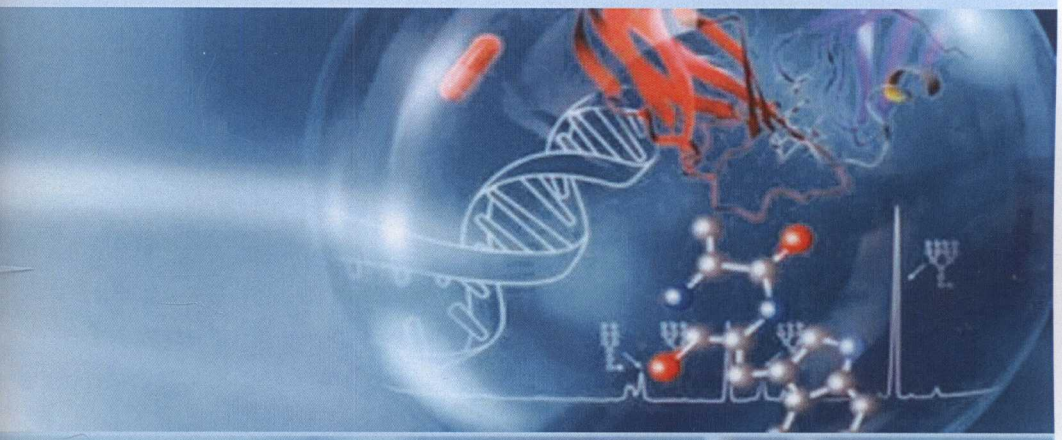
Ministry of Health of the Republic of Moldova
Nicolae Testemitanu State University of Medicine and Pharmacy
Drug Technology Department

**Cristina CIOBANU, Diana GURANDA**

Methodological guide for practical lessons at

# BIOPHARMACY AND PHARMACOKINETICS

for 5th – year students of the Faculty of Pharmacy



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**AUTHORS:**

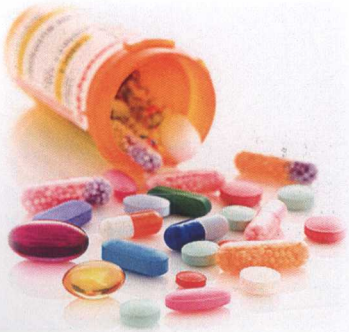
• **Cristina CIOBANU**, Doctor in pharmacy, Associate Professor, Drug Technology Department

• **Diana GURANDA**, Doctor in pharmacy, Associate Professor, Drug Technology Department

***REVIEWERS:***

• ***Eugen DIUG***, *Habilitated doctor in pharmacy, University Professor, Head of Drug Technology Department*

• ***Liliana DOGOTARI***, *Doctor in pharmacy, Associate Professor, Department of Social Pharmacy "Vasile Procopisin"*



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***of the Faculty of Pharmacy***



**Chisinau, 2019**

# CONTENTS

# INTRODUCTION

Biopharmacy is a pharmaceutical discipline which is used by the modern drug discovery, quality control and pharmaceutical attendance. The principles of biopharmacy and pharmacokinetics got a general distribution in pharmaceutical and medicinal investigations, but a small degree in clinical practice, which is tied to rational use of drugs or drug formulation on biopharmaceutical principle. Pharmacists, getting the opportunity of theoretical and practical preparations in a new specialty, the clinical pharmacy, need among pharmacology, pathology and drug therapy knowledge, a very strong cognation of drug substance pharmacokinetics with aim to formulation of adequate posology.

The teaching aids included in this volume, guide practical works in biopharmacy and pharmacokinetics shall allow a better understanding of pharmacokinetics movements of a drug substance within human body and what are the pharmaceutical factors what may influence it. This guide is reserved to students becoming pharmacists and even for pharmacists who are specialized or worked in the domain that requires biopharmacy and pharmacokinetics knowledge.

The activity of students in this direction offers them an efficient base for a better presentation of the drafts exposed at courses and also opportunity to apply the theoretical knowledge in practice.

Due to the fact that there is a real difficulty in conducting a pharmacokinetic study on experimental animals or volunteers in the practical training of pharmacist students, it is often the use of different *in vitro* pharmacokinetic models. These models allow simulation of the dissolution, absorption, distribution and elimination of the drug substance.

A basic model for laboratory experiments was the *in vitro* open-pharmacokinetic model. The model has been adapted to the existing device and its Experimental procedure to make it useful to students in the Biopharmacy and Pharmacokinetics Laboratory.

The model serves in the assessment of pharmacokinetics parameters, helps to understand their significance or in the estimation of the biopharmaceutical properties of drugs. The adding a number of selected problems of experimental and clinical pharmacokinetics, as well as of biopharmacy, suggests domains of practical application in the schemes formulation of drug introduction in the clinical situations and drug formulation on biopharmaceutical principles.

The methodical indication consists of the presentation of some theoretical aspects of the basic concept of biopharmacy and pharmacokinetics, followed by the description of practical work, problems, self-assessment tests, useful pharmacokinetic equations and individual work.

The works are scheduled for 3 hours. Each work is accompanied by a description of the purpose, the materials used, the Experimental procedure and the processing of the experimental results. The material has been verified for several years at the practical Biopharmaceutical and Pharmacokinetic Practice classes at the Drug technology Department.

***Biopharmacy*** - study of the factors influencing the bioavailability of a drug in man and animals and the use of this information to optimize pharmacological or therapeutic activity of drug products in clinical application. Study embracing this relationship between the physical, chemical and biological sciences as they apply to drugs, dosage forms, and to drug action.

The aim of biopharmaceutics is to adjust the delivery of drug to the general circulation in such a manner as to provide optimal therapeutic activity for the patient.

Pharmaceutical factors, also called biopharmaceuticals, are classified into 5 groups:

1. Chemical state of the drug;
2. Physical state of the drug;
3. Origin and quantity of auxiliary substances;
4. Type of pharmaceutical form and mode of administration;
5. Applied technological processes.

Biopharmaceutics is a major branch in pharmaceutical sciences which relates between the physicochemical properties of a drug in dosage form and the pharmacology, toxicology, or clinical response observed after its administration. Drug efficacy and safety are dependent on the dosing regimen. The optimal dosage and dosing intervals can be quite different for different drugs. Moreover, for a single drug, the optimal dosage can be different widely between patients.

It is not sufficient to know what the drug does to the body; it is also crucial to know what the body does to the drug. The knowledge of the pharmacodynamic and pharmacokinetic properties of the drug and its metabolites in humans and animals is crucial to understand its different effects among species and for adjusting drug dosing.

The plasma concentration of the drug is the basic concept of pharmacokinetics. Based on protein binding of the drug, the concentration of free drug available in the circulation influences greatly the dose calculations. The concentration of drug in the plasma is in equilibrium with some tissues in the body.

Human body is composed of a series of membrane barriers divided by aqueous-filled compartments. These membrane barriers are principally composed of the phospholipid bilayers resulting from the orientation of the lipids (phospholipids, glycolipids, and cholesterol) in the aqueous medium, which surround the cells and also form intracellular barriers around the organelles present in cells (mitochondria, nucleus, etc.). The phospholipids are amphipathic in nature and have aligned polar head groups and lipid "tails," so the polar head groups of phospholipid orientate toward the aqueous phases and the lipid tails form a highly hydrophobic inner core. Hence, the drug substance releases its hydration element and becomes hydrophobic. The drug disposition across the membrane depends on its lipophilicity and partition coefficient. Here, the protein binding plays an important role.

The polar molecules will be dissociated in an aqueous environment; thereby, the hydrophilicity arises and vice versa in the case of nonpolar molecules in a lipophilic environment. Every component of an organic compound has a defined lipophilicity.

Absorption and bile elimination rate are molecular weight dependent. Lower-molecular-weight compounds have better absorption and less bile excretion when compared to the higher-molecular-weight compounds. Drugs with higher lipophilicity can be better absorbed from the intestine.

Blood is the transporter of many vital substances and nutrients for the entire body and thus contains many endogenous and exogenous compounds in different concentrations. Biological samples (tissue extracts, plasma, serum, or urine) are extremely complex matrices comprised of many components that can interfere in estimation/quantification; hence, biological samples cannot normally be injected directly into the analyzing system for the determination of active principle. Sample pretreatment is required for achieving sufficient sensitivity and selectivity to determine the active principle. Chemical assays of high quality which include adequate sensitivity, selectivity and reproducibility are essential for obtaining valuable data.

Bioanalysis covers the quantitative measurement drugs and their metabolites in biological systems. Bioanalysis technique can provide a quantitative measure of the active drug and/or its metabolite(s) for the purpose of pharmacokinetics. Various analytical instrument methods such as high-performance liquid chromatography (HPLC) or gas chromatography (GC) or ultra performance liquid chromatography (UPLC) with variety of detectors such as UV, fluorescent, diode array, flame ionization, electron capture and mass spectrometry, and capillary electrophoresis–mass spectrometry may be used. For macromolecule, ELISA or RIA method can be used for quantification.

***Pharmacodynamics***- refers to the relationship between drug concentration at the site of action and the resulting effect, including the time course and intensity of therapeutic and its adverse effects. Studies are designed to investigate all primary and secondary effects related to the desired therapeutic effects, extensions of the therapeutic effect that might produce toxicity at higher doses, and effects related to interactions with other drugs.

***Pharmacokinetics***- refers to the study of the time course of a drug within the body (extent and duration of systemic exposure to the drug) and also incorporates the process about the drug's **absorption, distribution, metabolism and excretion** (ADME) pattern. In general, pharmacokinetic parameters are derived from the measurement of drug concentrations in blood or plasma.

*Parameters obtained from plasma concentrations*

- AUC=area under the curve of a plasma concentration versus time profile
- CL=total plasma clearance
- $V_D$=volume of distribution
- $t_{1/2}$=elimination half-life
- After an extravascular administration
- $t_l$=lag time
- $C_{max}$=maximum concentration
- $t_{max}$=time to reach $C_{max}$
- AUC=area under the curve of a plasma concentration versus time profile

- $t_{1/2}$=elimination half-life
- *Parameters obtained from urine concentrations (whatever the route of administration)*
- $M_U$=amount excreted in urine
- $CL_R$=renal clearance when plasma concentrations are known

## 1. ABSORPTION

Absorption studies generally involve serial determinations of drug concentration in blood and urine after dosing to indicate the rate and extent of absorption. Drug absorption refers to the passage of drug molecules from the site of administration into the circulation. Drug absorption requires that drugs cross one or more layers of cells and cell membranes. Solubility is manipulated mainly by the structure of the drug. In general, solubility is inversely proportional to the number and type of lipophilic functions within the molecule and tightness of the crystal packing of the molecule. Solubility decreases when there is increase in crystal packing or lipophilicity. The concentration of drug in solution is the driving force of the membrane transfer of drug into the body, and low aqueous solubility often continues to present itself as a problem even after formulation improvements.

Factors that influence drug absorption through oral route are:

1. Biological factors: Permeation of the drug across the membrane, GI transit, site specificity, first-pass metabolism, metabolism in the liver, excretion as bile, excretion through bladder, and protein binding of drugs
2. Pharmaceutical factors: Excipients, type of dosage forms, process of preparation, stability testing, and storage directions
3. Other factors: Solubility of the drug; partitioning properties; dissociation characteristics; salt formation; particle size, shape, volume, and its distribution; crystallinity; polymorphism; prodrugs; and stereotype and its formation.

Drugs may be either weak acids or bases that exist in both ionized and non-ionized forms in the body. Drug in the non-ionized form is sufficiently soluble in membrane lipids and can cross cell membranes. The rate of absorption depends upon the ratio of the two forms at a particular site and is also a factor in distribution and elimination. The protonated form of a weak acid is non-ionized, whereas the protonated form of a weak base is ionized. The pKa is the negative log of the ionization constant, particular for each acidic or basic drug. Protonated form predominates when the pH is less than the pKa, whereas nonprotonated form predominates when pH is greater than the pKa. In the stomach, with a pH of 1, weak acids and bases are highly protonated. At this site, the non-ionized form of weak acids (pKa = 4 ± 1) and the ionized form of weak bases (pKa = 9 ± 1) will prevail upon. Weak acids are absorbed without dissociation than weak base from the stomach and exactly opposite in the intestine where weak bases are absorbed readily than weakly acidic drugs. In intestine, weakly acidic drugs are also found to be absorbed even though they are ionized due to the large surface area.

Absorption takes place across the biological membrane by two methods. Lipid drugs are absorbed by transcellular mechanism where the drug distributes into the lipid core of the membrane which diffuses into the other side of the membrane. The solute may also

diffuse across the cell membrane and enter into the circulation. Another mechanism is the paracellular absorption. The aqueous-filled pores in between the cells aid absorption of the drugs. Water-soluble drugs are readily absorbed, but the molecule size of the particle plays an important role.

Transport across cell membranes:

***Passive diffusion.*** The concentration gradient provides energy for the transportation of the drug across the membrane, and also partitioning of the drug in favor of the lipid membrane decides the quantity of the drug absorbed. The unionized drug is absorbed markedly higher than the ionized form. Passive diffusion could be explained with Fick's first law which relates the diffusive flux to the concentration under the assumption of steady state. It postulates that the flux goes from regions of high concentration to regions of low concentration, with a magnitude that is proportional to the concentration gradient, or in simplistic terms, the concept that a solute will move from a region of high concentration to a region of low concentration across a concentration gradient.

***Active transport.*** Active transport is the movement of molecules across the lipid cell membrane against concentration gradient, i.e., moving from an area of lower concentration in the GIT to an area of higher concentration in the plasma. The absorption sites are at a specific place in the GIT. Active transport is usually associated with accumulating high concentrations of molecules that the cell needs, such as ions, glucose, and amino acids. This active transport process uses chemical energy, such as from adenosine triphosphate (ATP). These energy molecules are site specific – the drugs are transported at a particular site in the GIT, they are limited in number, and they act like a ferry service: it picks a molecule from the GIT, ferries across, leaves in the cytoplasm, and comes back to pick another molecule. The concentration of the drug in the plasma is maintained constant because of this "ferry" service, and the energy/carrier molecules are nothing but ATP-dependent proteins

***Endocytosis.*** Endocytosis is an energy-using process by which cells absorb molecules (such as proteins) by engulfing them. It is used by large polar molecules that cannot pass through the hydrophobic plasma or cell membrane. The opposite process is exocytosis. Phagocytosis is a specific form of endocytosis involving the vascular internalization of solids such as bacteria by an organism and is therefore distinct from other forms of endocytosis such as the vesicular internalization of various liquids (pinocytosis). Phagocytosis is involved in the acquisition of nutrients for some cells. Pinocytosis, otherwise known as cell drinking, fluid endocytosis, and bulk-phase pinocytosis, is a mode of endocytosis in which small particles are brought into the cell, forming an invagination and then suspended within small vesicles.

**Absorption rate constant:** "Fractional rate of drug absorption from the site of administration into the systemic circulation."

The oral absorption of drugs is often approximated assuming linear kinetics, typically when given in solution. The same is true for the absorption of drugs from many other extravascular sites, including subcutaneous tissue and muscle. Under these circumstances, absorption is characterized by an absorption rate constant and a corresponding absorption half-life.

However, the absorption of many drugs do not exactly follow linear kinetics. In some cases the drug may be absorbed at a constant rate so that the same amount of drug is absorbed during each time interval, mimicking constant rate intravenous infusion. Different factors may be responsible for nonlinear absorption such as controlled-release formulations or saturable transport mechanisms of the drug from the intestinal lumen to the portal circulation.

The rate of absorption determines the required time for the administered drug to reach an effective plasma concentration and may thus affect the onset of the drug effect. This rate influences both the peak plasma concentration ($C_{max}$) and the time it takes to reach this peak ($t_{max}$).

Variation of the rate of absorption can add to the global pharmacokinetic variability, particularly in patients with diseases affecting the absorption site (e.g. affections of the gastro-intestinal tract).

Flip-flop: In the most common situation, the absorption constant rate is greater than the elimination rate constant ($K_{el}$) and the terminal decline in plasma concentration is mainly driven by elimination. In some cases, the absorption rate can be smaller than the elimination rate. The drug cannot be eliminated faster than it is absorbed. The terminal slope of the plasma concentration-time curve then reflects the absorption constant rate. This phenomenon is typically used in controlled-release formulations, able to prolong the apparent half-life of the drug beyond its elimination half-life.

Controlled-release: In controlled-release formulations, drug release is much slower than from the conventional form. These preparations extend the apparent half-life of the drug and reduce the fluctuations in the plasma concentration at constant regimen. However, they may increase the variability in absorption.

The assessment of the $t_{max}$ depends on the value of both the absorption rate constant ($K_a$) and the elimination rate constant ($K_{el}$):

$$t_{max} = \ln (K_a) - \ln (K_{el}) / K_a - K_{el}$$

## 2. DISTRIBUTION

Distribution provides information on the extent and time course of tissue accumulation and the elimination of drug and/or its metabolites.

The disposition of drug into the organs and tissues via circulation depends upon the nature of the drug. The more lipophilic the drug is, the better will be the distribution into the organs and tissues. Hydrophilic drugs are normally concentrated in cells and they are referred to as ion trapping.

When a drug is introduced into the body, the rate of distribution is dependent upon the following:

1. Tissues with the highest blood flow receive the drug: The rate at which a drug is distributed to various organs after a drug dose is administered depends largely on the proportion of cardiac output received by the organs.
2. Protein binding: Binding to proteins is inevitable in the case of drugs particularly lipoproteins, glycoproteins, and β-globulins. The extent of binding depends on

the affinity of the drug molecule with the protein, and the maximum affinity could be 99 % also. Unbound drug diffuses in the liquids surrounding the cells.

3. Lipid solubility: Lipid solubility is a major factor affecting the extent of drug distribution, particularly to the brain, where the blood–brain barrier restricts the penetration of polar and ionized molecules. Highly lipid-soluble drug can enter the tissues.

4. Molecular size: Molecular size is a factor affecting the distribution of extremely large molecules.

5. Distribution depends upon the ionization of drug, whereas unionized drugs can go anywhere into the body.

Reasons for the variation in concentration of drug distribution are:

- Tissue differences in rates of uptake of drugs: Blood flow and capillary permeability;
- Differences in tissue/blood ratios at equilibrium: Dissolution of lipid-soluble drugs in adipose tissue, binding of drugs to intracellular sites, and plasma protein binding;
- Apparent volume of distribution (Vd).

**Volume of distribution**, also known as apparent volume of distribution, is a pharmacological, theoretical volume that the total amount of administered drug would have to provide the same concentration as it is in blood plasma.

If the amount of drug (X) and the resulting concentration (C) are known, then the volume of distribution (Vd) can be calculated using the simplified equations:

**X =Vd * C,**

where X = amount of drug in body, Vd = volume of distribution, and C= concentration in the plasma.

Lipid-insoluble drugs are mainly confined to the plasma and interstitial fluid; most do not enter the brain following acute dosing. Lipid soluble drugs reach all compartments and may accumulate in fat. For drugs that accumulate outside the plasma compartment, Vd may exceed the total body volume.

Factors involved in drug distribution and diffusion across blood tissue barrier are:

- Blood flow
- Permeability across blood tissue barrier
- Tissue solubility
- pH partition
- Protein binding within compartment

In our body, various structures are acting as reservoir for storage of drug substance. They are plasma proteins, erythrocytes, and cellular reservoir like muscles, fat tissue, bone, and transcellular compartments.

**Compartment models** are hypothetical structures used to describe the fate of a drug in a biological system after its administration into the body. When medications are administered to humans, the body acts as if it is a series of compartments. In many cases, the drug distributes from the blood into the tissues quickly, and a psuedoequilibrium of drug movement between blood and tissues is established rapidly. When this occurs, a

one-compartment model can be used to describe the serum concentrations of a drug. In some clinical situations, it is possible to use a one-compartment model to compute doses for a drug even if drug distribution takes time to complete. In this case, drug serum concentrations are not obtained in a patient until after the distribution phase is over.

Various compartment models in pharmacokinetic are:

- *One-compartment model:* Following drug administration, the body is depicted as a kinetically homogeneous unit.
- *Two-compartment model:* The two-compartment model resolves the body into a central compartment and a peripheral compartment.
- *Multi-compartment model:* In this model, the drug distributes into more than one compartment and the concentration–time profile shows more than one exponential.

Using compartment models, the body can be represented as a series of discrete sections (fig. 1). The simplest model is the one-compartment model that depicts the body as one large container in which drug distribution between blood and tissues occurs instantaneously.

Drug is introduced into the compartment, distributes immediately into a volume of distribution (V), and is removed from the body via metabolism and elimination via the elimination rate constant (Kel).

The simplest multi-compartment model is a two-compartment model that represents the body as a central compartment into which drug is administered and a peripheral compartment into which drug distributes. The central compartment 1 is composed of blood and tissues that equilibrate rapidly with blood. The peripheral compartment 2 represents tissues that equilibrate slowly with blood. Rate constants represent the transfer between compartments (k12, k21) and elimination from the body (k10).

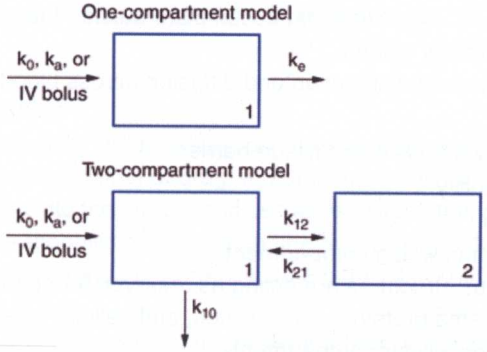


*Fig. 1.* One and two compartment model in linear pharmacokinetics
https://accesspharmacy.mhmedical.com/Content.aspx?bookid=1374&sectionid=74719519

## 3. BIOTRANSFORMATION / METABOLISM.

Biotransformation or drug metabolism is the enzyme-catalyzed conversion of drugs to their metabolites. Metabolism makes the drug less polar; lipid-soluble substance makes it more polar as well as water soluble, thus facilitating their excretion by the kidney. If a drug is already highly polar and water soluble, then it may not get metabolized and may get excreted as such. Liver is the chief organ for biotransformation of most drugs, but drug-metabolizing enzymes are found in many other tissues, including the gut, kidneys, brain, lungs, and skin. Lipophilic drug is converted to a hydrophilic one by extensive metabolism in the liver.Drug metabolism is traditionally carried out by phase I and phase II processes. Cytochrome P450 system has an important role and occupies a pivotal role in drug clearance in phase I.

**Phase I:** First step in biotransformation is the formation of product susceptible to phase II conjugative reaction. The phase I also involves unmasking a functional group like OH, NH2, and SH and conversion to more polar products which may be mostly inactive, less active, and modified activity.

**Phase II:** Coupling of drug or its oxidized metabolite to endogenous conjugating agent derived from carbohydrate, protein, or sulfur sources; generally products are more water-soluble and more readily excreted in urine or bile. Phase II involves conjugation reactions with glucuronic acid, sulfuric acid, acetic acid, and amino acid.

Biotransformation occurs somewhere between absorption and excretion; some may occur in the gut (digestion, decomposition in gastric acidity)

Role of enzymes in the biotransformation are drug metabolism; conversion of prodrug to active forms; synthesis of steroidal hormones, cholesterol, and bile acids; and finally formation and excretion of bilirubin. Biotransformation is mediated by cellular enzymes in the sarcoplasmic reticulum, mitochondria, cytoplasm, lysosomes, and nucleus.

Drug-metabolizing enzymes are classified into:

1. *Microsomal (inducible)*
2. *Non-microsomal (non-inducible)*

*Microsomal enzymes (inducible).* Microsomes are artificial spheres obtained from the endoplasmic reticulum by homogenization and fractionation, and they possess various drug-metabolizing enzymes. Glucuronyl transferase for conjugation. The drugs containing phenols, alcohols, and carboxylic acids are metabolized by conjugation method. The conjugates are mostly inactive and excreted in the bile and urine by anion carrier mechanism and enter into enterohepatic cycling (β-glucuronidase and sulfatase in the gut). Some enzymes are involved in reduction and hydrolysis. The modification of enzyme activity such as enzyme induction and enzyme inhibition was observed.

Majority of the drugs however are metabolized by the *non-microsomal enzymes* resulting in their activation, inactivation, or modification. The reactions are:

1. Inactivation by conjugation: Synthetic process by which a drug or its metabolite is combined with an endogenous substance.

2. Inactivation by oxidation: Involves introduction of a hydroxyl group into the drug molecule.

3. Inactivation by reduction: Many halogenated compounds and nitrated compounds are reduced by microsomal enzymes.

4.Inactivation by hydrolysis: Carried out by enzyme esterase; this hydrolyses the esters.

Drug metabolism is affected by various factors. The diseases that are categorized as acute and chronic liver diseases (reduces metabolism), liver cancer, cardiac diseases limiting blood flow to the liver, pulmonary diseases reducing hydrolysis of procainamide, and hyperthyroidism where metabolism are affected. And also metabolism increases t½ and hypothyroidism reduces metabolism t½ .

## 4. CLEARANCE (ELIMINATION)

**Drug clearance** (CL) is defined as- the volume of plasma in the vascular compartment cleared of drug per unit time by the processes of metabolism and excretion(only free, i.e., not protein bound). Extraction is the ratio of the clearance process (E) referring to the proportion of drug presented to the organ which is removed irreversibly (excreted) or altered to a different chemical form (metabolism) from the organ.

In order to be eliminated, a drug must be presented to the organs of elimination by the plasma flow. The higher the drug concentration, the more drug is presented to the organs and thus can be eliminated. In other words, the rate of drug elimination is proportional to the plasma concentration. Clearance is precisely this coefficient of proportionality. By contrast, the fraction of drug that is eliminated remains constant. Clearance of most drugs, for which excretion and metabolism are not saturated, is constant over the range of doses encountered clinically. Such drugs are said to undergo first order kinetics.

Clearance is the only factor determining the average drug concentration after the iv injection of a given dose. After an extravascular administration, the average drug exposure is determined both by clearance and by bioavailability. In a multiple dosage regimen, establishing the value of clearance is necessary to predict an average drug concentration within a therapeutic window. The individual factors that can influence clearance are the intrinsic functions of liver or kidneys. Therefore, variation of clearance can be anticipated when there is a major impairment of these organs. Blood flow to the organs of elimination can also affect clearance.

For every drug, each organ of elimination has its own clearance (e.g. hepatic clearance, renal clearance). The total body clearance results from the addition of these clearances:

**CL= Renal CL+ Hepatic CL+ other CL**

**Cl = Kel * Vd**

where: Kel = Elimination rate constant;
Vd = Volume of distribution.

Clearance for a drug is constant if the drug is eliminated by first-order kinetics.

**Half-life:** "Time it takes for the plasma concentration or the amount of drug in the body to be reduced by 50%."

By definition, the plasma concentration of a drug is halved after one elimination half-life. Therefore, in each succeeding half-life, less drug is eliminated. After one half-life the amount of drug remaining in the body is 50% after two half-lives 25%, etc. After 4 half-lives the amount of drug (6.25%) is considered to be negligible regarding its therapeutic effects.

The half-life of a drug depends on its clearance and volume of distribution. The elimination half-life is considered to be independent of the amount of drug in the body.

Half-life determines the length of the drug effect. It also indicates whether accumulation of the drug will occur under a multiple dosage regimen and it is essential to decide on the appropriate dosing interval.

**Elimination rate constant** (Kel): Fractional rate of drug removal from the body. This rate is constant in first-order kinetics and is independent of drug concentration in the body. λ is the slope of the plasma concentration-time line (on a logarithmic y scale).

Apparent half-life ($t_{1/2}$): In some cases, such as for controlled-release preparations, the rate of decline of the drug plasma concentration is not due to elimination alone. Other factors such as absorption rate or distribution rate influence plasma concentration decay. In such conditions, the observed half-life is called apparent half-life.

$$t_{1/2} = \frac{\ln(2)}{\lambda} = \ln(2) * \frac{Vd}{CL} = 0.693 * \frac{Vd}{CL}$$

where: Vd = volume of distribution

CL = clearance

Kel = elimination rate constant = CL/Vd

**Excretory organs:**

- Major routes: kidneys, liver, and lungs.
- Minor routes: sweat, saliva, tears, and breast milk.

Urine helps to quantitate the amount of drug excreted and is the most important excretory route for nonvolatile drugs and their metabolites (drug not bound to plasma proteins), proximal tubular active secretion, and passive tubular reabsorption.

Renal excretion: Small molecules with low molecular weight will appear in urine through glomerular filtration. Through tubular carrier systems (tubular secretion), a drug can be transported against the concentration gradient from the blood capillaries to the nephron lumen to be excreted in the urine.

Lipophilicity in drug clearance: Reduction in lipophilicity is observed when compared to the parent molecule during administration. For hydrophilic drugs (log D7.4 below 0), renal clearance is the predominant mechanism, whereas the drugs with log D7.4 values are above 0, renal clearance decreases with lipophilicity. Metabolic clearance increases with increasing log D, and this becomes the major clearance route of lipophilic compounds. The lowest clearance (negligible) is observed below log D7.4 values of 0 by

combined renal and metabolic processes (log D 7.4 Logarithm of the distribution coefficient (D) at pH 7.4).

Lipophilicity and reabsorption by the kidney: The degree of reabsorption (all along the nephron) depends on the physicochemical properties (degree of ionization and intrinsic lipophilicity) of the drug. After absorption, the equilibrium is reestablished in the kidney where the unbound drug in the urine and unbound drug in plasma are present on both sides of the membrane. The water-soluble drugs are absorbed easily, but lipophilic drugs will be reabsorbed by diffusion due to concentration gradient.

Effect of charge on renal clearance: Tubular pH is often more acidic (pH 6.5) than plasma; hence, acidic drugs are reabsorbed more extensively than basic. Greater rates of excretion/clearance can occur for these charged moieties due to the tubular active transport proteins.

Renal clearance: The unbound drug will be cleared by filtration, and the protein-bound drug will be cleared slowly as it dissociates after a long time. Drugs with increasing plasma protein binding have increased lipophilicity, which decreases the renal clearance. Renal clearance in drug design: Small molecules with relatively simple structures (molecular weights below 350) can successfully combine paracellular absorption and renal clearance.

Liver and biliary excretion: Liver is the organ where maximum metabolism takes place. The unabsorbed drugs and the metabolized drugs are excreted through fecal matter. Enzyme cytochrome is having a pivotal role in drug clearance by various oxidation reactions such as aromatic hydroxylation, aliphatic hydroxylation, N-dealkylation, O-dealkylation, S-dealkylation, N-oxidation, S-oxidation, and alcohol oxidation.

Lungs: The lungs are an important route for the excretion of gaseous anesthetics, alcohol, iodine, and iodates.

Other excretion routes are sweat, saliva, and tears which are generally pH dependent that mediate drug excretion by passive diffusion of lipophilic drugs.

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Milk: Milk is more acidic than plasma; hence, basic drugs tend to accumulate due to ionic trapping, whereas concentration of acidic drugs is lesser than in the plasma. Nonelectrolytes (ethanol, urea) enter milk in a pH-independent manner.

Hair and skin: Toxic metal may be excreted (murder, suicide).

**Elimination kinetics:**

**Definition Zero-order elimination kinetics**: "Elimination of a constant quantity per time unit of the drug quantity present in the organism."

**Definition First order elimination kinetics:** "Elimination of a constant fraction per time unit of the drug quantity present in the organism. The elimination is proportional to the drug concentration."

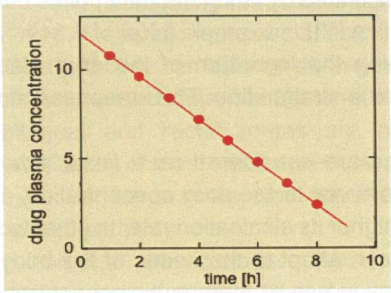



*Fig. 2.* Zero-order kinetics (linear y-axis)

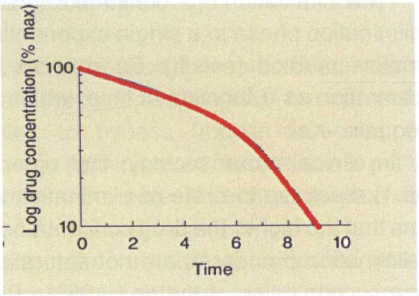



*Fig. 3.* Zero-order kinetics (log y-axis)

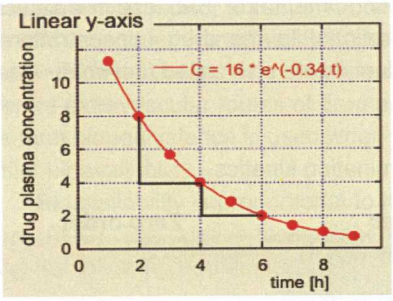



*Fig. 4.* First-order kinetics (linear y-axis)

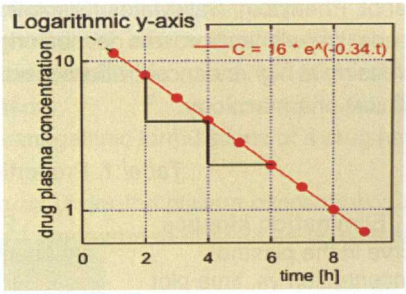



*Fig. 5.* First-order kinetics (log y-axis)

***Zero-order elimination kinetics:***

The plasma concentration – time profile during the elimination phase is linear (fig. 2,3). For example 1.2 mg are eliminated every hour, independently of the drug concentration in the body.Order 0 elimination is rather rare, mostly occurring when the elimination system is saturated. An example is the elimination of Ethanol.

***First-order elimination kinetics:***

For first order elimination, the plasma concentration – time profile during the elimination phase shows an exponential decrease in the plot with linear axes (fig. 4.) and is linear if plotted on a semi-logarithmic plot (plasma concentration on logarithmic axis and time on linear axis (fig. 5.).

For example, 1% of the drug quantity is eliminated per minute. Many drugs are eliminated by first order kinetics.

The time course of the decrease of the drug concentration in the plasma can be described by an exponential equation of the form:

$$C = C_0 * e^{-kel*t}$$

where: C = drug concentration;

$C_0$ = extrapolated initial drug concentration;

Kel = elimination rate constant;

t = time.

The elimination rate constant Kel can be calculated by fitting the data points during the elimination phase to a single exponential; yielding in this example a Kel of 0.34 $h^{-1}$. An alternative method (see fig. 5.) consists in plotting the logarithm of the drug plasma concentration as a function of time, which will yield a straight line. The steepness of this line equals –Kel.

In clinical pharmacology, first order kinetics are considered as a *linear process* (table 1), because the rate of elimination is proportional to the drug concentration. This means that the higher the drug concentration, the higher its elimination rate. In other words, the elimination processes are not saturated and can adapt to the needs of the body, to reduce accumulation of the drug. 95% of the drugs in use at therapeutic concentrations are eliminated by first order elimination kinetics. A few substances are eliminated by zero-order elimination kinetics, because their elimination process is saturated. Examples are Ethanol, Phenytoin, Salicylates, Cisplatin, Fluoxetin, Omeprazol. Because in a saturated process the elimination rate is no longer proportional to the drug concentration but decreasing at higher concentrations, zero-order kinetics are also called *non-linear kinetics* in clinical pharmacology.

*Tabel 1.* Properties of elimination kinetics

| Elimination kinetics | First order | Zero order |
|---|---|---|
| Curve in the plasma concentration vs. time plot after i.v. bolus | exponential decay (fig. 4) | linear (fig. 2) |
| Curve in the log plasma concentration vs. time plot after i.v. bolus | linear (fig. 5) | non-linear |
| Relation between elimination rate and drug concentration | elimination rate is proportional to drug concentration | elimination rate saturates with higher drug concentration< |
| Term in clinical pharmacology | linear kinetics | non linear kinetics |
| Concerns | 95 % of drugs, at therapeutic concentrations | the remaining 5 %, and ethanol |

**BIOAVAILABILITY** - fraction of a dose of drug that is absorbed from its site of administration and reaches, in an unchanged form, the systemic circulation.

The drug, its route of administration and its galenic formulation determine the amount of administered dose absorbed into the circulation. Patient dependant factors also influence bioavailability.

When the drug is administered orally the bioavailability depends on several factors:

1. Physicochemical properties of the drug and its excipients that determine its dissolution in the intestinal lumen and its absorption across the intestinal wall.
2. Decomposition of the drug in the lumen.
3. pH and perfusion of the small intestine.
4. Surface and time available for absorption.

5. Competing reactions in the lumen (for example of the drug with food).
6. Hepatic first-pass effect.

Bioavailability can also be determined for other extravascular routes of administration such as intramuscular, subcutaneous, rectal, mucosal, sublingual, transdermal etc. Sublingual and rectal routes are often used to bypass hepatic first-pass effect. Bioavailability of most small molecular weight drugs administered i.m. or s.c. are perfusion rate-limited. Large molecules administered i.m or s.c. enter the blood in part via the lymphatic pathway.

Clinical implications. When changing the route of administration or the formulation of a drug, the dose must be adapted with regard to the respective bioavailability of each route.

Bioavailability of a drug administered intravenously is by definition 100%. Bioavailability is less or equal to 100% for any other route of administration.

The term absolute bioavailability is used when the fraction of absorbed drug is related to its i.v. bioavailability. The term relative bioavailability is used to compare two different extravascular routes of drug administration.

The term bioequivalence is used when two different galenic formulations of a drug have a similar bioavailability.

Bioavailability is proportional to the total area under the plasma concentration-time curve (AUC). The relative bioavailability of drug 1 compared to drug 2 can be calculated using the following equation:

$$F = (AUC_2/AUC_1) * (D_1/D_2)$$

**Area under the plasma drug concentration-time curve** "Integral of the plasma drug concentration-time curve."

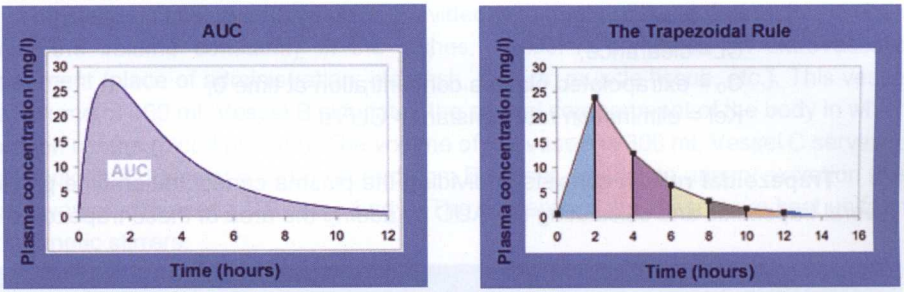



*Fig. 6.* Plasma curve after single extravascular dose administration

The area under the plasma drug concentration-time curve (AUC) reflects the actual body exposure to drug after administration of a dose of the drug and is expressed in mg*h/L (fig. 6).

This area under the curve is dependent on the rate of elimination of the drug from the body and the dose administered. The total amount of drug eliminated by the body may be assessed by adding up or integrating the amounts eliminated in each time interval, from time zero (time of the administration of the drug) to infinite time. This total amount corresponds to the fraction of the dose administered that reaches the systemic circulation.

The AUC is directly proportional to the dose when the drug follows linear kinetics. The AUC is inversely proportional to the clearance of the drug. That is, the higher the clearance, the less time the drug spends in the systemic circulation and the faster the decline in the plasma drug concentration. Therefore, in such situations, the body exposure to the drug and the area under the concentration-time curve are smaller.

During clinical trials, the patient's plasma drug concentration-time profile can be drawn by measuring the plasma concentration at several time points. The AUC can then be estimated. Knowing the bioavailability and the dose, the clearance of the drug may be calculated by dividing the dose absorbed by the AUC. The clearance calculated is relatively independent on the shape of the concentration-time profile. This method gives precious information on the pharmacokinetic behavior of a drug on trial. It can also be used to study a change in the clearance of a drug in specific clinical conditions, such as disease or concomitant drug administration.

Apparent clearance (CL'): In some pharmacokinetic trials, the bioavailability of the studied drug is not known. The apparent clearance, resulting from the dose divided by the AUC, reflects the drug's clearance that does not take into account the bioavailability of the drug.

$$\mathbf{AUC = F * D / Cl}$$

After an iv bolus injection, the AUC can be calculated by the following equation:

$$\mathbf{AUC = C_0 / Kel}$$

where: AUC= Area under the concentration-time curve;
F = bioavailability;
D = dose;
CL= clearance;
$C_0$ = extrapolated plasma concentration at time 0;
Kel = elimination rate constant = CL/Vd.

**Trapezoidal rule:** It consists in dividing the plasma concentration-time profile into several trapezoids and calculating the AUC by adding the area of these trapezoids.

| Time, hours | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_p$, mg/L | 5,5 | 9,6 | 11,8 | 12,5 | 12,0 | 11,0 | 9,2 | 7,5 | 5,8 | 4,3 | 2,6 | 0,5 |

**$AUC_0^{12}$ = area 1 + area 2 + area 3 + ... + area 11 + area 12 = ½ ($C_0$ + $C_1$) ($t_1 - t_0$) + ½ ($C_1$ + $C_2$) ($t_2 - t_1$) + ½ ($C_2$ + $C_3$) ($t_3 - t_2$) +.....+ ½ ($C_{10}$ + $C_{11}$) ($t_{11} - t_{10}$) + ½ ($C_{11}$ + $C_{12}$) ($t_{12} - t_{11}$).**

**$AUC_0^{12}$** = ½ (0 + 5,5)(1 – 0) + ½ (5,5 + 9,6) (2 –1) + ½ (9,6 + 11,8) (3 – 2) + ½ (11,8 + 12,5) (4 – 3) + ½ (12,5 + 12,0) (5 – 4) + ½ (12 + 11) (6 – 5) + ½ (11,0 + 9,2) (7 – 6) + ½ (9,2 + 7,5) (8 – 7) + ½ (7,5 + 5,8) (9 – 8) + ½ (5,8 + 4,3) (10 – 9) + ½ (4,3 + 2,6) (11 – 10) + ½ (2,6 + 0,5) (12 – 11) = 2,75 + 7,55 + 10,7 + 12,15 + 12,25 + 11,15 + 10,1 + 8,35 + 6,65 + 5,05 + 3,45 + 1,55 = **91,7 mg/mL h.**

## 1. Mono-compartmental open model description. Preparation of standard curve for the sodium salicylate determination

For simulation of plasma concentrations over time, a number of models based on the open-label single-compartment pharmacokinetic model are described. One of this model is described in fig. 7.

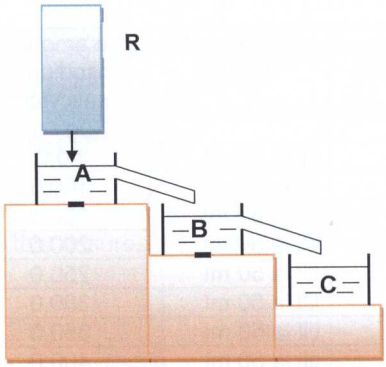



*Fig. 7.* Scheme of *in vitro* open mono-compartmental pharmacokinetic model

The system consists of 3 vessels, provided with a lateral liquid drain tube, in order to keep the volume constantly in the dishes. Vessel A simulates an extravascular compartment (place of administration: stomach, rectum, muscle tissue, etc.). This vessel has a volume of 150 ml. Vessel B simulates the central compartment of the body in which absorption occurs (blood plasma). The volume of the vessel is 300 ml. Vessel C serves to collect liquid that drains at a certain speed from B vessel, simulating urinary excretion (the primary way of eliminating MS in the body). The contents of the vessels are kept uniform with magnetic stirrers.

To maintain a constant flow rate of liquid, a reservoir fitted with a dropper with regulator is used to establish a certain flow rate. The liquid flows from one vessel to another at a constant speed. This system simulates blood circulation. Thus, mass transfer of a dissolved MS in water occurs. The administration of various extravascular (intravenous) (single-dose, single-dose, single-dose, intravenous, single-dose, intravenous, or intravenous) infusions (vessel B)

The rate of transfer of MS from one vessel to another will depend on its concentration in the vessel it leaves and the solvent flow rate. Thus, a first-order transfer kinetics, specific to the kinetic processes occurring in vivo, takes place.

The MS concentration in the vessels is determined by sampling (1 ml) at set intervals.

***Construction of the calibration curve for sodium salicylate.***

In experiments, sodium salicylate, which in Fe3 + acid medium forms a complex compound, the intensity of which is proportional to the salicylate concentration, will be used as the drug substance. The compound has a maximum absorption at the wavelength of 525 nm.

For building the calibration curve:

- Prepare a standard solution of 10% sodium salicylate (sol A);

1 ml of standard 10% sodium salicylate solution is transferred to a 100 ml volumetric flask and purified water is added to the solution (sol B);

- Make a series of dilutions.

| Nr. | Volume of solution B (ml) | Volume of dilution | The amount of sodium salicylate (µg / ml) | Absorbance (A) |
|---|---|---|---|---|
| 1 | 2,5 | till la 50 ml | 50,0 | 0,077 |
| 2 | 5,0 | till la 50 ml | 100,0 | 0,156 |
| 3 | 7,5 | till la 50 ml | 150,0 | 0,221 |
| 4 | 10,0 | till la 50 ml | 200,0 | 0,299 |
| 5 | 12,5 | till la 50 ml | 250,0 | 0,373 |
| 6 | 15,0 | till la 50 ml | 300,0 | 0,450 |
| 7 | 17,5 | till la 50 ml | 350,0 | 0,540 |
| 8 | 20,0 | till la 50 ml | 400,0 | 0,611 |
| 9 | 22,5 | till la 50 ml | 450,0 | 0,685 |

To 1 ml of the respective dilution add 2 ml of sol. HCl 0.01 mol / L and a drop of sol. $FeCl_3$ 50% and the absorbance is measured on photocolorimeter in visible with the green light filter.

Based on the data obtained, the calibration graph is compiled.

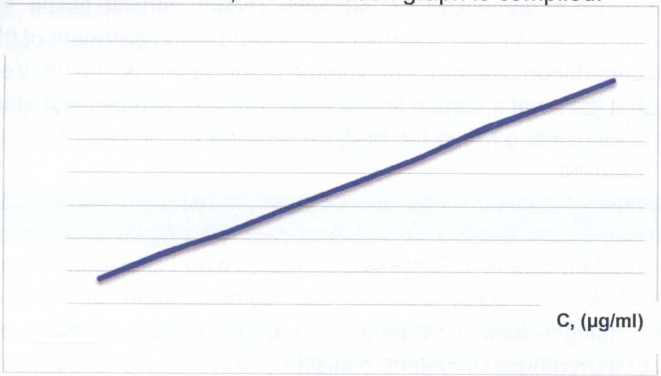



*Fig. 8.* Calibration curve for the determination of sodium salicylate

Evaluation of pharmacokinetic parameters of sodium salicylate using the *in vitro* pharmacokinetic model.

## 2. The pharmacokinetics of the unique dose using the pharmacokinetic model *in vitro.*

### *2. 1. Rapid intravenous administration*

**Objectives:**

- calculation of pharmacokinetic parameters: Kel, t½, Cl.
  for calculations, use:
- sodium salicylate concentrations after single dose administration in the central compartment (in vessel B).

**Apparatus, reagents:**

- Equipment: single-compartment pharmacokinetic model (vessel B); photocolorimeter; pipette 1, 2, 5 ml;
- Reagents: stock solution of 10% sodium salicylate; solution of HCl 0.01 mol/L; ground. FeCl3 50%.

**Experimental procedure:**

In vessel B pour the purified water up to the side drainage tube. Include the regulated magnetic stirrer at a speed that ensures the homogeneity of the contents of the vessel.

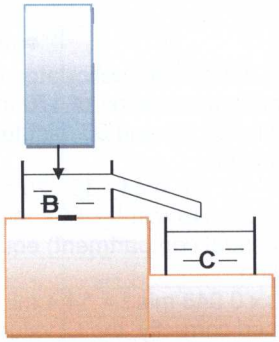



*Fig. 9.* Scheme of the open-label pharmacokinetic model for intravenous administration studies

Purified water is dripped from the tank at a flow rate of 14 ml / min ($k_0$). At the time when the excess water in vessel B begins to drip into vessel C, 1.5 ml of the stock solution of sodium salicylate is introduced into vessel B. Note the initial time $t_0$. At different time intervals, collect 1 ml of the solution from vessel B, in a tube add 2 ml of 0,01 mol / L HCl and 1 drop of sol. $FeCl_3$ 50%. Read absorbance on the photocolorimeter and identify the concentration values in the calibration graph.

The results are tabulated.

- The curve of sodium salicylate concentrations over time will be represented on the millimeter paper or computer, according to the obtained data.
- The graph of natural logarithms of sodium salicylate over time will be plotted.

• Based on the data from the table, calculate the pharmacokinetic parameters: Kel, t½ and Cl.

Concentrations of sodium salicylate in the samples in vessel B (blood compartment)

| Nr. | Sampling time (min) | A (λ = 525 nm) | C(μg/ml) | lnC |
|---|---|---|---|---|
| 1 | 0 | | 350 | 5,85 |
| 2 | 3 | | 330 | 5,79 |
| 3 | 6 | | 300 | 5,70 |
| 4 | 9 | | 270 | 5,59 |
| 5 | 12 | | 240 | 5,48 |
| 6 | 15 | | 210 | 5,34 |
| ***7*** | ***20*** | | ***175*** | ***5,16*** |
| 8 | 25 | | 125 | 4,82 |
| 9 | 30 | | 100 | 4,60 |
| ***10*** | ***35*** | | ***85*** | ***4,44*** |
| 11 | 40 | | 55 | 4,00 |

*Note:* The C (mg/ml) and lnC data are presented as an example for calculating pharmacokinetic parameters.

*Example of calculation:*

To calculate t½, a value of the sodium salicylate concentration is deduced by about half. From the table we take the concentration of 170 mcg / ml ($C_1$) and 85 mcg / ml ($C_2$) and the time respectively 20 minutes ($t_1$) and 35 minutes ($t_2$).

In the beginning Kel is calculated.

$Kel = lnC_1 - lnC_2 / t_2 - t_1 = 5.16 - 4.44 / 35 - 20 = 0.72 / 15 \text{ min} = 0.048 \text{ min}^{-1}$.

$t½ = 0.693 / Kel = 0.693 / 0.048 \text{ min}^{-1} = 14.4 \text{ min}$.

Vd is the volume of vessel B (central compartment) equal to 300 ml.

Cl is calculated from the equation:

$Cl = Vd \times Kel = 300 \text{ mL} \times 0.048 \text{ min}^{-1} = 14.4 \text{ mL / min}$

If the experiment is performed correctly, the Cl value will be close to the chosen water flow rate through the system vessels (14 ml / min).

## 2. 2. Extravascular administration.

**Objectives:**

• Calculation of pharmacokinetic parameters: $C_{max}$; $t_{max}$; kel, $t_{1/2}$, Cl.

For calculations, use:

• sodium salicylate concentrations after a single dose in the central compartment (vessel B).

**Apparatus, reagents:**

• Equipment: the monocomponent pharmacokinetic model (Figure 120); photocolorimeter; pipette 1, 2, 5 ml;

• Reagents: stock solution of 10% sodium salicylate; HCl salt 0.01 mol / L; ground. $FeCl_3$ 50%.

**Experimental procedure:**

Purified water is poured into the vessels A and B up to the side drainage tube. Included are magnetic stirrers set at a speed to ensure the homogeneity of the contents of the vessels.

Purified water is dripped from the tank into vessel A at a flow rate of 10 ml / min ($k_0$).When the excess water in vessel A begins to drip into vessel B, and from vessel B to vessel C, 1 ml of sodium salicylate stock solution is introduced into vessel A.

Note the initial time $t_0$. At different time intervals, sample 1 ml of the solution in vessel B is harvested. Add 2 ml of 0,01 mol/L HCl and 1 drop of sol. $FeCl_3$ 50%. Read the absorbance on the photocolorimeter and read the calibration graph for those concentrations. The results are tabulated.

- The curve of sodium salicylate concentrations over time will be represented on the millimeter paper or computer, according to the data in the table.
- The graph of natural logarithms of sodium salicylate over time will be plotted.
- Based on the table data, calculate the pharmacokinetic parameters: $C_{max}$; $t_{max}$; Ka; Kel; t½; Cl.

Concentrations of sodium salicylate in the samples in vessel B (blood compartment)

| Nr. | Sampling time (min) | A (λ = 525 nm) | C(μg/ml) | lnC |
|---|---|---|---|---|
| 1 | 5 | | 55 | 4,00 |
| *2* | *10* | | *90* | *4,49* |
| 3 | 15 | | 130 | 4,86 |
| *4* | *20* | | *165* | *5,11* |
| 5 | 25 | | 225 | 5,42 |
| ***6*** | ***30*** | | ***270*** | ***5,59*** |
| ***7*** | ***35*** | | ***245*** | ***5,50*** |
| 8 | 40 | | 210 | 5,35 |
| 9 | 45 | | 180 | 5,19 |
| 10 | 50 | | 150 | 5,01 |
| ***11*** | ***55*** | | ***125*** | ***4,82*** |
| 12 | 60 | | 75 | 4,32 |
| 13 | 65 | | 35 | 3,55 |

*Note:* The data from table - C (mg / ml) and lnC are presented as an example for calculating pharmacokinetic parameters.

*Example of calculation:*

$C_{max}$ - can be determined from the concentration curve corresponding to the peak and is equal to 270 (mg / ml);

$T_{max}$ = 30 min.

To calculate $t_{1/2}$, a sodium salicylate concentration value is taken after the maximum concentration has been reached, which is reduced by approximately half. From the table we take the concentration of 245 mg / ml ($C_1$) and 125 mg / ml ($C_2$) and 35 minutes ($t_1$) and 55 minutes ($t_2$) respectively.

In the beginning Kel is calculated.

Kel = lnC1 - lnC2 / t2 - t1 = 5.50 - 4.82 / 55 - 35 = 0.68 / 20 min = 0.034 $min^{-1}$.

$t_{½}$ = 0.693 / Kel = 0.693 / 0.034 $min^{-1}$ = 20.38 min

Vd is the volume of vessel B (central compartment) equal to 300 ml.

Cl is calculated from the equation:

Cl = Vd x Kel = 300 ml x 0.034 $min^{-1}$ = 10.2 ml / min

If the experiment is performed correctly, the Cl value will be close to the chosen water flow rate that passes through the system vessels (10 ml / min).

K abs. = lnC2 - lnC1 / $t_1$ - $t_2$ = 5.11 - 4.49 / 20 - 10 = 0.062 $min^{-1}$

### *2. 3. Pharmacokinetics of repeated doses*

**Objectives:**

- Determination of steady-state equilibrium values ($\bar{C}$); $C_{max}$ and $C_{min}$ in the single-compartmental pharmacokinetic model using the in vitro model and the pharmacokinetic parameters calculated for a single intravenous dose.
- Drug level simulation after repeated doses administered intravenously at a constant dosing interval.

**Apparatus, reagents:**

- Equipment: single-compartment pharmacokinetic model (vessel B); photocolorimeter; pipette 1, 2, 5 ml;
- Reagents: stock solution of 10% sodium salicylate; HCl salt 0.01 mol / L; ground. FeCl3 50%.

**Experimental procedure:**

In vessel B pour purified water up to the side drainage tube. Include the regulated magnetic stirrer at a speed that ensures the homogeneity of the contents of the vessel. From the tank in vessel B, purified water is dripped at a flow rate of 14 ml / min ($K_0$).

When the surplus of water in vessel B begins to drip into vessel C, 0.5 ml of sodium salicylate stock solution (10%) is introduced into vessel B at 10 minute intervals. Note the initial time $t_0$. Take 0.5 ml samples of vessel B according to the order in the table. For each sample, add 2 ml of 0.01 mol / L HCl and 1 drop of sol. $FeCl_3$ 50%. Read the absorbance on the photocolorimeter and read the calibration graph for those concentrations. The results are tabulated.

Concentrations of sodium salicylate in the samples in vessel B

| Nr. | Sampling time (min) | A (λ = 525 nm) | C(μg/ml) |
|---|---|---|---|
| | **0** | (0,5 ml stock solution) | |
| 1 | 0,5 | | |
| 2 | 5 | | |
| 3 | *9,5* | | |
| | **10** | (0,5ml stock solution) | |
| 4 | *10,5* | | |
| 5 | 15 | | |

| | | | |
|---|---|---|---|
| 6 | *19,5* | | |
| | **20** | (0,5 ml stock solution) | |
| 7 | *20,5* | | |
| 8 | 25 | | |
| 9 | 29,5 | | |
| | **30** | (0,5 ml stock solution) | |
| 10 | *30,5* | | |
| 11 | 35 | | |
| 12 | *39,5* | | |
| | **40** | (0,5 ml stock solution) | |
| 13 | *40,5* | | |
| 14 | 45 | | |
| 15 | *49,5* | | |
| | **50** | (0,5 ml stock solution) | |
| 16 | *50,5* | | |
| 17 | 55 | | |
| 18 | *59,5* | | |

• The curve of sodium salicylate concentrations over time will be represented on the millimeter paper or computer, according to the data in the table.
• Calculate steady-state equilibrium (Ĉ);
• Based on the data in the table, determine the pharmacokinetic parameters: $C_{max}$; $C_{min}$.

$$\hat{C} = D/V_d \times K_{el} \times \tau$$

where: Ĉ - steady state equilibrium;
D - administered dose; Vd - distribution volume;
Kel - elimination constant;
τ - the dosing interval between two successive doses.

*Example of calculation:*

**D** = 100 mg;
$\mathbf{V_d}$ = 300 ml;
$\mathbf{K_{el}}$ = 0,048 $min^{-1}$
**T**= 10 min
**Ĉ =** 100 mg/300 ml x 0,048 $min^{-1}$ x 10 min = **0,1584 mg/ml (158,4 µg/ml)**

$$C_{max} = D/V_d \times 1/1 - e^{-Kel \times \tau}$$

where: D = administered dose; Vd = vessel volume B;
kel = the speed of elimination constant (calculated at work No. 2);
t = dosing interval (between two successive doses); (e = 2.72).

$$C_{min} = D/V_d \times e^{-Kel \times \tau} / 1 - e^{-Kel \times \tau}$$

Compare the values obtained from the calculations with those observed experimentally.

## 2. 4. Intravenous perfusion

**Objectives:**

- Determining the period of accumulation of drug to steady state (plateau) and its concentration at any time during infusion.
- For this experiment, it is necessary to know the rate of elimination constant after administration of a single intravenous dose (vessel B) and vessel volume (Vd).

**Apparatus, reagents:**

- Equipment: single-compartment pharmacokinetic model (vessel B); photocolorimeter; pipette 1, 2, 5 ml;
- Reagents: stock solution of 10% sodium salicylate; solution of HCl 0.01 mol/L; solution of $FeCl_3$ 50%.

***The following calculations are used:***

1. Pharmacokinetic parameters following a single intravenous dose:

   Kel = 0.082 min-1

   Vd = 300 ml (vessel volume B)

2. Drug level in stationary state (CSS)

   CSS = 300 µg / ml

3. Set the infusion rate

   **$K_0$ = CSS x Kel x Vd** = 300 µg / ml x 0.082 min-1 x 300 ml = 7.38 mg / min

4. Calculation of the concentration of perfused solution (10% sodium salicylate)

   Infusion rate 16 ml / min

   16 ml ..... .7.38 mg

   1000 ml ..... X mg

   X = 461, 25 mg = 0.461 g

   or 4.6 ml of 10% solution and purified water to 1000 ml

**Experimental procedure:**

Introduce 1 liter of perfusion containing sodium salicylate into the reservoir in the calculated amount (4.6 ml of solution of 10%). Assure infusion rate of 16 ml / min.

Vessel B is filled with purified water. The magnetic stirrer is included and adjusted to a rate that ensures the homogeneity of the contents of the vessel. When liquid begins to drip from vessel B, note t0. Infusion continues for an hour. From vessel B samples of 1 ml are taken at the time intervals indicated in the table.

For each sample, add 2 ml of 0.01 mol / L HCl and 1 drop of sol. FeCl3 50%. Read the absorbance on the photocolorimeter and read the calibration graph for those concentrations. The results are tabulated.

Concentrations of sodium salicylate in the samples in vessel B

| Nr. | Sampling time (min) | A (525 nm) | C (µg/ml) |
|---|---|---|---|
| 1 | 0 | | |
| 2 | 3 | | |
| 3 | 6 | | |
| 4 | 9 | | |
| 5 | 12 | | |
| 6 | 15 | | |
| 7 | 20 | | |
| 8 | 25 | | |
| 9 | 30 | | |
| 10 | 35 | | |
| 11 | 40 | | |
| 12 | 45 | | |
| 13 | 50 | | |
| 14 | 55 | | |
| 15 | 60 | | |

*Graphic representations:*

Graphically plot concentrations over time. The sodium salicylate is accumulated in the graph to the concentration of the stationary state. Note the time of reaching it. Compare it to the theoretical value (t = 5 x t1 / 2).

The concentration of a drug substance in the central compartment (vessel B) CB at any time during the infusion of a drug solution can be calculated according to the equation:

$$C_B = k_p / k_{el} * V_B (1-e^{-Kel * t})$$

where:

Kp = the infusion rate constant, which expresses the rate at which the drug solution (and not the pure solvent) drips from the reservoir into the in vitro system (liquid flow was denoted by k0);

Kel = the rate of elimination constant (kel = k0 / VB);

Vb = vessel volume B (VB = dose / CB0).

At the time when the stationary state (plateau) is reached, the exponential is canceled and the expression becomes

$$C_{Bss} = k_0 / k_{el} * V_B$$

where:

$C_{BSS}$ is the steady state concentration achieved in a time period equal to about 5-6 x t½.

## 3. Evaluation of biopharmaceutical parameters of drug substances from different pharmaceutical forms using the pharmacokinetic *in vitro* model

### *3.1. Pharmaceutical forms with rapid disintegration*

**Sodium salicylate - 300 mg**
**Starch - 100 mg**
**Lactose - 100 mg**
**Total mass 500mg**

### *3.2. Pharmaceutical forms with slow disintegration*

**Sodium salicylate - 300 mg**
**Sodium carboxymethyl cellulose - 200 mg**
**Total mass 500 mg**

**Apparatus, reagents:**

- Equipment: pharmacokinetic mono-compartment open model (vessel B); photocolorimeter; pipette 1, 2, 5 ml;
- Reagents: stock solution of 10% sodium salicylate; solution of HCl of 0.01 mol / L; solution of $FeCl_3$ 50%.

**Experimental procedure:**

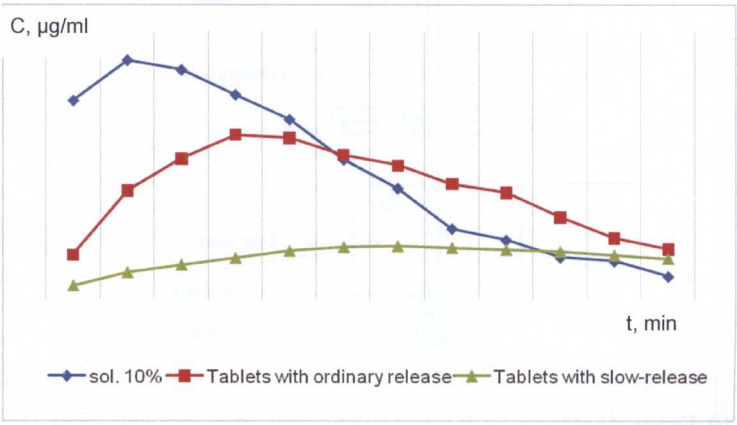
Purified water is poured into the vessels A and B up to the side drainage tube. Included are magnetic stirrers set at a speed to ensure the homogeneity of the contents of the vessels. Purified water is dripped from the tank into vessel A at a flow rate of 10 ml / min ($k_0$). When the surplus of water in vessel A begins to drip into vessel B, and from vessel B to vessel C, pharmaceutical forms placed in the stainless steel basket are introduced into container A. Note the initial time $t_0$. Take 1 ml samples from vessel B at intervals of 10 minutes. for 120 min.

For each sample, add 2 ml of 0.01 mol / L HCl and 1 drop of sol. $FeCl_3$ 50%. Read the absorbance on the photocolorimeter and read the calibration graph for those concentrations. The results are shown in the table (example).

| f/f | Sampling time, min / Concentration of sodium salicylate, μg / ml | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | **20** | 30 | **40** | 50 | 60 | **70** | **80** | 90 | 100 | 110 | 120 |
| 1 | 520 | **625** | 600 | 535 | 470 | 365 | 290 | 185 | 155 | 110 | 100 | 60 |
| 2 | 117 | 284 | 368 | **430** | 422 | 378 | 350 | 302 | 280 | 215 | 160 | 132 |
| 3 | 35 | 70 | 90 | 108 | 127 | 137 | **139** | 135 | 130 | 125 | 115 | 107 |

*Note:* The C (mg/ml) data from table is presented as an example for calculating pharmacokinetic parameters.

*Graphic representation:*




Calculate the relative bioavailability of sodium salicylate in tablets to the solution.

To begin with, calculate the area under the curve for all pharmaceutical forms by the equation:

$AUC_{0-12}$ = area 1 + area 2 + area 3 + ... + area 11 + area 12 = ½ (C0 + C1) (t1 - t0) + ½ (C1 + C2) ) (t3 - t2) + ..... + ½ (C10 + C11) (t11 - t10) + ½ (C11 + C12) (t12 - t11).

Calculate the relative bioavailability after the equation:

**BD (%) rel. = AUC test / AUC ref. x 100;**

Compare BD for the 2 tablet formulations.

Calculate Cmax; tmax .; kel, t½, Cl for all forms.
*Graphic representations:* plot concentrations over time. Conclusions.

## 4. Study the release of drug substance from different pharmaceutical forms. Dissolution testing

### *4. 1. Dissolution testing for capsules and pills*

Dissolution means dissolving. It is a vital first step when medicinal drugs are taken in the form of tablets and capsules. Rate of dissolution is an important property of a medicine as it indicates how quickly the drug in a formulation is released in the body and made available for absorption.

There are two main methods

- the rotating basket;
- the paddle.

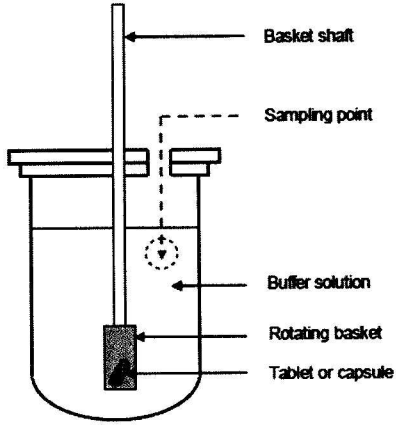



*Fig. 10.* Experimental set up for determining rate of dissolution using rotated basket method
*https://www.stem.org.uk/system/files/elibrary-resources/legacy_files_migrated/33410-RSCDissolutiontesting.pdf*

**Ojectives**

- Study of the dissolution rate of drug substances in tablets or capsules.

**Apparatus, reagents:**

- Dissolving Speed Determination Device, marketed by Erweka (see "bioavailability" section);
- 3 types of paracetamol produced by different manufacturers (or other preparation);
- 0.01 L hydrogen chloride solution; filter paper, funnels, tubes, pipettes of 5 ml, 2 ml and 1 ml, 100 ml bottles mol /.
- UV-VIS spectrophotometer.

**Experimental procedure:**

In the cylindrical vessel of the dissolution apparatus, 500 ml dissolving medium is introduced. Heat at 37°C. Introduce in the rotating basket: one tablet (0.5 g paracetamol) which is then placed in the dissolution medium. Adjust the shaking to 100 rpm. Take 5 ml samples after 10, 20, 30, 45 and 60 minutes with a pipette and filter through a paper filter, removing the first portions of the filter. For a dosing: 0,6 ml is placed in a 100 ml volumetric flask and made up to 0,01 mol/L (0,01 mol/l sodium hydroxide solution).

Read the absorbance of the solution on the spectrophotometer in UV at the wavelength of 257 nm in the cuvette of 10 mm layer thickness as the reference solution using 0,01 mol/L or 0,01 mol sodium hydroxide solution /IT.

The content of paracetamol (X,%) which has been given to the dissolution medium is calculated according to the formula:

$$X,\% = \frac{A_x \times m_{st} \times Vst_1 \times V_1 \times V_3 \times 100\ \%}{A_{st} \times Vst_2 \times Vst_3 \times m_x \times V_2}$$, where:

Axis - absorbance of the solution to be investigated;
Ast - the absorbance of the standard solution (0.5524);
Mst - mass of the standard sample of paracetamol (0,1557 g);
mx - mass of paracetamol in tablet (0.5 g);
Vst1 - volume of soil. standard taken for analysis (0.5 ml);
Vst2 - volume of the solution in which the standard sample was dissolved (100 ml);
Vst3 - volume of dilution of the standard solution (100 ml);
V1 - volume of dissolution medium (500 ml);
V2 - the volume of the filtrate taken for dosing (0.6 ml);
V3 - volume of the quenched flask in which the sample to be analyzed was diluted (100 ml)

The results are shown in the table below:

Dissolution results of 2 tablet products

| t, min | $Q_0$ (mg) | Assortment 1 | | | | Assortment 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | C,% | $Q_t$ (mg) | $Q_0-Q_t$ (mg) | ln $Q_0-Q$ | C,% | $Q_t$ (mg) | $Q_0-Q_t$ (mg) | ln $Q_0-Q$ |
| **10** | 500 | **35** | 175 | 325 | 5,78 | **23** | 115 | 385 | 5,95 |
| **20** | 500 | **48** | 240 | 260 | 5,56 | **28** | 140 | 360 | 5,88 |
| **30** | 500 | **69** | 345 | 155 | 5,04 | **38** | 190 | 310 | 5,73 |
| **45** | 500 | **80** | 400 | 100 | 4,60 | **42** | 210 | 290 | 5,66 |
| **60** | 500 | **92** | 460 | 40 | 3,68 | **58** | 290 | 210 | 5,34 |

*Note:* The data from table represent an example for calculation ofdissolution constant rate

*Graphic representation*

1. Curves of the percentage dissolved by time in numerical (cartesian) graph for all assortments researched in both dissolution media.
2. Determine the percentage of dissolution of paracetamol for each assortment in 45 minutes in both dissolution media.
3. In the semilogarithmic graph, plot the remaining percentage to dissolve (rest dissolving - RD), ($Q_0-Q_t$) by time, introducing RD on the logarithmic scale.

Calculate the dissolution rate constant

$$k_d = \ln C_1 - \ln C_2 / t_2 - t_1\ (min^{-1})$$

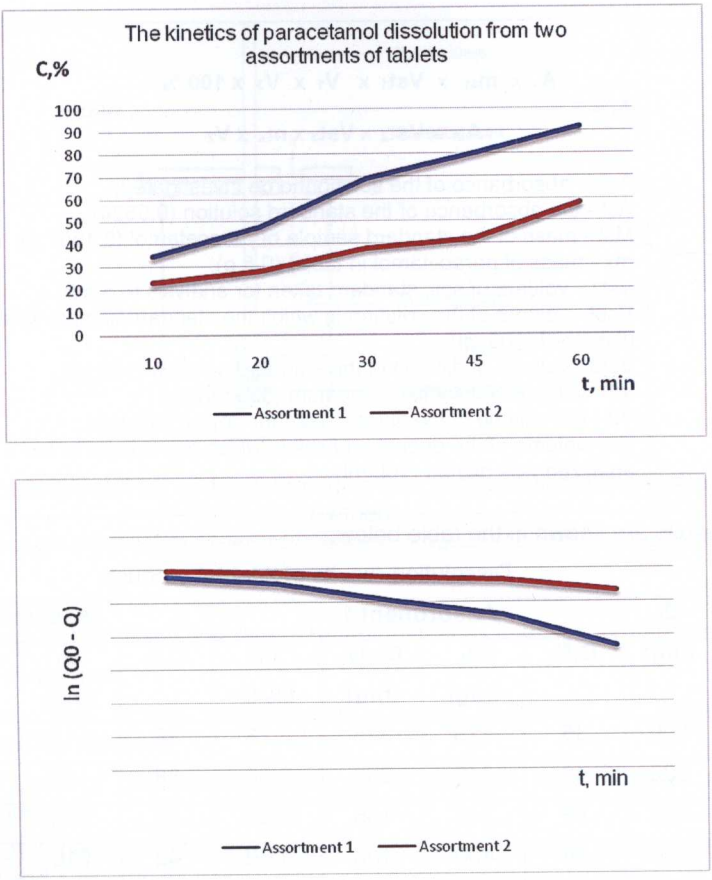



*Fig. 10.* The kinetics of dissolution of paracetamol from tablets

Kd calculation for both assortments:

$$k_d = lnC_1 - lnC_2 / t_2 - t_1 \ (min^{-1}); \text{ where } C = Q_0 - Q_t$$

**Assortment 1.**

$k_{d1} = 5,78 - 5,56 / 20 - 10 = 0,022\ min^{-1}$;

$k_{d2} = 5,56 - 5,04 / 30 - 20 = 0,052\ min^{-1}$;

$k_{d3} = 5,04 - 4,60 / 45 - 30 = 0,029\ min^{-1}$;

$k_{d4} = 4,60 - 3,68 / 60 - 45 = 0,061\ min^{-1}$;

$\mathbf{K_{d\ average} = k_{d1} + k_{d2} + k_{d3} + k_{d4} / 4 = 0,0410\ min^{-1}}$

**Assortment** 2.

$k_{d1} = 5,95 - 5,88 / 20 - 10 = 0,007\ min^{-1}$;

$k_{d2} = 5,88 - 5,73 / 30 - 20 = 0,015\ min^{-1}$;

$k_{d3} = 5,73 - 5,66 / 45 - 30 = 0,004\ min^{-1}$;

$k_{d4} = 5,66 - 5,34 / 60 - 45 = 0,021\ min^{-1}$;

$\mathbf{K_{d\ average} = k_{d1} + k_{d2} + k_{d3} + k_{d4} / 4 = 0,0117\ min^{-1}}$

The dissolution constant for assortment 2 is 3.5 times lower than for assortment 1.

Comparison of dissolution profiles of two products can be performed using the model independent model by means of two factors: difference factor ($f_1$) and similarity factor ($f_2$).

The difference factor expresses the percentage difference between 2 curves at each point and is an expression of the relative error between the two curves. It can be calculated by:

$$f_1 = \sum_{i=1}^{n} [\chi_r - \chi_t] / \sum_{i=1}^{n} \chi_r \times 100$$

in which:

n - the number of points of the profile;

$\chi_r$ - the average of the results at time t for the reference medicine;

$\chi_t$ - the average of the results at time t for the test drug.

The similarity factor is a logarithmic transformation of the reciprocal of the sum of the square errors and expresses the similarity in percent between the two curves.

$$f_2 = 50 \log \{[1 + (1/n) \sum_{i=1}^{n} (\chi_r - \chi_t)^2]^{-1/2} \times 100\}$$

in which:

n - the number of points of the profile;

$\chi_r$ - the average of the results at time t for the reference medicine;

$\chi_t$ - the average of the results at time t for the test drug.

Example of calculation:

Two types of tablet-containing paracetamol contain 500 mg of the active substance, giving the following dissolution profile results:

| Timp (min.) | Dissolved quantity (%) | |
|---|---|---|
| | $\chi_r$(assortment *1*) | $\chi_t$ (assortment *2*) |
| 10 | 35 | 23 |
| 20 | 48 | 28 |
| 30 | 69 | 38 |
| 45 | 80 | 42 |
| 60 | 92 | 58 |

Solution:

| $X_r$ | $X_t$ | $X_r - X_t$ | $(X_r - X_t)^2$ |
|---|---|---|---|
| 35 | 23 | 12 | 144 |
| 48 | 28 | 20 | 400 |
| 69 | 38 | 31 | 961 |
| 80 | 42 | 38 | 1444 |
| 92 | 58 | 34 | **1156** |

Factor $f_1$

$$f_1 = \frac{12 + 20 + 31 + 38 + 34}{35 + 48 + 69 + 80 + 92} \times 100 = \frac{135}{189} \approx 0{,}714 \times 100 = 41{,}6\%$$

Factor $f_2$

$$f_2 = 50 \log \frac{100}{\sqrt{1 + \sum (X_r - X_t)^2 (1/n)}} = \frac{100}{\sqrt{1 + 4105\,(1/5)}} =$$

$$= 50 \log \frac{100}{\sqrt{1 + 4105\,(0{,}2)}} = 50 \log \frac{100}{\sqrt{821{,}2}} = 50 \log \frac{100}{28{,}65} = 50 \times 0{,}54 = 27{,}0\%$$

U.S. Food and drug administration (FDA) recommend that these values to be included: $f_1 = 0$ -15% and $f_2 = 50$ ¬-100%.

According to the calculations, the difference factor ($f_1$ = 41.6%) and the similarity ($f_2$ = 27.0%) do not fall within the limits of the recommended values.

Thus, we can conclude that for assortment 2 (tested) there is no similarity to the reference one.

This result is also confirmed by the Dissolution Percentage, which is only 42% over 45 minutes. At the reference, this index is 80%, which corresponds to pharmacopoeial requirements (not less than 75% in 45 min).

### *4. 2. Dissolution testing for ointments and suppositories*

**Objectives:**

- Study of the dissolution rate of sodium salicylate in ointment, gels and suppositories.

**Apparatus, materials**:

- The dissolution rate determination apparatus, marketed by Erweka (fig. 12, fig. 13).
- Reagents: HCl salt 0.01 mol / L; solution of $FeCl_3$ 50%,

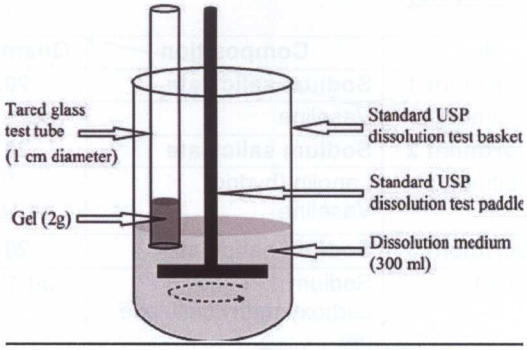



*Fig. 12.* Apparatus for the study of dissolution test in ointments
*gelshttps://www.researchgate.net/figure/Modi-fi-ed-USP-XXIII-in-vitro-dissolution-testing-apparatus_fig1_41563035*

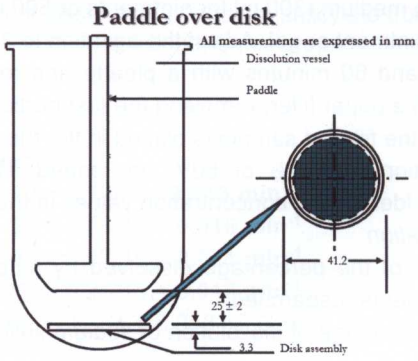



*Fig. 13.* Apparatus for the study of dissolution test in suppositories
*https://knowledgeofpharma.blogspot.com/2017/04/dissolution-apparatus-apparatus-5.html*

**3 assortments of suppositories:**

| Nr. | Composition | Quantity, g |
|---|---|---|
| **Assortment 1** | **Sodium salicylate** | **0,25** |
| | Cacao butter | ad 2,0 |
| **Assortment 2** | **Sodium salicylate** | **0,25** |
| | Semisynthetic glycerides | ad 2,0 |
| **Assortment 3** | **Sodium salicylate** | **0,25** |
| | PEG 6000<br>PEG 1500<br>PEG 400 (5:3:2) | ad 2,0 |

**3 assortments of ointments:**

| Nr. | Composition | Quantity, g |
|---|---|---|
| **Assortment 1** | **Sodium salicylate** | **20,0** |
| (ointment) | Vaseline | ad 100,0 |
| **Assortment 2** | **Sodium salicylate** | **20,0** |
| (ointment) | Lanolin (hydric)<br>Vaseline | 10<br>ad 100,0 |
| **Assortment 3** | **Sodium salicylate** | **20,0** |
| Gel | Sodium carboxymethylcellulose gel | ad 100,0 |

**Experimental procedure:**

In the dissolution medium (300 ml for ointments or 500 ml for suppositories) place a suppository or 1 g ointment or gel. Adjust the agitation to 25 rpm. Take 5 ml samples after 10, 20, 30, 45 and 60 minutes with a pipette and return with 5 ml of dialysis medium, filter through a paper filter, removing the first portions of the filtrate.

For a dosing: 1 ml of the filtered sample is placed in the tube, 2 ml of 0,01 mol / L HCl and 1 drop of solution of $FeCl_3$ of 50% are added. Read absorbance on the photocolorimeter and identify the concentration values in the calibration graph.

*Graphic representation*

1. Calculate curves of the percentage dissolved by time in numerical (cartesian) graph for all assortments researched.
2. Determine the percentage of dissolution of sodium salicylate for each assortment in 45 minutes.
3. In the semilogarithmic graph, plot the percentage remaining to dissolve (rest dissolving - RD), $(Q_0-Q_t)$ by time, introducing RD on the logarithmic scale. Calculate the dissolution rate constant.

$$k_d = \ln C_1 - \ln C_2 / t_2 - t_1 \ (min^{-1})$$

Example of calculation:

Dissolution results of 1 assortment of suppository

| t, min | Cacoa butter | | | |
|---|---|---|---|---|
| | C,% | $Q_t$ (mg) | $Q_0-Q_t$ (mg) | ln $Q_0-Q_t$ |
| 10 | 15,0 | 75,0 | 425,0 | 6,05 |
| 20 | 25,0 | 125,0 | 375,0 | 5,92 |
| 30 | 32,0 | 180,0 | 320,0 | 5,76 |
| 45 | 45,0 | 230,0 | 270,0 | 5,59 |
| 60 | 58,0 | 290,0 | 210,0 | 5,34 |

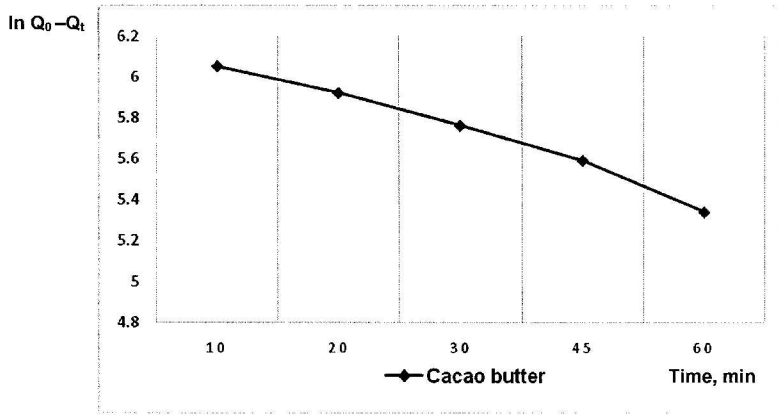



*Fig. 14.* Kinetics of dissolution of sodium salicylate from suppositories in semilogarithmic graph

**$k_d = \ln c_1 - \ln c_2 / t_2 - t_1$ ($min^{-1}$)**

$k_{d1}$ = 6,05 – 5,92 / 20 – 10 = 0,13/10= **0,013 $min^{-1}$**

$k_{d2}$ = 5,92 – 5,76 / 30 – 20 = 0,16/10 = **0,016 $min^{-1}$**

$k_{d3}$ = 5,76 – 5,59 / 45 – 30 = 0,17/15 = **0,011 $min^{-1}$**

$k_{d4}$ = 5,59 – 5,34 / 60– 45 = 0,25/15 = **0,016 $min^{-1}$**

$K_{d\ average}$= $k_{d1}$ + $k_{d2}$ + $k_{d3}$ + $k_{d4}$ /4 = 0,013 +0,016 +0,011 +0,016 /4 =

= 0,056/4 = **0,014$min^{-1}$**

Compare the values for all investigated assortments.

Conclusions should be made about the availability of sodium salicylate in semi-solid forms and the influence of the nature of the excipient.

# PROBLEMS

**Objectives**: In order to verify the knowledge of the biochemistry and pharmacokinetics obtained during the execution of the practical works, it is proposed to solve a number of problems.

**Problem models:**

**Problem nr. 1**

Calculate the pharmacokinetic parameters: t ½, Kel; $T_{max}$; Vd = 15 l. Indicate the route of administration of the drug:

| t, h | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C, µg/ml | 292 | 285 | 274 | 250 | 239 | 216 | 186 | 173 | 152 | 120 | 105 | 92 |
| lnC | 5,67 | 5,65 | 5,61 | 5,52 | 5,47 | 5,37 | 5,22 | 5,15 | 5,02 | 4,78 | 4,65 | 4,52 |

**Problem nr. 2**

Calculate the pharmacokinetic parameters: t ½, Kel, Kabs, Cl, $C_{max}$, $T_{max}$; Vd = 8 l. Indicate the route of administration of the drug.

| t, h | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C, µg/ml | 23,2 | 28,5 | 35.4 | 38,6 | 49,0 | 56,7 | 52,3 | 43,3 | 32,5 | 20,5 | 15,4 | 10,2 |
| lnC | 3,41 | 3,34 | 3,56 | 3,65 | 3,89 | 4,03 | 3,95 | 3,79 | 3,48 | 3,02 | 2,73 | 2,32 |

**Problem nr. 3**

Determine the relative bioavailability of acetaminophen (250 mg) from suppositories prepared on different excipients:

| Time, (hours) | Plasma concentration µg/ml | | |
|---|---|---|---|
| | Syrup | Suppositories | |
| | | Semisynthetic glycerides | Macrogols |
| 1 | 84 | 54 | 30 |
| 2 | 105 | 78 | 41 |
| 3 | 129 | 92 | 80 |
| 4 | 138 | 117 | 95 |
| 5 | 150 | 137 | 110 |
| 6 | 135 | 155 | 80 |
| 7 | 120 | 126 | 62 |
| 8 | 85 | 88 | 42 |
| 9 | 60 | 47 | 32 |
| 10 | 35 | 31 | 10 |

## Problem nr.4

Based on plasma concentration data, calculate the absolute bioavailability of diclofenac in suppositories. Calculate t50% of diclofenac for suppositories; indicate $C_{max}$.

| t, h | C, mg/ml, suppositories | ln C | C, mg/ml, injectable solution |
|---|---|---|---|
| 1 | 32,8 | 3,49 | 52,3 |
| 2 | 91,8 | 4,51 | 105,4 |
| 3 | 101,2 | 4,61 | 120,6 |
| 4 | 115,8 | 4,75 | 138,4 |
| 5 | 128,9 | 4,85 | 156,8 |
| 6 | 90,3 | 4,50 | 123,5 |
| 7 | 65,5 | 4,18 | 110,4 |
| 8 | 41,6 | 3,72 | 92,6 |
| 9 | 32,6 | 3,48 | 33,2 |
| 10 | 20,2 | 3,00 | 15,4 |
| 11 | 11,3 | 2,42 | 6,8 |

## Problem nr. 5

Calculate the absolute bioavailability of piroxicam in suppositories and capsules based on single-dose 20 mg; indicate $C_{max}$; $t_{max}$.

| time,h | **Concentration, µg/ml** | | |
|---|---|---|---|
| | injectable solution | suppositories | capsules |
| 1 | 370 | 210 | 146 |
| 2 | 460 | 315 | 168 |
| 3 | 390 | 340 | 199 |
| 4 | 290 | 360 | 218 |
| 5 | 230 | 395 | 256 |
| 6 | 180 | 360 | 287 |
| 7 | 160 | 305 | 298 |
| 8 | 130 | 275 | 265 |
| 9 | 100 | 205 | 215 |
| 10 | 95 | 170 | 187 |
| 11 | 80 | 133 | 120 |
| 12 | 62 | 106 | 89 |

## Problem nr 6

Based on plasma concentrations, compare the absolute bioavailability of diclofenac, given in volunteers at a single dose of 100 mg. Calculate t50% for both forms; $C_{max}$; $T_{max}$.

| Time, hours | C, µg/ml, tablets | lnC | C, µg/ml, injectable solution | lnC |
|---|---|---|---|---|
| 1 | 32,8 | 3,49 | 182,3 | 5,20 |
| 2 | 91,8 | 4,51 | 165,4 | 5,11 |
| 3 | 101,2 | 4,61 | 130,6 | 4,87 |
| 4 | 115,8 | 4,75 | 118,4 | 4,77 |
| 6 | 128,9 | 4,85 | 106,8 | 4,67 |
| 8 | 90,3 | 4,50 | 83,5 | 4,42 |
| 10 | 62,5 | 4,13 | 60,4 | 4,10 |
| 12 | 41,6 | 3,73 | 42,6 | 3,75 |
| 14 | 32,6 | 3,48 | 23,2 | 3,14 |

## Problem nr. 7

Based on plasma concentrations, compare the absolute bioavailability of etamsylate, given to volunteers at a dose of 250 mg once. Calculate t50% for both forms; $C_{max}$; $t_{max}$.

| Time, hours | C, µg/ml, injectable solution | lnC | C, µg/ml, capsules | lnC |
|---|---|---|---|---|
| 1 | 282,8 | 5,64 | 92,3 | 4,52 |
| 2 | 261,8 | 5,56 | 175,4 | 5,16 |
| 3 | 241,2 | 5,48 | 200,6 | 5,30 |
| 4 | 215,8 | 5,37 | 218,4 | 5,38 |
| 5 | 188,9 | 5,24 | 196,8 | 5,28 |
| 6 | 160,3 | 5,07 | 163,5 | 5,09 |
| 8 | 122,5 | 4,80 | 120,4 | 4,78 |
| 10 | 81,6 | 4,40 | 82,6 | 4,41 |
| 12 | 62,6 | 4,13 | 43,2 | 3,76 |
| 14 | 40,2 | 3,69 | 25,4 | 3,23 |
| 16 | 32,3 | 3,47 | 17,8 | 2,87 |

**Problem nr. 8**

Based on plasma concentrations, compare the bioavailability of phenoxymethylpenicillin in tablets to syrup given to volunteers at a single dose of 250 mg; $C_{max}$; $T_{max}$.

| Time, hours | C, mg/ml, tablets | C, mg/ml, syrup |
|---|---|---|
| 1 | 22,8 | 72,3 |
| 2 | 81,8 | 145,4 |
| 3 | 91,2 | 170,6 |
| 4 | 105,8 | 188,4 |
| 5 | 118,9 | 166,8 |
| 6 | 80,3 | 143,5 |
| 8 | 52,5 | 120,4 |
| 10 | 31,6 | 82,6 |
| 12 | 22,6 | 33,2 |
| 14 | 10,2 | 15,4 |
| 16 | 6,3 | 6,8 |

**Problem nr.9**

Based on plasma concentrations, compare the absolute bioavailability of diclofenac given to volunteers at a single dose of 100 mg. Calculate t50% for both forms; $C_{max}$; $T_{max}$.

| Time, hours | C, µg/ml, tablets | lnC | C, µg/ml, injectable solution | lnC |
|---|---|---|---|---|
| 1 | 32,8 | 3,49 | 182,3 | 5,20 |
| 2 | 91,8 | 4,51 | 165,4 | 5,11 |
| 3 | 101,2 | 4,61 | 130,6 | 4,87 |
| 4 | 115,8 | 4,75 | 118,4 | 4,77 |
| 6 | 128,9 | 4,85 | 106,8 | 4,67 |
| 8 | 90,3 | 4,50 | 83,5 | 4,42 |
| 10 | 62,5 | 4,13 | 60,4 | 4,10 |
| 12 | 41,6 | 3,73 | 42,6 | 3,75 |
| 14 | 32,6 | 3,48 | 23,2 | 3,14 |

**Problem nr.10**

Based on plasma data, compare the relative bioavailability of erythromycin, capsules and tablets, to volunteers at a dose of 250 mg once. Indicate Cmax; Tmax.

| Time, hours | C, mg/ml, tablets | C, mg/ml, capsules | C, mg/ml, suspension |
|---|---|---|---|
| 1 | 42,8 | 82,3 | 102,0 |
| 2 | 91,8 | 105,4 | 135,8 |
| 3 | 101,2 | 110,6 | 156,3 |
| 4 | 125,8 | 128,4 | 176,8 |
| 5 | 138,9 | 156,8 | 198,7 |
| 6 | 120,3 | 133,5 | 165,3 |
| 8 | 92,5 | 100,4 | 128,3 |
| 10 | 61,6 | 82,6 | 103,5 |
| 12 | 22,6 | 33,2 | 45,6, |
| 14 | 10,2 | 15,4 | 23,6 |

**Problem nr. 11**

Based on plasma concentrations, compare the ibuprofen bioavailability of tablets to capsules given at 400 mg once-a-day volunteers. Calculate t50%. for both forms; $C_{max}$; $T_{max}$.

| Time, hours | C, mg/ml, tablets | lnC | C, mg/ml, capsules | lnC |
|---|---|---|---|---|
| 1 | 32,8 | 3,49 | 92,3 | 4,52 |
| 2 | 91,8 | 4,51 | 115,4 | 4,74 |
| 3 | 111,2 | 4,71 | 130,6 | 4,87 |
| 4 | 125,8 | 4,83 | 148,4 | 4,99 |
| 5 | 138,9 | 4,93 | 166,8 | 5,11 |
| 6 | 140,3 | 4,94 | 173,5 | 5,15 |
| 7 | 132,5 | 4,88 | 180,4 | 5,19 |
| 8 | 121,6 | 4,80 | 192,6 | 5,26 |
| 9 | 102,6 | 4,63 | 163,2 | 5,09 |
| 10 | 80,2 | 4,38 | 150,4 | 5,01 |
| 12 | 71,3 | 4,26 | 126,8 | 4,84 |
| 14 | 60,5 | 4,10 | 92,6 | 4,52 |
| 16 | 46,0 | 3,82 | 63,2 | 4,14 |

**Problem nr. 12**

Calculate the infusion rate of the 150 mg / 3ml amiodarone (infusion concentrate) solution for injection to provide a steady-state plasma concentration of 1.58 mg% (Css). t1 / 2 = 5.3 hours; Vd = 26 L. What will be the volume of concentrate required to be added to the infusion?

**Solution:**

$K_0$ = Css * Kel * Vd = 15.8 mg / l * 0.130 $hour^{-1}$ * 26 l = 53.4 mg / hr

150 mg ---------- 3 ml
53.4 mg ---------- X ml
X = 1.068 ml ~ 1 ml added to the solvent (physiological solution) for infusion over 1 hour.

**Problem nr. 13**

Calculate the steady state steady state of the drug given at 500 mg at 8-hour intervals if the half-life (T½) is 3 hours and has a volume of distribution (Vd) of 20 liters and the bioavailability (BD ) of 70%.

**Problem nr. 14**

Calculate the sustained dose for 24 hours for the sustained release pharmaceutical form. Stationary stationary concentration of 0.5 mg% (Css). The initial fast-delivery dose that provides effective concentration is 150 mg ($C_O$); t½ = 5.6 hours; Vd = 28 L.

**Solution:**

$K_0 = \hat{C} * Vd * Kel$
$K_0$ - drug flow
$\hat{C}$ - 0.5 mg% (5 mg / l)
Vd - 28 l
t½ - 5.6 hours (Kel = 0.693 / t½)
K0 = 5 mg / l x 28 x 0.693 / 5 = 19.4 mg / hr
for 24 hours (24 x 19.4) = 465.6 mg
A - initial dose = 150 mg
B - support dose = 465.5 mg
C = A + B = 150 + 465 = 615 mg

**Problem nr. 15**

Itraconazole should be given in capsules (100 mg) to a 70 kg patient. The absorption of the preparation is 90% (BD = F-bioavailability, or fraction of the absorbed dose). t1 / 2 = 6 hours; Vd = 0.36 l / kg, and the steady-state (effective) plasma concentration is 10 mg / l. The doctor wants to administer the drug at 6-hour intervals. What dose will you prescribe?

**Solution:**

Vd = 0.36 l x 70 kg = 25.2 l.
Kel = 0.693 / 6 = 0.1155 hr-1
D = Css x Kel x Vd x t / F = 10 mg / l x 0.1155 hr-1 x 25.2 l x 6 hours / 0.9 (0.066) = 192 ~ 200 mg
2 capsules of 100 mg once every 6 hours will be given.

**Problem nr. 16**

Calculate the dissolution rate (Kd) of acetylsalicylic acid (500 mg) from tablets manufactured by different manufacturers. Which assortment of tablets does not meet the dissolution test requirements?

| Manufacturer's name | Time of sampling | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | 30 | 45 | 60 |
| | Concentration of dissolved substance,% | | | | | | | |
| Manufacture A | 10 | 20 | 35 | 47 | 58 | 65 | 85 | 93 |
| Manufacture B | 5 | 7 | 15 | 25 | 40 | 56 | 68 | 82 |
| Manufacture C | 8 | 15 | 28 | 31 | 45 | 60 | 72 | 86 |

**Problem nr. 17**

Calculate the dissolution rate (Kd) of acetaminophen (500 mg) from tablets manufactured by different manufacturers. Which assortment of tablets does not meet the dissolution test requirements?

| Manufacturer's name | Time of sampling | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | 30 | 45 | 60 |
| | Concentration of dissolved substance,% | | | | | | | |
| Manufacture A | 15 | 25 | 40 | 57 | 68 | 75 | 88 | 96 |
| Manufacture B | 10 | 15 | 25 | 40 | 56 | 68 | 82 | 92 |
| Manufacture C | 12 | 24 | 30 | 42 | 50 | 62 | 71 | 84 |

**Problem nr. 18**

Calculate the difference factor ($f_1$) and the similarity factor ($f_2$) of 2 assortments of Captopril, tablets, 50 mg. Conclude about compliance within the limits recommended by the FDA USA. And if the tested product has the same pharmaceutical availability as the reference (percentage of the substance dissolved at 45 min).

| Sampling time, min | Dissolved amount (%) | |
|---|---|---|
| | Captopril-Ferein (reference product) | Captopril-KMP (tested product) |
| 10 | 30 | 25 |
| 20 | 40 | 36 |
| 30 | 62 | 58 |
| 45 | 85 | 70 |
| 60 | 90 | 86 |

## SELF-ASSESSMENT TESTS

1. The rate of drug transport across a cell membrane by lipid diffusion depends on all of the following EXCEPT:
   a. Drug size (diffusion constant)
   b. Surface area of absorption
   c. Lipid partition coefficient
   d. Density of transporters
   e. Concentration gradient

2. The major mechanism of drug transport involved in the transport of drug out of the blood into tissues is:
   a. Aqueous diffusion
   b. Lipid diffusion
   c. Active transport
   d. Facilitated transport
   e. Receptor-mediated endocytosis

3. The distribution of drugs into the central nervous system (brain) usually depends on:
   a. Aqueous diffusion
   b. Lipid diffusion
   c. Active transport
   d. Facilitated transport
   e. Receptor-mediated endocytosis

4. Following intravenous administration, drugs are distributed fastest to:
   a. the skin, kidney, and brain
   b. the liver, kidney, and brain
   c. the liver, adipose, and brain
   d. the liver, kidney, and adipose
   e. the adipose, skin, and brain

5. At pH 5.0, the ratio of the protonated to unprotonated forms of morphine (a weak base containing an ionizable amine group, pKa = 7.0) would be:
   a. 1:100
   b. 1:10
   c. 1:1
   d. 10:1
   e. 100:1

6. Which of the following characteristics is most likely to be associated with a high apparent volume of distribution?
   a. High hepatic extraction ratio
   b. Penetration across the blood:brain and blood:testes barriers
   c. Extensive binding to plasma protein
   d. Distribution into total body water
   e. Extensive binding to tissue constituents

7. The volume of distribution of gentamicin, a highly polar water-soluble drug, is 14 L per 70 kg. This reflects the distribution of gentamicin into:
   a. Plasma
   b. Plasma and Blood
   c. Plasma, blood, and interstitial fluid (extracellular water)

d. Total body water
e. Adipose tissue

8. The half life of a drug eliminated by first order elimination kinetics will be LONGER in individuals who have an:
a. increased volume of distribution or increased clearance
b. increased volume of distribution or decreased clearance
c. decreased volume of distribution or increased clearance
d. decreased volume of distribution or decreased clearance

9. A drug x was administered to a patient through i.v bolus injection. A plasma drug concentrations of 0.78 mg/L was measured after 4 hours. A plasma drug concentration of 0.195 mg/L was measured after 8 hours. The drug's distribution is instantaneous. Assuming a first order process, calculate the half-life of the drug.
a. 1 h
b. 1.5 h
c. 2 h
d. 34 h
e. None of the above

10. If food decreases the rate but not the extent of the absorption of a particular drug from the gastrointestinal tract, then taking the drug with food will result in a smaller
a. area under the plasma drug concentration time curve
b. maximal plasma drug concentration
c. time at which the maximal plasma drug concentration occurs
d. fractional bioavailability
e. total clearance

11. If a drug exhibits first-order elimination, then
a. the elimination half-life is proportional to the plasma drug concentration
b. the drug is eliminated at a constant rate
c. hepatic drug metabolizing enzymes are saturated
d. drug clearance will increase if the plasma drug concentration increases
e. the rate of drug elimination (mg/min) is proportional to the plasma drug concentration

12. After a person ingests an overdose of an opioid analgesic, the plasma drug concentration is found to be 32 mg/L. How long will it take to reach a safe plasma concentration of 2 mg/L if the drug's half-life is 6 hours?
a. 12 hours
b. 24 hours
c. 48 hours
d. 72 hours
e. 1 week 4.

13. What dose of a drug should be injected intravenously every 8 hours to obtain an average steady-state plasma drug concentration of 5 mg/L if the drug's volume of distribution is 30 L and its clearance is 8 L/h?
a. 40 mg
b. 80 mg
c. 160 mg
d. 320 mg
e. 400 mg

14. The volume of distribution of a drug will be greater if the drug

a. is more ionized inside cells than in plasma
b. is administered very rapidly
c. is highly ionized in plasma
d. has poor lipid solubility
e. has a high molecular weight

15. Imagine a drug that is given as an intravenous bolus. The dose was 80 mg. The elimination follows first order principles. 2 hours after administration the drug a concentration C1 of 1.48 µg/ml is observed. Four hours after the administration the concentration C2 was 0.74 µg/ml. What is the elimination rate constant of this drug?

a. 0.346 h-1
b. 0.693 h
c. 0.693 h-1
d. 0.346 µg/(ml*h)
e. 0.370 h-1

***ANSWERS:***

*1. d, e; 2. a;3. b;4.b; 5. e;6. e;7. c; 8. b; 9. c ; 10.b 11. e; 12. b; 13. d; 14. a; 15. a.*

### *Answer True (T) or False (F)*

1. Lipophilic unionized drugs are likely to enter tissues relatively fast.
2. The uptake of a hydrophilic drug into tissue can be increased significantly by increasing the blood flow through the tissue.
3. The volume of distribution will be reduced with increased clearance of a drug.
4. A characteristic of absorption by lipid diffusion is its saturability at high drug concentrations.
5. A slower absorption might be advantageous for a drug with a narrow therapeutic window.
6. For bioequivalence tests, AUC is a relevant measure to assess whether test and reference formulation deliver the same dose.
7. For bioequivalence tests, AUC is a relevant measure to assess whether test and reference formulation have the same volume of distribution.
8. For bioequivalence tests, Cmax is a relevant measure to assess whether test and reference formulation have the same rate of absorption.
9. A characteristic of drugs eliminated by zero order kinetic processes is that the half-life is not constant.
10. For a drug eliminated by first order kinetics, the time required for continuous intravenous administration to achieve the steady state plasma drug concentration depends on the rate of drug administration.
11. The plasma drug concentration versus time curve for a drug eliminated by zero order kinetics is linear.
12. Drug metabolism is the process that converts chemicals into less polar metabolites so that they are more difficult to excrete.
13. A fundamental characteristic of all first order pharmacokinetic processes is that the rate of the process is proportional to drug concentration
14. Competition between two drugs for binding to plasma protein(s) can result in a change in the concentration of free drug and potential drug toxicity.
15. At pH 9.0, morphine (a weak base containing an ionizable amine group, pKa of 7.0) would exist predominantly in the charged form.

***ANSWERS:***

*1. T; 2. F; 3. F; 4. F; 5. T; 6. T; 7. F; 8. T; 9. T; 10. F; 11. T; 12. F; 13.T; 14.T; 15. F.*

## Symbols

D = dose
$\tau$ = dosing interval
CL = clearance
Vd = volume of distribution
$k_e$ = elimination rate constant
$k_a$ = absorption rate constant
F = fraction absorbed (bioavailability)
$K_0$ = infusion rate
T = duration of infusion
C = plasma concentration

## General

**Elimination rate constant**

$$k_e = \frac{CL}{Vd} = \frac{\ln\left(\frac{C_1}{C_2}\right)}{(t_2 - t_1)} = \frac{\ln C_1 - \ln C_2}{(t_2 - t_1)}$$

**Half-life**

$$t_{1/2} = \frac{0.693 \cdot Vd}{CL} = \frac{\ln(2)}{k_e} = \frac{0.693}{k_e}$$

## Intravenous bolus

**Initial concentration**

$$C_0 = \frac{D}{Vd}$$

**Plasma concentration (single dose)**

$$C = C_0 \cdot e^{-k_e \cdot t}$$

**Plasma concentration (multiple dose)**

$$C = \frac{C_0 \cdot e^{-k_e \cdot t}}{\left(1 - e^{-k_e \cdot \tau}\right)}$$

**Peak (multiple dose)**

$$C_{max} = \frac{C_0}{\left(1 - e^{-k_e \cdot \tau}\right)}$$

**Trough (multiple dose)**

$$C_{min} = \frac{C_0 \cdot e^{-k_e \cdot \tau}}{\left(1 - e^{-k_e \cdot \tau}\right)}$$

**Average concentration (steady state)**

$$\overline{Cp}_{ss} = \frac{D}{CL \cdot \tau}$$

## Oral administration

**Plasma concentration (single dose)**

$$C = \frac{F \cdot D \cdot k_a}{Vd(k_a - k_e)} \cdot \left(e^{-k_e \cdot t} - e^{-k_a \cdot t}\right)$$

**Time of maximum concentration (single dose)**

$$t_{max} = \frac{\ln\left(\frac{k_a}{k_e}\right)}{(k_a - k_e)}$$

**Plasma concentration (multiple dose)**

$$C = \frac{F \cdot D \cdot k_a}{Vd(k_a - k_e)} \cdot \left(\frac{e^{-k_e \cdot t}}{\left(1 - e^{-k_e \cdot \tau}\right)} - \frac{e^{-k_a \cdot t}}{\left(1 - e^{-k_a \cdot \tau}\right)}\right)$$

**Time of maximum concentration (multiple dose)**

$$t_{max} = \frac{\ln\left(\frac{k_a \cdot \left(1 - e^{-k_e \cdot \tau}\right)}{k_e \cdot \left(1 - e^{-k_a \cdot \tau}\right)}\right)}{(k_a - k_e)}$$

**Average concentration (steady state)**

$$\overline{C} = \frac{F \cdot D}{CL \cdot \tau}$$

**Clearance**

$$Cl = \frac{Dose \cdot F}{AUC}$$

$$Cl = k_e \cdot V_d$$

## Constant rate infusion

**Plasma concentration (during infusion)**

$$C = \frac{k_0}{CL} \cdot \left(1 - e^{-k_e \cdot t}\right)$$

**Plasma concentration (steady state)**

$$C = \frac{k_0}{CL}$$

**Calculated clearance (Chiou equation)**

$$CL = \frac{2 \cdot k_0}{(C_1 + C_2)} + \frac{2 \cdot Vd \cdot (C_1 - C_2)}{(C_1 + C_2) \cdot (t_2 - t_1)}$$

## Short-term infusion

**Peak (single dose)**

$$C_{max(1)} = \frac{D}{CL \cdot T} \cdot \left(1 - e^{-k_e \cdot T}\right)$$

**Trough (single dose)**

$$C_{min(1)} = C_{max(1)} \cdot e^{-k_e \cdot (\tau - T)}$$

**Peak (multiple dose)**

$$C_{max} = \frac{D}{CL \cdot T} \cdot \frac{\left(1 - e^{-k_e \cdot T}\right)}{\left(1 - e^{-k_e \cdot \tau}\right)}$$

**Trough (multiple dose)**

$$C_{min} = C_{max} \cdot e^{-k_e \cdot (\tau - T)}$$

**Calculated elimination rate constant**

$$k_e = \frac{\ln\left(\frac{C^*_{max}}{C^*_{min}}\right)}{\Delta t}$$

with $C_{max}^*$ = measured peak and $C_{min}^*$ = measured trough,
measured over the time interval $\Delta t$

## Volume of Distribution

$$V = V_p + V_T \cdot K_p$$

$$V = V_p + V_T \cdot \frac{fu}{fu_T}$$

## Clearance

$$Cl = \frac{Dose}{AUC}$$

$$Cl = k_e \cdot V_d$$

**Calculated peak**

$$C_{max} = \frac{C^*_{max}}{e^{-k_e \cdot t^*}}$$

with $C_{max}^*$ = measured peak, measured at time $t^*$ after the end of the infusion

**Calculated trough**

$$C_{min} = C^*_{min} \cdot e^{-k_e \cdot t^*}$$

with $C_{min}^*$ = measured trough, measured at time $t^*$ before the start of the next infusion

**Calculated volume of distribution**

$$Vd = \frac{D}{k_e \cdot T} \cdot \frac{\left(1 - e^{-k_e \cdot T}\right)}{[C_{max} - (C_{min} \cdot e^{-k_e \cdot T})]}$$

**Calculated recommended dosing interval**

$$\tau = \frac{\ln\left(\frac{C_{max(desired)}}{C_{min(desired)}}\right)}{k_e} + T$$

**Calculated recommended dose**

$$D = C_{max(desired)} \cdot k_e \cdot V \cdot T \cdot \frac{\left(1 - e^{-k_e \cdot \tau}\right)}{\left(1 - e^{-k_e \cdot T}\right)}$$

## Two-Compartment-Body Model

$$C = a \bullet e^{-\alpha} + b \bullet e^{-\beta}$$

$$AUC_{\infty} = a / \alpha + b / \beta$$

$$Vd_{area} > Vd_{ss} > Vc$$

**Creatinine Clearance**

$$CL_{crea}(male) = \frac{(140 - age) \bullet weight}{72 \bullet Cp_{crea}}$$

$$CL_{crea}(female) = \frac{(140 - age) \bullet weight}{85 \bullet Cp_{crea}}$$

With weight in kg, age in years, creatinine plasma conc. in mg/dl and $CL_{crea}$ in ml/min

## INDIVIDUAL WORK

***Topics for writing papers:***

| | |
|---|---|
| 1 | Biopharmacy and pharmacokinetics - generalities. Biopharmaceuticals. |
| 2 | Phases of drug biotransformation. The biopharmaceutical phase. |
| 3 | Biopharmaceutical aspect of the tablets. |
| 4 | Biopharmaceutical aspect of capsules. |
| 5 | The biopharmaceutical aspect of suppositories. |
| 6 | Ionization of drug substances. Basic principles. |
| 7 | Influence of pH on the ionization of drug substances with weak acidity or weak base. |
| 8 | Absorption of drugs in the gastrointestinal tract. Absorption in the buccal mucosa. |
| 9 | Absorption in the gastric mucosa. |
| 10 | Absorption in the small intestine mucosa. |
| 11 | Absorption in the large intestine mucosa. |
| 12 | Rectal absorption. |
| 13 | Parenteral drug absorption. Vascular absorption. Intramuscular and subcutaneous absorption. |
| 14 | Percutaneous absorption. |
| 15 | Absorption from implants. |
| 16 | Vaginal and uterine absorption. |
| 17 | The Biopharmaceutical Classification of Drugs. |
| 18 | Gastrointestinal solubility and permeability of drug substances. Lipinski's rule (the rule of 5). |
| 19 | Bioavailability of drugs. Bioequivalence of drugs. |
| 20 | Determining area under the curve. Methods of calculation. |
| 21 | Absolute and relative bioavailability. Methods of calculation. |
| 22 | Objectives of a bioavailability study. |
| 23 | Determination of in vitro dissolution rate. Machinery. Calculation of the dissolution rate constant. |
| 24 | Comparison of the dissolution profiles of two products, using the model independent model. The difference factor (f1) and the similarity factor (f2). |
| 25 | Classical pharmacokinetics. One-compartment model. |
| 26 | Intravenous use. Calculation of pharmacokinetic parameters: Kel; t½; Cl; Vd. |
| 27 | Extravascular administration. Calculation of pharmacokinetic parameters: K abs; Kel; T½; Cl. |
| 28 | Classical pharmacokinetics. Two-part model. |
| 29 | Principles of drug release in pharmaceutical forms and systems. Classification. |
| 30 | Modified Release Medicines. Classification. Taking repeated doses. |
| 31 | Types of drug release from prolonged and sustained release forms. |
| 32 | Rapid release pharmaceutical forms. |
| 33 | Oral medicinal products with prolonged action. |
| 34 | Controlled release pharmaceutical systems. Classification. |
| 35 | Pre-programmed release pharmaceutical systems. |
| 36 | Pharmaceutical systems activated. |
| 37 | Vectorized or Target Transport Systems. Classification. |
| 38 | Classification of medical transporters. Principles for selecting transporters. Generations of transporters. |

**Biopharmaceutical and pharmacokinetic assessment of medicines**

| No. | EXAMINATION PARAMETERS: |
|---|---|
| 1. | **International Nonproprietary Name** (INN) |
| 2. | **Synonyms** |
| 3. | **ATC Code** |
| 4. | **IUPAC name** |
| 5. | **2D** and **3D** Structure |
| 6. | Molecular mass (Da) |
| 7. | ***DESCRIPTION:*** |
| 8. | Physical properties |
| 9. | *meltingpoint |
| 10. | ***pKa** (or pKb) |
| 11. | **pH** of **Saturated Solution** |
| 12. | Solubility (in water and other solvents), **LogS** |
| 13. | **Log P (**Partition coefficient) |
| 14. | **Log D** (distribution coefficient) |
| 15. | **MlogP** (Moriguchi octanol-water partition coefficient.) |
| 16. | Polar Surface Area **($A^2$)** |
| 17. | Hydrogen Donor Count H **(OH + NH)** |
| 18. | Hydrogen Acceptor Count H **(N + O)** |
| 19. | Assess the rate of absorption and intestinal permeability according to Lipinski's rule (**score 0, 1, 2, 3, 4**). |
| 20. | Determine the Biopharmaceutical Class according to the **Biopharmaceutics Classification System.** |
| 21. | ***PHARMACOKINETICS:*** |
| | * **Bioavailability (%)** for different pharmaceutical forms. Assess bioavailability for different routes of administration. |
| | ***Protein binding** |
| | ***Metabolism** |
| | **Pharmacokinetic parameters: *Ka;*Kel; *t50%; *Vd; *Cl; *Cmax; *tmax** |
| 22. | **Calculate the percentage of non-ionized form** in different segments of the Gastrointestinal Tract (the results you write in the table) and conclude about the absorption capacity. Present the calculations. |
| 23. | Existing pharmaceutical forms of the medicinal substance (their characteristic). Evaluate administration paths. |
| 24 | **Sides effects** and their treatment. Overdose. |
| 25. | **Precautions** in drug administration. |
| 26. | **Drug interactions**. |
| 27. | **Indications and contraindications**. |
| 28. | **General conclusions** on treatment optimization, based on the results of the biopharmaceutical and pharmacokinetic evaluation. |

# REFERENCES

References used for making the methodological guide are in the quoted order: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