Course Description

Practical Biopharmaceutics focuses on the experimental and applied aspects of biopharmaceutics, providing hands-on experience in evaluating drug absorption, bioavailability, and pharmacokinetics. This course equips students with the practical skills necessary to assess and optimize drug formulations, dosage forms, and delivery systems. Students will engage in laboratory experiments, case studies, and data analysis exercises to bridge the gap between theoretical concepts and real-world applications.

Learning Outcomes:

By the end of this course, students will be able to:

1. Prepare and analyze calibration curves for quantitative drug analysis.
2. Evaluate the performance of pharmaceutical dosage forms, including antacids, bulk-forming laxatives, and compressed tablets, using in-vitro techniques.
3. Analyze dissolution profiles and their implications for drug bioavailability.
4. Understand the kinetics of drug reactions and their relevance to drug stability.
5. Calculate pharmacokinetic parameters following intravenous bolus drug administration

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Lab (1):

Introduction to Biopharmaceutics

To illustrate the importance of the drug substance and the drug formulation on absorption, and distribution of the drug to the site of action, one must first consider the sequence of events that precede elicitation of a drug's therapeutic effect.

First, the drug in its dosage form is taken by the patient either by an oral, intravenous, subcutaneous, transdermal, etc., route of administration.

Next, the drug is released from the dosage form in a predictable and characterizable manner.

Then, some fraction of the drug is absorbed from the site of administration into either the surrounding tissue, into the body (as with oral dosage forms), or both.

Finally, the drug reaches the site of action.

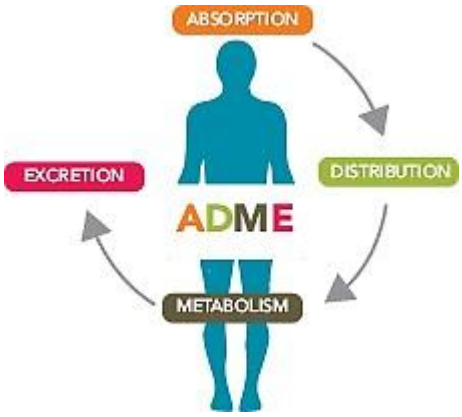
❖ Biopharmaceutics

Is the science that examines the interrelationship of the physicochemical properties of the drug, the dosage form in which the drug is given, and the route of administration on the rate and extent of drug absorption.

Thus, biopharmaceutics involves factors that influence the:

1. Protection and stability of the drug within the product;
2. The rate of drug release from the product;
3. The rate of dissolution of the drug at the absorption site; and
4. The availability of the drug at its site of action.

Pharmacokinetics is the science of the kinetics of drug absorption, distribution, and elimination (ie, metabolism and excretion). The distribution through and elimination of the drug in the body varies for each patient but can be characterized using mathematical models and statistics. The description of drug distribution and elimination is often termed drug disposition.



Pharmacodynamics refers to the relationship between the drug concentration at the site of action (receptor) and pharmacologic response

➢ Measurement of drug concentration

Drug concentrations are measured in biologic samples, such as milk, saliva, plasma, urine, faeces and expired air.

➢ Plasma Level–Time Curve

Minimal effective concentration (MEC) reflects the minimum concentration of drug needed at the receptors to produce the desired pharmacologic effect.

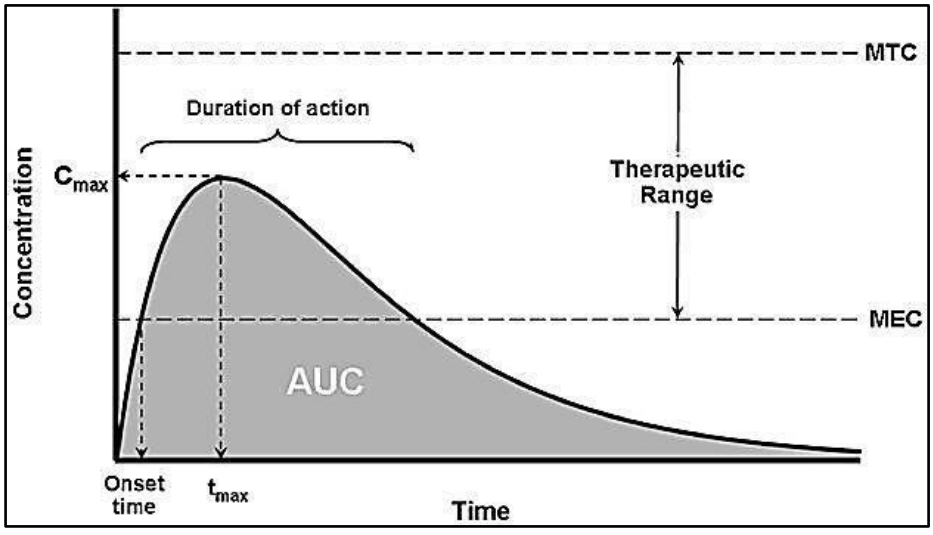
Similarly, the **minimal toxic concentration** (MTC) represents the drug concentration needed to just barely produce a toxic effect.

The intensity of the pharmacologic effect is proportional to the number of drug receptors occupied, which is reflected in the observation that higher plasma drug concentrations produce a greater pharmacologic response, up to a maximum.

The **onset time** corresponds to the time required for the drug to reach the MEC.

The **duration of drug action** is the difference between the onset time and the time for the drug to decline back to the MEC.

Area under the curve (AUC) is related to the amount of drug absorbed systemically.



The actual **dosing regimen** (dose, dosage form, dosing interval) was carefully determined in clinical trials to provide the correct drug concentrations at the site of action.

This sequence of events is profoundly affected—in fact, sometimes orchestrated—by the design of the dosage form, the drug itself, or both.

The study of biopharmaceutics is based on fundamental scientific principles and experimental methodology. Studies in biopharmaceutics use both *in-vitro* and *in-vivo* methods.

***In-vitro* methods** are procedures employing test apparatus and equipment (in the lab) without involving laboratory animals or humans.

***In-vivo* methods** are more complex studies involving human subjects or laboratory animals.

By applying the principles of biopharmaceutics, patient care can be improved by the pharmacist. Knowing the physicochemical properties of a drug can help the healthcare team as a whole make better drug therapy choices and anticipate drug interactions.

Lab (2):

Calibration Curve

The construction of a curve or straight line by plotting observed or experimental data on a graph is an important method of visualizing relationships between variables.

In pharmacokinetics, time is the independent variable and is plotted on the abscissa (*x* axis), whereas drug concentration is the dependent variable and is plotted on the ordinate (*y* axis).

Two types of graph paper are usually used in pharmacokinetics. These are Cartesian or rectangular coordinate graph paper and semilog graph paper.

Calibration curve is prepared from a series of standard solutions. When making solutions for a calibration curve, each solution can be made separately. However, that can take a lot of starting material and be time consuming. Another method for making many different concentrations of a solution is to use serial dilutions. With serial dilutions, a concentrated sample is diluted down in a stepwise manner to make lower concentrations using dilution equation:

$$C_1 * V_1 = C_2 * V_2$$

Calibration curves are used to obtain the concentration of an unknown sample of the same drug (quantitative analysis).

Spectrophotometry is the method used to measure the concentration of samples depending on the quantity of light absorbed.

When the sample is placed in the cell of the instrument and a light is projected on it, some of the light will be absorbed and the some will be transmitted or reflected. 100%

transmitted light means zero absorption and vice versa. Every chemical substance has the ability to absorb light.

The Beer-Lambert law (or Beer's law) is the linear relationship between absorbance and concentration of an absorbing species. It is only applied for diluted solutions. The BeerLambert law is written as:

$A = a * b * c$

Where a is the absorptive constant, b is the path length, and c is the analyte concentration.

When working in concentration units of molarity, the Beer-Lambert law is written as: **$A = \varepsilon * b * c$**

where ε is the wavelength-dependent molar absorptivity (extinction) coefficient with units of M^{-1} cm^{-1} .

- **Instrument used in the measurement of spectrophotometry**

1. Spectron-20 or Visible spectrophotometer for (400-700) nm, colored solutions should be used.
2. UV spectrophotometer for (200-400) nm

A **blank solution** is used for zeroing the instrument. It is composed of all the constituents of the solutions except the active ingredient which is required to be measured.

➢ Straight line equation

Physiological variables are not always linearly related. However, the data may be arranged or transformed to express the relationship between the variables as a straight line. The general equation of a straight line is

y = a + mx

where:

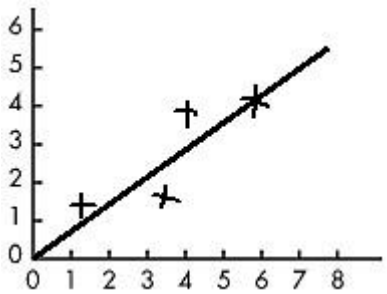
y = the dependent variable.

a = the *y* intercept. *m* = the slope of the line. *x* = the independent variable.

R^2 is the square of the correlation coefficient (r) and provides information about how far away the *y* values are from the predicted line. A perfect line would have an R^2 value of 1, and most R^2 values for calibration curves are over 0.95.

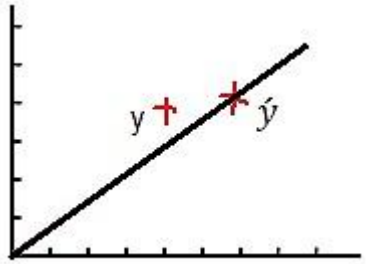
➢ Curve Fitting

1- Eye fitting: not a reliable method which involve drawing a straight line among the detected scattered points.



2- Least square method: more reliable which minimizes the sum of the square of deviation of observed value from the line

$$\Sigma(y - \acute{y})^2$$



$$y' = bx + c$$

$$b = \frac{n\,\Sigma(x.y) - (\Sigma x).(\Sigma y)}{n(\Sigma x^2) - (\Sigma x)^2}$$

$$c = \frac{\Sigma y - b(\Sigma x)}{n}$$

where:

$\acute{y}$ = calculated value
y = observed value x = concentration c = intercept b = slope
n = number of standard solutions

For each value of y we obtain $\acute{y}$.

For each value of x we calculate y.

Experiment:

Preparation of Standard Curve (Calibration Curve) of salicylic acid and calculating the concentration of an unknown sample.

Aim of the experiment:

To determine the unknown concentration of a substance (salicylic acid) in a sample by comparing the sample with a series of standard samples whose concentrations are known. to establish a relationship between the known concentrations of and the corresponding measured response from an instrument or method absorbance in spectroscopy. This curve is used to:

1. **Quantify unknown samples**: By comparing the response from unknown samples to the calibration curve, the concentration of the substance in the unknown sample can be determined.
2. **Ensure accurate measurements**: It helps in ensuring that the instrument or method is working correctly and provides reliable results.
3. **Correct for instrument variability**: Calibration curves allow the correction of potential biases or drifts in the measurement system, making the measurements more accurate and precise.
4. **Validate analytical methods**: The calibration curve serves as a quality control tool to ensure that the analytical procedure is performing within the expected accuracy and precision limits.

In summary, the calibration curve aims to provide a reliable reference that allows for the determination of concentrations in unknown samples by relating their measurement responses to known standards.

Experimental Work:

Since salicylic acid is sparingly soluble in water (equivalent to 200 mg/ 100 mL) sodium salicylate will be used because it is readily soluble.

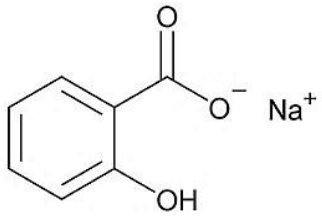
mg S.A	mL water
200	100
X	250

X = 500 mg = 0.5 g of salicylic acid in 250 mL water

So, the quantity of sodium salicylate required for the preparation of stock solution is:

M.wt of sodium salicylate	M.wt of salicylic acid
160 g	138 g y
0.5 g	

y = 0.5797 g of sodium salicylate ≈ 0.5 g



1. Prepare100 mL stock solution (100mg / 100mL) of sodium salicylate .
2. Find the lambda max (λ_{max}) of sodium salicylate in the UV-Spectrophotometer using water as the blank solution.
3. Prepare 10 mL of (50, 40, 30, 20 and 10) mg/ 100 mL standard (diluted) solutions of salicylic acid using the relationship:

$$C_1 * V_1 = C_2 * V_2$$

3. Run the serial diluted solutions in the UV-Spectrophotometer at the detected λ_{max} and record the results.
4. Plot the observed data using excel program and find the linear equation.

5. Use the UV-Spectrophotometer to get the absorbance of the unknown sample.
6. Find the concentration of the given unknown sample.
7. Fulfill the following table:

Sample no.	Na salicylate conc. (mg/100mL)	UV absorbance at λ_{max} ( ) nm
1	0	
2	10	
3	20	
4	30	
5	40	
6	50	
Unknown sample		

Lab (3):

In vitro evaluation of antacid

Antacids are chemical substances which on ingestion act by neutralization of gastric juice to form a neutral salt. They could form a protective layer on the mucosa and inhibit pepsin activity.

Antacids could be used to relieve the symptoms of Dyspepsia and Gastro-oesophageal reflux disease.

Antacids are best given when symptoms occur or are expected, usually between meals and at bedtime, 4 or more times daily.

Phases of gastric acid secretion:

The basal rate of hydrochloric acid secretion varies diurnally, being highest in the evening and lowest in the morning. After ingestion of a meal, the rate of acid secretion in the stomach increases. The three phases of increased acid secretion in response to food are the cephalic phase (before food reaches the stomach), the gastric phase (elicited by the presence of food in the stomach), and the intestinal phase (elicited by input from the duodenum and upper jejunum).

The pH range of the stomach is usually (1 – 3) or (2.5 – 3.5). Antacids could raise the pH of the stomach to (3.5 - 5.5). In cases of hyperacidity the pH range is (1 – 2).

Types of antacid according to their solubility:

1- Water soluble antacids which contain sodium bicarbonate or sodium chloride like (Heno) ® and (Citrogran) ®.

Their properties:

- Rapid onset of action but short duration.
- Systemically absorbed which could cause systemic alkalosis on prolonged use.
- React with gastric juice (HCl) resulting in the evolution of carbon dioxide leading to flatulence.

So, they should be avoided in hypertension, edema and pregnancy.

2- Water insoluble antacids which contain aluminium hydroxide and magnesium carbonate, hydroxide and trisilicate like (Maalox) ® and (Moxal) ®.

Their properties:

- Delayed onset of action but long duration.
- They will not cause systemic alkalosis since they won't be absorbed systemically.
- They will not cause evolution of carbon dioxide during their reaction with gastric juice (HCl).

Magnesium-containing antacids tend to be laxative whereas aluminium-containing antacids may be constipating; antacids containing both magnesium and aluminium may reduce these colonic side-effects.

Alginate taken in combination with an antacid increases the viscosity of stomach contents and can protect the oesophageal mucosa from acid reflux. Some alginate containing preparations form a viscous gel ('raft') that floats on the

surface of the stomach contents, thereby reducing symptoms of reflux. Example (Gaviscon) ®

FDA: 5 mg/ dose of acid neutralizing agent should raise the pH of the stomach to 3.2 or greater within 10 minutes of administration.

H.W./ What are the properties of an ideal antacid?

Aim of the Experiment:

The aim of this study is to evaluate the acid-neutralizing capacity and pH changes of selected antacids under simulated gastric conditions. This is achieved by:

1. Assessing the effect of water-insoluble and water-soluble antacids on the pH of Artificial Gastric Juice (AGJ) over time.

2. Simulating stomach conditions by periodically adding fresh AGJ to the reaction mixture, while withdrawing a sample for analysis.

3. Measuring the pH changes at 5-minute intervals to monitor the neutralizing ability of the antacids

4. Comparing the performance of different antacid formulations based on their ability to restore and maintain the desired pH, thereby simulating their action in the human stomach.

Through this procedure, the study aims to provide a detailed understanding of the antacid's performance in neutralizing stomach acid and its potential for practical use in treating acid-related gastric disorders.

Experimental Work:

1. Add 150 mL of Artificial Gastric Juice (AGJ) in a 250 mL beaker.
2. Fill 50 mL burette with AGJ and place it with a stand in position to add solution to the beaker within specified time intervals.
3. Record the initial pH of the AGJ (at time zero).
4. Add the selected antacid to the beaker and mix with the juice from the burette.
5. Record the pH at 5 min intervals for water insoluble and water soluble antacid using pH meter.
6. In order to simulate stomach condition, add 2 mL of fresh AGJ from the burette every 2 min and withdraw 5 mL using a pipette.
7. Continue recording for 50 min.
8. Fill the below table with your readings.

Time (min)	pH of water insoluble antacid	pH of water soluble antacid
0		
5		
10		
15		
20		
25		
30		
35		
40		
45		
50		

Lab (4):

In vitro Evaluation of bulk forming laxatives

Before prescribing laxatives it is important to be sure that the patient is constipated and that the constipation is not secondary to an underlying undiagnosed complaint. It is also important for those who complain of constipation to understand that bowel habit can vary considerably in frequency. Hence, it could be explained to the patient that constipation is the passage of hard stools less frequently than the patient's own normal pattern.

Uses of laxatives:

Laxatives are medications commonly used to:

1. Treat constipation by accelerating the movement of food through the gastrointestinal tract.
2. They are of value in drug-induced constipation.
3. They are used for the expulsion of parasites after anthelmintic treatment.
4. Laxatives are also used to clear the alimentary tract before surgery and radiological procedures.

Drawbacks of laxatives:

1. They all have a risk of being habit-forming.
2. Laxatives increase the potential of loss of pharmacologic effect of poorly absorbed, delayed-acting, and extended-release oral preparations by accelerating their transit through the intestines.
3. They may cause electrolyte imbalances when used chronically.

These drugs can be classified on the basis of their mechanism of action as bulk-forming agents, irritants or stimulants of the gut, osmotic laxatives and stool or fecal softeners.

A. Bulk-forming laxatives

They increase fecal mass in the large intestine, causing water retention and intestinal distension, thereby increasing peristaltic activity. These laxatives include hydrophilic colloids (from indigestible parts of fruits and vegetables) such as methylcellulose, ispaghula, and sterculia, *psyllium* seeds and bran. Methylcellulose also acts as a fecal softener.

Bulk-forming laxatives can be used in the management of patients with colostomy, ileostomy, haemorrhoids, anal fissure, chronic diarrhoea associated with diverticular disease, irritable bowel syndrome, and as adjuncts in ulcerative colitis.

Bulk-forming laxatives should be used cautiously in patients who are bed-bound, due to the potential for intestinal obstruction. They should be contraindicated in patients having difficulty in swallowing, intestinal obstruction, colonic atony and fecal impaction. Patients should be advised that the full effect may take some days to develop. Adequate fluid intake must be maintained to avoid intestinal obstruction.

B. Stimulant laxatives

Stimulant laxatives act on the nerve fibers in the mucosa of the colon leading to increased intestinal motility and often cause abdominal cramp. These laxatives include *Senna* and cascara (both are anthraquinones), bisacodyl, sodium picosulfate, and castor oil. Docusate sodium probably acts both as a stimulant and as a softening agent. Glycerol suppositories act as a rectal stimulant by virtue

of the mildly irritant action of glycerol. Stimulant laxatives should be avoided in intestinal obstruction.

C. Saline and osmotic laxatives

Osmotic laxatives increase the amount of water in the large bowel, either by drawing fluid from the body into the bowel or by retaining the fluid they were administered with. Lactulose (a semi-synthetic disaccharide), Macrogols (inert polymers of ethylene glycol PEG) are mainly used osmotic laxatives. Saline purgatives such as magnesium salts are useful where rapid bowel evacuation is required. Sodium salts should be avoided as they may give rise to sodium and water retention in susceptible individuals.

D. Stool softeners (emollient laxatives or surfactants)

Surface-active agents that become emulsified with the stool produce softer feces and ease passage. These include docusate sodium, docusate calcium, and docusate potassium. Such drugs are useful for oral administration in the management of haemorrhoids and anal fissure while glycerol is useful for rectal use.

Faecal softeners may take days to become effective. They should not be taken together with mineral oil because of the potential for absorption of the mineral oil.

E. Lubricant laxatives

Mineral oil and glycerine suppositories are considered to be lubricants. They facilitate the passage of hard stools. Mineral oil should be taken orally in an upright position to avoid its aspiration and potential for lipid or lipoid pneumonia.

Enemas containing arachis oil lubricate and soften impacted feces and promote a bowel movement.

Aim of the Experiment:

To evaluate the activity of various commercially available Bulk-Type Laxatives *In Vitro* indifferent PH media (acidic, neutral, and basic media) under simulated condition to the of GIT. This will give an indication of how a bulk-forming laxative might behave in the body. By conducting these in vitro evaluations, students can assess the properties and effectiveness of bulk laxatives, which helps to understand how they may perform in the human body. However, for a comprehensive understanding of efficacy and safety, in vivo studies (human or animal) are also typically required.

Experimental Work:

1. Weigh three samples of 1 g bran.
2. Add the measured amounts in 100 mL cylinders.
3. Pour 10 mL of water in one cylinder and stir gently for 15 seconds.
4. Fill the cylinder with further solvent up to 40 mL and leave the cylinder undisturbed in a water bath at 37 °C.
5. Repeat the same procedure using 1N NaOH and 1N HCl as solvents.
6. Record the volume of the laxative (in mL) in a Table at regular time intervals (each 5 minutes) for 50 minutes.
7. Draw a curve between laxative volumes (mL) versus time (min) and compare the efficacy of the laxative within the studied media.

Time (min)	Volume (mL) of laxative in acidic media	Volume (mL) of laxative in neutral media	Volume (mL) of laxative in basic media
0			
5			
10			
15			
20			
25			
30			
35			
40			
45			
50			

Lab (5):

In vitro dissolution of compressed tablet

Dissolution is the process by which a solid solute enters a solution. In the pharmaceutical industry, it may be defined as the amount of drug substance that goes into solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition.

Dissolution is considered as one of the most important quality control tests performed on pharmaceutical dosage forms and is now developing into a tool for predicting bioavailability, and in some cases, replacing clinical studies to determine bioequivalence. Dissolution behavior of drugs has a significant effect on their pharmacological activity. In fact, a direct relationship between in vitro dissolution rate of many drugs and their bioavailability has been demonstrated and is generally referred to as in vitro-in vivo correlation, IVIVC.

Solid dosage forms may or may not disintegrate when they interact with gastrointestinal fluid following oral administration depending on their design as explained in Figure 1.

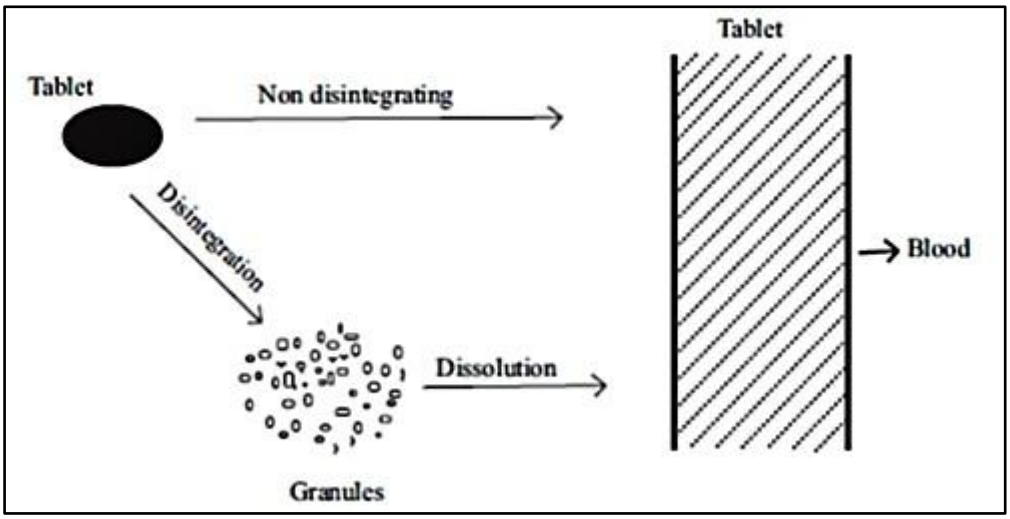


Figure 1: Schematic diagram of the dissolution process.

Dissolution kinetics is important in determining the bioavailability of a drug. The dissolution rate controls rate of build-up (accumulation) of certain drugs in the blood stream. It was thus recognized that in-vitro dissolution kinetics provides useful information on the availability of drugs and their subsequent therapeutic effects in-vivo. This led to the inclusion of dissolution tests in the United States NF (1970) and USP (1970) monographs for one capsule and twelve tablet preparations. In 1975, dissolution tests were included in the British Pharmacopoeia (amendment to BP 1973) for Digoxin tablets. Various pharmacopoeias contain specifications on the dissolution requirements of various drugs. A variety of designs of apparatus for dissolution testing have been proposed and tested, varying from simple beaker with stirrer to complex systems with lipid phases and lipid barrier where an attempt is made to mimic the biological milieu. The choice of the apparatus to be used depends largely on the physicochemical properties of the dosage form.

Compressed tablets are the standard uncoated tablets made by either direct compression or wet granulation or dry granulation or double compaction. They may be used for local action in gastro-intestinal tract or systemic action. When tablet exert local action, they are formulated as more water insoluble by means of selecting slow dissolving excipients and thus provides local action for long time period. e.g., antacids and adsorbents. The drugs that produce systemic action have some aqueous solubility and are designed to disintegrate and dissolve quickly so that the drug can be quickly absorbed and produce systemic action. Generally, an active pharmaceutical ingredient (API) exhibits bioavailability depending upon biopharmaceutical class, which is based on water solubility and gastro-intestinal membrane permeability criteria. But, it can be altered by appropriate selection of excipients and processing technology.

Aim of the experiment:

to determine the rate and extent to which the active pharmaceutical ingredient (API) in a drug product dissolves into a solution under standardized conditions.. This test simulates how the drug would dissolve and become available for absorption in the body, which is essential for understanding the drug's bioavailability. By conducting this test, pharmaceutical manufacturers can predict how the drug will behave in vivo and make necessary adjustments to improve its efficacy and safety. The dissolution test also plays a key role in product development, quality control, and regulatory approval processes.

Experimental Work:

A. Determination of λ_{max} of Albendazole

The UV-Visible spectrophotometric scan of Albendazole in 0.1N HCl (pH 1.2) should show a peak at 295 nm as shown in Figure 2.

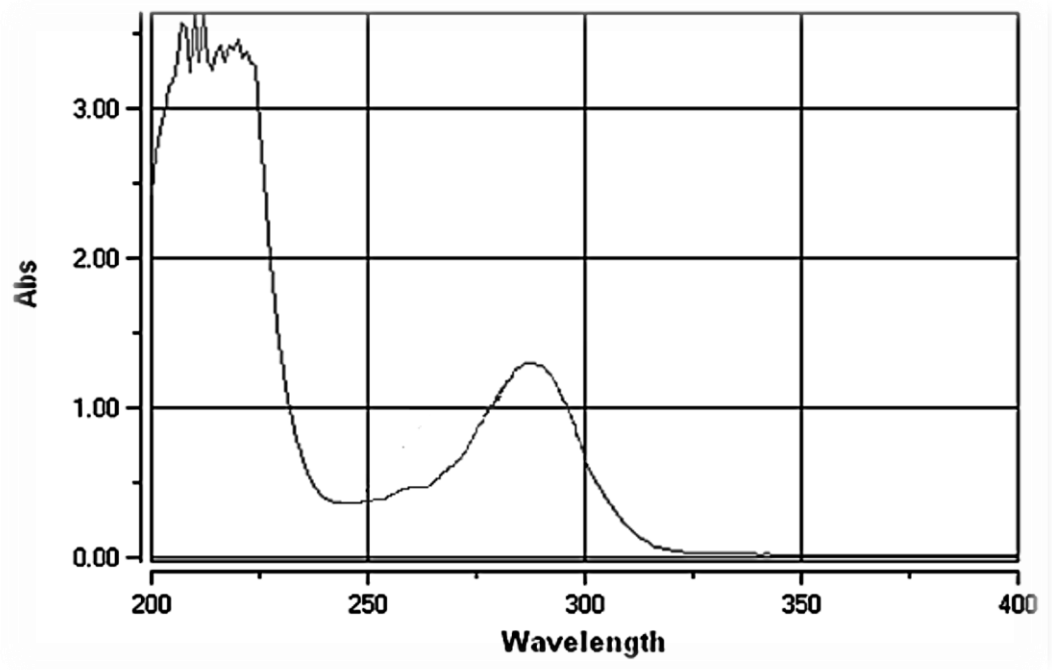


Figure 2: UV scan of Albendazole within the range (200-400) nm in 0.1N HCl (pH =1.2) showing λ_{max} at 295 nm.

B. Calibration curve of Albendazole

1. Preparation of standard stock solution: Weigh accurately 10 mg of pure Albendazole and transfer it into 100 mL volumetric flask and adjust the volume with 0.1 N HCl.

2. Preparation of working solutions: From stock solution, calculate the amount of Albendazole stock solution that should be measured to get serial dilutions of the drug having the concentrations of 2.5, 5, 10, 15, 20 and 25 µg\mL.

3. Measurement of absorbance: Measure the absorbance of the respective dilutions at λ_{max} 295 nm using UV-Visible spectrophotometer. Plot the graph of absorbance of Albendazole against concentration in MS Excel and determine the slope and intercept.

Calibration curve of Albendazole is shown below in Figure 3.

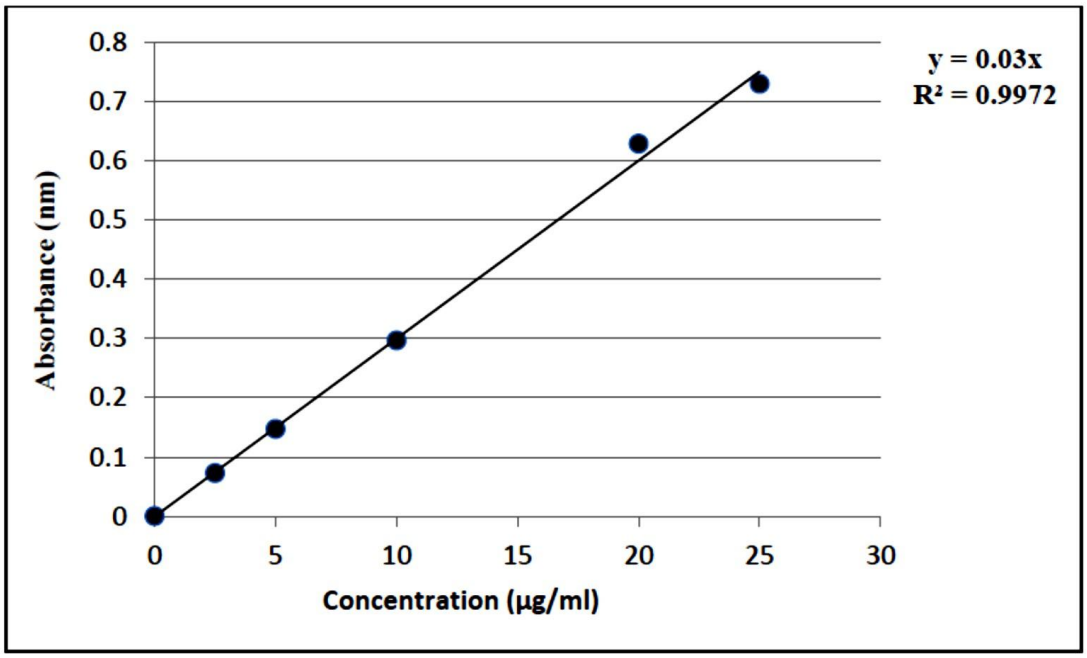


Figure 3: Calibration curve of Albendazole in 0.1N HCl at λ_{max} of 295 nm.

C. Procedure for dissolution

1. Perform dissolution test for the tablets using Type II (paddle) dissolution apparatus.
2. The apparatus should be adjusted at constant stirring speed of 50 rpm and the temperature should be maintained at 37°C ± 0.5 °C.

3. Place one tablet in the 1000-mL glass vessel of the apparatus which has to be prefilled with 900 mL of 0.1 N HCl as dissolution medium.

4. Withdraw five mL sample of the medium and replace it by the same volume of fresh dissolution medium at specified time intervals for 30 minutes.

5. Filter the samples through microfilter and analyze them spectrophotometrically at the λ_{max} of Albendazole.

6. From the absorbance values determine concentration of drug (µg/mL) and percent drug released.

7. With the help of MS Excel, plot the graph of percent drug released versus time.

D. Calculations

1. Determination of concentration of dissolved drug (µg/mL)

Y=mX+ c

Where,Y=absorbance, m=slope, X=concentration (µg/mL), c= intercept.

2. Amount of drug released (mg)

Amount of drug released =

[Concentration (µg/mL) x (volume of dissolution medium) x (dilution factor)]/1000

3. Dilution factor

Dilution factor = volume of diluted sample (mL)/ volume of sample removed (mL)

4. Percent cumulative drug released

Percent cumulative drug release = (amount of drug released) x 100 /strength of tablet

Time (min)	Absorbance	Amount of drug released (mg)	Percent cumulative drug released
5			
10			
15			
20			
30			

Lab (6)

Pharmacokinetic rate and order of reaction

Introduction:

When a drug is administered via injection as a sterile solution, key points to consider are:

1. The intravenous (IV) route ensures the entire dose reaches circulation.

2. The desired drug concentration is quickly achieved.

3. Doses must be carefully calculated to avoid adverse or toxic effects.

One-Compartment Open Pharmacokinetic Model:

The one-compartment open model assumes the body is a single unit with no barriers to drug movement. The plasma drug concentration reflects that in all body tissues. This model applies to drugs that distribute rapidly. After a rapid IV bolus, complete circulation occurs within 1 to 3 minutes, and absorption is not considered. The drug is eliminated 100% via renal excretion, with no metabolism. Pharmacokinetic parameters are derived assuming first-order elimination and passive diffusion.

Aim:

To calculate the pharmacokinetic parameters of a drug following intravenous bolus administration using the one-compartment model.

Experimental work:

1.Collect Plasma Concentration Data

After the IV bolus injection, collect blood samples at different time points.

Measure the plasma concentration of the drug at each time point using an appropriate analytical method (e.g., HPLC, UV spectroscopy).

2. Plot the Plasma Concentration vs. Time Curve

Plot the plasma drug concentration (C) on the y-axis and time (t) on the x-axis.

The curve is expected to exhibit a biexponential decay, with the initial steep drop followed by a slower decline.

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3. pharmacokinetic parameters Calculation:

1. Elimination half-life

The elimination half-life is sometimes called "biological half-life" of a drug which may be defined as the time at which the amount of unchanged drug becomes half (or 50%) of the initial amount of drug. Elimination half-life can be calculated from following equation:

$$t^{1/2} = \frac{0.693}{K}$$

Alternatively, half-life can be determined graphically as explained below in Figure 3.

❖ **Graphical determination of half-life:**

a. On Y axis near to elimination phase select initial concentration as (C, 20). From that point draw a straight line which intersects plotted line (See Figure 3). This point would be the first point of intersection. From intersected point draw perpendicular straight line on X axis which gives the time (t_1).

b. On Y axis draw a second straight line from half concentration of initial (C/2, 10) which intersects plotted line. This will be the second point of intersection. From that point draw perpendicular on X axis which gives the time (t_2).

c. Calculate half -life by subtracting t_2 form t_1 .

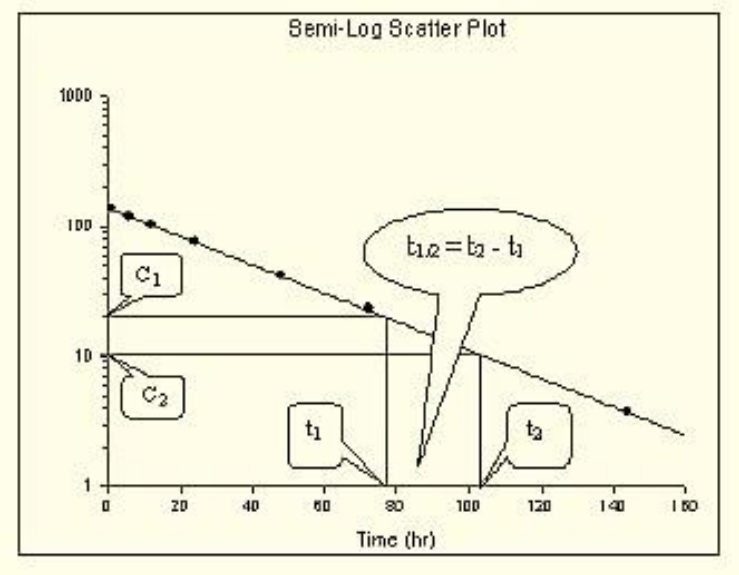

Figure 3: Semilog plot of plasma concentration versus time showing determination of half-life.

2. Elimination rate constant

Speed at which drug eliminates from body is known as elimination rate constant. The elimination or removal of drug from the body is the sum of urinary excretion, metabolism, biliary excretion, pulmonary excretion etc. Thus, the elimination rate constant is an additive property of all rate constants.

Total elimination rate constant can be calculated from following equations:

$$Slope = \frac{-K}{2.303} = \frac{\log C_2 - \log C_1}{t_2 - t_1}$$

K = – (Slope * 2.303)

$$K = \frac{0.693}{t1/2}$$

3. Area Under Curve (AUC)

AUC expresses the total amount of drug that comes into the systemic circulation after its administration. By using concentration at zero time AUC can be calculated from following equation (Y intercept is C_0 concentration).

$$AUC_{0-\infty} = \frac{C_0}{K}$$

4. Apparent volume of distribution (V)

Concentrations (amount per unit volume) and not masses (mg), are usually measured in plasma or serum. Therefore, a term is needed to relate the measured concentration (C) at a time to the mass of drug (A) at that time. This term is defined as the apparent volume of distribution (V). It is the measure of the extent of distribution of drug in compartment. It is defined as the hypothetical volume of body fluid into which a drug is dissolved or distributed.

In order to determine the apparent volume of distribution of a drug, it is necessary to have plasma/serum concentration versus time data. It is important to note that the apparent volume of distribution is a constant for a given drug and is independent of the administered dose and route of drug administration.

Now, from equation: $C = \frac{A}{V}$, $V = \frac{A}{C}$

where (A) = amount of drug; V= apparent volume of distribution; and (C) = plasma concentration at time, t.

When t = 0, the equation can be written as:

$$V = \frac{A_0}{C_0} = \frac{Dose}{C_0}$$

Where A = administered dose of a drug, (C_0) = plasma concentration at time t = 0.

5. Clearance

Clearance is the most important parameter in clinical drug applications and is useful in evaluating the mechanism by which a drug is eliminated by the whole organism or by a particular organ. It is defined as theoretical volume of body fluid containing drug from which the drug is completely removed in a given period of time. Clearance for a drug is constant if the drug is eliminated by first-order kinetics. Mathematically, clearance (L/hr) is the product of the first-order elimination rate constant (K) and the apparent volume of distribution (V). Thus,

$$\textbf{Clearance (Cl)} = \frac{\textbf{Rate of elimination}}{\textbf{Plasma drug concentration}} = \frac{dA/dt}{C}$$

Substituting *dA/dt* = *KA* in above equation, we get

$$Cl = \frac{KA}{C}$$

Since *A / C* = *V*, the clearance equation can be written as:

$$Cl = KV$$

Applications

1. Various pharmacokinetic parameters can be estimated.
2. Pharmacokinetic parameters are very useful in calculating the dose of new drug.
3. Bioequivalence study can be undertaken based on plasma data of various brands.

Exercises:

For all the following questions:

a. Prepare a semilogarithmic plot and estimate the half-life of drug.

b. Estimate the total AUC.

c. Calculate volume of distribution.

d. Calculate total clearance.

Given data1: Plasma concentration profile after a single 600 mg intravenous dose of a medication to an adult is given below:

Time (hr)	1	2	3	5	7	8
Conc. (mg/L)	37	21.5	12.5	4.5	2.6	1.2

Given data2: Plasma data obtained after a bolus dose of 184 mg of a drug in a newborn infant is summarized below:

Time (hr)	1	6	12	24	48	72
Conc. (mg/L)	137	120	103	76	42	23

Given data3: Plasma data obtained after a bolus dose of 500 mg of a drug is summarized in table below.

Time (hr)	1.5	2	4	6	10	16
Conc. (mg/L)	9	8.2	7.9	6.6	6.2	4.6

Given data4: Plasma data obtained after a bolus dose of 50 mg of a drug is given in following table:

Time (hr)	1	3	5	7	10	18
Conc. (mg/L)	20	11.3	7	4.3	2	1.5

Given data5: A 500 mg dose of a drug was given by IV bolus dose and the data below was collected:

Time (hr)	0.5	1	2	4	6	8
Conc. (mg/L)	13	13.5	10	7.6	5.1	3.4

Results:

Write down the pharmacokinetic parameters calculated from given plasma data of ceftriaxone in the below table:

No.	Parameter	Result
1	Biological half-life	
2	Elimination rate constant	
3	Total AUC	
4	Volume of distribution	
5	Clearance	

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