**Subject Name: Physical Pharmaceutics –II Module -V**

**Subject Code: BP 403T**

## Objectives of the course

- Know the principles of chemical kinetics and to use them for stability testing and determination of expiry date of formulations

## Learning outcomes

- Students learnt about the chemical kinetics, physical and chemical properties, nature of pharmaceutical products their stability.

# Structure of Module –V BP 403T

# Learning Material

**Drug Stability**

- Reaction kinetics: zero, pseudo-zero, first and second order, units of basic rate constants, determination of reaction order.
- Physical and chemical factors influencing the chemical degradation of pharmaceutical product: temperature, solvent, ionic strength, dielectric constant, specific and general acid base catalysis, Simple numerical problems.
- Stabilization of medicinal agents against common reactions like hydrolysis and oxidation.
- Accelerated stability testing in expiration dating of pharmaceutical dosage forms. Photolytic degradation and its prevention.

# Kinetics and Drug Stability

# CONTENTS

# Introduction
# (Zero, 1st, 2nd, Pseudo 1st)

- Chemical kinetics provide the basis to predict drug stability.
- The extent of inactivation of drug due to various environmental adverse conditions can be understood from the drug stability studies.
- It is expressed as a rate process.
- These studies help to predict the expiry period (shelf life) of a product.

# Rate and order (Zero, 1st, 2nd, Pseudo 1st)

- The rate, velocity or speed of a reaction is given by ± *(dc/dt)*. Here *dc* is the small change in the concentration within a given time interval *dt*.

- Order of a reaction is defined as the number of concentration terms on which the rate of a reaction depends when determined experimentally.

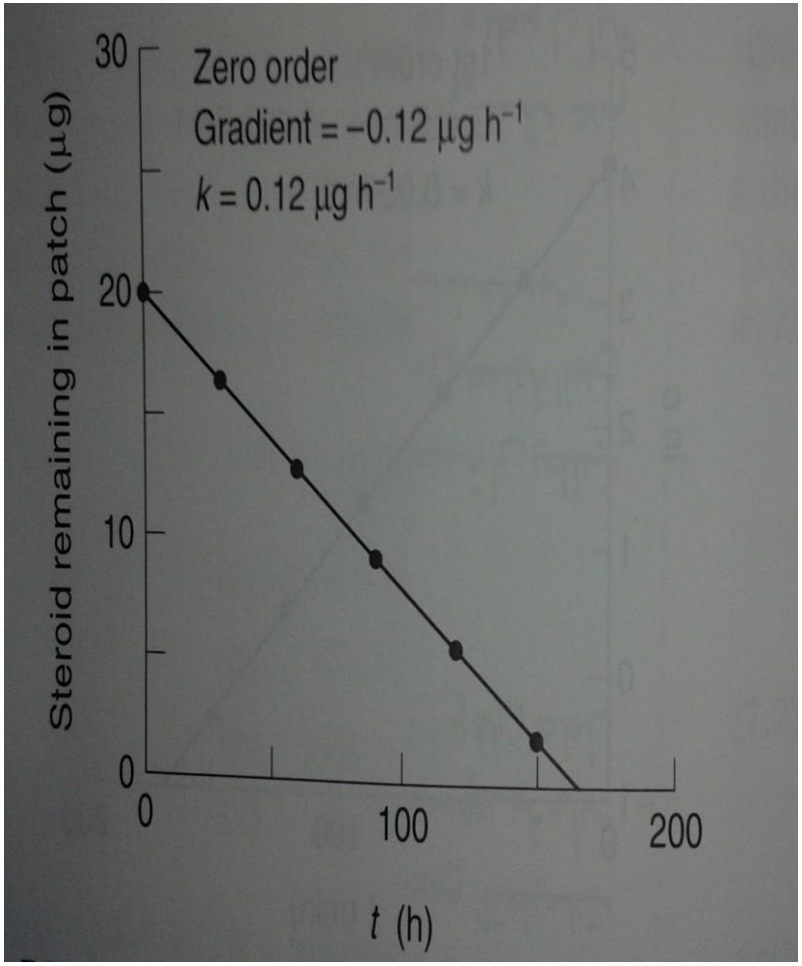
# Zero Order Reaction

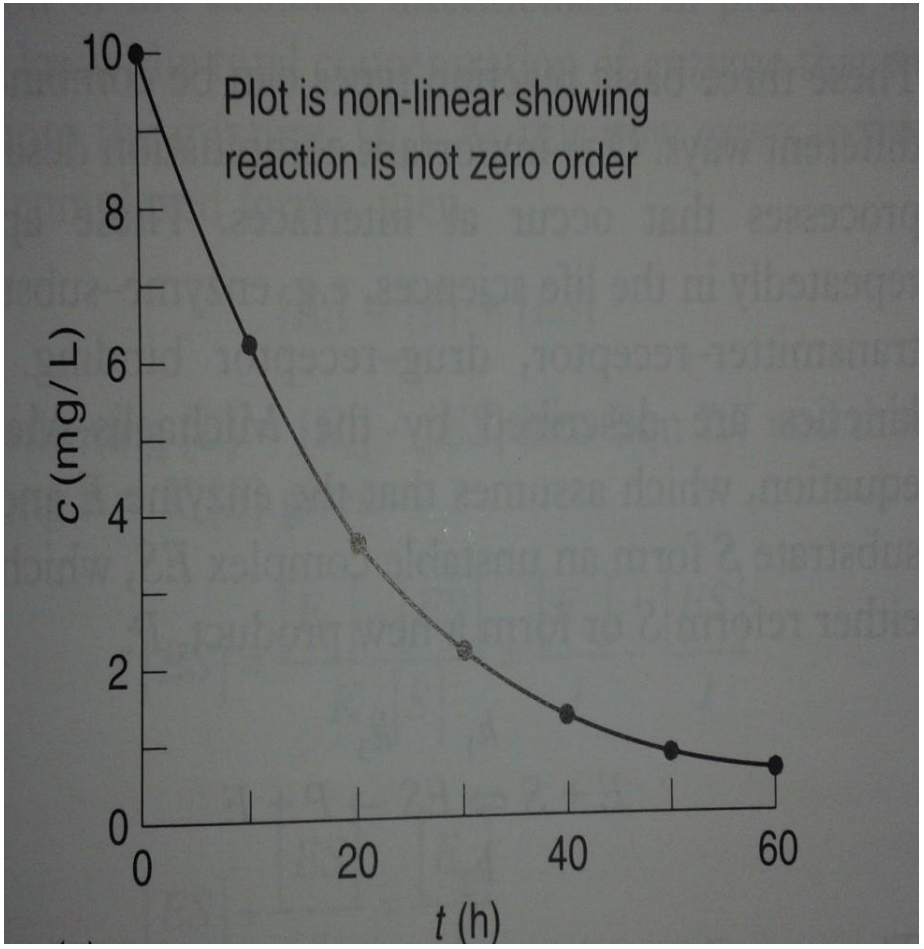
- Zero order reaction is defined as a reaction in which the rate does **not depend** on the concentration terms of the reactants.
- This is mathematically expressed as:

$$\frac{-dc}{dt} = k_o$$

- Where $k_0$ is the specific rate constant for a zero order

# Zero Order Reaction



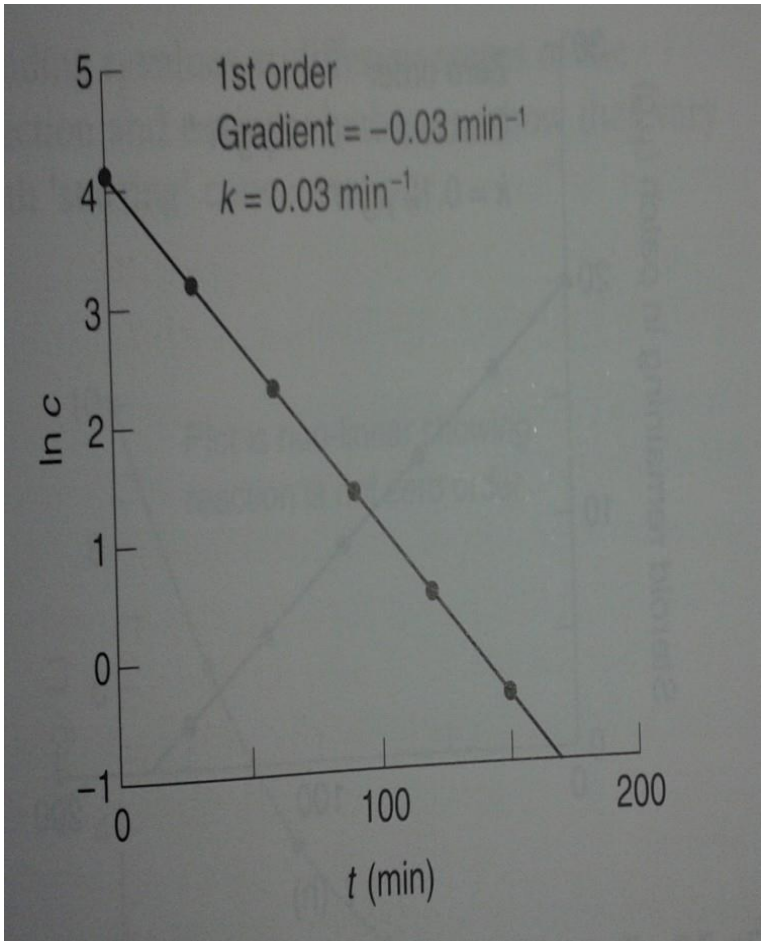
# First Order Reaction

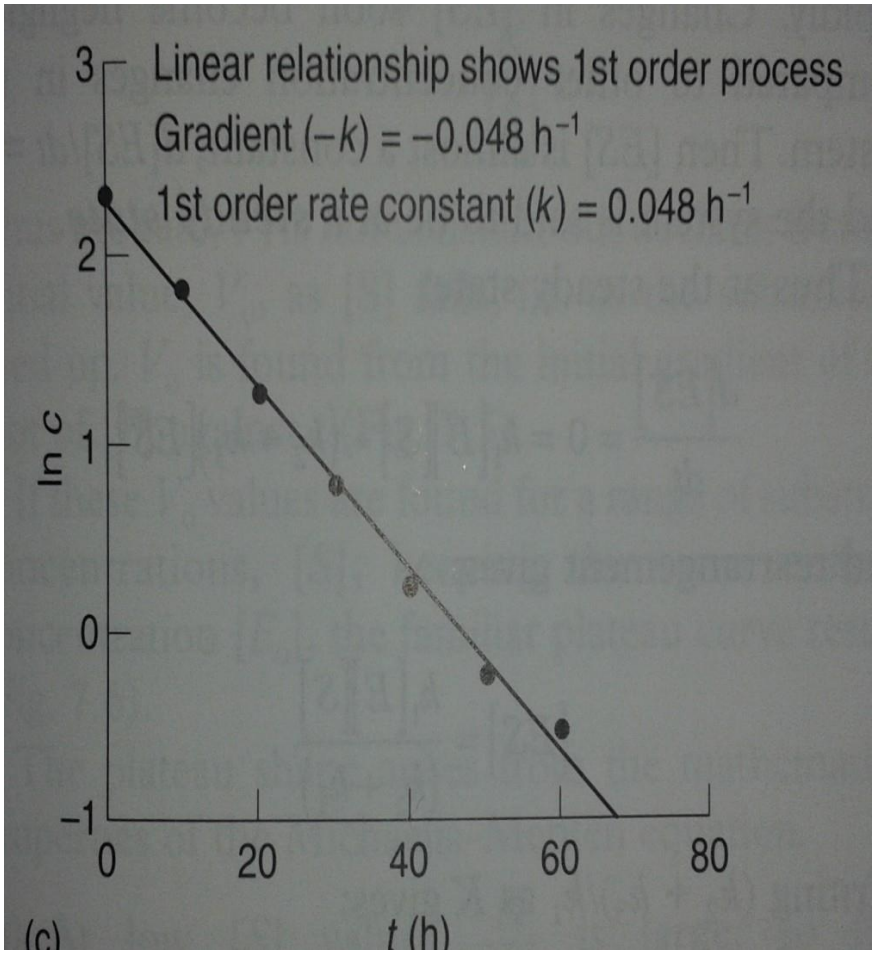
- First order reaction is defined as a reaction in which the rate of reaction **depends** on the concentration of one reactant.
- Mathematically, the first order rate equation can be written as:

$$\frac{-dc}{dt} \propto c \qquad \frac{-dc}{dt} = k_1 c$$

- Where c is the concentration of the reactant and $k_1$ is the specific rate constant for first order

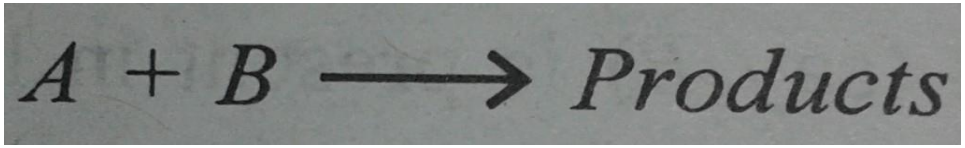
# First Order Reaction



# Second Order Reaction

- Second order reaction is defined as a reaction in which the rate depends on the concentration terms of two reactants each raised to the power one.
- Consider the following reaction



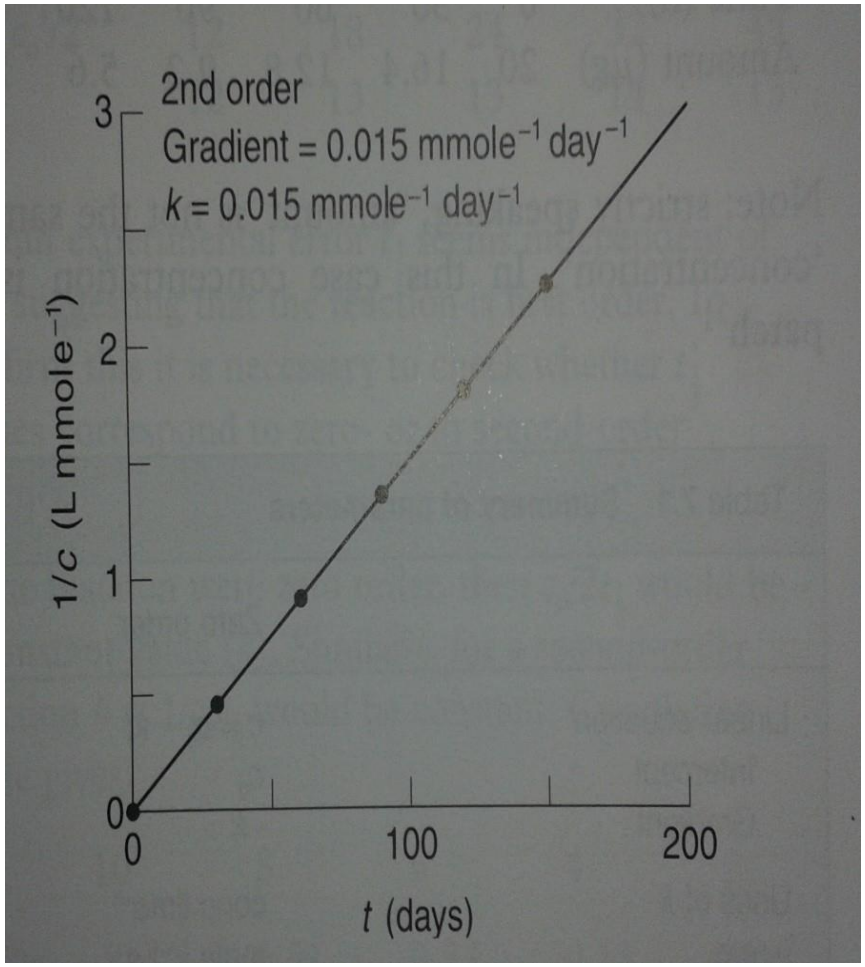
$$A + B \longrightarrow Products$$

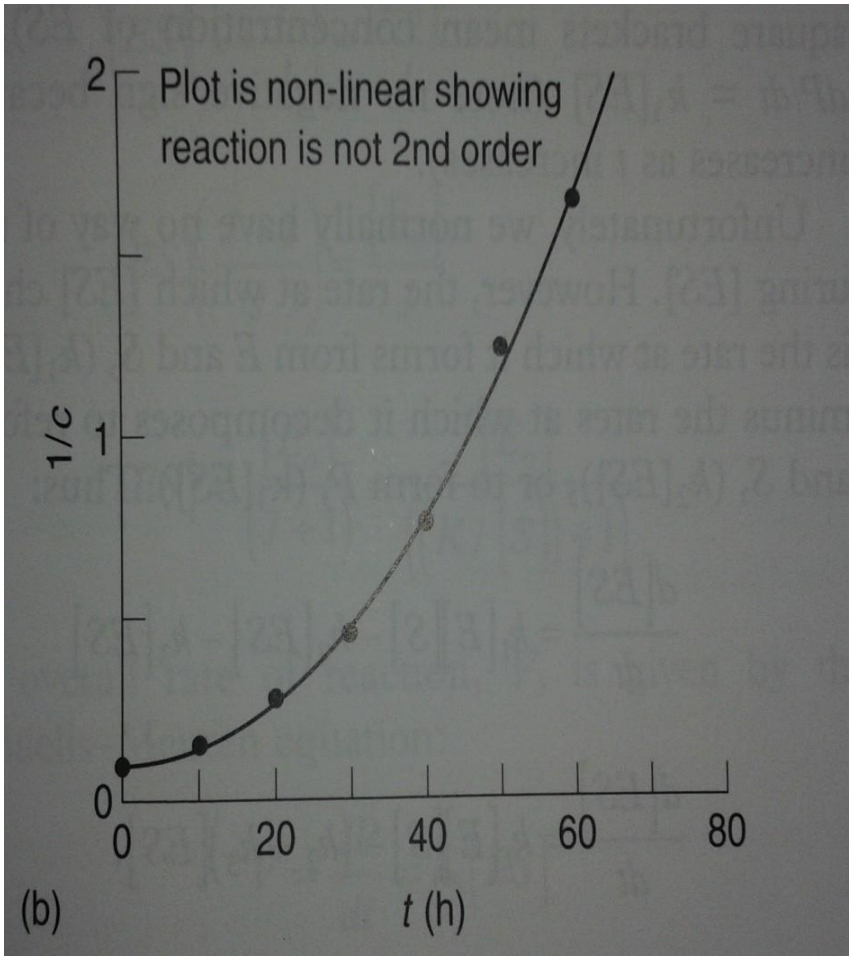
- The rate equation can be written as

$$-\frac{dA}{dt} = -\frac{dB}{dt} = k_2[A]^1[B]^1$$

- Where [A] and [B] are the concentration of A and B, respectively, and k2 is the specific rate constant for second order. In other words, the rate of reaction is first order with respect to A, and again first order with respect to B. So the overall order of this reaction is second order.

# Second Order Reaction



# Pseudo First Order Reaction

- Pseudo first order reaction is defined as a reaction which is originally a second order, but is made to behave like a first order reaction.
- In second order reaction, the rate depends on the concentration terms of two reactants. Therefore the rate equation would be

$$\frac{-dc}{dt} = k_2[A][B]$$

$$\frac{-dc}{dt} = k_2[A][constant] = k_1[A]$$

# Methods of determination or reaction order

- There is no straight forward method to theoretically know the order of a reaction.
- The exact order can be determined experimentally. The following methods are employed to decide the order of a reaction.

**1. Graphic Method**

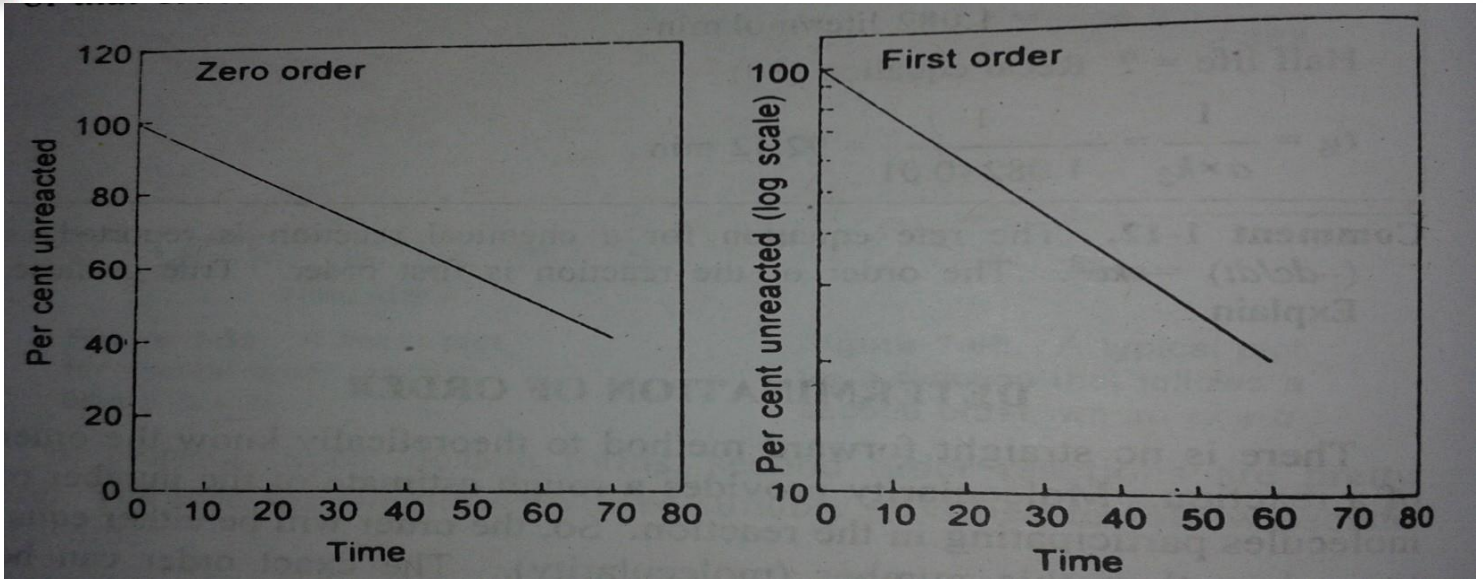
**2. Substitution Method**

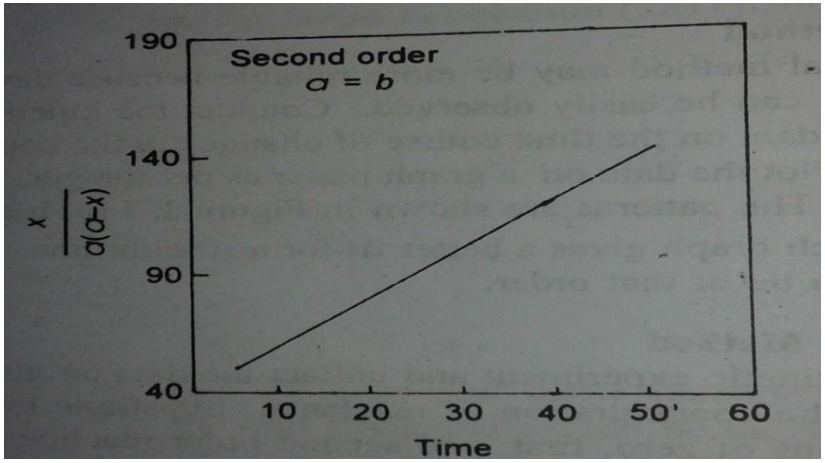
**3. Half Life Method**

# 1.Graphic Method

- This pictorial method may be more reliable because deviations from the best fit line can be easily observed. Conduct the kinetic experiment and collect the data on the time course of changes in the concentration of the reactants. Plot the data on a graph paper as per the general principles of each order.
- Decide which graph gives a better fit for a straight line. The reaction is considered to be of that order.

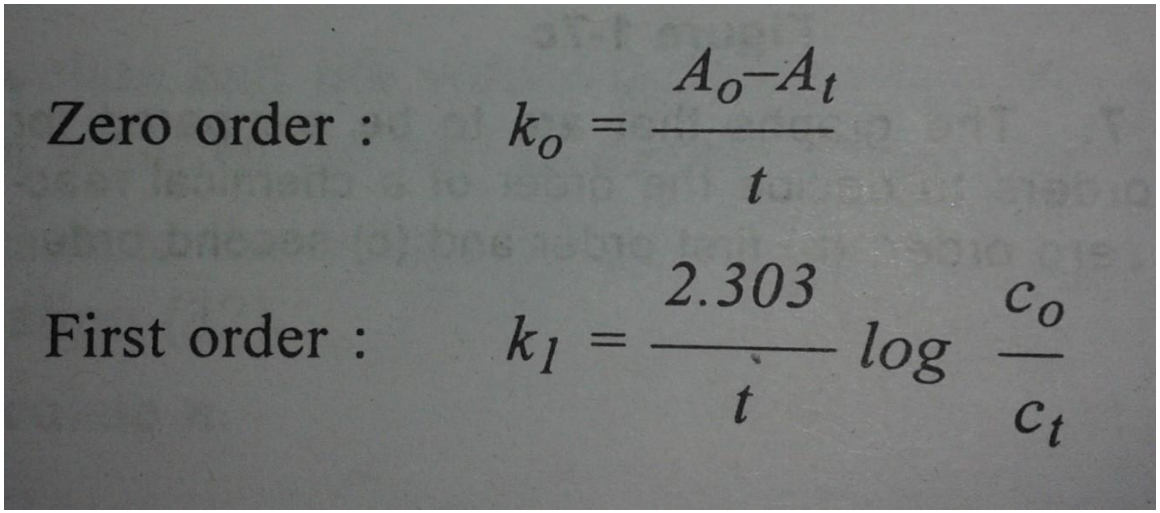
# 1.Graphic Method



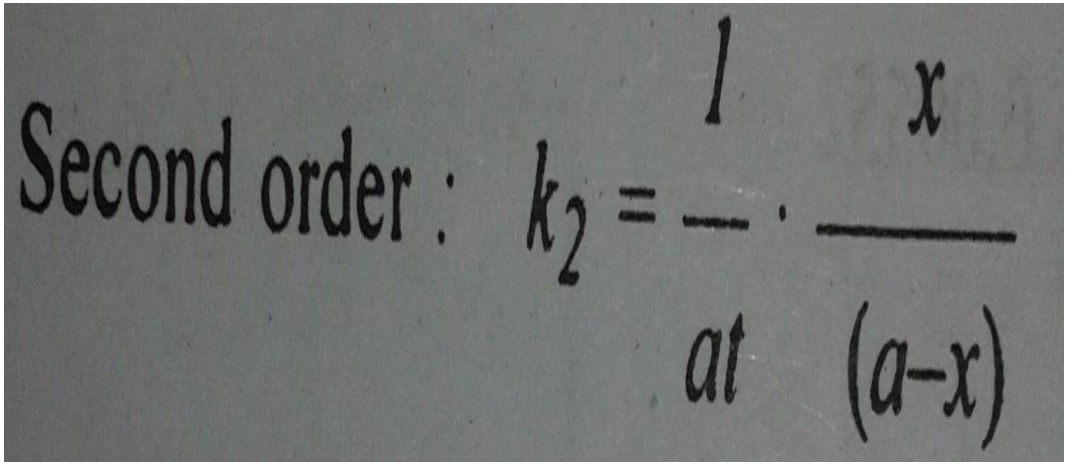
# 2. Substitution Method

- Conduct a kinetic experiment and collect the data on the time course of changes in the concentration of reactants. Substitute the data in the integral equation of zero, first, and second order reactions to get k values.



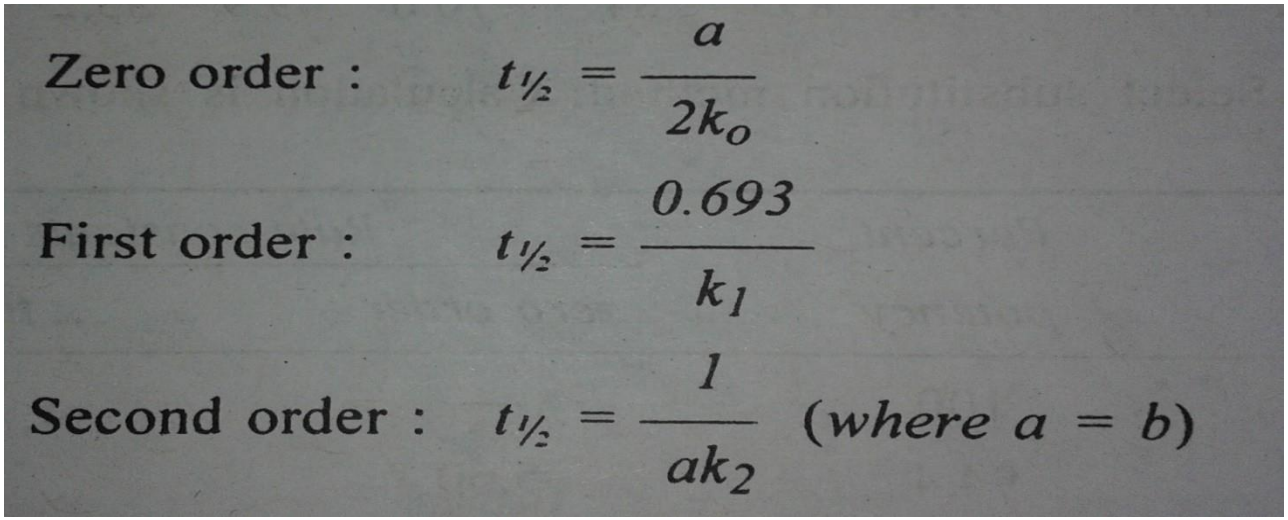
# 2. Substitution Method

- Select the order in which k values at different time periods remain constant within the experimental errors. The reaction is considered to be of that order.

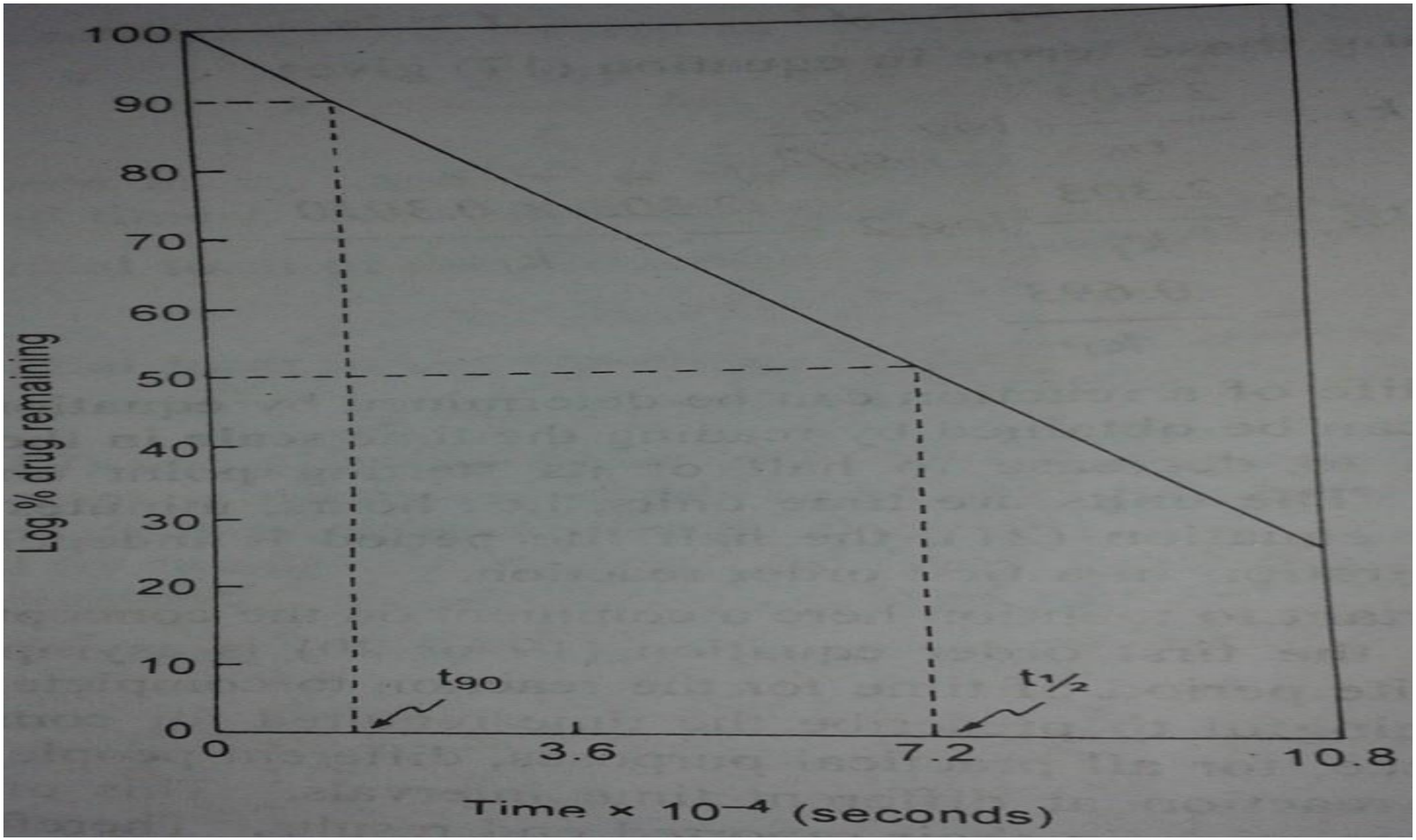


# 3. Half Life Method

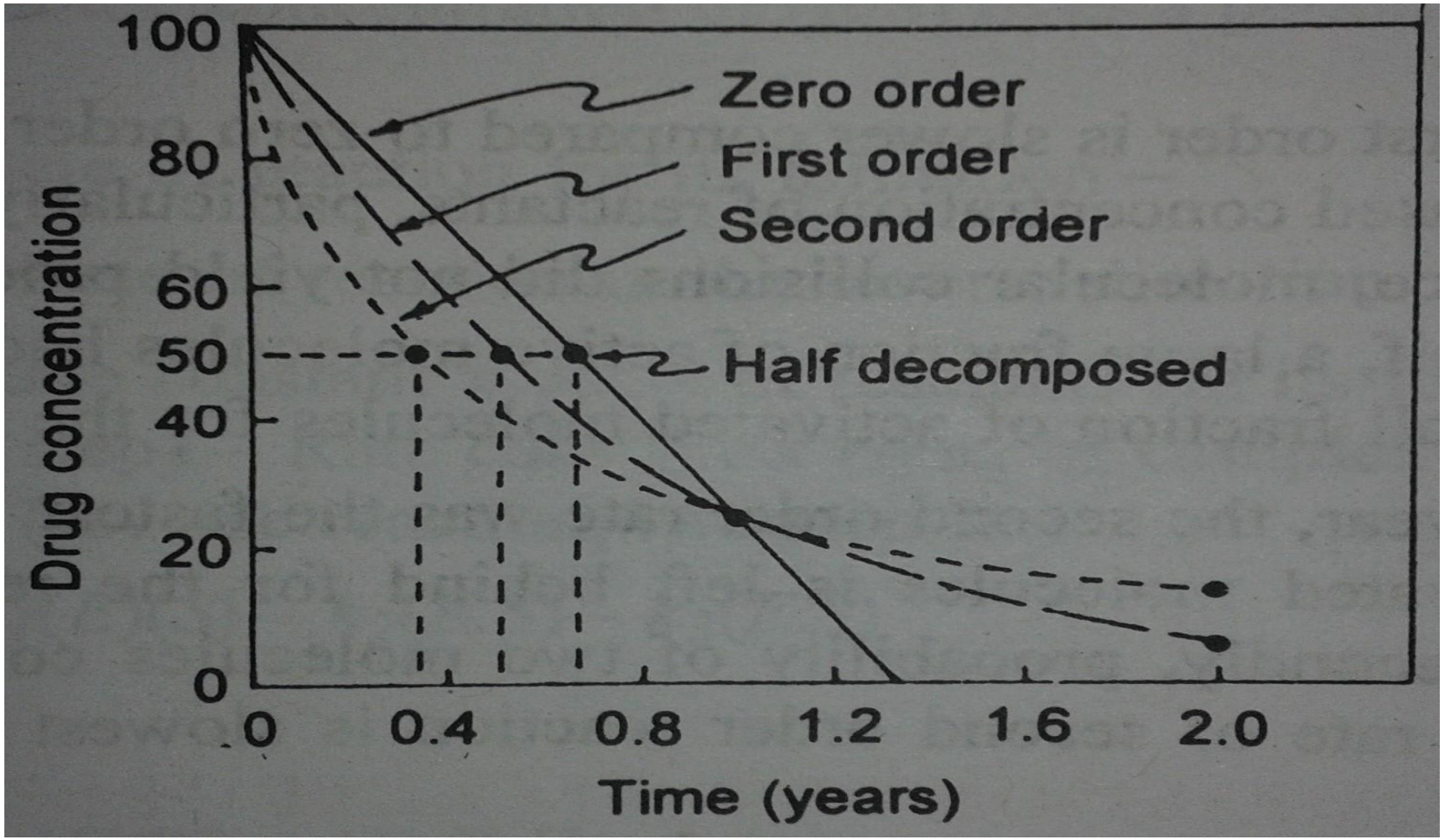
- Calculate the average k value using the data for zero, first, and second orders as given in substitution method or graphic method. Then, estimate the $t_{1/2}$ values for each time period in the kinetic study.
- Equations are as follows.



# 3. Half Life Method



# 3. Half Life Method



# Factors affecting reaction rate

- It is the time course of changes in the concentration of the reactants in a reaction.

1. Drug stability
2. Dissolution
3. Drug release
4. Pharmacokinetics
5. Drug action

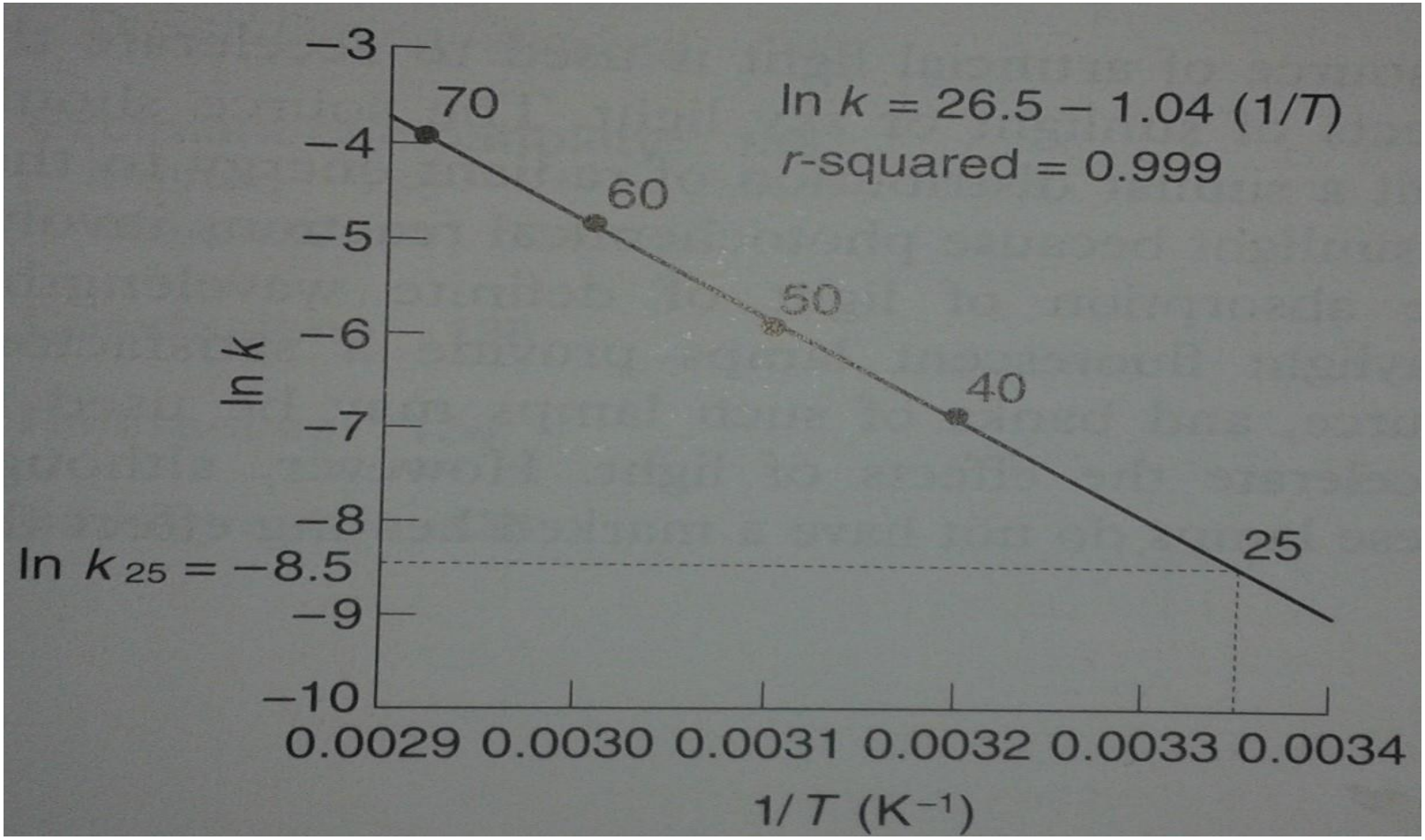
# Kinetics of solid state and stability testing

- In all solid dose formulations there will be some free moisture (contributed by excipients as well as the drug), and certainly in tablets a significant percentage, typically 2% w/w, is required to facilitate good compression.
- The ionic equilibria are quite different and comparison is meaningless.
- They should not be extrapolated glibly to the solid state.

# Prediction of Shelf-Life

- The mathematical prediction of shelf-life is based on the application of the Arrhenius equation.
- If the slope of this line is determined from the results of accelerated tests at high temperatures.
- Substitution of this value of k into the appropriate order of reaction allows the amount of decomposition after a given time to be calculated.
- This approach involves, and preliminary experiments are therefore necessary to determine this order.

# Prediction of Shelf-Life



# Storage of pharmaceutical products

- Appropriate storage of raw materials which includes drugs, excipients and finished products is necessary.
- Storage of medicinal products maintains the physical, chemical and biological properties.
- Major areas which demand careful consideration of storage are hospitals, marketplace of retailers and wholesalers.
- All medicinal products should be stored in such a way as to avoid contamination.

# Methods for Storage of pharmaceutical products

- Some of the methods are discussed in the following sections.

1. Storing of products in well-closed containers
2. Storing the products by protection from light
3. Storing the products in a cool place
4. Storing the products by the addition of other substances

# Kinetics and Thermodynamics

- **Kinetics:** Chemical kinetics involve the study of the rate of a chemical process. The rate of a reaction can be understood by studing the time course of changes in the concentration.

- **Thermodynamics:** Thermodynamics deals with the quantitative relationships of interconversion of the various forms of energy, including mechanical, chemical, electric, and radiant energy.

# Drug Stability and Stabilization Techniques

# CONTENTS

- Stability
- Physical And Chemical Instability
- Stabilization of polymorph in formulation
- Chemical degradation pathways of drugs and their stabilization technique.
  - Arrhenius equation and calculation of shelf-life

# DRUG STABILITY

- Stability of pharmaceutical product may be defined as the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological therapeutic and toxicological specification.

- Assurance that the packed product will be stable for its anticipated self life must come from an accumulation of valid data on the drug in its commercial package.

- These stability data involves selected parameters that taken together from the stability profile. Pharmaceutical products are expected to meet their specification for identifying purity, quality and strength throughout their defined storage period at specific storage condition.

# DRUG STABILITY

- The stability of pharmaceutical product is investigated throughout the various stages of the development process.
- The stability of the drug substance is first assessed in the preformulation stage.
- At this stage, pharmaceutical scientists determine the drug substance.
- Stability/ compatibility with various solvents, buffered, solutions, and excipents considered for formulation developments

# DRUG STABILITY

- Optimization of the stable of formulation of a pharmaceutical product is built upon the information obtained from the performulation stage and continues during the formulation development stages.

- Once a pharmaceutical product has gained regulatory approved and is marketed, the pharmacist must understand the proper storage and handling of the drug.

# DRUG STABILITY

- In some cases a pharmacist may need to prepare stable compounded preparations from this product.

- It is the responsibility of the pharmacist via the information of the manufacture to instruct the patient in the proper storage and handling of the drug product.

# DRUG STABILITY

- The USP defines the stability of pharmaceutical product as "extent to which a product retains within specified limits" and throughout its period of storage and use(i.e its shelf life) the same properties and characteristics that it possessed at the time of its manufacturer"

# DRUG STABILITY

- There are five types of stability that must be consider for each drug

| Type of Stability | Conditions Maintained Throughout the Shelf-Life of the Drug Product |
|---|---|
| Chemical | Each active ingredient retains its chemical integrity and labeled potency, within the specified limits. |
| Physical | The original physical properties, including appearance, palatability, uniformity, dissolution, and suspendability are retained. |
| Microbiological | Sterility or resistance to microbial growth is retained according to the specified requirements. Antimicrobial agents that are present retain effectiveness within the specified limits. |
| Therapeutic | The therapeutic effect remains unchanged. |
| Toxicological | No significant increase in toxicity occurs. |

# DRUG STABILITY

- Stability of drug also can be defined as the time from the date of manufacture and packaging of the formulation until its chemical or predetermined level of labelled potency and its physical characteristics have not changed appreciably.

# WHY STABILITY…

- Provide a evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as….. temperature, Humidity and light.

- Establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions.

- Because physical, chemical or microbiological changes might impact the efficiency and security of the final product.

# Where and Why?

## Stability Studies are preformed on ...

- **Drug Substances (DS)** The unformulated drug substance that may subsequently be formulated with excipients to produce the dosage form.

- **Drug Products (DP)** The dosage form in the final immediate packaging intended for marketing..... controlled and documented determination of acceptable changes of the drug substance or drug product

# What are changes?

- Physical changes
  - Appearance
  - Melting point
  - Clarity and color of solution
  - moisture
  - Crystal modification (Polymorphism)
  - Particle size
- Chemical changes
  - Increase in Degradation
  - Decrease of Assay
- Microbial changes

# Forced degradation studies

- Acidic & Basic conditions.
- Dry heat exposure
- UV radiation exposure
- Influence of pH
- Influence of temperature
- Influence of ionic strength

# STABILITY

- Ideally any commercial pharmaceutical product should have a shelf life of 5 yrs and should not fall below 90-95% potency under recommended storage.

- In designing a solid dosage form it is necessary to know the inherent stability of the drug substance, excipients to be used, formulation procedure.

- For a drug substance, we need to study 3 categories of stabilities-

1. Solid state stability of drug only
2. Compatibility studies ( drug+ excipients )
3. Solution phase stability

# 1. SOLID STATE STABILITY

- It includes both physical and chemical stability
- Physical changes caused by Polymorphic transitions and Hygroscopicity.
- Chemical changes such as solvolysis, oxidation, photolysis, pyrolysis.
- Examination of the chemical structure.
- Example- presence of unsaturation makes the compound susceptible to free radical mediated or photocatalyzed oxidation.
- Strained rings are more prone to pyrolysis.

# PHYSICAL CHANGES/INSTABILITY

- Solubility
- $pK_a$
- Melting point
- Crystal form
- Equilibrium moisture content.

- Example- amorphous materials are less stable than their crystalline counterparts.

- A relatively dense material may better withstand ambient stresses aminobenzylpenicillin trihydrate is more denser and stable than its amorphous form.

# CHEMICAL CHANGES/INSTABILITY

- Solid state reactions are generally slow and it is customary to use stress conditions in investigation of stability.
- Data obtained under stress is then extrapolated to make prediction of stability.
- High temperature can drive moisture out of a sample and render the material apparently stable otherwise prone to hydrolysis.
- Example- Above 65% relative humidity the beta form of chlortetracycline hydrochloride transforms into alpha form.

# CHEMICAL DEGRADATION STUDY

- **Hydrolysis-** usually drugs such as esters, amides and lactams undergo hydrolysis.

- **Oxidation Reduction-** loss of electrons, gain of electrons. Auto oxidation also is responsible. Eg- tetracyclines, vit A, vit D, morphine.

- **Photolysis**- Compounds such as ascorbic acid, riboflavin, cyanacobalamine, folic acid undergo degradation on exposure to light. Sometimes coupled with thermal reactions.

- **Isomerisation**-Compounds get converted into a less effective form. Eg-Adrenaline solutions at low pH lose activity since its levo form is more stable than dextro form

# ELEVATED TEMPERATURE STUDIES

- Tests are usually performed at 40 ,50 ,60$^{o}$C in conjuction with ambient humidity.
- Higher temperatures are also used, samples kept at highest temperature examined for chemical and physical changes at weekly intervals- if no change is seen after 30 days at 60$^{o}$C Stability prognosis is excellent.
- Arrhenius Treatment is used to determine the degradation rate at lower temperature.

# ARRHENIUS EQUATION (Effect of temperature)

- $K = Se^{-Ha}/RT$

where..k = specific rate of degradation.

R = gas constant ( 1.987 calories degree$^{-1}$mole).

T = absolute temperature.

S = frequency factor.

**Logarithmically ,**

$\ln k = -Ha/RT + \ln S$

**converting to log $_{10}$**

$\text{Log } k = -\Delta Ha/2.303\ R\ .1/T + \log S$

log k = specific rate of degradation

S = constant

# ARRHENIUS EQUATION

- Plot of log K v/s 1/T….yields a slope equal to -ΔHa/2.303 R ….. From which heat of activation (ΔHa) can be calculated.

- Log k2/k1 = ΔHa/2.303 R . ( T2 – T1 )/ T2.T1

# Mean Kinetic Temperature

$$Tk = \frac{\Delta H/R}{-\ln\left( e - DHRT_1 + e^{-\Delta H/R\, T_2} + \ldots + e^{-\Delta H/R\, T_n} \right)}{n}$$

Tk = Mean kinetic temp

H = Heat of activation (83.144 KJ/mole)

R = Universal gas constant ($8.3144 \cdot 10^{1}$ – KJ/mole/degree )

T1 = average storage temp during first time period ( months)

T2 = average storage temp during second time period (months)

Tn = average storage temp during $n^{th}$ time period ( months)

n = no of average temp recorded (min )

T = temp in $^{o}$ k ( degree kelvin )

# STABILITY UNDER HIGH HUMIDITY CONDITIONS

- In presence of moisture, many drug substances hydrolyze react with other excipients or oxidize.
- These tests are performed by exposing the drugs to different relative humidity conditions
- Preformulation data of this type is helpful in determining if the material should be protected and stored in a controlled low-humidity environment or if aqueous based granulation should be avoided.

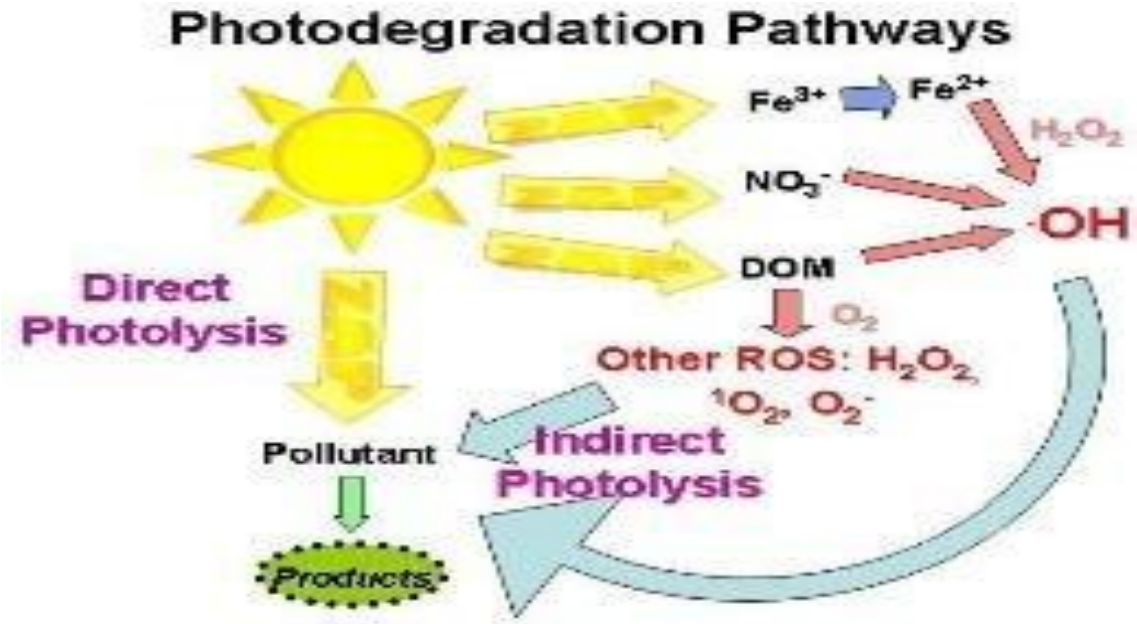
# PHOTOLYTIC STABILITY

- Many drugs fade or darken on exposure to light and this leads to an aesthetic problem which can be controlled by using

1 Amber Glass Container

2 Opaque Container

3 Incorporating a Dye



# STABILITY TO OXIDATION

- Stability to oxygen must be evaluated to establish that the final product should be packaged under inert atmosphere or it requires an antioxidant.
- A 40% oxygen atmosphere allows for rapid evaluation
- The samples are kept in dessicators.
- Process is repeated 3-4 times to assure 100% of desired atmosphere.

# 2. COMPATIBILITY STUDIES

- Three different techniques are employed in Drug-Excipient Compatibility Screening

1. TLC
2. Differential thermal analysis
3. Diffuse Reflectance Spectroscopy

# A. DRUG EXCIPIENT TLC

- Involves storage of both drug+excipient mixture as such and another one granulated with water at elevated temperature.
- Unstressed sample as control.
- Samples kept in ampoules to prevent escape of moisture.
- Tested for appearance/ decomposition using TLC.
- If any change in chromatogram such as appearance of spot/ change in Rf values is indicative of an interaction.

# B. DIFFERENTIAL THERMAL ANALYSIS

- Useful for investigation of solid-solid interactions.
- Thermograms are obtained for pure drugs and for mixtures using different ratios.
- In absence of any interaction thermograms of mixture show pattern corresponding to that of individual components.
- But if interactions occur it is indicated in thermograms by appearance of one/ more peaks corresponding to those components.

# C. DIFFUSE REFLECTANCE SPECTROSCOPY

- Drug+ excipient mixtures are exposed to incident radiations
- A portion of this incident radiation is partly absorbed and partly diffused.
- DRS depends upon packing density, particle size, crystal form.
- Helps to investigate physical and chemical changes occuring at surface
- Shift in diffuse reflectance spectrum- physical adsorption
- Appearance of new peak- chemisorption/ formation of a new degradation product.

# 3. SOLUTION PHASE STUDY

- This study assures that the drug substance does not degrade intolerably when exposed to gastrointestinal fluids.
- Stability of dissolved drug in buffers ranging from pH 1-8 is investigated.
- Example- if it degrades in acidic solutions a less soluble form will show increased bioavailability.

# EFFECT OF pH

- Most of the drugs are stable at pH 4 – 8.
- Weakly acidic and basic drugs are most soluble in ionized form and instability is likely as they are charged.
- This dilemma that potent drugs being poorly soluble, pH ionisation being best solution.
- Inclusion of a water miscible solvent increases stability thus suppressing ionization

# SOLVOLYSIS

- When the reacting solvent is not water then the breakdown is termed solvolysis
- It involves Transition State Theory, where reactants must attain a higher transitional energy state before a reaction proceeds
- Eg- if a compound produces more polar degradation products then addition of less polar solvent will stabilize formulation.
- If products are less polar, vehicle should be highly polar.

# MISCELLANEOUS PROPERTIES

- **DENSITY-**it is useful for the idea about size of final dosage form, critical for low potency drugs, also affects flow properties.

- **HYGROSCOPICITY**- equilibrium moisture content has to be calculated that influences the flow and compression characteristics and hardness of final tablet

# MISCELLANEOUS PROPERTIES

- **FLOWABILITY**- Flow properties are critical for efficient tabletting operation.
- Angle of repose has to be calculated which should be within 25 – $45^0$.
- Example- Acicular crystal materials with low density, and with static charge exhibit poor flow. Grinding of acicular crystals results in improvement of flow properties

- **COMPRESSABILITY**- powders that form hard compacts under applied pressure without exhibiting tendency to cap or chip are readily compressible

# POSSIBLE CHANGES (Visible and invisible)

- Loss of active ingredient
- Alteration in bioavailability
- Loss of content uniformity
- Decline of microbiological status
- Loss of pharmaceutical elegance
- Formation of toxic degradation products
- Loss of package integrity
- Reduction of label quality
- Modification of any factor of functional relevance( dissolution, release, etc)

# Stability studies at different stages

- Stability on pre-formulation batches
- Accelerated and long term testing for registration
- On-going Stability testing
- Follow-up Stabilities

# STRESS TESTING

- Helps to identify the likely degradation products and establish degradation pathways and intrinsic stability of molecule.
- Carried out on single batch.
- Effect of temperature ( every $10^0$ C)
- Humidity

# TRANSITION OF PRODUCT FROM IDEALITY TO NON IDEALITY AFTER MANUFACTURE

- Ideal production environment
- Regulations and controls
- GMP
- GLP
- Non-ideal shipment and storage due to- transport, wholesalers, retailers, hospital stores, patients.

# ROLE OF STABILITY TESTING

- Provides evidence on how the drug substance or product quality varies with time under environmental conditions during distribution.
- Helps to recommend storage conditions including establishment of shelf life, expiry date or retest period
- Key assurance of quality of pharmaceuticals.

# STAGES OF DRUG AND PRODUCT DEVELOPMENT AND STABILITY TESTING

- Pre-clinical studies
- Clinical studies
- Pre- formulation
- Formulation development
- Scale up
- Commercial manufacturing
- Distribution and shipping
- Post approval changes
- Market surveillance

**Selection of samples**

- API, excipient, batches

**Scope**

- Appearance
- Appropriate physical-chemical parameter
- Assay / Degradation products

**Up to 3 month**

**Scope**

- Solubility Profile
- Hygroscopicity
- Thermal stability (Melting point, Polymorphism)
- Chemical stability

1 Batch

**Up to 3 month**

**Scope**

- Determination of expire date
- Determination of preliminary specifications
- Release of clinical batches
- Monitoring of samples during the clinical phases
- Definition of storage conditions
- Definition of Tests for registration stability

**Up to 36 month**

# ICH

- ICH stands for International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use

- Objectives of ICH

- Harmonization of registration applications within the three regions of the EU, Japan and the United States.

- ICH is a joint initiative involving both regulators and industry as equal partners in the scientific and technical discussions of the testing procedures which are required to ensure and assess the safety, quality and efficacy of medicines.

- Tripartite guideline on stability testing of new drug substances and products (Q1A) in 1993, has become standard for stability evaluation in Japan, US, Europe.

# ICH Guidelines

- Quality Guidelines "**Q**" (chemical and pharmaceutical QA)
  - details see next slide

- Safety Guidelines "**S**" (in vitro and in vivo pre-clinical studies)
  - covering Carcinogenicity Testing, Genotoxicity Testing, Toxicokinetics and Pharmacokinetics ….. etc.

- Efficacy Guidelines "**E**" (clinical studies in human subject)
  - Covering clinical safety, Dose Response Studies, Good Clinical Practices, Clinical evaluation …. etc.

- Multidisciplinary Guidelines "**M**"
  - Covering Medical Terminology, Electronic Standards for Transmission of Regulatory Information …… etc.
  - Important for Stability !
    - » Guideline **M4**: The Common Technical Document (CTD)

# ICH Q-Guidelines (Quality)

- Stability Testing in Climatic Zone I and II (Q1A)
- Photostability Testing (Q1B)
- Stability Testing for New Dosage Forms (Q1C)
- Bracketing and Matrixing Designs (Q1D)
- Evaluation of Stability Data (Q1E)
- Stability Testing in Climatic Zones III and IV (Q1F)
- Validation of Analytical Procedures (Q2)
- Impurities (Q3)
- Q4- Pharmacopoeial Harmonization
- Biotechnological Products (Q5)
- Specifications (Q6)

# GUIDELINES

- $50^0$ C/ ambient humidity( to cover extremely hot and dry conditions, $30^0$ C/ 80% RH) to cover extremely high humidity conditions ; one batch for 3 months.

- WHO DRAFT GUIDELINE 2007

  For API, exposing a solid sample to elevated temperatures such as 60- $120^0$ C or 5- $10^0$ C below the melting point can generate a different degradation profile.

This approach usually generates degradation products that can be used as a worst case to assess the performance of analytical method

# TYPE, SIZE, NUMBER OF BATCHES

- ICH/ WHO GUIDELINES-

- At least 3 primary batches of drug product, should be of the same formulation, packaged in same container as proposed for marketing

- 2 out of 3 batches should be pilot scale batches.

- Stability to be performed on each strength, container size.

# LONG TERM STABILITY STUDIES

- Study is performed at 25$^0$C/60% or 30$^0$C/ 65%.
- Ideally 12 months data is to be generated but 6 months data is also acceptable in circumstances for submission of registration dossier, continued till end of shelf life.
- For parenterals stability has to carried out at 2-8$^0$ C for drugs to be stored in freezer testing should be done at -20$^0$ C

# ACCELERATED STABILITY STUDIES

- Storage condition of 40$^0$C and relative humidity of 75% has been recommended for all the four zones for drug substances and drug products.
- Studies carried out for 6 months.
- Accelerated storage conditions must be at least 15$^0$C above the expected actual storage temperature and appropriate relative humidity

# Climatic Zones / Storage conditions

| Climatic Zone | Calculated data | | Derived data | |
|---|---|---|---|---|
| Countries | Temp. °C | MKT °C | Humidity % RH | Temp °C | Humidity % RH |
| Climatic Zone I<br>"Temperate"<br>Japan, United Kingdom, Northern Europe,<br>Canada, Russia, United States | **20** | **20** | **42** | **21** | **45** |
| Climatic Zone II<br>"Mediterranean, Subtropical"<br>Japan, United States, Southern Europe | **26.4** | **22** | **52** | **25** | **60** |

# Climatic Zones / Storage conditions

| Climatic Zone Countries | Calculated data | | | Derived data | |
|---|---|---|---|---|---|
| | Temp. °C | MKT °C | Humidity % RH | Temp °C | Humidity % RH |
| Climatic Zone **III** "Hot, dry" Iran, Iraq, Sudan | **26,4** | **27,9** | **35** | **30** | **35** |
| Climatic Zone **IV** "Hot, humid" Brazil, Ghana, Indonesia, Nicaragua, Philippines | **26,7** | **27,4** | **76** | **30** | **70** |

# Climatic Zones / Storage conditions

| Study | Storage condition | Minimum time period covered by data at submission |
|---|---|---|
| Long term | **25°C ± 2°C / 60% ± 5% r.h or 30°C ± 2°C / 65% ± 5% r.h.** | **12 months** |
| Intermediate | **30°C ± 2°C / 65% ± 5% r.h.** | **6 months** |
| Accelerated | **40°C ± 2°C / 75% ± 5% r.h.** | **6 months** |

**Drug substances - intended for storage in a Refrigerator**

| Study | Storage condition | Minimum time period covered by data at submission |
|---|---|---|
| Long term | **5°C ± 3°C** | **12 months** |
| Accelerated | **25°C ± 2°C / 60% ± 5% r.h.** | **6 months** |

# Climatic Zones / Storage conditions

## Drug substances/Product- intended for storage in Freezer

| Study | Storage condition | Minimum time period covered by data at submission |
|---|---|---|
| Long term | **-20°C ± 5°C** | **12 months** |

## Drug products - General case

| Study | Storage condition | Minimum time period covered by data at submission |
|---|---|---|
| Long term | **25°C ± 2°C / 60% ± 5% r.h. or 30°C ± 2°C / 65% ± 5% r.h.** | **12 months** |
| Intermediate | **30°C ± 2°C / 65% ± 5% r.h.** | **6 months** |
| Accelerated | **40°C ± 2°C / 75% ± 5% r.h.** | **6 months** |

# Drug products - packaged in Semi-permeable containers

| Study | Storage condition | Minimum time period covered by data at submission |
|---|---|---|
| Long term | **25°C ± 2°C / 40% ± 5% r.h.**<br>**or**<br>**30°C ± 2°C / 35% ± 5% r.h.** | **12 months** |
| Intermediate | **30°C ± 2°C / 65% ± 5% r.h.** | **6 months** |
| Accelerated | **30°C ± 2°C / 65% ± 5% r.h.** | **6 months** |

# PROTECTION AGAINST HYDROLYSIS

- Good packaging practices like moisture resistant packs. Eg- strip packs stored in controlled humidity and temperature conditions, even using desiccant such as silica gel.
- Buffering agents for pH control
- Alteration of dielectric constant
- Addition of complexing agents like caffeine
- Use of Surfactants ,Good Refrigeration

# PROTECTION AGAINST OXIDATION

- Incorporation of antioxidants such as BHA, BHT, Propyl gallate, Tocopherol
- Chelation using EDTA, Citric acid, Tartaric acid
- Use of inert gas like Nitrogen
- Protection from light by use of amber colored container
- Storage at low temperature

# Testing scope for Solid dosage

- **Physical-chemical properties**
  - Appearance
  - Elasticity
  - Mean mass
  - Moisture
  - Hardness
  - Disintegration
  - Dissolution

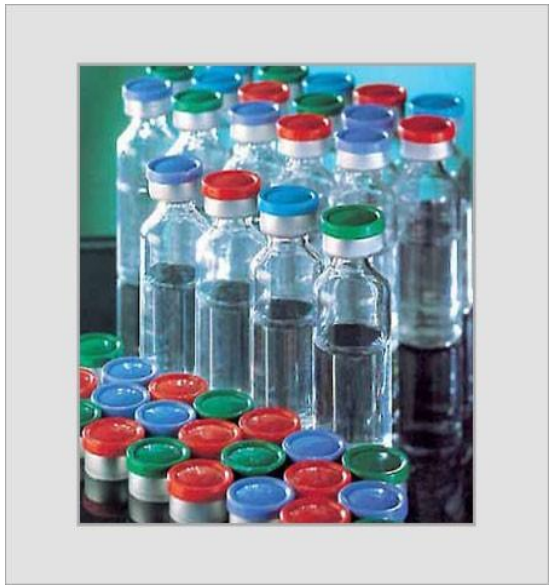
- **Chemical properties**
  - Assay
  - Degradation


- **Microbial properties**

- **Container closure system properties**
  - Functionality tests (e.g. extraction from blister)

# Testing scope for LIQUID FORMS for inj. and PARENTRAL

- Physical-chemical properties
  - pH
  - Loss on weight
  - Color & clarity of solution
  - Sterility Tests
- Chemical properties
  - Assay
  - Degradation products
  - Degradation preservatives
  - Content antioxidants
- Microbial properties
  - Pyrogen Testing
- Container closure system properties
  - Functionality tests
  - Leakage test



# Testing scope for Oral liquid form

- Physical-chemical properties
  - pH
  - Color & clarity of solution
  - Viscosity
  - Particle size distribution (for oral suspensions only)
- Chemical properties
  - Assay
  - Degradation products
  - Degradation preservatives
  - Content antioxidants
- Microbial properties
- Container closure system properties
  - Functionality tests

# Testing scope for SEMI LIQUID FORMS

- Physical-chemical properties
  - Appearance, odor, homogeneity, consistency
  - Loss on weight, Viscosity
  - Content uniformity (within the container)
- Chemical properties
  - Assay
  - Degradation products & preservatives
  - Content preservatives
  - Degradation– Content antioxidants
- Microbial properties
- Container closure system properties
  - Functionality tests

# Recent development in ICH Guidelines

- In February this year two new ICH Guidelines on the topic of stability testing were published. They can now be commented. Q1E Draft Consensus Guideline Evaluation of Stability Data

- Q1F Draft Consensus Guideline
  Stability Data Package for Registration in Climatic Zones III and IV

- Both new Drafts refer to the revised ICH Guideline Q1A(R) – "Stability Testing of New Drug Substances and Products."

# Recent development in ICH Guidelines

- The Guideline "Evaluation of Stability Data" describes when and how an extrapolation of the data can be undertaken in order to establish the re-test period for a drug substance or the shelf life for a drug product beyond the observed range itself, based on the data resulting from the long-term stability testing.

- The Guideline on stability testing for Climatic Zone III and IV takes up a proposal made by WHO and now defines not only storage conditions for stability testing relevant for the ICH tripartite regions (Europe, USA, Japan), but also completes the recommendations for the standardization of the storage conditions for the Climatic Zones III (dry-hot) and IV (very hot/humid).

# Recent development in ICH Guidelines

- For these Climatic Zones, the following standard conditions are recommended:
- Long-term testing: 30°C / 65% RH
- Accelerated conditions: 40°C / 75% RH
- This means that the "accelerated conditions" remain the same as in the Q1A(R) Guideline and only the "long-term storage conditions" have to be modified.

# SHELF LIFE

- Self life (referred to as expiration dating period) is the time period during which a drug product is expected to remain within the approved specification for use, provided that it is stored under the conditions defined on the container label.

# SHELF LIFE

*Maximum and Minimum time at which potency must be at least 90% of label claim at the temperature indicated in order to predict a shelf life of two years at Room Temperature.*

| Temperature | Maximum time for Study | Minimum time for Study |
|---|---|---|
| 37° | 12 Months | 6.4 Months |
| 45°C | 8.3 Months | 2.9 Months |
| 60°C | 4.1 Months | 3 Weeks |
| 85°C | 06Weeks | 2.5 Days |

# Calculation of shelf life

- Example:- self life of Aspirin suspension:
- A prescription for a liquid aspirin is called for, It contains 325mg/5ml or 6.5g/ 100ml.
- Solubility of aspirin at $25^0C$ is 0.33g/100ml. Therefore the suspension will definitely be a suspension.
- Other ingredients in the prescription cause the product to have a pH of 6.
- The first order rate constant for aspirin degradation in the solution is $4.5\times10^{-6}$ $sec^{-1}$.
- Calculate the zero order rate constant.
- Determine the self life, $t_{90}$ for the liquid preparation, assuming that the product is satisfactory until at the time at which it has decomposed to 90% of its original concentration (i.e 10% decomposition) at $25^0C$.

# Calculation of shelf life

Ans:- $K_0 = K \times$ [ aspirin in solution],

Thus $K_0 = [4.5 \times 10^{-6}\ sec^{-1}] \times [0.33g/100ml]$

$K_0 = 1.5 \times 10^{-6}\ g/100ml\ sec^{-1}$

$$t_{90} = \frac{0.10[A]_0}{K_0}$$

$$= \frac{(0.10)(6.5g/100ml)}{1.5 \times 10^{-6} g/100ml\ sec^{-1}}$$

$= 4.3 \times 10^{5}$ sec

= 5 days

# STABILITY OF DRUG

# Overview :

# Drug Stability
# Factors effecting drug stability
# Objective of drug stability
# Various types of drug stability
# Various types of drug instability
# Stability Testing
# Stability studies for Pharmaceutical Products

## Drug Stability:

The capability of a particular drug formulation in a specific container to remain within a Particular chemical, microbiological, therapeutically, physical & Toxicological specification in a specified period of time.

## Drug Instability:

The incapacity or incapability of a particular formulation in a specific container to remain within a particular chemical, microbiological, therapeutically, physical & toxicological specification.

# Shelf life:

Shelf life may be defined as the time required to degrade a pharmaceutical product to 10% which is pharmaceutically acceptable. It is indicated as t90 and the unit is time/conc.

$$t90 = \frac{(a-0.9a)}{ko} = \frac{0.1\,a}{ko}$$

Where, a = initial concentration of drug product .
$k_o$ = specific rate constant for zero order reaction.

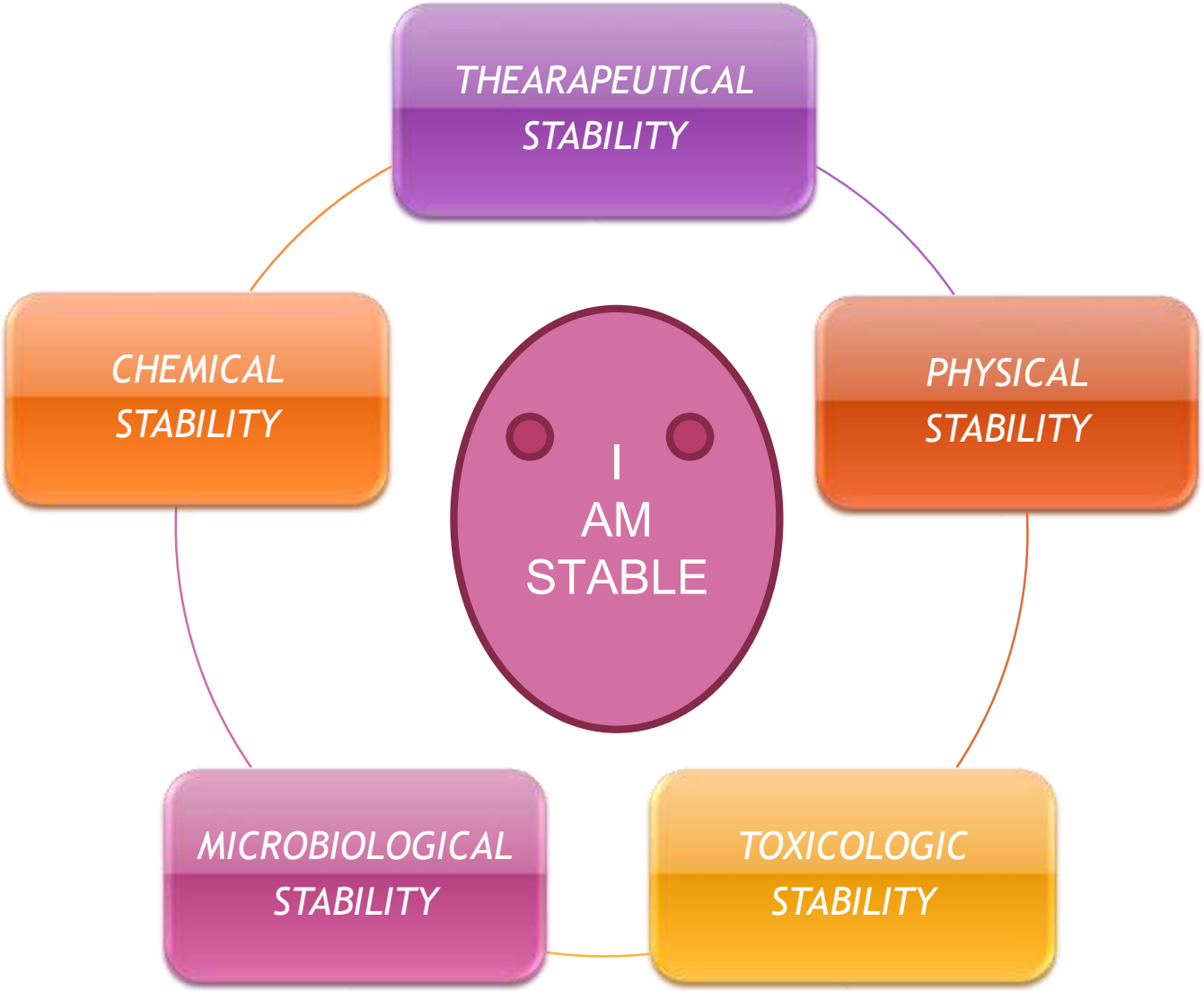
# Factors effecting drug stability:

- PH
- Temperature
- Moisture
- Humidity
- Light
- Storage closure and containers
- Oxygen
- Particle size  (suspension and emulsion)
- Additives
- Molecular binding
- Diffusion of drugs and excipients .

# Objective of the drug stability:

- To determine maximum expiration date/ shelf life.
- To provide better safety to the patients.
- To provide better storage condition.
- To determine the packaging components.
- To gather information during preformulation stage to produce a stable product.

# TYPES OF STABILITY



# TYPES OF STABILITY CONSIDERED FOR ANY DRUG :

- **Chemical :** Heat of combustion, enthalpy, chemical stability.
- **Physical :** appearance , palatability ,uniformity & suspendability.
- **Microbiological :** resistance to microbial growth.
- **Therapeutic :** unchanged.
- **Toxicological :** No significant change.

# TYPES OF DRUG INSTABILITY:

2 major types-

i. Physical degradation
ii. Chemical degradation

## i. Physical degradation:

"Degradation, which results into the change of physical nature of the drug."

**Types:** Types of physical degradation are as under :

1. Loss of volatile components
2. Loss of $H_2O$
3. Absorption of $H_2O$
4. Crystal growth
5. Polymorphic changes
6. Color changes

# 1. Loss of Volatile Components:

*Examples:*

a. Aromatic waters

b. Elixirs

c. Spirits

## 2. Loss of $H_2O$:

a. Saturated solution: become supersaturated and precipitate as crystals.

b. Emulsions: leads to separation & change to other type.

c. Creams: especially oil/water, they become dry.

d. Pastes.

e. Ointments: especially aqueous base ointments.

***Humectant*** *can* prevent this. ex: Glycerin

## 3. Absorption of $H_2O$:

Hygroscopic drugs causes the physical degradation which depends on temperature and humidity.

Can be seen in-

a. Powders
b. Suppositories made from Glycerin, Gelatin, polyethylene glycol.
c. Calcium chloride, potassium citrate.

## 4. Crystal Growth:

In solutions after super saturation crystal growth occurs. E.g. Injection of calcium glucconate.

In suspensions crystals settle down e.g.  Ophthalmic preparations.

## 5. Polymorphic Changes:

In polymorphic changes a stable crystal form loosens. This may cause alteration in solubility and possibly crystalline growth in aqueous suspensions.

## ii. Chemical Degradation:

Change in the chemical nature of the drug is called as chemical degradation.

It can occurs through several pathways like –

hydrolysis ,oxidation , decarboxylation , photolysis.

# 1. Hydrolysis:

It is defined as the reaction of a compound with water.

- Most important systems containing water are emulsion , suspension , solutions , etc.
- It is usually catalyzed by hydrogen ion(acid) or hydroxyl ion(base).
- In this process active drug is decomposed with solvent.
- Main classes of drugs that undergo hydrolysis are the Esters ,Amide ,Alkali, Acid.

## Ester Hydrolysis:

R-COOR (ester) + $H_2O$ → R-COOH (acid) + R-OH(alcohol)

Example of drugs: aspirin, atropine, procaine.

## Amide Hydrolysis:

RCONHR(amide) + $H_2O$ → RCOOH + R-$NH_2$(AMINE)

Example of drugs : chloramphenicol, barbiturates.

**Types of Hydrolysis:** It has two types :

- Ionic hydrolysis
- Molecular hydrolysis

**Ionic hydrolysis:**

It occurs when the salts of the weak acids & bases interact with water to give either alkaline or acidic solutions. e.g. $CH_3COOK$ gives alkaline while codeine phosphate gives acidic Sol when interact with water.

**Molecular Hydrolysis:**

It is much slower and irreversible process. It is catalyzed by hydrogen or hydroxyl ion. e.g. the local anaesthetics, amethocaine and benzocaine.

# Protection against Hydrolysis :

- Avoiding contact with moisture at time of manufacture.
- In liquid dosage form optimum PH for max stability
- Hydrolysis of certain drugs such as benzocaine and procaine can be decreased by the addition of specific complexing agent like caffeine to the drug solutions .
- Hydrolysis susceptible drugs such as penicillin and derivatives can be prevented by formulating them in the dry powder form instead of a liquid dosage form such as solutions or suspensions.

# Preventive Measures for Hydrolysis:

## (i) Adjustment of pH:

Rate of decomposition is critically dependent upon pH. In the case of acid-base catalyzed hydrolysis at minimum pH the drug stability is maximum.

## (ii) Choice of solvent:

- Aspirin is unstable in aq. Sol. So it is formulated in alcohol.
- In some cases non-aq. Solvent increases the instability of product e.g. Cyclamic acid in aq. sol. Hydrolyze in slow rate while in alcohol high rate.

**(iii) Addition of surfactants:**

Addition of surfactants results into significant improvement of drug stability. Because of micelles formation. Surfactants are of two types cationic and anionic.

**(iv) Production of insoluble form of drug:**

Hydrolysis occur only with that portion of drug which is in aq. Sol.

Hydrolysis can be minimized by

- making suspensions
- pH adjustment of the aq. Vehicle.
- preparing insoluble salt of the drug.

## (v) Modification of chemical structure:

Change of chemical structure of a chemical drug may prevent the hydrolysis.

e.g. Alkyl to alkyl chain.

## (vi) Presence of complexing agent:

Complexing agent form water soluble complex with drug so that the rate of decomposition may be decreased. e.g. caffeine decrease the rate of decomposition of local anesthetics such as benzocaine, procaine & amethocaine.

# 2. Oxidation:

Removal of an electropositive atom, radical or electron, or the addition of an electronegative atom or radical.

- Oxidation is controlled by environment i.e, light ,trace elements , oxygen and oxidizing agent.
- Occurs when exposed to atmospheric oxygen.

## Types:

Oxidation has two types

· Auto-oxidation

· Photo-oxidation

## (i) Auto-oxidation:

- Oxidation in which the oxygen present in the air is involved. This process proceeds slowly under the influence of atmospheric oxygen e.g. Oil, fats & unsaturated compound can undergo auto- oxidation.

- Free radicals produced during initial reaction are highly reactive and further catalyze the reaction produced additional free radicals and causing a chain reaction.

# (ii) Photo-oxidation:

Oxidation in which removal of the electron is involved without presence of $O_2$. This type is less frequently encountered e.g. It occurs in adrenaline, riboflavin & ascorbic acid etc.

## STEPS INVOLVED OXIDATION REACTION:

- **INITIATION** : Formation of free radicals is taken place .

  R--H → R. + [H. ]
- **PROPOGATION** : free radical is regenerated and react with more oxygen .

  R. + O2 → R.—O2

  R.O2 + RH → ROOH + R.
- **HYDROPEROXIDE DECOMPOSITION**

  ROOH → RO. + OH.
- **TERMINATION** : free radicals react with each other resulting in inactive products.

  R.--O2 + X → Inactive product

  RO2 + RO2 → Inactive product

## EXAMPLE OF DRUGS DECOMPOSED BY OXIDATION PATHWAYS

clove oil , ethyl oleate ,Heparin , Ascorbic acid , Morphine ,Vitamin A , Vitamin B12 , etc.

# Protection against oxidation:

**(i) Use of anti-oxidants:** antioxidants are Mainly of 3 types :

1. The first group probably inhibits the oxidation by reacting with free radicals.

Example – tocopheral , butylated hydroxyl anisole (BHA) , butylated hydroxyl toluene's (BHT). Concentration 0.001 – 0.1%.

2. The second group comprising the reducing agents , have a lower redox potential than the drug or other substance that they should protect and are therefore more readily oxidized.Example –ascorbic acid and iso ascorbic acid , potassium or sodium salts of metabisulfite.

3. The third group, little antioxidant effect themself but enhance the action of true antioxidant Example -- Citric acid , tartaric acid , disodium edetate and lecithin .

## (ii) Use of chelating agents :

when heavy metals catalyze oxidation .

Example -- EDTA , citric acid , tartaric acid form complexes.

- The presence of reducing agent:
  Oxidation of pharmaceutical products can be retarded by the addition of reducing agents they are equally effective against oxidizing agents and atmospheric oxygen.
  e.g.
    - potassium metabisulphites
    - sodium metabisulphites

**(iii) Removal of oxygen:**

By limiting the contact of drug with the atmosphere oxidation may be often minimized.

**(iv) The presence of surface active agent:**

Presence of surface active agents such as oil soluble vitamins,essential oils and unsaturated oils can reduce the oxidation.

# 3. Photolysis :

Exposure to light cause substantial degradation of drug molecule.

- When molecules are exposed to electromagnetic radiation they absorb light which cause increase in energy which can :

- Cause decomposition.
- Retained or transferred.
- Be converted to heat .
- Result in light emission at a new wavelength (fluorescence , phosphorescence).

**Example of phototoxic drugs:**

Furosemide, acetazolamide, cynocobalamine.

**Protection from photolysis :**

1. Use of amber colored bottles.
2. Storing the product in dark , packaging in cartons.
3. Coating of tablets with polymer films.

## 4. Decarboxilation:

Elimination of $CO_2$ from a compound.

e.g.
- When sol. Of $NaHCO_3$ is autoclaved.
- autoclaving the tuberculostatic agent sodium aminosalicylate

## 5. Isomerization:

Conversion of an active drug into a less active or inactive isomer having same structural formula but different stereochemical configuration.

Types:

- Optical isomerization
- Geometrical isomerization

## 6. Polymerization:

Combination of two or more identical molecules to form a much larger and more complex molecule.

e.g. Degradation of antiseptic formulations and aldehydes is due to polymerization.

# Regulatory Requirements:

Stability study requirement and expiration dates are covered in the current GMP , USP and FDA

- GMP (Good Manufacturing Practice) states that there will be written testing program design to access the stability characteristics of drug products . And result of such stability testing will be used to determine appropriate storage condition and expiration dates.

# ICH guideline for stability Testing:

The ICH has so far released six guidelines for stability studies as indicated in table :

| ICH GUIDELINES | TITLE |
|---|---|
| Q 1 A | Stability testing of new drug substances and products (second revision) |
| Q1B | Stability testing : photo stability testing of new drug substance and products. |
| Q1C | Stability testing for new dosage forms |
| Q1D | Bracketing and matrixing designs for stability testing of drug substances and products |
| Q1E | Evaluation of stability data |
| Q1F | Stability data package for registration application in climatic zones III and IV |

# Climatic zones:

AS per ICH and WHO guidelines ,world has been divided into four zones :

1. Zone i – Temperate.
2. Zone ii - Subtropical with possible high humidity.
3. Zone iii – Hot, dry.
4. Zone iv – Hot, humid.

Bangladesh belongs to “IVa climatic zone”.

# Long term stablity testing :

According to WHO, long term stability testing during and beyond expected shelf life under storage conditions in the intended market.

## Recommended condition for long term stability testing:

| STORAGE CONDITIONS | | |
|---|---|---|
| TEMPERATURE (‘C) | RELATIVE HUMIDITY% | MINIMUM TIME |
| 25’C+/- 2’C | 60 +/- 5% | 12 MONTHS |
| 30’C +/- 2’C | 30+/- 5% | 6 MONTHS |

# ACCELERATED STABILITY STUDIES:

| STORAGE CONDITIONS | | |
|---|---|---|
| TEMPERATURE (‘C) | RELATIVE HUMIDITY% | MINIMUM TIME |
| 40’C +/- 2’C | 75 +/-5% | 6 MONTHS |

- Must be 15’C above the actual storage temperature and relative humidity.

**Table 3 Test conditions for accelerated physical stability tests for capsule dosage forms**

| Test conditions | Observation |
|---|---|
| 80 % RH at room temperature in an open container. | Capsules are observed periodically for 2 weeks, both gross and subtle effects of the storage conditions are noted and recorded. The control capsule should not be affected except at the 80% RH station. |
| 40° C in an open container. | |
| 40° C in a closed container (glass bottle with tight screw-cap). | |

# Stability studies for pharmaceutical products:

## (i) Tablets :

- **FRIABILITY TEST :** Studies revel the physical instability if any in tablet. Maximum weight loss should not be more than 1%.
- **HARDNESS TEST :** Shows resistance to crushing.
- **CONTENT UNIFORMITY TEST** : The Tablet pass the test if 9 of the 10 tablets must contain not less than 85% and not more than 115% of the labeled drug content.
- **DISINTEGRATION TEST :** Uncoated tablet: 5-30 minutes, Coated tablet: 1-2 hours.
- **DISSOLUTION TEST :** Basket apparatus (U.S.P.-I), Paddle apparatus (U.S.P.-II)

## (ii) Gelatine capsules :

- Gelatin capsules are largely effected by temperature and humidity and susceptibility to microbial degradation .

- soft gelatin capsule have Relative Humidity 20 to 30% at 21 to 24’C.

- hard gelatin capsule contain 13 to 16% moisture.

- . Hard gelatin capsule are tested for Brittleness , dissolution , water content and level of microbial contamination.

## (iii) Emulsions :

Tested for phase separation , PH , viscosity , level of microbial contamination , and distribution of dispersed globules.

## (iv) Oral solutions & suspensions :

- Formation of precipitate , clarity for solutions , PH , viscosity , microbial contamination.
- Additionally for suspensions , redispersibility , rheological properties ,mean size and distribution of particles should be considered .

## (v) Nasal sprays:

- **Solution and Suspensions**

Clarity , level of microbial contamination , PH , particulate matter , unit spray medication , content uniformity , particle size distribution , weight loss.

- Microscopic evaluation ,(for suspension) , foreign particulate matter and extractable/ leachable from components of the container , closure and pump.

## (vi) Topical, Opthalmic & Otic preparation :

- Included in this broad category are ointments ,creams , lotions ,paste , gel , solutions ,eye drops and cutaneous sprays.

- **TOPICAL**
  - Clarity , homogenesity , PH , resuspendibility for lotions , consistency , viscosity , particle size distribution , sterility and weight loss.

- **FOR OPTHALMIC OR OTIC PREPRATION**
  - Sterility , particulate matter ,and extractable.

- **SUPPOSITORIES**
  - Softening range , dissolution (at 37'C)

- **PARENTERALS**
  - Color , clarity (for solutions) , particulate matter , PH, sterility , pyrogen / endotoxins .

  - Color monitoring , reconstitution time and water content ,to be performed at regular intervals.

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- ICH Q1B : “Photostability Testing of New Drug Substances and Products”.

- ICH Q1C : Stability Testing of New Dosage Forms”.


Thank you