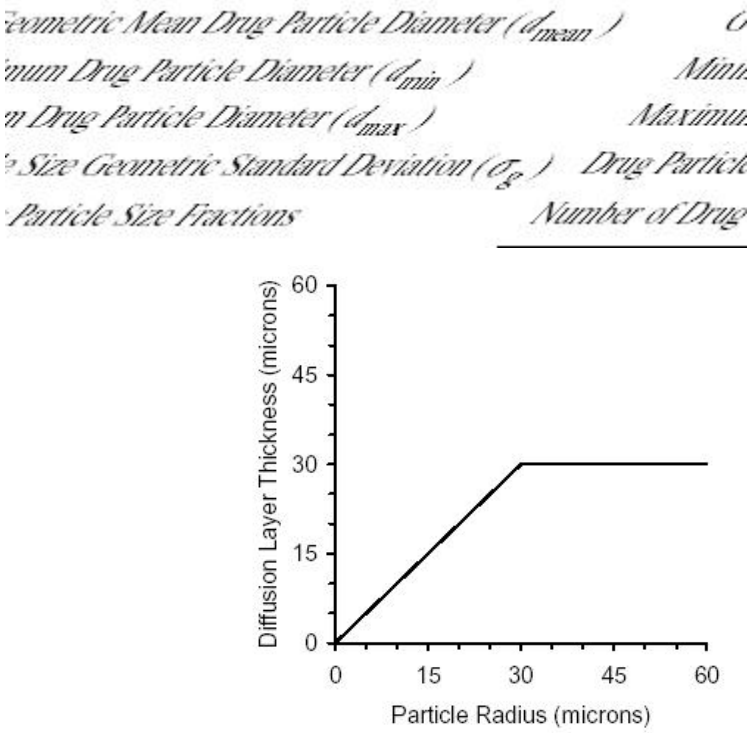


*Figure 4.3.13(1) Plot of Diffusion Layer Thickness and Particle Radius*

The above group of drug particle size parameters allows the simulation of a polydisperse drug powder as a log-normal mass distribution shown above and to the right.

The following equations are used to calculate the minimum and maximum particle diameters, which are updated automatically anytime a change is made to either the mean or standard deviation:

$$d_{\min} = \frac{d_{\text{mean}}}{\sigma_g^3}$$

$$d_{\max} = d_{\text{mean}}\,\sigma_g^3$$

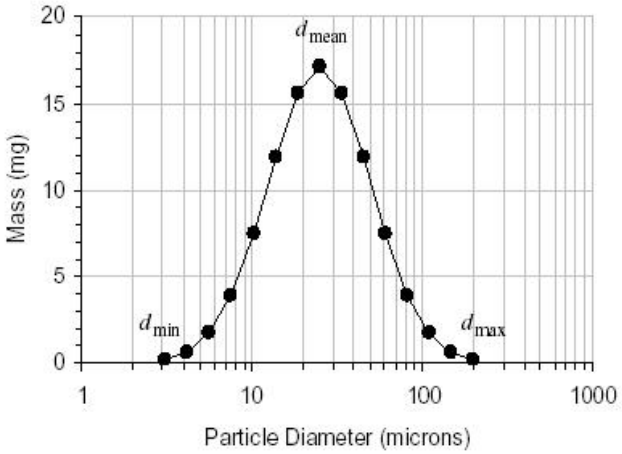


*Figure 4.3.13(2) Plot of Mass and Particle Radius*

A geometric standard deviation of one yields a monodisperse distribution while increasing its value widens the distribution. In the figure below, the number of particle size fractions is 15. Mechanistically, treating a polydisperse powder as a collection of monodisperse fractions of various sizes is more realistic and simulates dissolution better. The choice of the number of particle size fractions is a tradeoff between a better simulation of the dissolution data versus the increased time required to complete the simulation.

## 4.3.14 Simulation Time:

Simulation Time is the desired duration of the simulation entered in units of minutes. For example, if one wished to simulate the dissolution of a drug powder for one hour, 60 should be entered for the simulation time. The actual time to complete the simulation will only be a small fraction of real time.

## 4.3.15 Dilution Factor (DF):

It is the factor which helps in calculation of percentage release of drug in vitro method.diltion can be carrid out for measurement of absorbance or for assay method acording to which instrument is used.

### 4.3.16 Withdrawal Volume of Dissolution Media (ml):

Withdrawal volume of dissolution media has been done according to the release rate of drug in the body and for in vitro dissolution preffered withdrawal volume according to dilution and described in assay method.

### 4.3.17 Dose (mg):

It is the quantity of something that may be eaten by or administered to an organ. Dose is the initial mass of solid drug in mg used in the simulation. The dose will be distributed by mass into the various particle size fractions *oi X* according to the geometric mean drug particle diameter and standard deviation, or if desired, according to the actual experimental particle size distribution.

### 4.3.18 Print Frequency (mg/cc):

During a simulation run, a Microsoft Excel spreadsheet will be created to store the simulated data for future viewing and graphing. Simulated data is calculated approximately every second of simulated time. However, to save every calculated time point would result in an extremely long spreadsheet. The Print Frequency allows the user to select the interval between simulated data saved to the spreadsheet.

### 4.3.19 Use Experimental Drug Particle Size:

Checking this box allows the use of experimental drug particle size data. When selected, all other drug particle size inputs on the user interface will be ignored except the number of particle size fractions. When the simulation is initiated, an Excel spreadsheet will appear to allow the user to enter particle size data either manually or by pasting from the clipboard.

Particle diameter in micrometers must be entered in column 1 and the associated percent volume or mass must be entered in column 2. The number of rows of data entered into the spreadsheet must also equal the number of drug particle size fractions entered in the main user interface.

### 4.3.20 Drug Density (mg/cc):

Drug Density is the true density of the drug and is a necessary conversion factor between drug particle size (volume) and drug mass. Typically, a gas pycnometer would be used to measure the true density of a drug powder.

### 4.3.21 Diffusion Coefficient (cm2/min):

Diffusion coefficient is a parameter expressing the transfer rate of a substance by random molecular motion. Mathematically, it is defined as the specific transfer rate under a unit driving concentration gradient.

### 4.3.22 Human or Animal Body Weight (Kg):

Clearance and Volume of Distribution are inputted on a per kilogram basis. In equation 18, it can be seen that because clearance is divided by the volume of distribution, the body weight will cancel out as both clearance and volume of distribution are divided by body weight. However, when calculating blood/plasma concentration of drug, the mass in the central blood/plasma is divided by the volume of distribution in L/kg multiplied by the body weight in kg.

### 4.3.23 Bioavailability:

Bioavailability is entered in fractional form ranging from 0 to 1. As seen in equation 18, it reduces the amount of drug reaching the systemic circulation. Bioavailability does not affect the calculation of the amount of drug absorbed as shown in equation 14. As a result, it is possible to have a drug that is well absorbed, that is, it crosses the intestinal membrane rapidly compared to other drugs, but does not completely reach the systemic circulation due to metabolism by the liver or by the intestinal enterocytes.

### 4.3.24 Clearance (ml/min/kg):

Clearance is the rate drug removal from the central blood/plasma compartment entered in units of ml/min/kg. The Clearance (Cl) of a drug is the volume of plasma from which the drug is completely removed per unit time. The amount eliminated is proportional to the concentration of the drug in the blood.

## 4.3.25 Volume of Distribution (L/Kg):

Volume of Distribution is the hypothetical volume of the central blood/plasma compartment and is entered in units of L/kg. The Volume of Distribution (Vd) is the amount of drug in the body divided by the concentration in the blood. Drugs that are highly lipid soluble, such as digoxin, have a very high volume of distribution (500 litres). Drugs which are lipid insoluble, such as neuromuscular blockers, remain in the blood, and have a low Vd.

## 4.3.26 K12 (1/min) & K21 (1/min):

K12 and K21 are first order rate constants used to calculate the rate of drug transfer in and out of the peripheral compartment respectively (see Figure 1). Setting K12 equal to zero reduces the pharmacokinetic model shown in Figure 1 to a one-compartment pharmacokinetic model.

## 4.3.27 Metastable Factor:

During the simulation of drug dissolution, either in vitro or in vivo, it is possible that drug solubility could decrease, most likely for a basic drug exiting the stomach and entering the higher pH environment of the small intestine. As a result, the concentration of drug dissolved in solution could be higher than the solubility of the drug. If this happens, the overall sign of the following term in equations 12 and 13 becomes negative:

$$\left(C_s(t) - \frac{X_{dT}}{V(t)}\right)$$

If not all of the solid drug has dissolved, there will be crystalline drug surface on which crystal growth can occur, or at least, a very high concentration of supersaturated drug near the solid/liquid interface where nucleation and crystal growth would likely occur. In this case, precipitation will be simulated automatically as the reverse of dissolution using equations 12 and 13. It is also possible that the entire drug powder has dissolved, leaving no solid surface for crystal growth. In this case, the metastable factor provides options for initiating nucleation for further precipitation. The metastable factor is defined as follows:

$$metastable\ factor = \frac{\frac{X_{dT}}{V}}{C_s} = \frac{concentration\ of\ drug\ in\ solution}{solubility} \quad meta$$

If conditions are such that the metastable factor is exceeded, then nucleation will be initiated to allow for precipitation. For example, if the metastable factor were one, nucleation and precipitation would occur as soon as the solubility fell below the concentration of drug in solution. If the metastable factor were 10, the solubility would have to be more than 10 times smaller than the concentration of drug in solution before nucleation and precipitation would occur. This allows the existence of a metastable supersaturated state if so desired.

The figure below shows a simulation of precipitation in an in vitro situation. Dissolution is complete by 90 minutes when solubility is decreased from 0.1 to 0.01 mg/ml. As soon as solubility falls below the concentration of dissolved drug, precipitation occurs and the amount of dissolved drug decreases with the concomitant increase in the amount of solid drug. In the illustration below, the metastable factor was one, so that precipitation occurred immediately. Increasing the metastable factor would delay precipitation. At some point, continuing to increase the metastable factor further will prevent precipitation entirely.

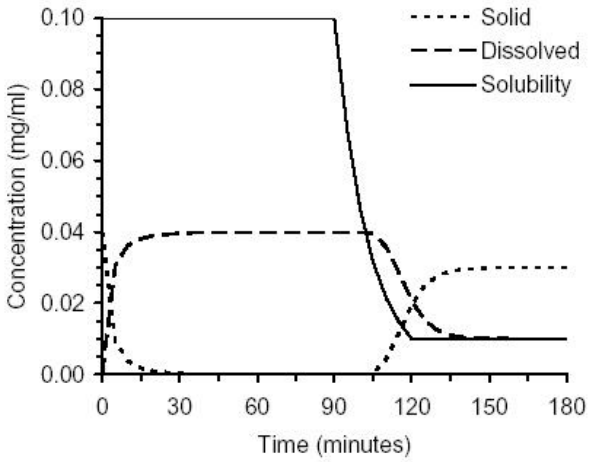


*Figure 4.3.27(1) Simulation of precipitation in an in vitro situation*

# 4.4 Tables

## 4.4.1 Cumulative Percentage Release:

### 4.4.1.1 Absorbance:

In spectroscopy, the absorbance A is defined as

—

where I is the intensity of light at a specified wavelength ë that has passed through a sample (transmitted light intensity) and I0 is the intensity of the light before it enters the sample or incident light intensity. Absorbance measurements are often carried out in analytical chemistry, since the absorbance of a sample is proportional to the thickness of the sample and the concentration of the absorbing species in the sample, in contrast to the transmittance I / I0 of a sample, which varies logarithmically with thickness and concentration.

Outside the field of analytical chemistry, e.g. when used with the Tunable Diode Laser Absorption Spectroscopy (TDLAS) technique, the absorbance is sometimes defined as the natural logarithm instead of the base-10 logarithm, i.e. as

—

The S.I. units for absorbance are absorptions (ab). No customary units exist.

#### 4.4.1.2 Time:

Time is a basic component of the measuring system used to sequence events, to compare the durations of events and the intervals between them, and to quantify the motions of objects.

#### 4.4.1.3 Concentration:

Concentration is the measure of how much of a given substance there is mixed with another substance. This can apply to any sort of chemical mixture, but most frequently the concept is limited to homogeneous solutions, where it refers to the amount of solute in a substance.

Concentration is the strength of a solution; number of molecules of a substance in a given volume (expressed as moles/cubic meter). If the concentration of the drug increases in the body then the concentration gradient decreases leading to a decreases rate of the dissolution of the drug.

### 4.4.2 Cumulative Percentage:

Calculated by dividing the cumulative release by the number of observations and multiplying it by 100. The last value will always be equal to 100%. This allows for easier comparison of the data. Cumulative percentage release is the cumulative release of drug from the dosage form into the solution.

### 4.4.3 Calibration Curve:

In analytical chemistry, a calibration curve is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration.

Here, the calibration curve is used for measurement of concentration from the data of time and absorbance. So it can be used for calculation of concentration for dissolution testing and help in finding release of the drug

#### 4.4.3.1 How to Create Calibration Curve:

To find the best-fit straight line we use linear regression analysis. From the equation $y = mx + c$ you substitute in the y value (response) and solve for x. Many different variables can be used as the analytical signal, in Figure 1 analysis was of Chromium(III) by chemiluminescence. The detector (PMT) detects light, voltage increases with intensity of light, this creates a peak and the peak area is the analytical signal. For most analytical techniques, the ultimate goal is to obtain a calibration curve; and there are a number of advantages to this method:

The calibration curve not only gives you your answer it also gives you an idea about how good that answer is. Provided you are operating in the linear response range the plot should be a straight line, deviations from this straight line give a good indication about the precision of the result. That is to say, if the data points are spread out, there is less certainty in the result.

The calibration curve provides you with an empirical relationship, as opposed to a theoretical one. Instrumental response is usually highly dependent on the condition of the analyze, solvents used and impurities it may contain; it could also be affected by external factors such as pressure and temperature. Many theoretical relationships, such as fluorescence, require the determination of an instrumental constant anyway; in which case a calibration curve is the only way to do the determination. By making standards as similar

as possible to the unknown and creating the calibration curve, what is created is a custom relationship, which takes into account all the above factors and many others for the specific conditions of the experiment. The disadvantages are that a set of standards needs to be made, for which you must have a source of analyte material of known composition. This is a time-consuming process, may use significant quantities of expensive chemicals, and results in an increased amount of waste for disposal. Also, you need to make a rough estimate of the composition of your unknown sample, in order to enable interpolation the concentrations of the standards should fall above and below the expected unknown concentration.

In short, there is a lot of information about the analysis in a calibration curve and it is a simple way to account for all the influencing factors that could change the analytical signal.

### 4.4.3.2 Applications of Calibration Curve:

- Analysis of concentration
- Verifying the proper functioning of an analytical instrument or a sensor device such as an ion selective electrode

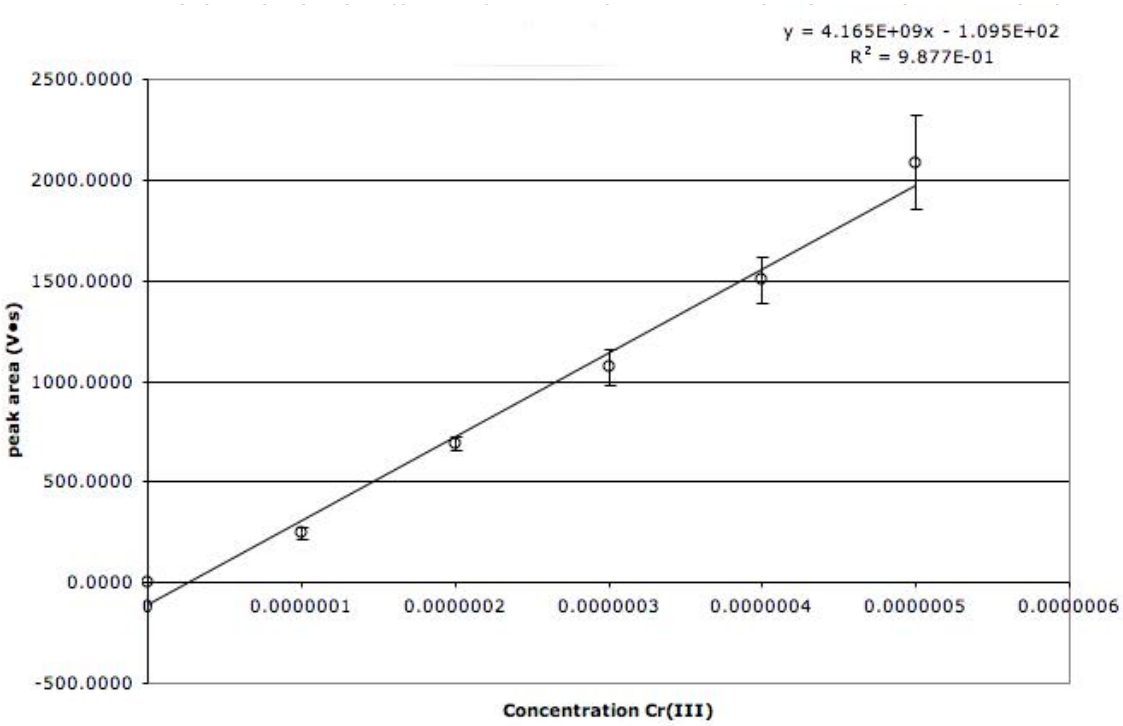


*Figure 4.4.3.1(1) Calibration Curve*

### 4.4.4 Controlled Release Rate:

The controlled release rate determines the amount of drug that is released every minute, either as a solid powder when running in the controlled release mode, or as dissolved drug when running in the controlled release no dissolution mode. The release rate can either be constant or change with time.

Due to the complexity of simulating the controlled release of a polydisperse powder, running in the controlled release mode can take several hours, depending on the length of the simulation. This time can be reduced by decreasing the number of drug particle size fractions.

Running in the controlled release no dissolution mode is very fast and can be used to quickly determine the release rate needed to achieve a desired drug plasma concentration. Once the target release rate has been determined, running the same release rate in the controlled release rate mode will give insight into whether solubility and/or drug particle size will have an impact on the desired drug plasma concentration profile. [49-50].

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# Chapter 5: INTEGRATED PROTOTYE SOFTWARE DESIGN, IMPLEMENTATION & RESULT ANALYSIS

## 5.1 Introduction:

Dissolution testing plays an important role in pharmaceutical quality control and in the development of solid, semi-solid, and transdermal pharmaceutical forms. The dissolution kinetic is reexamined under simulated physiological conditions, which are specified in both the U.S. Pharmacopeia (USP) and the European Pharmacopeia (EP) dissolution testing regulations. As such, these analytics are performed in a highly regulated Good Manufacturing Practice (GMP) environment, and present particular challenges for related software applications. The role of computer based information systems has considerably increased in pharmacy as well as clinical practice in the last decade. However, the use of such systems in dissolution test is still not widespread. Several mathematical systems are commercially available for dissolution parameters computations. In addition, comprehensive systems customized to specific needs have also been developed. The use of such systems in dissolution parameters calculations, questions regarding the role of structured data *versus* free text input, standardization of nomenclature, and compatibility with other systems, are hotly debated. We describe the development of simple software for dissolution parameters computations records, which attempts to resolve some of these issues. The prototype described herein was specifically designed to meet the requirements of the dissolution parameters calculations programme of a tertiary referral and teaching with a high volume of procedures. After appropriate coding, for storage and subsequent analysis at a later date, and *(b)* to the report generator. An additional module was included to allow modification and update of previously recorded data. A unique number assigned to each test record was to be used as a primary identifier throughout the record.

The method implemented as this software provides Graphical User Interface (GUI) interface to the user for taking input for the drug concentration, instrument response and time data. After taking input it display list where user can opt for specific set of computations and can get the results for desired set of computation. The software supplements visualization along with computation. The user can opt for reports to be provided by the software.

It generate calibration curve, cumulative percentage release, dissolution efficiency, comparison of any number of drug profiles through similarity and dissimilarity factors. It is also facilitate to work with various steps before comparing number of drug profiles, which includes bioavailability, Mean Residence Time (MRT), Volume of Distribution, Clearance, Dissolution Efficiency from this prototype. The prototype has various modules for input and modification of data, computation of various parameters and visualization with facilities to generate reports of dissolution parameters.

The use of interface is designed for work with much ease in respecting. With little practice, scientists soon became adept at entering details correctly and quickly. The slightly increased time of data entry into the computer was more than made up by uniform and complete report generation. A user-friendly prototype providing computation and visualization parse drug dissolution parameters. The analytical scientists can utilize the prototype for intensive research as wide variety of parameter computation at simple key stroke.

## 5.2 Materials & Method:

### 5.2.1 Requirements:

The central objective of this project was to create a data model capable of accurately representing the calculations for dissolution parameters computations in a computer-suitable format. Our main requirements included that the system should *(a)* be simple enough to be directly operated by the analytical scientist(s) in analytical research laboratory, *(b)* run on personal computers, *(c)* allow comprehensive data entry conforming to well recognized procedures which are currently carried out, *(d)* generate a printed report, *(e)* allow modification and update of data at a later date, and *(f)* permit subsequent statistical analysis of records in a tabular format. As dissolution data were to be handled by

analytical scientists with minimal previous computer experience, an emphasis was laid on a user-friendly interface.

## 5.2.2 Software Construction:

The software was written in Visual Basic.NET 2005 and MATLAB 7 and generated a series of successive screens for data entry. Two screens, relating to *(a)* Various tables data and *(b)* Parameters. Data flow was designed in two directions: *(a)* to a database, after appropriate coding, for storage and subsequent analysis at a later date, and *(b)* to the report generator. An additional module was included to allow modification and update of previously recorded data. Another module was designed for filling test reports on specimens obtained during the procedure, as and when these results became available. A unique identifier assigned to each test record was to be used as a primary identifier throughout the record.

## 5.2.3 Data Entry:

Modules were developed to allow easy user access and facilitate data entry. On completion of one module, automatic transfer to the subsequent module was envisaged. The basic module was structured as a large window, with smaller sub-windows appearing only on demand. The entire software was kept menu driven, with a simple and consistent hierarchical structure.

This system will enable us to have Data acquisition from remote places like drug dissolution machine or remote computer that attached directly to the drug dissolution machine. There may be number of places where human presence is not desirable or human involvement is not desirable and for that this data acquisition system is useful for this developed prototype.

All the industry will find this product useful for them, as it covers basic data acquisition system which regulates basic parameter measurement like Calibration Curve, Cumulative Percentage Release, Dissolution Volume etc with having fast response, highly user interactive console and easy to use,

### 5.2.3.1 Serial Communications:

Serial is a very common protocol for device communication that comes standard on just about every PC.

5.2.3.1.1 Data Entry through Communication Device:

There are two basic types of serial communications synchronous and asynchronous. With synchronous communications, the two devices initially synchronize themselves to each other, and then continually send characters to stay in sync. Even when data is not really being sent, a constant flow of bits allows each device to know where the other is at any given time. That is, each character that is sent is either actual data or an idle character. Synchronous communications allows faster data transfer rates than asynchronous methods, because additional bits to mark the beginning and end of each data byte are not required. The serial ports on IBM-style PCs are asynchronous devices and therefore only support asynchronous serial communications.

Asynchronous means "no synchronization", and thus does not require sending and receiving idle characters. However, the beginning and end of each byte of data must be identified by start and stop bits. The start bit indicates when the data byte is about to begin and the stop bit signals when it ends. The requirement to send these additional two bits causes asynchronous communication to be slightly slower than synchronous however it has the advantage that the processor does not have to deal with the additional idle characters.

An asynchronous line that is idle is identified with a value of 1. By using this value to indicate that no data is currently being sent, the devices are able to distinguish between an idle state and a disconnected line. When a character is about to be transmitted, a start bit is sent. A start bit has a value of 0 (also called a space state). Thus, when the line switches from a value of 1 to a value of 0, the receiver is alerted that a data character is about to be sent.

Most computers include two RS-232 based serial ports. Serial is also a common communication protocol that is used by many devices for instrumentation; numerous GPIB-compatible devices also come with an RS-232 port. Furthermore, serial communication can be used for data acquisition in conjunction with a remote sampling device.

The concept of serial communication is simple. The serial port sends and receives bytes of information one bit at a time. Although this is slower than parallel communication, which allows the transmission of an entire byte at once, it is simpler and can be used over longer distances. For example, the IEEE 488 specifications for parallel communication state that the cabling between equipment can be no more than 20 meters total, with no more than 2 meters between any two devices; serial, however, can extend as much as 1200 meters.

Typically, serial is used to transmit ASCII data. Communication is completed using 3 transmission lines: (1) Ground, (2) Transmit, and (3) Receive. Since serial is asynchronous, the port is able to transmit data on one line while receiving data on another. Other lines are available for handshaking, but are not required. The important serial characteristics are baud rate, data bits, stop bits, and parity. For two ports to communicate, these parameters must match.

a) Baud rate:

A speed measurement for communication. It indicates the number of bit transfers per second. For example, 300 baud is 300 bits per second. When we refer to a clock cycle we mean the baud rate. For example, if the protocol calls for a 4800 baud rate, then the clock is running at 4800Hz. This means that the serial port is sampling the data line at 4800Hz.

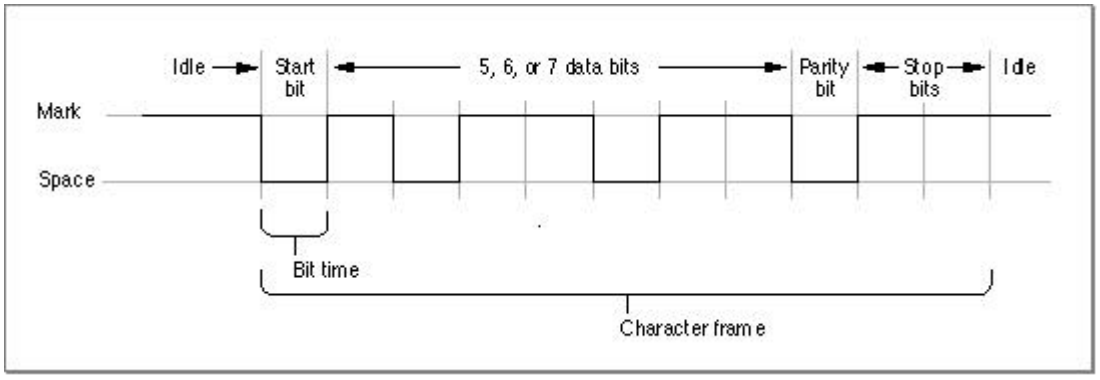



*Fig. 5.2.3.1.1(1) Braud Rate*

b) Data bits:

A measurement of the actual data bits in a transmission. When the computer sends a packet of information, the amount of actual data may not be a full 8 bits. Standard values for the data packets are 5, 7, and 8 bits. Which setting you choose depends on what information you are transferring. For example, standard ASCII has values from 0 to 127 (7 bits). Extended ASCII uses 0 to 255 (8 bits). If the data being transferred is simple text (standard ASCII), then sending 7 bits of data per packet is sufficient for communication. A packet refers to a single byte transfer, including start/stop bits, data bits, and parity. Since the

numbers of actual bits depend on the protocol selected, the term packet is used to cover all instances.

c) Stop bits:

It is used to return the signal for end of communication of a single packet. Typical values are 1, 1.5, and 2 bits.

d) Parity:

A simple form of error checking that is used in serial communication. There are four types of parity: even, odd, marked, and spaced. For even and odd parity, the serial port will set the parity bit (the last bit after the data bits) to a value to ensure that the transmission has an even or odd number of logic high bits.

5.2.3.1.2 Rs-232 Standard For Communication:

The example serial waveforms in above figure show the waveform on a single conductor to transmit a byte (0x41) serially. The upper waveform is the TTL-level waveform seen at the transmit pin of 8051. The lower waveform shows the same waveform converted to RS232C levels. The voltage level of the RS232C is used to assure error-free transmission over greater distances than would be possible with TTL levels.

It is first step to collect values for computations. Its also help to scientist to automation of fetching the values from the machine.

Free text was allowed in some fields, such as the information beyond the fixed choices available to the user. To allow complete data acquisition in each test, all data fields were marked mandatory, and the user was not allowed to proceed to a subsequent field without recording data in such fields.

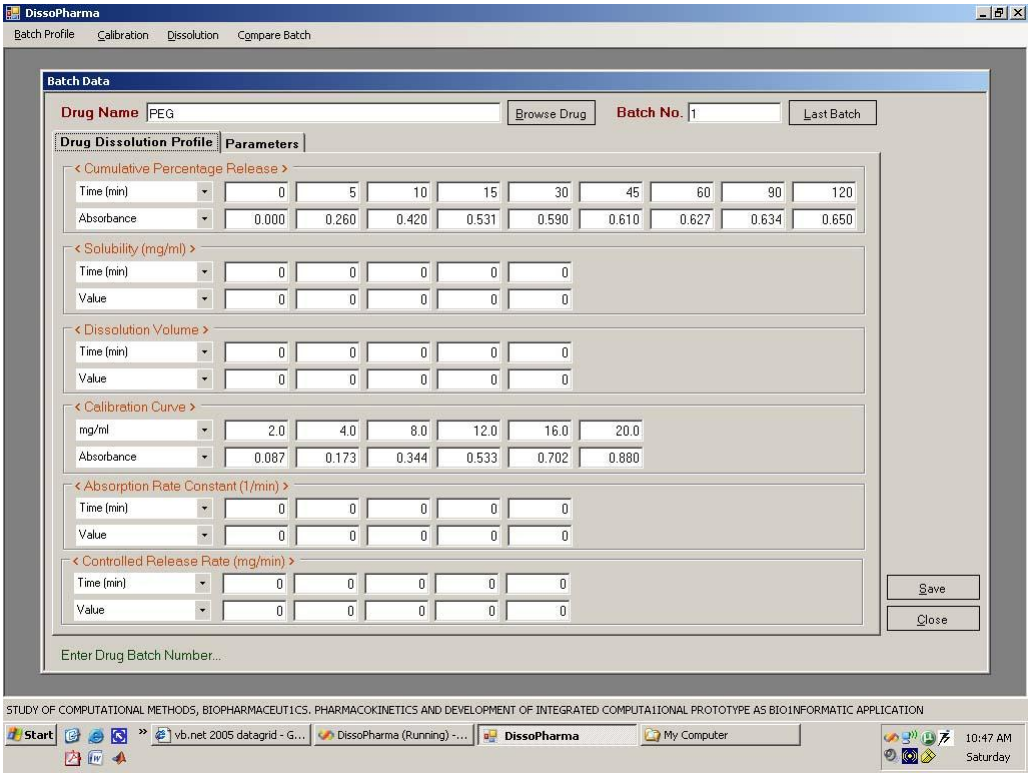



*Fig. 5.2.3.1.2(1) Input form for Batch Data Profiles*

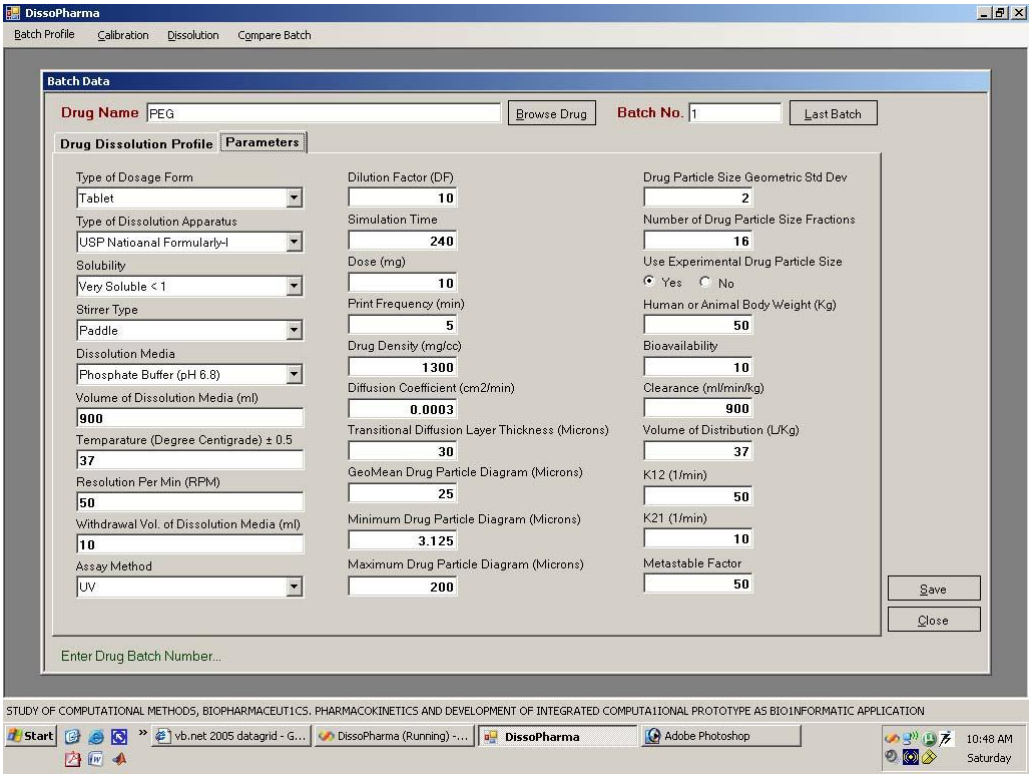



*Fig. 5.2.3.1.2(2) Batch Data Profiles Parameters*

### 5.2.4 Debugging and Modification:

After initial development, the software was tested over a four-week period by input of data. An attempt was made to rectify problems faced initially by the users. Opinion was sought from faculty members regarding possible modifications and improvements. Inconsistencies in the programming script, which gave rise to error messages during operation of software, were corrected.

### 5.2.5 Software Validation:

To evaluate the actual utility of the software, all consecutive test records were entered using this computer software. Analytical scientists were asked to assess the overall quality of the reports and the content of information. After entry of data for 60 consecutive test procedures, these details were subjected to statistical analysis to evaluate the robustness of the database component.

### 5.2.6 Linearity or calibration curve:

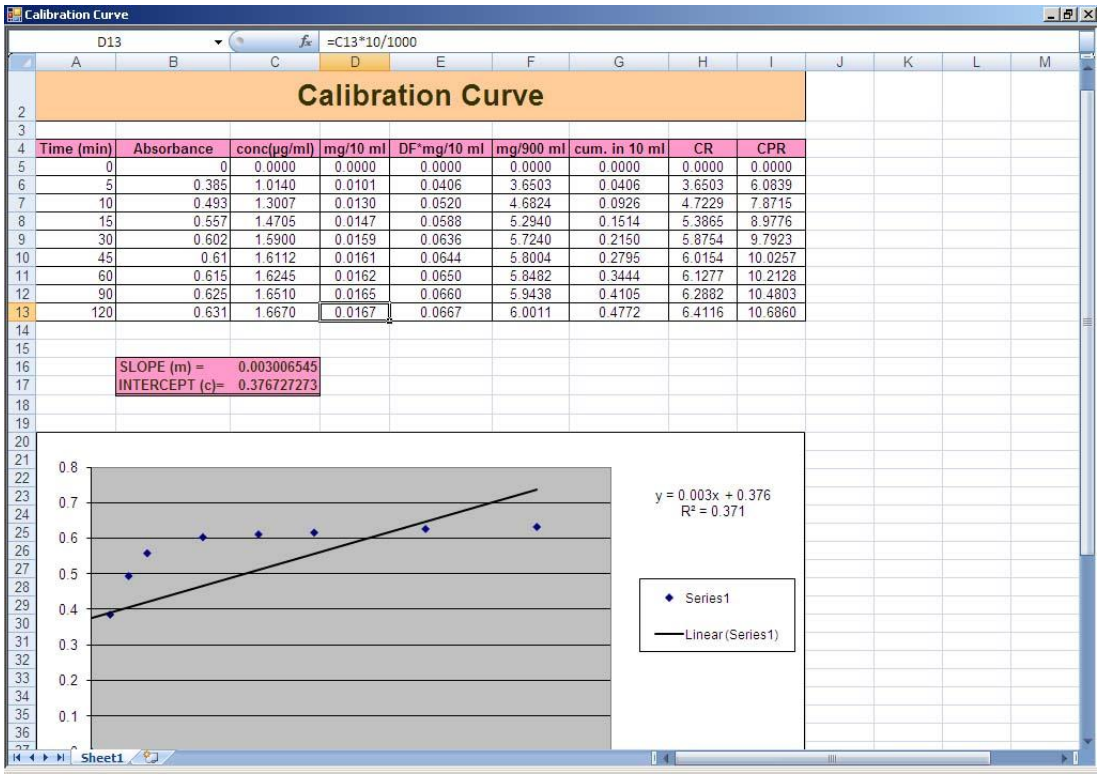
The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyze in the sample.

A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighing of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range.

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyze concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may need to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity.

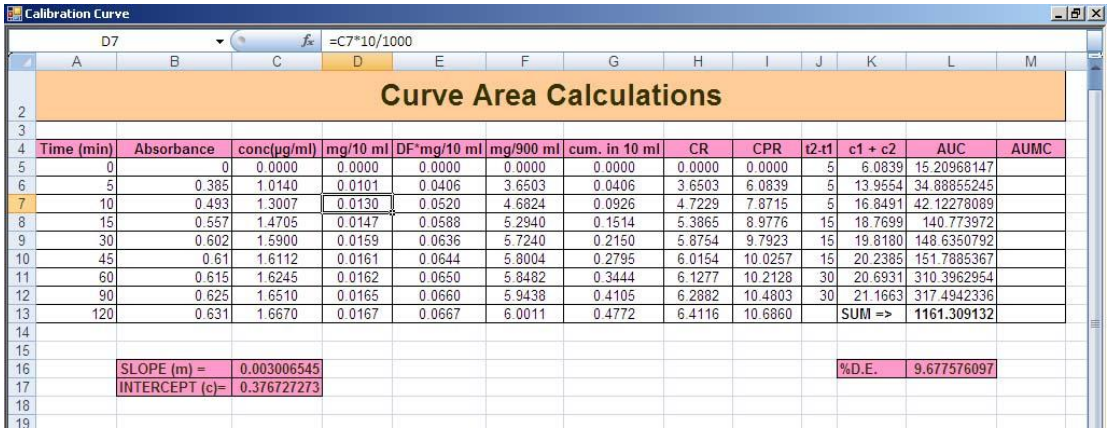
The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be

helpful for evaluating linearity. For the establishment of linearity, a minimum of 5 concentrations is recommended. For the dissolution calculation concentrations were calculated from the respective calibration curve.




| Time (min) | Absorbance | conc(µg/ml) | mg/10 ml | DF*mg/10 ml | mg/900 ml | cum. in 10 ml | CR | CPR |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 5 | 0.385 | 1.0140 | 0.0101 | 0.0406 | 3.6503 | 0.0406 | 3.6503 | 6.0839 |
| 10 | 0.493 | 1.3007 | 0.0130 | 0.0520 | 4.6824 | 0.0926 | 4.7229 | 7.8715 |
| 15 | 0.557 | 1.4705 | 0.0147 | 0.0588 | 5.2940 | 0.1514 | 5.3865 | 8.9776 |
| 30 | 0.602 | 1.5900 | 0.0159 | 0.0636 | 5.7240 | 0.2150 | 5.8754 | 9.7923 |
| 45 | 0.61 | 1.6112 | 0.0161 | 0.0644 | 5.8004 | 0.2795 | 6.0154 | 10.0257 |
| 60 | 0.615 | 1.6245 | 0.0162 | 0.0650 | 5.8482 | 0.3444 | 6.1277 | 10.2128 |
| 90 | 0.625 | 1.6510 | 0.0165 | 0.0660 | 5.9438 | 0.4105 | 6.2882 | 10.4803 |
| 120 | 0.631 | 1.6670 | 0.0167 | 0.0667 | 6.0011 | 0.4772 | 6.4116 | 10.6860 |

*Fig. 5.2.6(1) Calibration Curve*



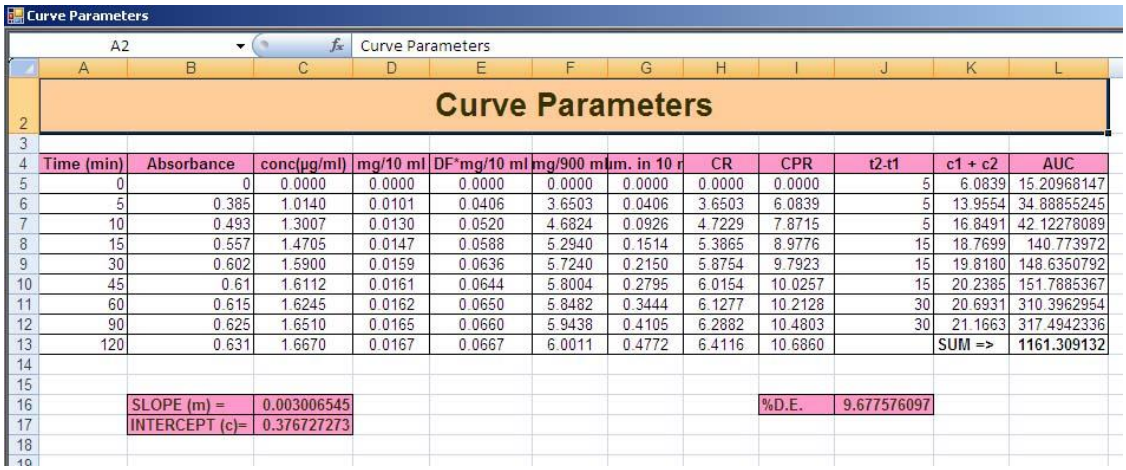

| Time (min) | Absorbance | conc(µg/ml) | mg/10 ml | DF*mg/10 ml | mg/900 ml | cum. in 10 ml | CR | CPR | t2-t1 | c1 + c2 | AUC | AUMC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 5 | 6.0839 | 15.20968147 | |
| 5 | 0.385 | 1.0140 | 0.0101 | 0.0406 | 3.6503 | 0.0406 | 3.6503 | 6.0839 | 5 | 13.9554 | 34.88855245 | |
| 10 | 0.493 | 1.3007 | 0.0130 | 0.0520 | 4.6824 | 0.0926 | 4.7229 | 7.8715 | 5 | 16.8491 | 42.12278089 | |
| 15 | 0.557 | 1.4705 | 0.0147 | 0.0588 | 5.2940 | 0.1514 | 5.3865 | 8.9776 | 15 | 18.7699 | 140.773972 | |
| 30 | 0.602 | 1.5900 | 0.0159 | 0.0636 | 5.7240 | 0.2150 | 5.8754 | 9.7923 | 15 | 19.8180 | 148.6350792 | |
| 45 | 0.61 | 1.6112 | 0.0161 | 0.0644 | 5.8004 | 0.2795 | 6.0154 | 10.0257 | 15 | 20.2385 | 151.7885367 | |
| 60 | 0.615 | 1.6245 | 0.0162 | 0.0650 | 5.8482 | 0.3444 | 6.1277 | 10.2128 | 30 | 20.6931 | 310.3962954 | |
| 90 | 0.625 | 1.6510 | 0.0165 | 0.0660 | 5.9438 | 0.4105 | 6.2882 | 10.4803 | 30 | 21.1663 | 317.4942336 | |
| 120 | 0.631 | 1.6670 | 0.0167 | 0.0667 | 6.0011 | 0.4772 | 6.4116 | 10.6860 | | SUM => | 1161.309132 | |

*Fig. 5.2.6(2) Curve Area Calculations*

## 5.2.7 Dissolution Study:

In vitro dissolution specifications are established to guarantee batch-to-batch consistency and to indicate potential bioavailability problems. For new drug products, dissolution specifications must be based on data obtained from the batch used in the bioavailability assay (bio-batch). For generic drugs, the dissolution specifications are generally the same of the reference drug product. These specifications are confirmed by testing the performance of the bio-batch dissolution. If the generic drug dissolution is substantially different from the reference drug product dissolution, and the in vivo study had proved the bio-equivalence between them, a different dissolution specification for the generic drug can be established, provided it is based upon a validated IVIVC. In that case, the specification must be fulfilled throughout the permanence of the generic drug in the market. The specifications must be based on the bio-batch dissolution characteristics. If the formulation developed for commercialization differs significantly from the bio-batch, the comparison of the dissolution profiles and the bio-equivalence study between these two formulations is recommended.

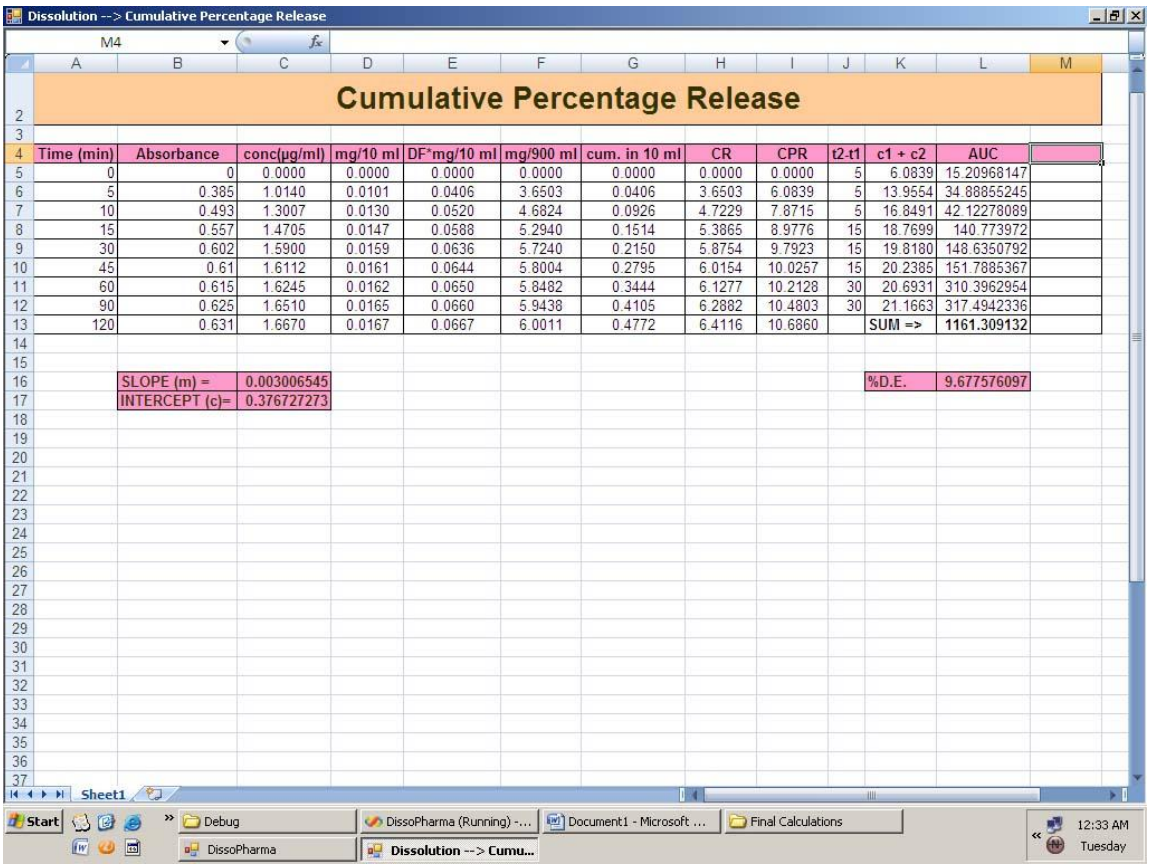
The dissolution tests must be undertaken under such conditions as: basket method at 50/100 rpm or paddle method at 50/75/100 rpm. To generate a dissolution profile, at least five sampling points must be obtained of which a minimum of three must correspond to percentage values of dissolved drug lower than 65% (when possible) and the last point must be relative to a sample period of time equal to, at least, the double of the former period of time. For drug products of rapid dissolution, samples at shorter intervals (5 or 10 minutes) may be necessary. For drug products with highly soluble drugs that present rapid dissolution (cases I and III of BCS), a dissolution test of a single point (60 minutes or less) that proves a dissolution of, at least, 85% is sufficient for batch to batch uniformity control. For drug products containing drugs poorly soluble in water, which dissolve very slowly (case II of BCS), a two points dissolution test, that is, one at 15 minutes and another at 30, 45 or 60 minutes, to ensure 85% of dissolution is recommended.



Curve Parameters

| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | **Curve Parameters** | | | | | | | | | | | |
| 3 | | | | | | | | | | | | |
| 4 | Time (min) | Absorbance | conc(µg/ml) | mg/10 ml | DF*mg/10 ml | mg/900 ml | um. in 10 r | CR | CPR | t2-t1 | c1 + c2 | AUC |
| 5 | 0 | 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 5 | 6.0839 | 15.20968147 |
| 6 | 5 | 0.385 | 1.0140 | 0.0101 | 0.0406 | 3.6503 | 0.0406 | 3.6503 | 6.0839 | 5 | 13.9554 | 34.88855245 |
| 7 | 10 | 0.493 | 1.3007 | 0.0130 | 0.0520 | 4.6824 | 0.0926 | 4.7229 | 7.8715 | 5 | 16.8491 | 42.12278089 |
| 8 | 15 | 0.557 | 1.4705 | 0.0147 | 0.0588 | 5.2940 | 0.1514 | 5.3865 | 8.9776 | 15 | 18.7699 | 140.773972 |
| 9 | 30 | 0.602 | 1.5900 | 0.0159 | 0.0636 | 5.7240 | 0.2150 | 5.8754 | 9.7923 | 15 | 19.8180 | 148.6350792 |
| 10 | 45 | 0.61 | 1.6112 | 0.0161 | 0.0644 | 5.8004 | 0.2795 | 6.0154 | 10.0257 | 15 | 20.2385 | 151.7885367 |
| 11 | 60 | 0.615 | 1.6245 | 0.0162 | 0.0650 | 5.8482 | 0.3444 | 6.1277 | 10.2128 | 30 | 20.6931 | 310.3962954 |
| 12 | 90 | 0.625 | 1.6510 | 0.0165 | 0.0660 | 5.9438 | 0.4105 | 6.2882 | 10.4803 | 30 | 21.1663 | 317.4942336 |
| 13 | 120 | 0.631 | 1.6670 | 0.0167 | 0.0667 | 6.0011 | 0.4772 | 6.4116 | 10.6860 | | SUM => | 1161.309132 |
| 14 | | | | | | | | | | | | |
| 15 | | | | | | | | | | | | |
| 16 | | SLOPE (m) = | 0.003006545 | | | | | | %D.E. | 9.677576097 | | |
| 17 | | INTERCEPT (c)= | 0.376727273 | | | | | | | | | |
| 18 | | | | | | | | | | | | |

*Fig. 5.2.7(1) Curve Parameters*



Dissolution --> Cumulative Percentage Release

| | A | B | C | D | E | F | G | H | I | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | **Cumulative Percentage Release** | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | |
| 4 | Time (min) | Absorbance | conc(µg/ml) | mg/10 ml | DF*mg/10 ml | mg/900 ml | cum. in 10 ml | CR | CPR | t2-t1 | c1 + c2 | AUC | |
| 5 | 0 | 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 5 | 6.0839 | 15.20968147 | |
| 6 | 5 | 0.385 | 1.0140 | 0.0101 | 0.0406 | 3.6503 | 0.0406 | 3.6503 | 6.0839 | 5 | 13.9554 | 34.88855245 | |
| 7 | 10 | 0.493 | 1.3007 | 0.0130 | 0.0520 | 4.6824 | 0.0926 | 4.7229 | 7.8715 | 5 | 16.8491 | 42.12278089 | |
| 8 | 15 | 0.557 | 1.4705 | 0.0147 | 0.0588 | 5.2940 | 0.1514 | 5.3865 | 8.9776 | 15 | 18.7699 | 140.773972 | |
| 9 | 30 | 0.602 | 1.5900 | 0.0159 | 0.0636 | 5.7240 | 0.2150 | 5.8754 | 9.7923 | 15 | 19.8180 | 148.6350792 | |
| 10 | 45 | 0.61 | 1.6112 | 0.0161 | 0.0644 | 5.8004 | 0.2795 | 6.0154 | 10.0257 | 15 | 20.2385 | 151.7885367 | |
| 11 | 60 | 0.615 | 1.6245 | 0.0162 | 0.0650 | 5.8482 | 0.3444 | 6.1277 | 10.2128 | 30 | 20.6931 | 310.3962954 | |
| 12 | 90 | 0.625 | 1.6510 | 0.0165 | 0.0660 | 5.9438 | 0.4105 | 6.2882 | 10.4803 | 30 | 21.1663 | 317.4942336 | |
| 13 | 120 | 0.631 | 1.6670 | 0.0167 | 0.0667 | 6.0011 | 0.4772 | 6.4116 | 10.6860 | | SUM => | 1161.309132 | |
| 14 | | | | | | | | | | | | | |
| 15 | | | | | | | | | | | | | |
| 16 | | SLOPE (m) = | 0.003006545 | | | | | | | | %D.E. | 9.677576097 | |
| 17 | | INTERCEPT (c)= | 0.376727273 | | | | | | | | | | |
| 18 | | | | | | | | | | | | | |

Sheet1

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*Fig. 5.2.7(2) Cumulative Percentage Release*

## 5.2.8 Dissolution Efficiency:

Khan[16] suggested Dissolution Efficiency (D.E.) as a suitable parameter for the evaluation of in vitro dissolution data. D.E. is defined as the area under dissolution curve up to a certain time 't' expressed as percentage of the area of the rectangle described by 100% dissolution in the same time. $T_{50}$ and D.E. 120 min values were calculated from the dissolution data.

Dissolution efficiency (D.E.) = $\frac{0\int^{t} y.dt}{y100^{t}} x100$

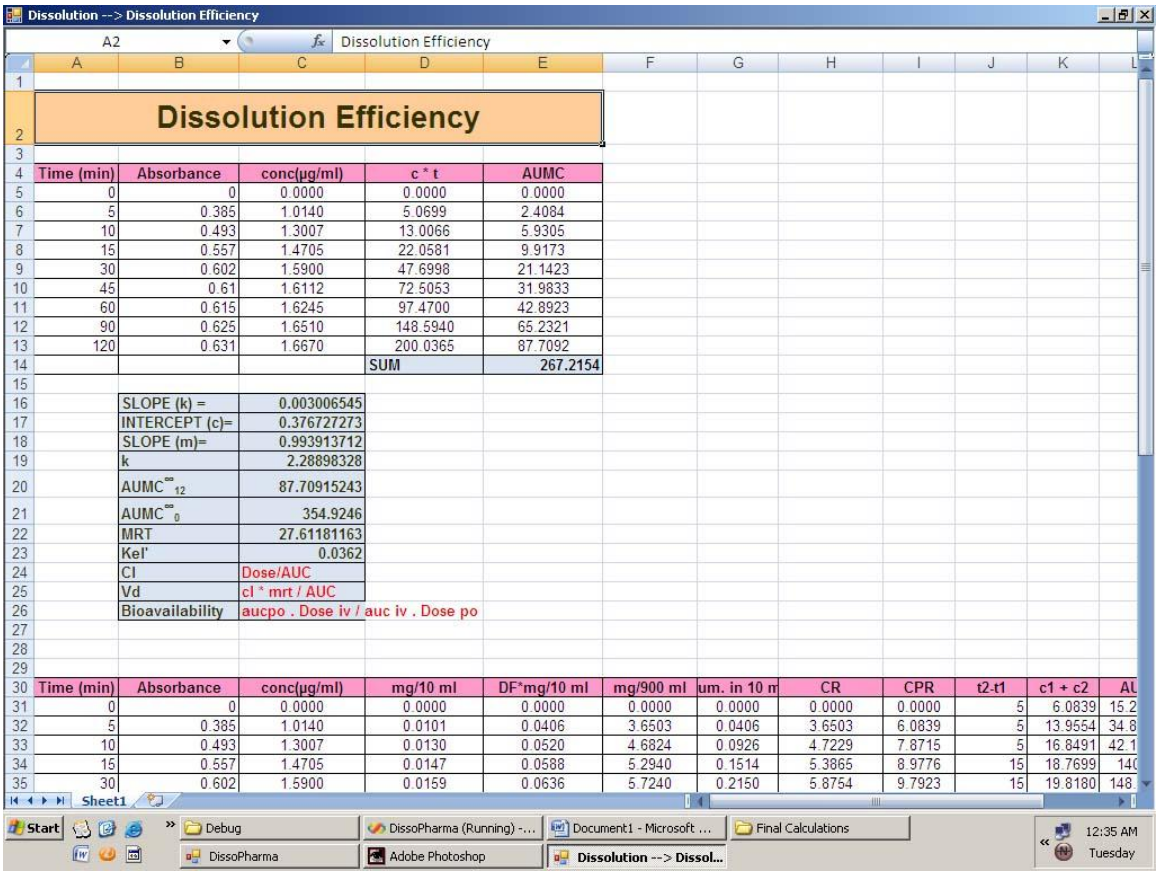



*Fig. 5.2.8(1) Dissolution Efficiency*

## 5.2.9 Comparison of dissolution profiles by similarity and dissimilarity factor:

To avoid the requirement of bioequivalence studies of the immediate release pharmaceutical forms of lower dosage, when several presentations with the same

formulation exist, the dissolution profiles must be compared and must be identical among all dosages.

Until recently, single point dissolution tests and specifications have been employed to evaluate scale-up and post-registration changes. When minor alterations are carried out, the single point dissolution test may be adequate to ensure drug product quality and performance. For major alterations, the comparison of dissolution profiles obtained in identical conditions between the altered formulation and original one, is recommended. In this comparison, the curve is considered as a whole, in addition to each sampling point of the dissolution media, by means of independent model and dependent model methods. Independent Model Method employing the Similarity factor. A simple independent model method employs a difference factor ($f_1$)

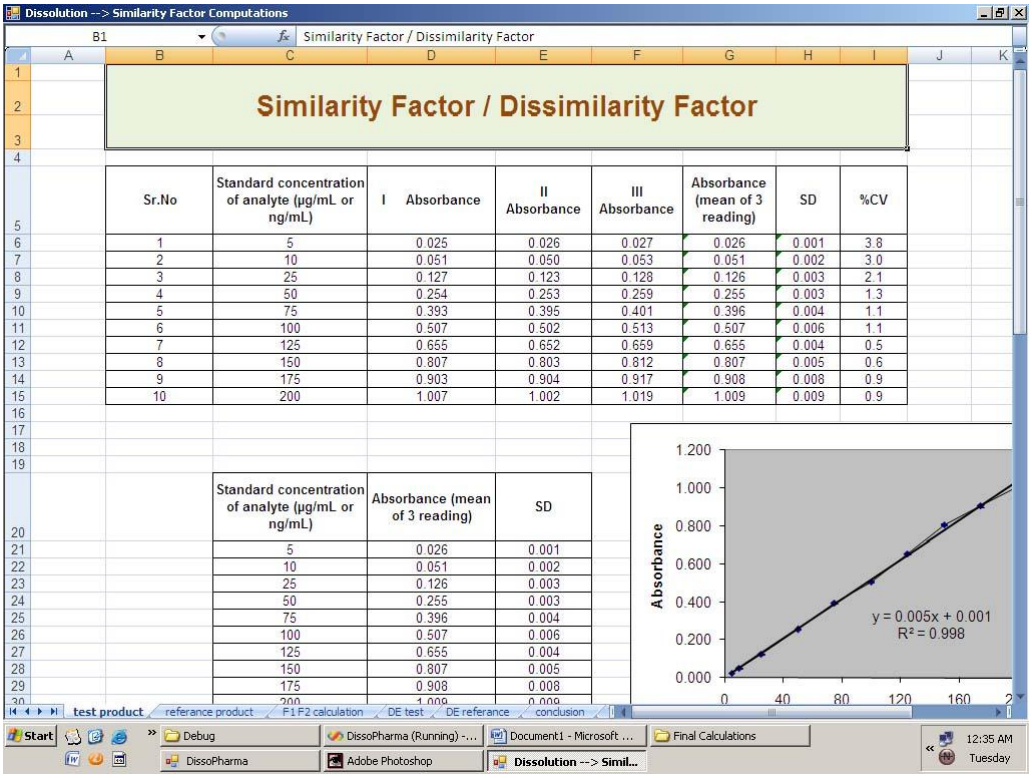



*Fig. 5.2.9(1) Similarity/Dissimilarity Factor - 1*

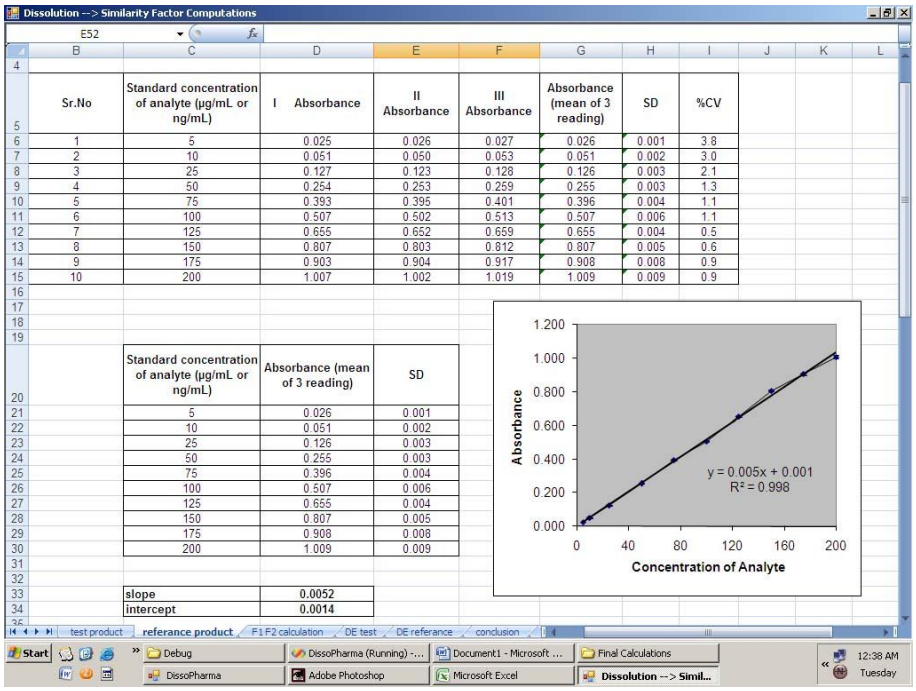



*Fig. 5.2.9(2) Similarity/Dissimilarity Factor –2*

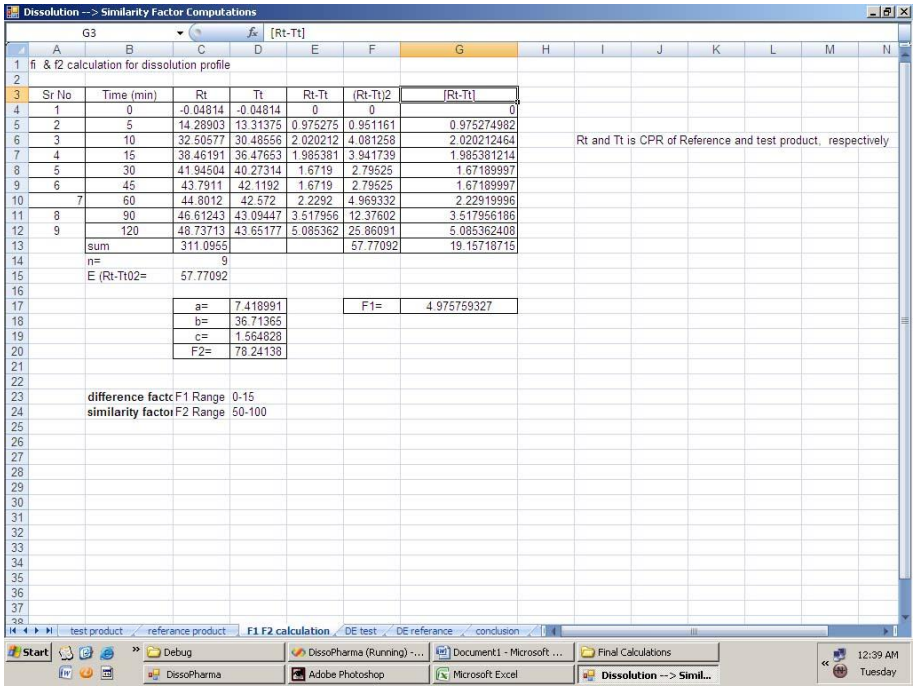



*Fig. 5.2.9(3) Similarity/Dissimilarity Factor - 3*

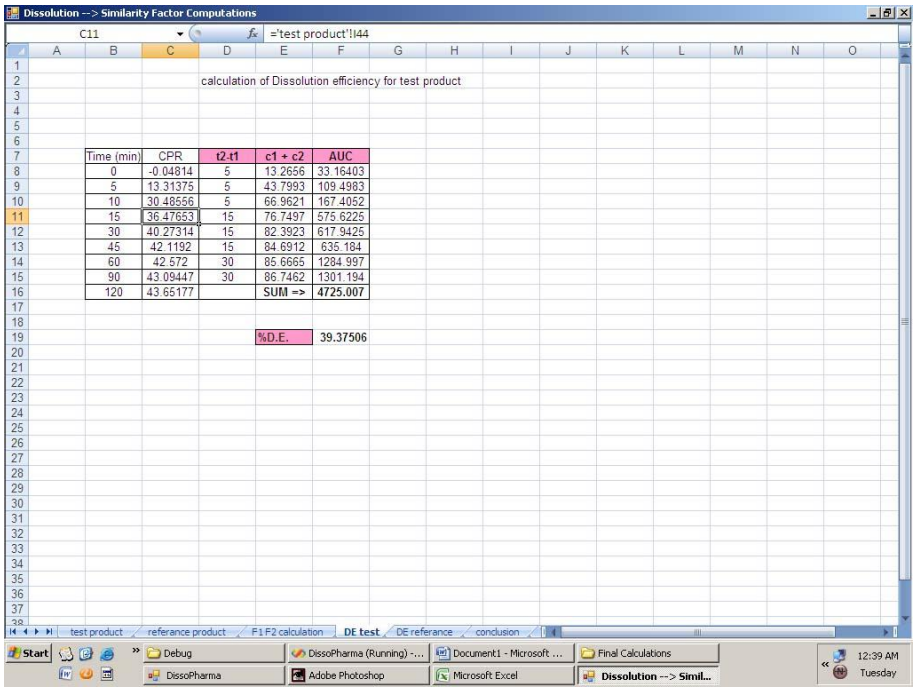



*Fig. 5.2.9(4) Similarity/Dissimilarity Factor - 4*

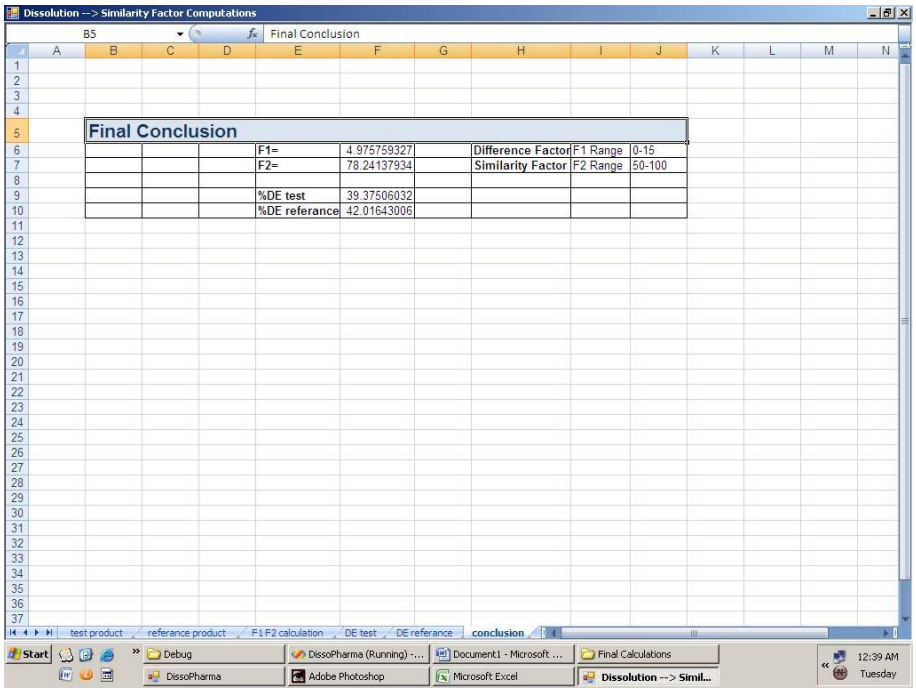



*Fig. 5.2.9(5) Similarity/Dissimilarity Factor –5*

and a similarity factor ($f_2$) to compare dissolution profiles. Factor $f_1$ calculates the percentage difference between two the profiles at each sampling point and corresponds to a relative error measure between the profiles:

$$f_1 = \{\lceil \Sigma^n{}_{t-1} \mid R_t - T_t \mid \rceil + \lceil \Sigma^n{}_{t-1} R_t \rceil\} \times 100$$

where:

n = number of sampling points

Rt = value dissolved in time t (percentage), obtained with the reference product or with the original formulation (before the alteration)

Tt = percentage value dissolved from the altered formulation, in time t.

Factor $f_2$ corresponds to a similarity measure between the two curves:

$$f_2 = 50 \times \log \{\lceil 1 + (1/n) \Sigma^n{}_{t-1} (R_t - T_t)^2 \rceil^{-0,5} \times 100\}$$

The procedure is described as follows:

- Determine the dissolution profile of products, test and reference, using twelve units of each.
- Calculate factors $f_1$ and $f_2$ using the equations presented previously.
- Criteria for two dissolution profiles to be considered similar. The nominal range of $f_1$ and $f_2$ values are 0 to 15 and 50 to 100, respectively.

The procedure is also to be considered:

- Use a minimum of five sampling points;
- Consider only one point above 85% of dissolution for both products;
- To allow use of averages, the coefficients of variation for the first points (15 minutes, for example) should not exceed 20%. For the remaining points a maximum of 10% is allowed;
- The average values of Rt may derived from the last reference batch, without alteration, or from two or more consecutive batches, without alteration.
- In cases where dissolution is very quick, presenting a value equal to or higher than 85% of the drug dissolved in 15 minutes, factors $f_1$ and $f_2$ loose their distinguishing power and therefore it is not necessary to calculate them.

# 5.3 Bioavailability:

Bioavailability is entered in fractional form ranging from 0 to 1. As seen in equation 18, it reduces the amount of drug reaching the systemic circulation. Bioavailability does not affect the calculation of the amount of drug absorbed as shown in equation 14. As a result, it is possible to have a drug that is well absorbed, that is, it crosses the intestinal membrane rapidly compared to other drugs, but does not completely reach the systemic circulation due to metabolism by the liver or by the intestinal enterocytes.

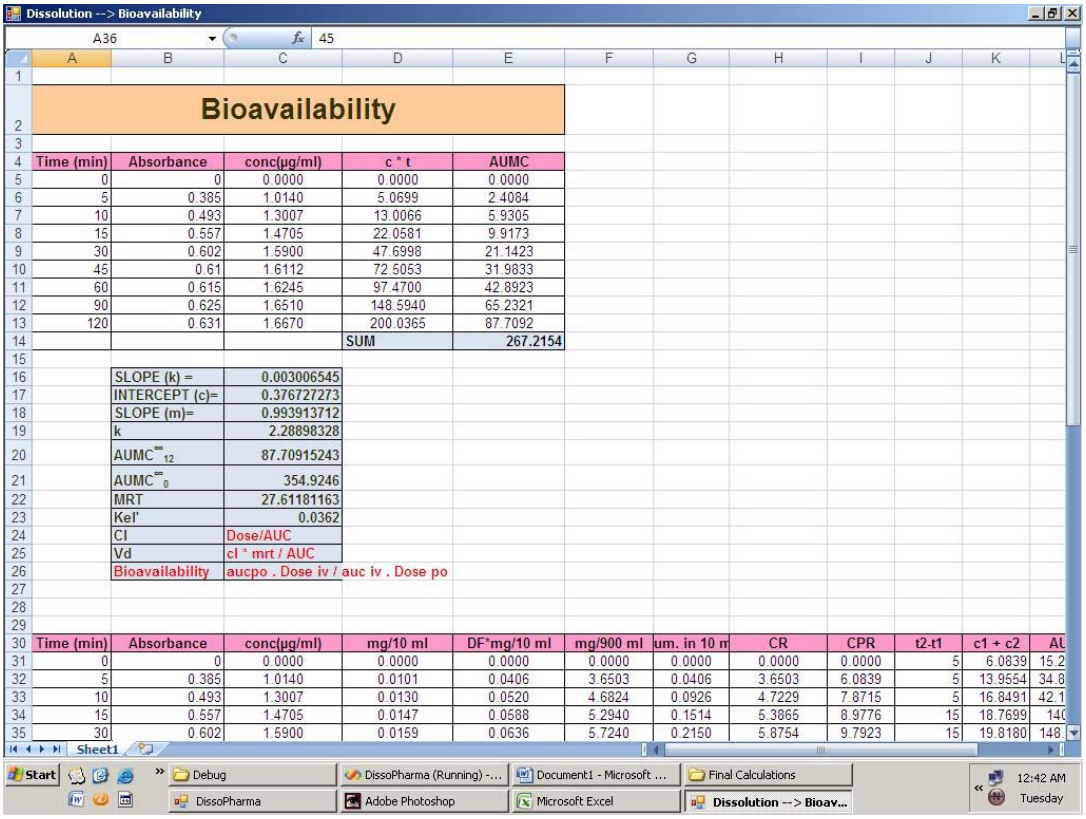
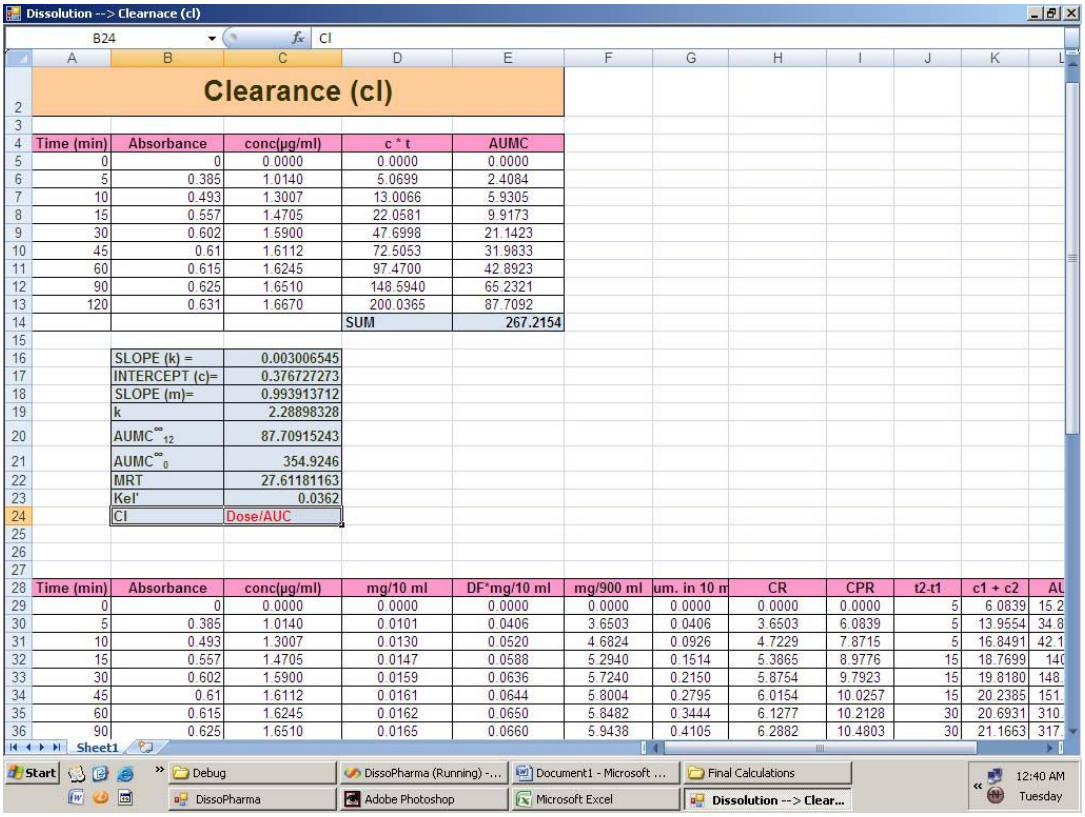



*Fig. 5.3(1) Bioavailability*

# 5.4 Clearance (ml/min/kg):

Clearance is the rate drug removal from the central blood/plasma compartment entered in units of ml/min/kg. The Clearance (Cl) of a drug is the volume of plasma from

which the drug is completely removed per unit time. The amount eliminated is proportional to the concentration of the drug in the blood.



Dissolution --> Clearnace (cl)

**Clearance (cl)**

| Time (min) | Absorbance | conc(µg/ml) | c * t | AUMC |
|---|---|---|---|---|
| 0 | 0 | 0.0000 | 0.0000 | 0.0000 |
| 5 | 0.385 | 1.0140 | 5.0699 | 2.4084 |
| 10 | 0.493 | 1.3007 | 13.0066 | 5.9305 |
| 15 | 0.557 | 1.4705 | 22.0581 | 9.9173 |
| 30 | 0.602 | 1.5900 | 47.6998 | 21.1423 |
| 45 | 0.61 | 1.6112 | 72.5053 | 31.9833 |
| 60 | 0.615 | 1.6245 | 97.4700 | 42.8923 |
| 90 | 0.625 | 1.6510 | 148.5940 | 65.2321 |
| 120 | 0.631 | 1.6670 | 200.0365 | 87.7092 |
| | | | SUM | 267.2154 |

| | |
|---|---|
| SLOPE (k) = | 0.003006545 |
| INTERCEPT (c)= | 0.376727273 |
| SLOPE (m)= | 0.993913712 |
| k | 2.28898328 |
| $AUMC^{\infty}_{12}$ | 87.70915243 |
| $AUMC^{\infty}_{0}$ | 354.9246 |
| MRT | 27.61181163 |
| Kel' | 0.0362 |
| Cl | Dose/AUC |

| Time (min) | Absorbance | conc(µg/ml) | mg/10 ml | DF*mg/10 ml | mg/900 ml | um. in 10 m | CR | CPR | t2-t1 | c1 + c2 | AU |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 5 | 6.0839 | 15.2 |
| 5 | 0.385 | 1.0140 | 0.0101 | 0.0406 | 3.6503 | 0.0406 | 3.6503 | 6.0839 | 5 | 13.9554 | 34.8 |
| 10 | 0.493 | 1.3007 | 0.0130 | 0.0520 | 4.6824 | 0.0926 | 4.7229 | 7.8715 | 5 | 16.8491 | 42.1 |
| 15 | 0.557 | 1.4705 | 0.0147 | 0.0588 | 5.2940 | 0.1514 | 5.3865 | 8.9776 | 15 | 18.7699 | 140 |
| 30 | 0.602 | 1.5900 | 0.0159 | 0.0636 | 5.7240 | 0.2150 | 5.8754 | 9.7923 | 15 | 19.8180 | 148. |
| 45 | 0.61 | 1.6112 | 0.0161 | 0.0644 | 5.8004 | 0.2795 | 6.0154 | 10.0257 | 15 | 20.2385 | 151. |
| 60 | 0.615 | 1.6245 | 0.0162 | 0.0650 | 5.8482 | 0.3444 | 6.1277 | 10.2128 | 30 | 20.6931 | 310. |
| 90 | 0.625 | 1.6510 | 0.0165 | 0.0660 | 5.9438 | 0.4105 | 6.2882 | 10.4803 | 30 | 21.1663 | 317. |

Sheet1

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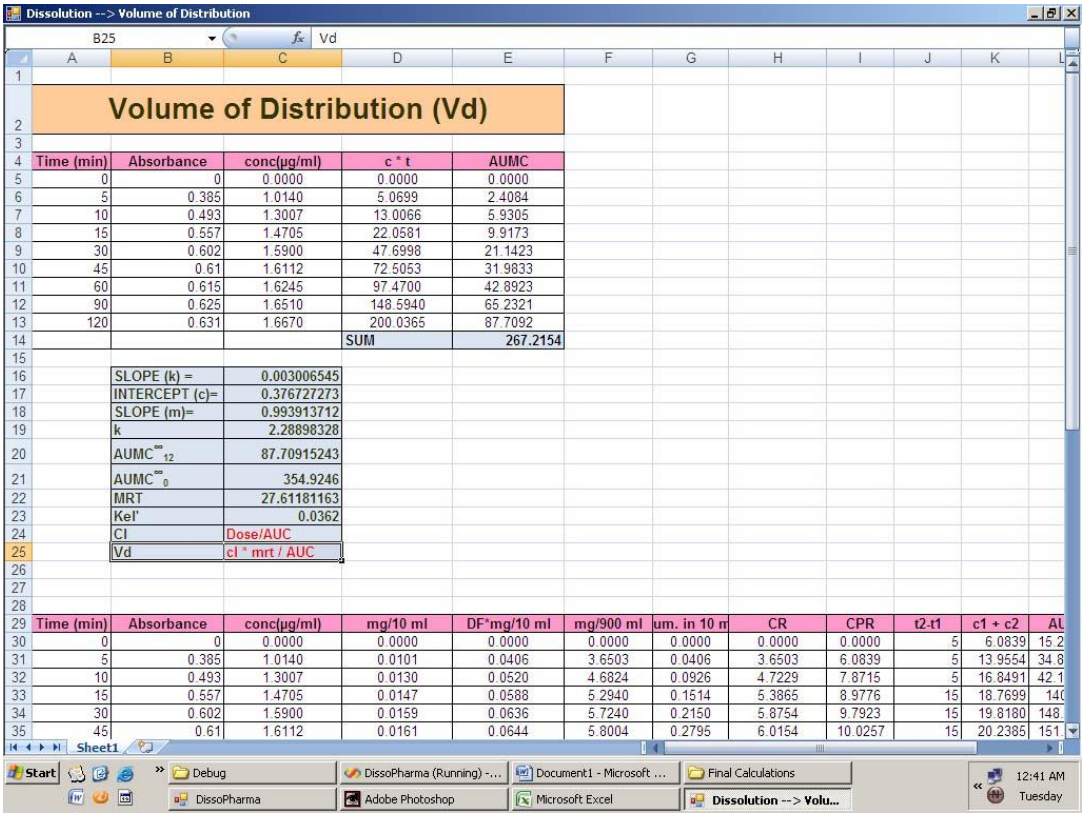
*Fig. 5.4(1) Clearance*

## 5.5 Volume of Distribution (L/Kg):

Volume of Distribution is the hypothetical volume of the central blood/plasma compartment and is entered in units of L/kg. The Volume of Distribution (Vd) is the amount of drug in the body divided by the concentration in the blood. Drugs that are highly lipid soluble, such as digoxin, have a very high volume of distribution (500 litres). Drugs which are lipid insoluble, such as neuromuscular blockers, remain in the blood, and have a low Vd.



Dissolution --> Volume of Distribution

B25 Vd

**Volume of Distribution (Vd)**

| Time (min) | Absorbance | conc(µg/ml) | c * t | AUMC |
|---|---|---|---|---|
| 0 | 0 | 0.0000 | 0.0000 | 0.0000 |
| 5 | 0.385 | 1.0140 | 5.0699 | 2.4084 |
| 10 | 0.493 | 1.3007 | 13.0066 | 5.9305 |
| 15 | 0.557 | 1.4705 | 22.0581 | 9.9173 |
| 30 | 0.602 | 1.5900 | 47.6998 | 21.1423 |
| 45 | 0.61 | 1.6112 | 72.5053 | 31.9833 |
| 60 | 0.615 | 1.6245 | 97.4700 | 42.8923 |
| 90 | 0.625 | 1.6510 | 148.5940 | 65.2321 |
| 120 | 0.631 | 1.6670 | 200.0365 | 87.7092 |
| | | | SUM | 267.2154 |

| | |
|---|---|
| SLOPE (k) = | 0.003006545 |
| INTERCEPT (c)= | 0.376727273 |
| SLOPE (m)= | 0.993913712 |
| k | 2.28898328 |
| $AUMC^{\infty}_{12}$ | 87.70915243 |
| $AUMC^{\infty}_{0}$ | 354.9246 |
| MRT | 27.61181163 |
| Kel' | 0.0362 |
| Cl | Dose/AUC |
| Vd | cl * mrt / AUC |

| Time (min) | Absorbance | conc(µg/ml) | mg/10 ml | DF*mg/10 ml | mg/900 ml | um. in 10 m | CR | CPR | t2-t1 | c1 + c2 | AU |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 5 | 6.0839 | 15.2 |
| 5 | 0.385 | 1.0140 | 0.0101 | 0.0406 | 3.6503 | 0.0406 | 3.6503 | 6.0839 | 5 | 13.9554 | 34.8 |
| 10 | 0.493 | 1.3007 | 0.0130 | 0.0520 | 4.6824 | 0.0926 | 4.7229 | 7.8715 | 5 | 16.8491 | 42.1 |
| 15 | 0.557 | 1.4705 | 0.0147 | 0.0588 | 5.2940 | 0.1514 | 5.3865 | 8.9776 | 15 | 18.7699 | 140 |
| 30 | 0.602 | 1.5900 | 0.0159 | 0.0636 | 5.7240 | 0.2150 | 5.8754 | 9.7923 | 15 | 19.8180 | 148. |
| 45 | 0.61 | 1.6112 | 0.0161 | 0.0644 | 5.8004 | 0.2795 | 6.0154 | 10.0257 | 15 | 20.2385 | 151. |

Sheet1

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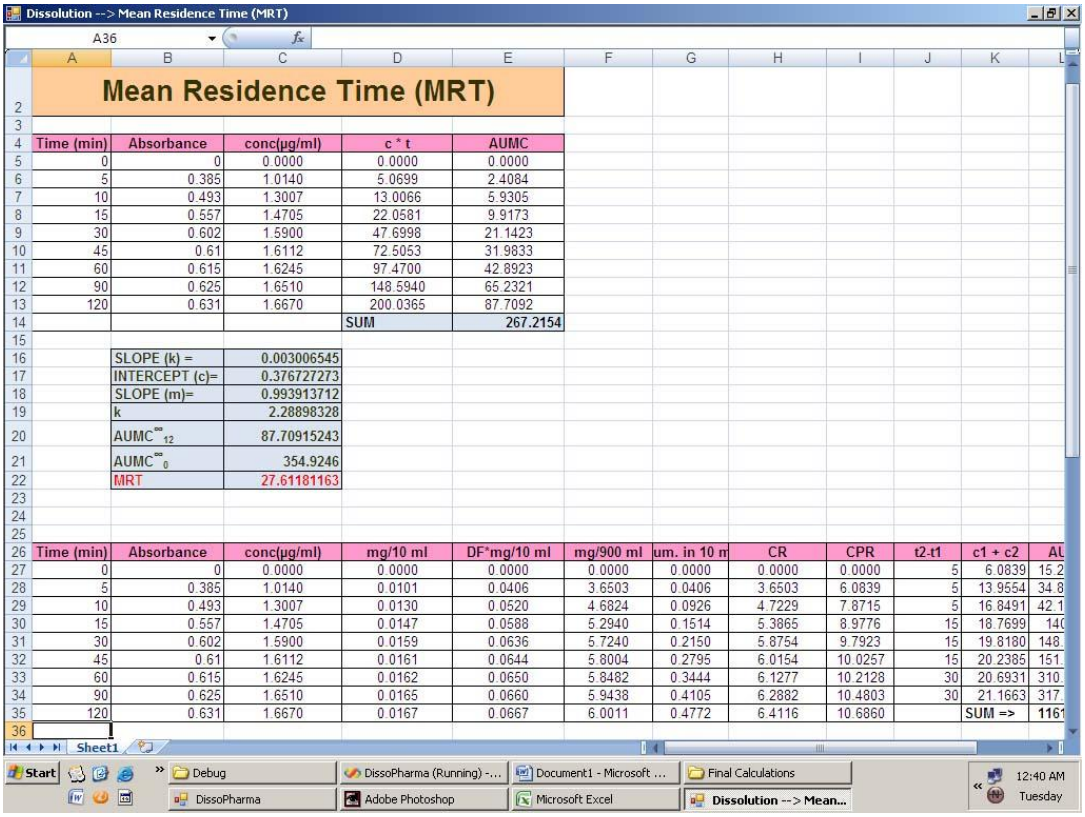
*Fig. 5.5(1) Volume of Distribution*

# 5.6 Mean Residence Time (MRT):

Equations for the mean residence time (MRT) of drug in the body and related functions are derived for drugs which are intravenously administered into a one- or two-compartment system with Michaelis-Menten elimination. This MRT is a function of the steady-state volume of distribution and time-average clearance obtained from the dose and area under the curve (dose/AUC). The differences between the MRT calculated by the proposed method and by using the moment theory method (AUM/AUC) are demonstrated both mathematically and by computer simulations.



Dissolution --> Mean Residence Time (MRT)

**Mean Residence Time (MRT)**

| Time (min) | Absorbance | conc(µg/ml) | c * t | AUMC |
|---|---|---|---|---|
| 0 | 0 | 0.0000 | 0.0000 | 0.0000 |
| 5 | 0.385 | 1.0140 | 5.0699 | 2.4084 |
| 10 | 0.493 | 1.3007 | 13.0066 | 5.9305 |
| 15 | 0.557 | 1.4705 | 22.0581 | 9.9173 |
| 30 | 0.602 | 1.5900 | 47.6998 | 21.1423 |
| 45 | 0.61 | 1.6112 | 72.5053 | 31.9833 |
| 60 | 0.615 | 1.6245 | 97.4700 | 42.8923 |
| 90 | 0.625 | 1.6510 | 148.5940 | 65.2321 |
| 120 | 0.631 | 1.6670 | 200.0365 | 87.7092 |
| | | | SUM | 267.2154 |

| | |
|---|---|
| SLOPE (k) = | 0.003006545 |
| INTERCEPT (c)= | 0.376727273 |
| SLOPE (m)= | 0.993913712 |
| k | 2.28898328 |
| $AUMC^{\infty}_{12}$ | 87.70915243 |
| $AUMC^{\infty}_{0}$ | 354.9246 |
| MRT | 27.61181163 |

| Time (min) | Absorbance | conc(µg/ml) | mg/10 ml | DF*mg/10 ml | mg/900 ml | um. in 10 m | CR | CPR | t2-t1 | c1 + c2 | AU |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 5 | 6.0839 | 15.2 |
| 5 | 0.385 | 1.0140 | 0.0101 | 0.0406 | 3.6503 | 0.0406 | 3.6503 | 6.0839 | 5 | 13.9554 | 34.8 |
| 10 | 0.493 | 1.3007 | 0.0130 | 0.0520 | 4.6824 | 0.0926 | 4.7229 | 7.8715 | 5 | 16.8491 | 42.1 |
| 15 | 0.557 | 1.4705 | 0.0147 | 0.0588 | 5.2940 | 0.1514 | 5.3865 | 8.9776 | 15 | 18.7699 | 140 |
| 30 | 0.602 | 1.5900 | 0.0159 | 0.0636 | 5.7240 | 0.2150 | 5.8754 | 9.7923 | 15 | 19.8180 | 148. |
| 45 | 0.61 | 1.6112 | 0.0161 | 0.0644 | 5.8004 | 0.2795 | 6.0154 | 10.0257 | 15 | 20.2385 | 151. |
| 60 | 0.615 | 1.6245 | 0.0162 | 0.0650 | 5.8482 | 0.3444 | 6.1277 | 10.2128 | 30 | 20.6931 | 310. |
| 90 | 0.625 | 1.6510 | 0.0165 | 0.0660 | 5.9438 | 0.4105 | 6.2882 | 10.4803 | 30 | 21.1663 | 317. |
| 120 | 0.631 | 1.6670 | 0.0167 | 0.0667 | 6.0011 | 0.4772 | 6.4116 | 10.6860 | | SUM => | 1161 |

Sheet1

Start | Debug | DissoPharma (Running) -... | Document1 - Microsoft ... | Final Calculations | DissoPharma | Adobe Photoshop | Microsoft Excel | Dissolution --> Mean... | 12:40 AM Tuesday

*Fig. 5.6(1) Mean Residence Time (MRT)*

# 5.7 Compare Dissolution Profiles:

The Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA) recently released a guidance which set forth application information that should be provided to CDER to assure continuing product quality and performance characteristics of immediate release oral solid dosage formulations for specific post-approval changes. Commonly called SUPAC IR, this guidance has the major intent to reduce the number of manufacturing changes that require preapproval by FDA. Consequently, dissolution testing is a prominent feature of SUPAC IR requirements. In order for a pharmaceutical sponsor to take advantage of the benefits offered in the SU-PAC IR guidance where Case B or Case C dissolution testing is required, a sponsor will need to demonstrate that the dissolution profiles of the product pre-change and post-change are “similar.” The objective of the work was to apply several profile comparison approaches to dissolution

data of tablet formulations in order to quantify each method's metric for comparing dissolution profiles.

### 5.7.1 Model-independent Methods:

The methods investigated to compare profiles can be classified into two categories: model-independent approaches and model-dependent approaches. The percent dissolved at 60 and 90 minutes for the fast test formula, and the medium test formula were assigned values of 100% for each sample.

Model-independent approaches can be further differentiated as ANOVA-based procedures, ratio test procedures, or pair-wise procedures. Two ANOVA-based procedures were evaluated: ANOVA on the percent dissolved at each time point and the level and shape approach. For the ANOVA testing of the percent dissolved data, one-way ANOVA plus Tukey's test for times 5–45 minutes were conducted. For 60 and 90 minute data, the one-sample t-test, which compared the percent dissolved of the slow formula against 100%, was conducted. Each of the "level" and "shape" portions of the lev and shape approach were performed. Prior to analysis by the level and shape approach, the data were transformed in terms of first difference.

### 5.7.2 Model-dependent Methods:

The model-dependent approaches included zero-order, first-order, Hixson-Cro-well, Higuchi, quadratic, Weibull, Gompertz, and logistic models. The procedure to compare two dissolution profiles using a model-dependent approach follows and resembles the above ratio test procedures since a 90% confidence interval is ultimately constructed. For each of the two formulations being compared, the model under study was fitted to each individual dissolution profile using our prototype. From the mean ratio of the model parameter and the SE of the mean ratio, a 90% confidence interval was constructed.

The result of the one-way ANOVA for each time point from 5–60 minutes was a p-value <0.001. The results of the one-sample t- test at 60 and 90 minutes were p-values of 0.389 and 0.560. While these results are informative, ANOVA may serve as a conservative method. The level and shape approach appeared to possess the same limitation as ANOVA of the percent dissolved at each time point, in that the level and shape approach addresses a question concerning the statistical sameness rather than pharmaceutical sameness. When dissolution data out to 30, 45, and 60 minutes were considered, the p-values for the "level"

test statistic and th “shape” test statistic were always<0.0002. When dissolution out to 90 minutes was considered, the level and shape p-values were 0.467 and <0.0002, respectively.

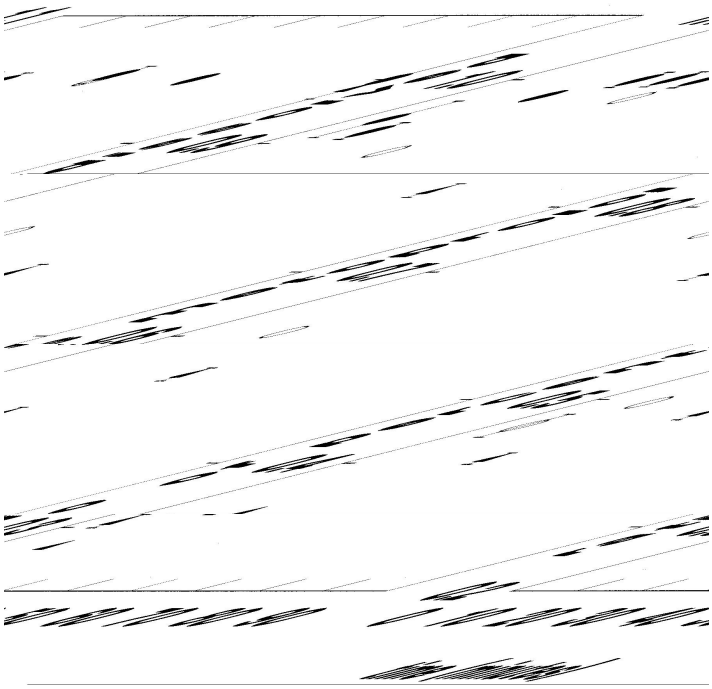


*Fig. 5.7.2(1) Ratio of mean percent dissolved for closed circle versus open circle. Error bars denote SE from n = 6.*

Throughout the dissolution, the ratio of the percent dissolved from the fast formulation was always within 90%. During the first 10 minutes, the medium dissolving test formula was less than half of that; but by 25 minutes it was about 90% and by 45 minutes it was fully dissolved. The ratio was below 0.8 for the first 45 minutes; only until 60 minutes did the ratio reach a value greater than 0.8. The 90% confidence intervals for the mean ratio of percents dissolved were about twice as wide as the SE bars [10-13].

## 5.8 Results:

In the calibration curve module, the number identifies each test record uniquely. The date of procedure is automatically derived from the system date maintained by the computer clock, but can be changed manually. The user has to enter the number of observations of concentration and instrumental response. The possible locations in the

dissolution parameters tree are represented by a cascading hierarchy of tables. An additional table listing the appropriate divisions/ segments appears.

On completion of data entry, the user is transferred to the print module, where he can preview the report prior to printing. The printed report contains all the information entered in the database. It also contains standard set post- procedure instructions for the test, and also has space for signatures for the analytical scientist carrying out the procedure. Problems initially faced by users were primarily related to data entry. Scientists, not having any working knowledge of computers, encountered problems such as a slow speed of data entry and failure to enter data in mandatory fields (with a consequent error message that did not allow the user to proceed further without rectifying the mistake).

With little practice, they became adept at entering details correctly and quickly. Almost all the analytical scientists reported a slightly increased time of data entry into the computer, in comparison to writing reports on a standard printed Performa. However, all agreed that the report and data generated through the software were uniformly complete, and more than made up for the extra time spent. The new report has a uniform and easily understood structure, and is free of any inadvertent omissions.

The database component was evaluated by analyzing 60 consecutive records entered over a 4-month period. Statistics were generated on the demographic variables and complications. Data access and analysis were easily and quickly performed. Data were found to have been completely transferred from data entry screens to the database and no missing values were encountered.

## 5.9 Discussion:

Structured input and free-text input represent two fundamentally different ways of entering data into a computer. Initial reports of test databases relied heavily on text based tools. Such input facilitates personalized style and flexibility in description of test records, and generates a well readable report. However, free-text input weakens the utility of the database, as it is not suited to subsequent analysis. Structured input and the resulting categorical data offer an important advantage in this regard. Data thus entered is more likely to be complete and is well suited for research and analysis, as well as for the generation of administrative reports and for quality control. It has been estimated that use of computerized test records improves completeness of data entry by more than 50 per

cent[5]. However, a major trade-off for structure is flexibility. We therefore used a basic structured data entry protocol, supplemented by use of free text only under special situations. Experience with previously designed bronchoscopes software has shown that the reporting procedure is slightly lengthened using computerized data entry[3]. This increase in time is variable. Besides operator related factors, it is related to the amount of free text entered and the number of tables accessed during structured data entry[4]. However, the additional effort is rewarded by a more comprehensive and accurately documented report, and constant availability and better management[3-4].

A major feature of the software is the powerful database component. We had laid particular stress on this aspect in view of the stress on academic and research activities. This portion of the software has been built as a set of two interrelated databases in Microsoft Access, which can easily handle large databases (and is thus suited to the volume of test procedures performed at our department) and also offers a wide range of analytical tools through a versatile query system. We have evaluated the robustness of this module of the software through an analysis of 60 consecutive test records.

Although such analysis requires some working knowledge of the database system, it is easy of learn. No data was lost and statistical analysis could be easily performed. Both user-friendliness of the software and completeness of data entry are critical to the success and acceptance of such software. We allowed easy integration of buttons, text boxes, check boxes and fields for free text to achieve this end. The format for data input was optimized through continuous interaction between scientists and the programmer. Scientists and other faculties were involved early and frequently during the development of the software, so that they were able to contribute ideas and advice. The software has now been under routine use for over a year, and has performed well in areas of data entry, report generation and data analysis. Successful development and routine application of the database is, however, only a short-term achievement. More importantly, continuous improvements need to be made as and when new areas emerge. The system is adaptable and capable of keeping pace with new technological advances.

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# Chapter 6: Contribution of the Research Work & Suggested Future Work

## 6.1 Contribution of the Research Work: A Discussion

Software Technologies are providing reduced drug dissolution computation time e-process solutions for accelerating global pharmaceutical development. Software developers thus far believe that they have the potential to cut 1 to 2 years off the current 7 to 9 years it takes to bring new drugs to market, enabling them to increase their revenue potential. Many of them use Internet technologies to conduct and manage dissolution testing and computation efficiently and effectively. They are providing service solutions to allow sponsors of drug dissolution computation to eliminate the need to transcribe and process traditional paper data forms, to access global drug dissolution testing data in real-time, and to begin data analysis.

Now that drug dissolution of drug profiles and bioinformatics associated computational methods are reality. Somehow, the resulting compounds still have to be delivered in the most effective manner. An injectible or solid oral immediate or sustained released are “no brainers” for pharma in terms of dosage form selection. Now go have fun with solubility and stability, and its headache time, depending where you are in the world and your choice of vice. To combine the right chemical ingredients in the right proportions, driving the right reactions under the right conditions to achieve the right end product with the right properties requires an incredible amount of information and an incredible amount of knowledge. All this information and knowledge has to be available at the point of formulation as well as at the analysis stage to find whether particular drug batches are efficient of drug or not. Managing the formulation process without iteration is the secret to reducing development time. Having software to provide the right information at the right place at the right time and you avoid costly, embarrassing mistakes and eliminate duplicate work. Today, a drug dissolution testing could even identify promising leads, store, and manage a pharma company’s testing portfolio or library. Modeling and simulation software

can now allow researchers to screen a wide variety of materials and process variables in silico, fail early, and focus on the most promising lead candidates for further experimental work. Such methods enable substantial time and cost savings.

Drug dissolution testing and informatics software platforms also provide great science from easy-to-use desktop environments that interact with the databases throughout a corporate IT infrastructure. They can combine high-quality modeling and visualization with powerful methods to analyze and predict the parameters and behavior of chemical and biological systems. These powerful methods include molecular mechanics, quantum mechanics, mesoscale modeling, instrument simulation, and statistical correlations. This new trend is being reinforced by many of scientific papers and has been successfully applied across industry and academia.

With the convergence of drug dissolution testing and IT, there clearly will be growing pains. Despite the challenges ahead, the union of high-tech and Pharma is sure to unleash novel tools and products that today can't be envisioned. As drug dissolution testing companies struggle to understand and use numerous data points and bits of information they are generating, IT companies are eager to meet the demands of this new market opportunity. Future computing solutions will require the capability to store vast quantities of data.

In this evolving area, the ability to avoid chasing down a dead-end street will become increasingly important. To quickly discard all but the leading options and eliminating time wasted on low probability routes while identifying the best and least expensive ingredient mix can save money and time. In the final analysis, this will allow drug dissolution testing companies to be in a greater position of strength.

The resulting speed-to-market advantage they can offer will take them beyond the innovation of their testing systems alone and become integral with their strategies. Positioning will be easy and feed into the "you can have it now" instead of in 18 months. And, by getting their drug company prospects to market sooner, drug dissolution testing companies can improve their odds of solidifying licensing and development deals on better terms

The anticipated prototype is to provide both scientific computational power and visualization power particularly with use of tools such as MATLAB, .NET and set of bioinformatics tools and database. The environment brings ease to use to get desired computation, data analysis with visualization.

The main objective of this work was to apply several drug profiles comparison approaches to one dissolution data set in order to quantify each method's metric for comparing dissolution profiles through the developed prototype. In spite of the need to compare dissolution profiles, methods to compare dissolution profiles are well developed.

This work was conducted with the intent to compare all batch profiles and to gain familiarity with the numerical values of those methods. It is concluded that the ratio test procedures, the pair-wise procedures, and several of the model-dependent approaches yielded numerical results which can possibly serve as objective and quantitative metrics for comparing entire dissolution profiles.

## 6.2 Suggested Future Work:

Growth will become the result of the intersection of life sciences and IT period. Sun Microsystems, for example, has major research programs on informatics. Merck scientists are becoming computer experts themselves. Oracle developed a database capable of organizing tons of drug information moving from research through the drug dissolution testing process. And, this is just the beginning. They can now assure that vast quantities of data generated from experiments can be rapidly studied and correlated with billions of other data bits streaming from Merck's other labs. Looking at data in real time permits company scientists to make fast calls about which compounds to push into development. Information technology provides the only means for drug companies to automate and truncate the development process. Drug companies spend huge amount per year on IT.

The future application of Drug Dissolution Parameters Computations (DDPC) will become more important when the present framework gains increased recognition, which will probably be the case if the DDPC borders for certain Class II (high permeability, low solubility) and Class III (low permeability, high solubility) drugs are extended. Revision of the DDPC guidelines by the regulatory agencies in communication with academic and industrial scientists is exciting and will hopefully result in an increased applicability in drug development.

Finally, scientists in this field emphasize the usefulness of DDPC as a simple tool in early drug development to determine the rate-limiting step in the oral absorption process, which has facilitated the exchange of information between experts involved in the overall drug development process. In the future, this increased awareness of a proper

biopharmaceutical characterization of new drugs may result in drug molecules with sufficiently high permeability, solubility and dissolution rate properties, that will automatically help increase the importance of BCS as a regulatory tool.

# Appendix A: FORMULAS DESCRIPTION

| Single Dose Pharmacokinetics | |
|---|---|
| General Disposition Parameters and Constants | |
| Dose Amount | *D* |
| Fraction of dose absorbed<br>Used to correct dose amount for some oral dose calculations. | *F* |
| Exponential Summation<br>Expression for sum of 1st order kinetic terms. | $C = \sum C_n e^{-\lambda_n t}$<br>for *n* exponential terms |
| Y-Intercept<br>Coefficient of each exponential term. Note the sign of the absorption coefficient is negative. | $C_n$ |
| Slope | $s = \frac{-\lambda_n}{2.303}$ |
| Rate constant | $\lambda_n = -2.303s$ |
| Elimination rate constant | $\lambda_z$ |
| Half-life | $t_{1/2} = \frac{0.693}{\lambda_n}$ |
| Descriptive Curve Parameters | |
| $C_{initial}$<br>Initial concentration extrapolated to time zero for i.v. dose. | $C_0 = \sum C_n$ |
| Tmax (obs)<br>Usually applies to oral doses only. | $T_{max} = time\ point\ at\ C_{max}$ |
| Cmax (calculated)<br>For biexponential oral data only. | $C_{max} = \frac{FD}{V} e^{-\lambda_z t_{max}}$<br>where *V* is Vd (area). |
| Tmax (calculated)<br>For biexponential oral data only. | $t_{max} = \frac{2.303}{\lambda_a - \lambda_z} \log \frac{\lambda_a}{\lambda_z}$<br>where $\lambda_a$ and $\lambda_z$ are the apparent absorption and elimination rate constants, respectively. |

| Curve Area Calculations | |
|---|---|
| AUC(0-t) (obs area)<br>Trapezoid calculation of AUC using observed data points only. Useful when final concentration values tend to exaggerate total AUC. | $AUC_{(0-t)} = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2}(C_i + C_{i+1})$<br>where *n* is the number of data points. |
| AUC (area)<br>Total AUC computed by combining AUC(0-t) with an extrapolated value. | $AUC_\infty = AUC_{(0-t)} + \frac{C_n}{\lambda_z}$<br>where $C_n$ is the last concentration. |
| AUC (expo)<br>Total AUC computed using exponential terms. | $AUC_\infty = \sum \frac{C_n}{\lambda_n}$ |
| % of AUC (expo)<br>Percent each exponential term contributes to the total AUC. | $\%\,AUC_\infty = 100\frac{(C_n/\lambda_n)}{AUC_\infty}$ |
| **Statistical Moment Calculations** | |
| AUMC (area)<br>Calculation of total area under the first-moment curve (plot of *Ct* vs *t*) by combining trapezoid calculation of $AUMC_{(0-t)}$ and extrapolated area. | $AUMC_{(0-t)} = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2}(C_i t_i + C_{i+1} t_{i+1})$<br>$+ \frac{C_{last} \cdot t_{last}}{\lambda_z} + \frac{C_{last}}{\lambda_z^2}$ |
| AUMC (expo)<br>Total AUMC computed using exponential terms. | $AUMC_\infty = \sum \frac{C_n}{\lambda_n^2}$ |
| % of AUMC (expo)<br>Percent each exponential term contributes to the total AUMC. | $\%\,AUMC_\infty = 100\frac{(C_n/\lambda_n^2)}{AUMC_\infty}$ |
| MRT (area)<br>Mean Residence Time calculated using trapezoid area calculations extrapolated to infinity. | $MRT = \frac{AUMC_\infty}{AUC_\infty}$<br>where both area terms use trapezoidal calculations |
| MRT (expo)<br>Mean Residence Time calculated using exponential terms. | $MRT = \sum \frac{1}{\lambda_n}$ |
| **Volume of Distribution Calculations** | |
| Vc (initial central compartment)<br>Apparent volume of the central compartment for i.v. doses only. | $V_c = \frac{D}{\sum C_n}$ |
| Vd (obs area)<br>Apparent volume of distribution based on $AUC_{(0-t)}$ trapezoid calculation and elimination rate. Use when total AUC (area) is exaggerated due to high terminal concentration values. | $V = \frac{FD}{AUC_{(0-t)}\lambda_z}$ |

| | |
|---|---|
| Vd (area)<br>Apparent volume of distribution based on trapezoid AUC (area) and elimination rate. Applies mainly to i.v., but also to oral if complete absorption (*F*=1) is assumed. | $V = \frac{FD}{AUC_{\infty}\lambda_z}$ |
| Vd (area) / kg<br>Apparent volume of distribution normalized by animal weight. Uses same equation as Vd (area). | $V_{normalized} = \frac{V}{Body\ Weight\ (kg)}$ |
| Vd (expo)<br>Apparent volume of distribution calculated from exponential terms. | $V = \frac{FD}{\lambda_z \sum \frac{C_n}{\lambda_n}}$<br>where $\lambda_z$ is the elimination rate |
| Vss (area)<br>Apparent volume of distribution at steady state estimated graphically from trapezoidal total area measurements. Applies to iv dose. | $V_{ss} = \frac{D \cdot [AUMC_{\infty}]}{[AUC_{\infty}]^2}$ |
| Vss (expo)<br>Apparent volume of distribution at steady state estimated from exponential terms. Applies only after iv and assumes elimination from central compartment. | $V_{ss} = D \cdot \frac{\sum \frac{C_n}{\lambda_n^2}}{\left(\sum \frac{C_n}{\lambda_n}\right)^2}$ |
| Systemic Clearance Calculations | |
| CL(sys) (obs area)<br>Systemic clearance based on $AUC_{(0\text{-}t)}$ trapezoid calculation. Use when total AUC (area) is exaggerated due to high last concentration. | $CL = \frac{FD}{AUC_{(0-t)}}$ |
| CL (area)<br>Systemic clearance based on trapezoid AUC (area). Applies mainly to i.v. data. Limited to oral data only if complete absorption (*F*=1) is assumed. | $CL = \frac{FD}{AUC_{\infty}}$ |
| CL (area) / kg<br>Systemic clearance normalized by animal weight. Uses same equation as CL (area). | $CL_{normalized} = \frac{CL}{Body\ Weight\ (kg)}$ |
| CL (expo)<br>Systemic clearance calculated using exponential terms. | $CL = \frac{FD}{\sum \frac{C_n}{\lambda_n}}$ |

| | |
|---|---|
| Half-life based on Vd and CL<br>Alternate calculation of half-life using Vd (area) and CL (area). For i.v. data only. | $t_{\frac{1}{2}} = \frac{0.693 \cdot V}{CL}$ |
| Two-compartment Open Model Microconstants | |
| k12<br>Microconstant calculated using exponentials. Applies to 2 compartment i.v. dose data only. | $k_{12} = \lambda_1 + \lambda_z - k_{21} - k_{10}$ |
| k21<br>Microconstant calculated using exponentials. Applies to 2 compartment i.v. dose data only. | $k_{21} = \frac{C_1\lambda_z + C_z\lambda_1}{C_1 + C_z}$ |
| Multiple Intravenous Dose Pharmacokinetics | |
| General | |
| Dose Interval (*tau*)<br>Time span between dosing intervals. Distinguish from time after dose (*t*). | *tau*<br>Assume constant dose interval |
| First Dose Concentration Calculations | |
| C1(max)<br>Maximum concentration after first dose interval (*tau*). Equal to $C_{initial}$ | $C_{1(max)} = \sum C_n$ |
| C1(min)<br>Minimum concentration at end of first dose interval (*tau*). | $C_{1(min)} = \sum C_n e^{-\lambda_n \tau}$ |
| C1(ave)<br>Average concentration during first dose interval (*tau*). | $C_{1(ave)} = \sum \frac{C_n(1 - e^{-\lambda_n \tau})}{\lambda_n \tau}$ |
| Prediction of Steady State Parameters | |
| Css(min)<br>Minimum concentration during any dosing interval at steady state. | $C_{ss(min)} = \sum \frac{C_n e^{-\lambda_n \tau}}{1 - e^{-\lambda_n \tau}}$ |
| Css(min)<br>Minimum concentration during any dosing interval at steady state. Included on graph. | $C_{ss(min)} = \sum \frac{C_n e^{-\lambda_n \tau}}{1 - e^{-\lambda_n \tau}}$ |
| Css(max) - Css(min)<br>Difference between peak and trough concentration during steady state. | $\Delta_{\overline{C}_{ss}} = C_{ss(max)} - C_{ss(min)}$ |
| Css(ave)<br>Average concentration at steady state. | $\overline{C}_{ss} = \sum \frac{C_n}{\lambda_n \tau}$ |
| Css(ave) (area)<br>Average concentration at steady state calculated from trapezoidal AUC data for a single dose. | $\overline{C}_{ss} = \frac{AUC_\infty}{\tau}$ |

| Accumulation Factors | |
|---|---|
| R based on Css(max)/C1(max)<br>Accumulation ratio based on maximum concentrations after first dose and at steady state. | $R = \frac{C_{ss(max)}}{C_{1(max)}}$ |
| R based on Css(min)/C1(min)<br>Accumulation ratio based on minimum concentrations after first dose and at steady state. | $R = \frac{C_{ss(min)}}{C_{1(min)}}$ |
| R based on Css(ave)/C1(ave)<br>Accumulation ratio based on average concentrations after first dose and at steady state. | $R = \frac{\overline{C}_{ss}}{C_{1(ave)}}$ |
| **Time to Reach Percent of Steady State** | |
| To reach 95% Css(ave)<br>Time required to reach 95% of average steady state concentration. Assumes one-compartment characteristics apply. | $t_{0.95\overline{C}_{ss}} = -3.32 \cdot t_{1/2} \cdot \log(1 - f_{ss})$<br>where $f_{ss}$ is the fraction of the steady state concentration. |
| To reach 99% Css(ave)<br>Time required to reach 95% of average steady state concentration. Assumes one-compartment characteristics apply. | $t_{0.99\overline{C}_{ss}} = -3.32 \cdot t_{1/2} \cdot \log(1 - f_{ss})$<br>where $f_{ss}$ is the fraction of the steady state concentration. |
| **General and Graphing Functions** | |
| Dose Interval (tau)<br>Constant time span between dosing intervals. Distinguish from time after dose (*t*). | (*tau*) Assumes equal dose intervals |
| Graphing Function<br>The graphing function is based on a mathematical generalization of the graphical superimposition principle. It involves the addition of a decay function ($C_N$) to the initial concentration ($C_1$)at repeated time points for a progressive series of doses (N). Assumes constant dose intervals during the postdistribution phase. | $C_{(N,t)} = C_{1(t)} + C_{N(t)}$<br>where<br>$C_{1(t)} = \sum C_n e^{-\lambda_n t}$<br>and<br>$C_{N(t)} = \frac{C_g e^{-\lambda_z \tau}(1 - e^{-(N-1)\lambda_z \tau}) e^{-\lambda_z t}}{1 - e^{-\lambda_z \tau}}$ |
| **First Dose Concentration Values** | |
| C1(max)<br>Observed maximum concentration taken from data set. | $C_{max}$ |
| C1(min)<br>Minimum concentration at end of first dose interval (*tau*). | $C_{1(min)} = \sum C_n e^{-\lambda_n \tau}$ |

| | |
|---|---|
| C1(ave)<br>Average concentration during first dose interval (*tau*). | $C_{1(ave)} = \sum \frac{C_n(1-e^{-\lambda_n \tau})}{\lambda_n \tau}$ |
| Prediction of Steady State Parameters | |
| Css(max)<br>Computed from a simplification of the graphing function to a steady state form as shown. The Css(max) is evaluated as the maximum concentration during the steady state dosing interval. | $C_{ss(t)} = C_{1(t)} + \frac{C_g \cdot e^{-\lambda_z \tau} \cdot e^{-\lambda_z t}}{1-e^{-\lambda_z \tau}}$<br>where<br>$C_{1(t)} = \sum C_n e^{-\lambda_n t}$ |
| Css(min)<br>Computed using same steady state equation as Css(max) and evaluating the minimum concentration during a steady state dose interval. | Same as above. |
| Css(max) - Css(min)<br>Difference between peak and trough concentration during steady state. | $\Delta_{\overline{C}_{ss}} = C_{ss(max)} - C_{ss(min)}$ |
| Css(ave)<br>Average concentration at steady state. | $\overline{C}_{ss} = \sum \frac{C_n}{\lambda_n \tau}$ |
| Css(ave) (area)<br>Average concentration at steady state calculated from trapezoidal AUC data for a single dose. | $\overline{C}_{ss} = \frac{AUC_\infty}{\tau}$ |
| Additional Oral Dose Calculations | |
| Tmax (1st dose, observed)<br>Observed time of largest concentration value from data set. | $t_{max}$ |
| Tmax (1st dose, calculated)<br>Calculation of time at which maximum concentration occurs after a single dose. Applies to 1-compartment characteristics, but calculated also to illustrate magnitude for 2-compartments. | $t_{max\ calculated} = \frac{2.303}{\lambda_a - \lambda_z} \log \frac{\lambda_a}{\lambda_z}$<br>where $\lambda_a$ is the absorption rate and $\lambda_z$ is the elimination rate. |
| Tmax(ss)<br>Calculation of time at which maximum concentration occurs after dosing during steady state. Applies to 1-compartment characteristics, but calculated also to illustrate magnitude for 2-compartments. | $t_{max_{ss}} = \frac{2.303}{\lambda_a - \lambda_z} \log \frac{\lambda_a(1-e^{-\lambda_z \tau})}{\lambda_z(1-e^{-\lambda_a \tau})}$<br>where $\lambda_a$ is the absorption rate and $\lambda_z$ is the elimination rate. |

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# Appendix B: DEFINITIONS OF TERMS

Batch:

A specific quantity of a drug or other material produced according to a single manufacturing order during the same cycle of manufacture and intended to have uniform character and quality, within specified limits *(21 CFR 210.3(b)(2))*.

Batch formula (composition):

A complete list of the ingredients and their amounts to be used for the manufacture of a representative batch of the drug product. All ingredients should be included in the batch formula whether or not they remain in the finished product.

Bioavailability:

The rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action.

Biobatch:

A lot of drug product formulated for purposes of pharmacokinetic evaluation in a bioavailability/bioequivalency study. This lot should be 10% or greater than the proposed commercial production batch or at least 100,000 units, whichever is greater.

Bioequivalent drug products:

Pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose of the therapeutic moiety under similar experimental conditions, either single dose or multiple dose. Some pharmaceutical equivalents or pharmaceutical alternatives may be equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on chronic use, or are considered medically insignificant for the particular drug product studied.

Correlation:

As used in this guidance, a relationship between in vitro dissolution rate and in vivo input (absorption) rate.

Development:

Establishing an in vitro/in vivo correlation.

Drug product:

A finished dosage form, e.g., tablet, capsule, or solution, that contains a drug substance, generally, but not necessarily, in association with one or more other ingredients.

Extended release dosage form:

A dosage form that allows a reduction in dosing frequency as compared to that presented by a conventional dosage form, e.g., a solution or an immediate release dosage form.

Evaluation:

In the context of in vitro/in vivo correlation, a broad term encompassing experimental and statistical techniques used during development and evaluation of a correlation which aid in determining the predictability of the correlation.

Formulation:

A listing of the ingredients and composition of the dosage form.

In vitro/in vivo correlation:

A predictive mathematical model describing the relationship between an in vitro property of an extended release dosage form (usually the rate or extent of drug dissolution or release) and a relevant in vivo response, e.g., plasma drug concentration or amount of drug absorbed.

In vivo dissolution:

The process of dissolution of drug in the gastro-intestinal tract.

In vitro release:

Drug dissolution (release) from a dosage form as measured in an in vitro dissolution apparatus.

In vivo release:

In vivo dissolution of drug from a dosage form as determined by deconvolution of data obtained from pharmacokinetic studies in humans (patients or healthy volunteers).

Level A correlation:

A predictive mathematical model for the relationship between the entire in vitro dissolution/release time course and the entire in vivo response time course, e.g., the time course of plasma drug concentration or amount of drug absorbed.

Level B correlation:

A predictive mathematical model for the relationship between summary parameters that characterize the in vitro and in vivo time courses, e.g., models that relate the mean in vitro dissolution time to the mean in vivo dissolution time, the mean in vitro dissolution time to the mean residence time in vivo, or the in vitro dissolution rate constant to the absorption rate constant.

Level C correlation:

A predictive mathematical model of the relationship between the amount dissolved in vitro at a particular time (or the time required for in vitro dissolution of a fixed percent of the dose, e.g., T %) and a summary parameter that characterizes the in vivo time 50 course (e.g., C or AUC).

Lot:

A batch, or a specific identified portion of a batch, having uniform character and quality within specified limits or, in the case of a drug product produced by continuous process, a specific identified amount produced in a unit of time or quantity in a manner that assures its having uniform character and quality within specified limits.

Mean absorption time:

The mean time required for drug to reach systemic circulation from the time of drug administration. This term commonly refers to the mean time involved in the in vivo release and absorption processes as they occur in the input compartment and is estimated as MAT = MRT - MRT oral i.v.

Mean in vitro dissolution time:

The mean time for the drug to dissolve under in vitro dissolution conditions.

Mean in vivo dissolution time:

For a solid dosage form: MDT = MRT - MRT . This solid solution reflects the mean time for drug to dissolve in vivo.

Mean residence time:

The mean time that the drug resides in the body. MRT may also be the mean transit time. MRT = AUMC/AUC.

Narrow therapeutic index drugs:

Drugs having, for example, less than a two-fold difference in the minimum toxic concentrations and the minimum effective concentrations.

Nonrelease controlling excipient (noncritical compositional variable):

An inactive ingredient in the final dosage form that does not significantly affect the release of the active drug substance from the dosage form.

Release mechanism:

The process by which the drug substance is released from the dosage form.

Release rate:

Amount of drug released per unit of time as defined by in vitro or in vivo testing.

Statistical moments:

Parameters that describe the characteristics of the time courses of plasma concentration (area, mean residence time, and variance of mean residence time) and of urinary excretion rate.