4) Solvent Effect:

In order to prepare a many of dosage forms, the active substance must be dissolved in water or water-mixed solvents. Most used are ethanol, propylene glycol, polymeric alcohols such as PEG, and low molecular weight alcohols such as glycerin.

The degradation reactions that occur if the reactant is not water is called «Solvolysis».

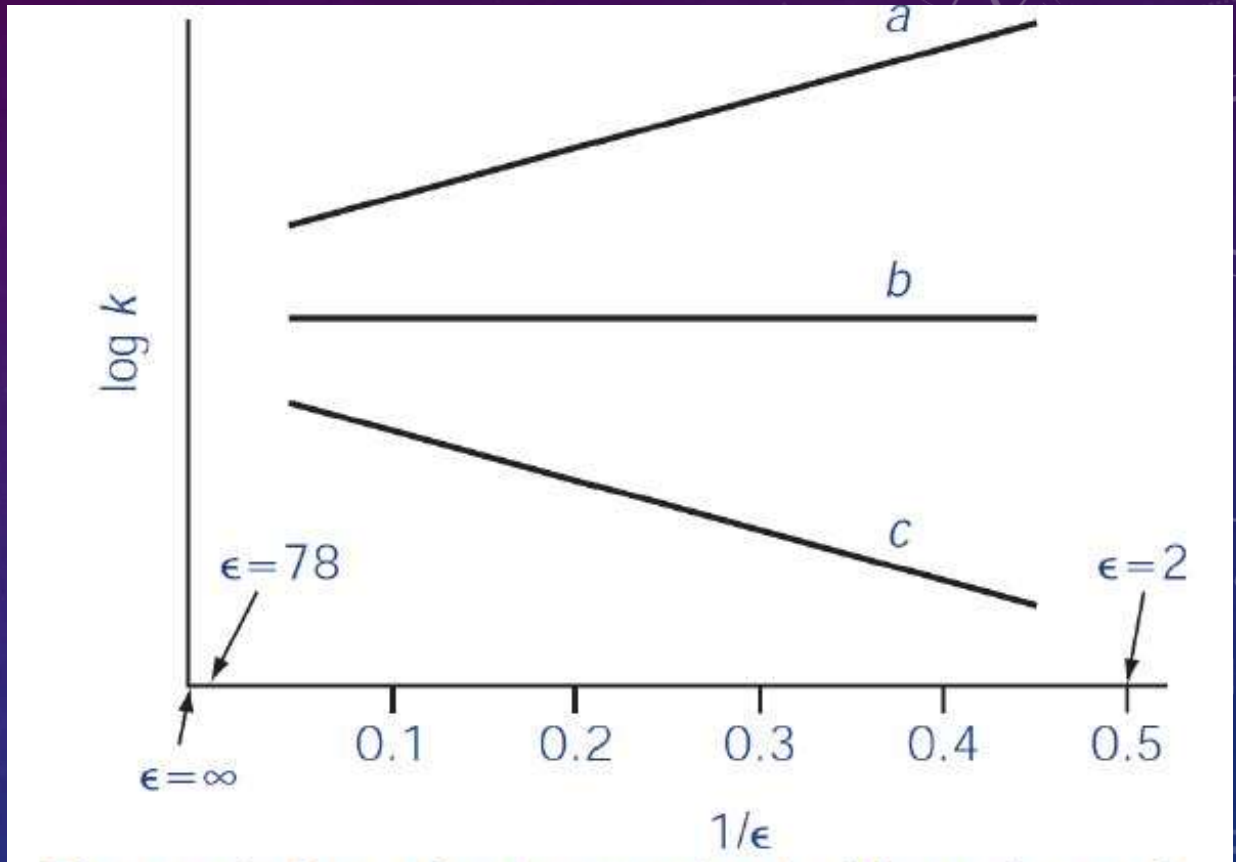
- Polar substances are soluble in polar solvents.

- If a substance is degredated in the solvent, the polarity of the solvent should be reduced if more polar material is formed.

- If less polar material is formed when the material is degraded, increasing the polarity of the solvent slows down the degredation.

Example: Salicylic acid is formed as the decomposition product in aqueous solutions of aspirin. Salicylic acid is less polar than aspirin and polar solvent should be added. However, aqueous solutions of aspirin cannot be prepared since there is no more polar solvent than water. The market product is therefore not available. The degredation rate can be reduced by using the active substance in the form of a suspension, water-insoluble salt or ester.

- For ions of like charge, an increase in dielectric constant of the solvent results in an increase in the rate of the reaction.
- For a reaction between ions of opposite sign, an increase in dielectric constant results in a decrease in the rate of the reaction.



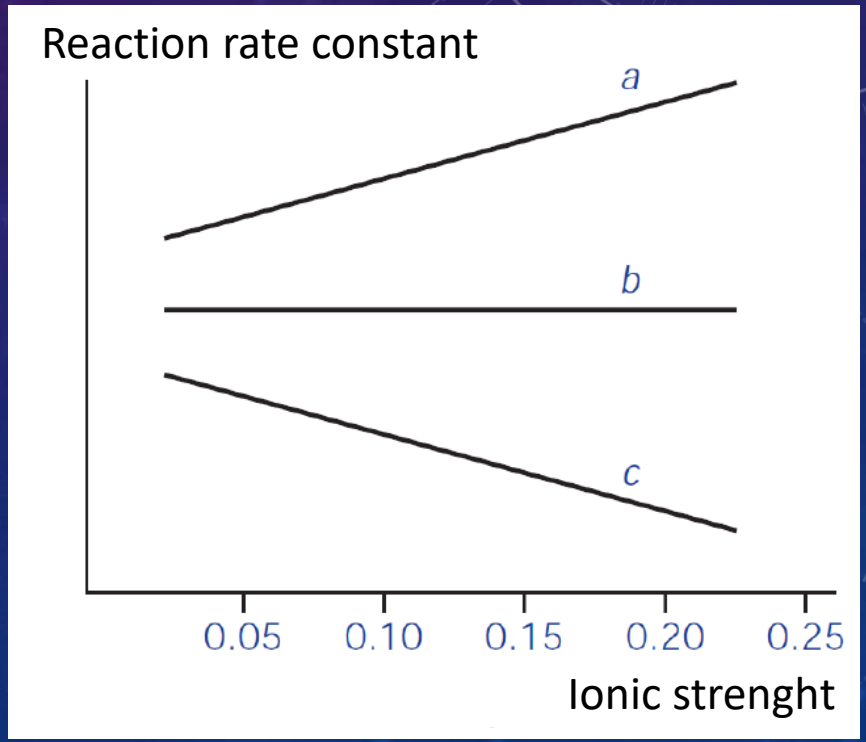
The variation of rate constant with reciprocal of dielectric constant for reaction between:
a: ions of opposite charge,
b: ion and uncharged molecule
C: ions of similar charge

5) Ionic Resistance and the Effect of Strenght:

In many drug solutions buffer salts are used to keep the pH of the formulation at optimum levels. These salts affect the rate of degradation of the drug in various ways.

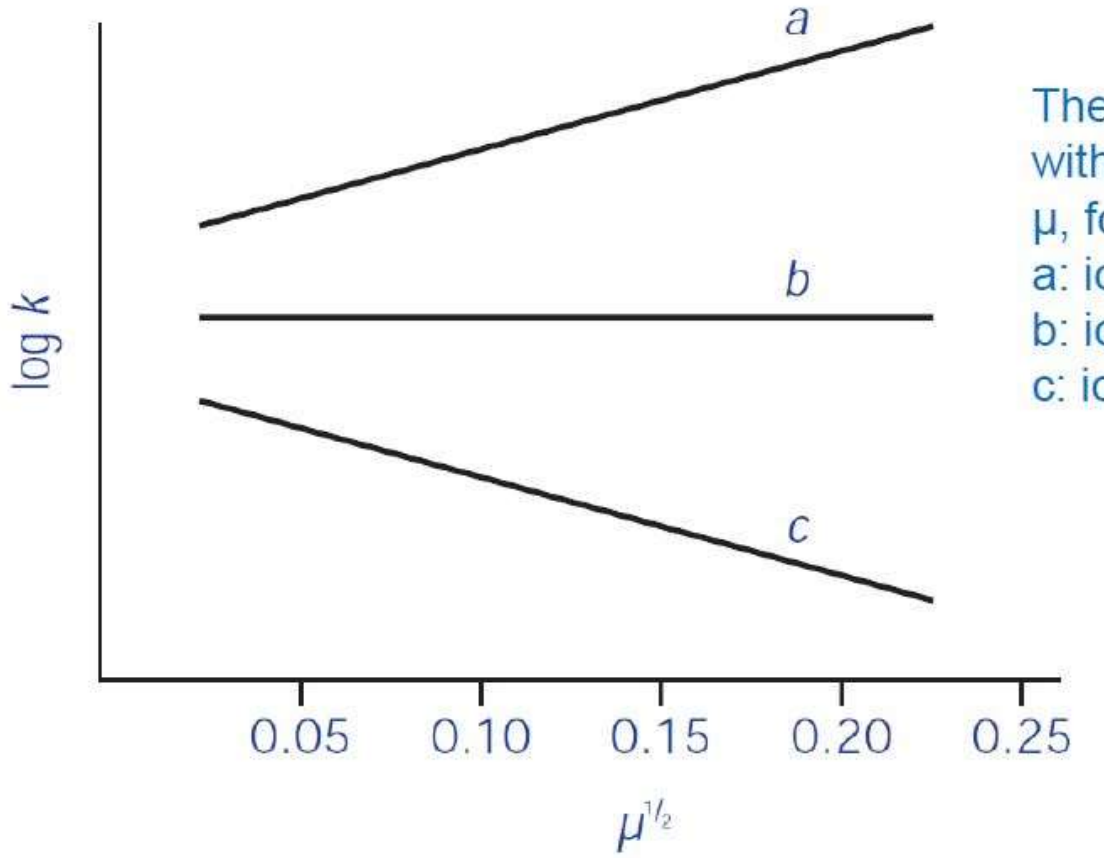
In this context, 3 cases are encountered:

a. If the reactants (ions) share the same charge, the activated complex will be more highly charged than the reactants. Increasing the ionic strenght of the solution will therefore have a greater stabilizing effect upon the complex than on the reactants, and will thus increase the rate constant by lowering the activation energy.



b. If the reactants are oppositely charged, the charge on the activated complex will be lower than the charges on the reactants, and the rate constant will decrease with ionic strength.

c. If one of the reactants is uncharged, there will be no change in the rate constant with ionic strenght.



The variation of rate constant, *k*, with square root of ionic strength, μ, for reaction between:
a: ions of similar charge,
b: ion and uncharged molecule and
c: ions of opposite charge.

6) Effect of dielectric constant :

The dielectric constant of an object is the ratio that reduces the attraction force between two electric charges.

When the two ions with different electric charges react, the degredation rate will decrease as the dielectric constant increases. When the ions in a similar charge react, the degredation rate increases as the dielectric constant of the medium increases due to the repulsion between the ions increases.

7) Effect of pH :

The pH of the solution is one of the most important factors affecting the reaction rate in aqueous solutions. The degradation of many drugs consists of extreme pH variations.

The rate of reaction is increased by the catalytic effect of hydrogen and hydroxyl ions (specific acid-base catalysis), and by the components of the buffer system (general acid-base catalysis).

To determine the degradation rates of the active substances, depending on the pH, the solution is prepared at a pH of 0-14 in a series of different pHs and the active substance is added. By determining the degredetion rates of this material, pH-rate profiles are formed. Using these profiles, the pH value of the active substance is determined to be the most stable.

8) Catalyst Effect :

Catalyst is a substance that increases the speed of a reaction without itself being altered chemically, by lowering the activation energy. The catalysts remain unchanged at the end of the reaction.

The catalysts generally increase the reaction rate by lowering the activation energy which is the minimum energy required for the reaction. Some catalysts may reduce the reaction rate.

If a catalyst inhibits the reaction, it is called a «negative catalyst».

There are 6 types of catalyst effects in chemical reactions:

1. ***Specific acid catalysts:*** *refers to the catalysis with H + ion from water.*
2. ***Specific base catalysts*** *are catalyzed by OH-ion from water.*
3. ***General acid catalysts:*** *refers to catalysis with protons from buffers outside the H + ion from water.*
4. ***General base catalysts:*** *refers to the catalysis with other OH- ions other than the OH-ion from water.*

5. ***Nucleophilic catalysts (Lewis Base):*** ***Electron-yielding substances. It refers to the catalysis by nucleophilic substances formed by the sharing of an electron pair with an atom other than the proton (usually C atom).***

6. ***Electrophilic catalysts (Lewis Acid):*** ***Electron field substances. It refers to the catalysis with acid catalysts that are involved in the formation of an electron pair.***

9) Effect of Solubility of Active Substance :

In order for an active substance to be degraded, it must first be dissolved.

Depending on the nature of the active substance, degredation may occur.

Example: Penicillins are easily hydrolyzed by cleavage of the β-lactam ring. In order to prevent hydrolysis, the water insoluble salts of penicillin were synthesized and the suspension was prepared. As the solubility of penicillin is reduced, degradation in suspension is delayed. While the half-life of the penicillin-Na or -K salts with a solubility of 10mg/ml in solution is one year, the half-life of the penicillin procaine monohydrate or benzoate tetrahydrate salts with solubility values of 1mg/ml in suspension is 7.3 years.

10) Effect of Excipients :

The efficacy of each excipient must be evaluated upon degradation of the active substance in the formulation development stage. For example, the surfactants are added to the formulations because they increase the solubility and protect the active substance from degradation by keeping it in the micelles. But;

- Polysorbate 80 increases the degradation of Prednisol.
- The protective activity of methyl parabens is eliminated if polysorbate 80 is present in the medium.
- If the talc is used in combination with thiamine HCl, the degredation rate of the substance increases.
- If ascorbic acid solution is used with sucrose, its stability increases and degredation rate decreases.

Hydrotropy: *Increasing the solubility of insoluble materials by forming an intermolecular complex with soluble substances.*

- Caffeine reduces the hydrolysis of the substance by creating steric obstruction and increases its solubility. For this reason, the stability of the active substances was increased by 2-3 times by adding to Prokaine, Benzocaine, Tetrakaine.

- Cyclodextrins increase stability when combined with the active agent.

- When used in combination with other drugs, antacids reduce the effectiveness of drugs and affect their shelf life. For example; If aspirin is formulated with antacid substances, its stability decreases and shelf life decreases.

DRUG DECOMPOSITION

- In the formulation of drug dosage forms, stability consideration for the active pharmaceutical ingredients and the excipients is critical.
- This is because degradation and decomposition processes lead to loss of efficacy, making the drug in a specific packaging not to remain in the specified chemical, physical, microbiological, therapeutic and toxicological specifications.

- Therefore, understanding the degradation pathways in order to achieve stability of both the drug substance and drug products is a key quality goal.
- Pharmaceutical products tends to deteriorate on storage, even though it is expected to retain acceptable chemical, physical and microbiological stability.
- To get desired effect from any pharmaceutical product is has to be stable throughout its shelf life.

- Drug substances used as pharmaceuticals have diverse molecular structures, therefore, they are susceptible to different kinds of degradation pathways.
- Degradation of drugs occur through three principal pathways namely

❖ Chemical Degradation

❖ Physical Degradation

❖ Microbial Degradation.

CHEMICAL DEGRADATION

- Drugs may break down in solution and also in the solid state (for example, in tablet or powder form).

- It is often possible to predict which drugs are likely to decompose by looking for specific chemical groups in their structures.

The main ways in which drugs break down are as follows:

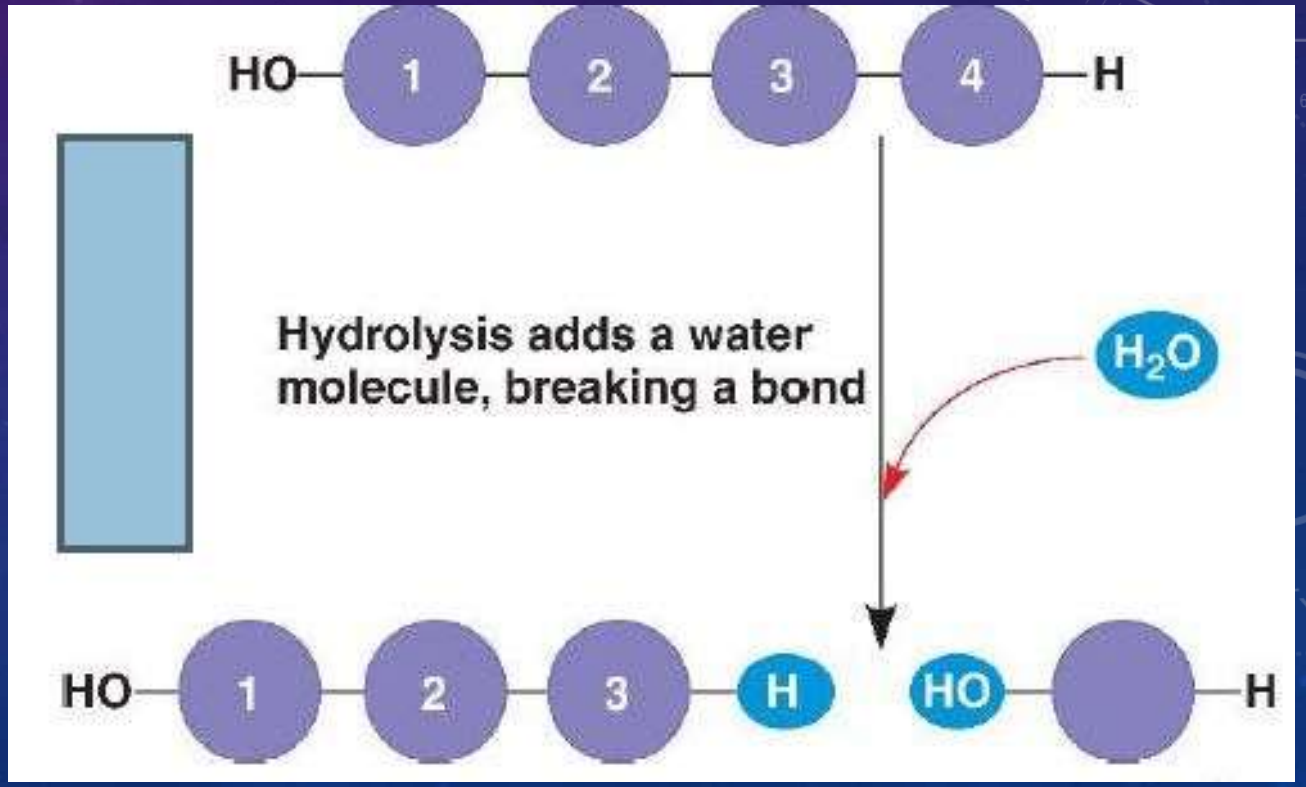
1. Hydrolysis / Solvolysis (very common)
2. Oxidation (very common)
3. Isomerization
4. Photodegradation (Photochemical decomposition)
5. Polymerization of drugs

1. Hydrolysis:

- ✓ Hydrolysis is one of the most common reactions seen with pharmaceuticals, since water is part of many products and moisture is everywhere.
- ✓ Hydrolysis means the cleavage of chemical bonds by the addition of water.
- ✓ Hydrolysis reactions usually depend on pH and temperature and it can be catalysed by hydrogen ions (specific acid catalysis) or hydroxyl ions (spesific base catalysis).
- ✓ Drugs containing ester, amide, lactam, imide or carbamate groups are susceptible to hydrolysis.

Solutions can be stabilised by:

- formulating at the pH of maximum stability.
- altering the dielectric constant by the addition of non-aqueous solvents.



2. Oxidation:

- ✓ Oxidation reaction is the greatest cause of chemical degradation.
- ✓ Oxidation involves the removal of an electropositive atom (e.g. hydrogen) or electron, or the addition of an electronegative atom (e.g. oxygen).
- ✓ Drugs that are susceptible to oxidation include steroids, polyunsaturated fatty acids, and drugs that contain conjugated double bonds.

- ✓ Sometimes molecular oxygen is involved at room temperature. This reaction is known as «auto-oxidation».

Three primary mechanisms exist for oxidative degradations:

- Nucleophilic and electrophilic oxidations are typically mediated by peroxides.
- Electron transfer process via catalysis by transition metal such as cupper ions.
- Autoxidation involves free-radical initiated chain reactions. A single free-radical can cause oxidation of many drug molecules.

- ✓ Some functional groups subject to oxidation are phenols, aldehydes, alcohols and unsaturated fats and oils.

Formulations can be stabilized by:

- replacing the oxygen in pharmaceutical containers with nitrogen or carbon dioxide.
- avoiding contact of the drug with heavy-metal ions such as iron, cobalt or nickel because they catalyze oxidation.
- antioxidants should be included in the formulation.

3. Isomerization:

- ✓ Isomerization is the process of conversion of a drug such as adrenaline, into its optical or geometric isomers, which are often of lower therapeutic activity.
- ✓ There are two types of isomerization:
- ❖ Optical isomerism,
- ❖ Geometric isomerism.

- **Optical isomerism is divided into**
  - **Racemization** like epimerization, it is a reversible conversion between optical isomers also known as enantiomers. Thalidomide is racemic. The R-thalidomide causes birth defect while the S- thalidomide is active against morning sickness. For example; penicillins, cephalosporins, benzodiazepines...
  - **Epimerization** is observed in compounds having more than one asymmetric carbon atom in the molecule. Pilocarpine epimerises by base catalysis. Tetracyclines (to epitetracycline) and ergortamine manifest epimerization by acid catalysis.
- **Geometric isomerism** forms CIS and Trans isomers of the compounds. For example; vitamin A forms the *cis–trans* isomers.

4. Photodegradation (Photochemical Decomposition):

- ✓ Light energy, like heat, may provide the activation necessary for the degradation of some drugs, such as phenothiazines..

- ✓ Photodegradation occurs when molecules absorb light wavelength, especially 300-400 nm. UV light causes more damage than red or orange light and shorter wavelengths cause more damage than longer ones.

- ✓ Photodecomposition involves oxidation mechanism, although others like polymerization or ring opening may occur. Once initiated can progress in the absence of light in a chain reaction.
- ✓ It occurs during manufacture, storage and during the use of the product.
- ✓ In susceptible compounds, photodecomposition creates free radical intermediates, which can perpetuate chain reactions.

- ✓ To avoid photochemical reactions, photo labile formulations are packaged in coloured containers.
- ✓ Yellowish green glass is best protector against UV radiation; amber colour gives only a little protect ion from infrared radiation.
- ✓ The addition of an antioxidant like sodium thiosulfate or sodium metabisulfate hinders the photodegradation of sulfacetamide.

5. Polymerization:

- ✓ Polymerization is the process by which two or more identical drug molecules, like ampicillin, combine together to form a much larger and more complex molecule.
- ✓ The reactants are called monomers and the products are called polymers.
- ✓ For example; aminopenicillin, such as ampicillin sodium in aqueous solution and also formaldehyde.

- ✓ Formaldehyde solution may result into a formation of white deposit when kept in cold.
- ✓ In order to avoid polymerisation on storage, glutaraldehyde needs to be formulated at an acidic pH, where the process does not occur.

PHYSICAL DEGRADATION

- Polymorphism
- Particle size
- Vaporization
- Evaporation
- Temperature
- Efflorescence
- Hygroscopy
- Deliquescence

Polymorphism:

- ✓ Polymorphs are different crystal forms of the same compound caused by exposure to changes in temperature, pressure, relative humidity, drying, granulation, milling and compression.
- ✓ Polymorphs differ in their crystal energy, insolubility, dissolution rate and melting point. The meta stable seeks to revert to the most stable form. Steroids, sulphamides and barbiturates are notorious for their propensity to form polymorphs.
- ✓ Examples of drugs that polymerise include amino-penicillins, such as ampicillin sodium in aqueous solution, and also formaldehyde.

Particle size:

- ✓ Particle size affects solubility and dissolution, and absorption rate, also, the flowability of powder.
- ✓ Decrease in particle size increases surface area of the drug
- ✓ Suspension and emulsion are more stable at lower particle size.

Vaporization:

- ✓ Volatile components such as alcohol, ether, ketones, aldehydes iodine, volatile oils, camphor and cosolvent of lower molecular weight etc., escape from formulation through vaporization, even at room temperature, leading to drug loss.

- ✓ Such product should be placed in well closed containers, at proper temperature. For example; nitroglycerin, chloroform and volatile oil..

Evaporation:

- ✓ Evaporation of water from liquid preparation will cause the drug concentration to change with the possibility of crystallization, if the solubility of the drug in the solvent is exceeded. Water loss from emulsion will cause it to crack or suspension to cake.

Temperature:

- ✓ Increase in temperature degrades thermo-labiles, it enhances degradation chemically and physically..

Efflorescence:

- ✓ Efflorescence is the process where some drugs löse water to the atmosphere resulting in increased concentration of the drug.
- ✓ Saturated solution becomes supersaturated, crystallization.

Hygroscopic:

- ✓ Drugs absorb water from the atmosphere causing physical degradation, like glycerol and plant extract.

Deliquescente:

- ✓ Absorbs water from the atmosphere and turns to liquid. For example; clasium chloride, potassium citrate, ammonium chloride….

MICROBIOLOGICAL DEGRADATION

- Micro-organisms are every where: air, food, water and humans, raw materials and finished products.
- Degradation due to micro-organisms can render the product harmful to the patient or have an adverse effect on the product properties.
- Once opened, a product degrades microbiologically shortening the shelf life, except there is addition of preservatives.
- For example; injectable need to be used immediately the container is opened.

STABILITY STUDIES

When developing a new drug, various stages are passed. After carrying out pharmacological and toxicological tests on the drug, clinical studies are performed.

In this context, stability studies are divided into 3 phases.

PHASE I: In addition to the normal toxicological and thought clinical trials of the drug, the initial stability information is collected for the active substance and the formulations that can be used in clinical trials. This information should be shown that the drug is stable throughout the clinical trials.

PHASE II: The stability of the research formulations used in clinical trials is evaluated and these data are used to prepare the desired formulation. The results are made to determine the shelf life of the formulation.

PHASE III: Tests on the final product are carried out in this phase. The preparation of stability protocols and new drug application is also done in this phase.

Stability Studies and Stability Tests

Stability tests are performed to determine how much the quality of an active substance or a pharmaceutical product in the planned packaging material changes over time under the influence of various environmental factors such as temperature, humidity, light, so as to determine a re-test period for the active substance and a shelf life for the final product and to recommend storage conditions.

Stability studies are carried out in 5 areas:

- Chemical stability analysis
- Physical stability examination
- Microbiological stability analysis
- Therapeutic stability examination
- Toxicological stability analysis

Chemical Stability Investigations:

These tests include degredetion of the active substance, the mechanism of degradation, the degradation products and the identification and quantification of the impurities resulting from the synthesis of the active substance.

There are two important points in chemical stability tests:

- ✓ The products formed during the degradation of the active substance should not be toxic. If there is any toxic effect, it should be within the limits specified in the official guidelines. Impurities and levels should be determined and acted within these limits.
- ✓ The decrease in the amount of active substance in the drug to 90% should not be less than 1 year. Generally, 2-5 years are suitable for shelf life.

Physical Stability Investigations:

Physical stability properties change according to different drug forms. Physical changes are as important as chemical change. The active substance and the activity of the drug may change. These variates;

- Particle size,
- Polymorphism,
- Crystal structure,
- Moisture content,
- Viscosity,
- Phase decomposition,
- Sedimentation,
- Appearance, color, smell, taste,
- Cake in suspension,
- Spotting on tablet,
- Adhesion in capsule,
- Homogeneity,
- pH
- Clarity,
- Dispersibility, hardness, erosion,

Microbiological Stability Analysis:

Microbiological proliferation occurs especially in liquid and semi-solid dosage forms (solution, suspension, emulsion, cream, ointment).

In order to prevent microbiological proliferation, the effects of the antimicrobial agents added to the product must be maintained within predetermined limits.

- ✓ There are no antimicrobial agents in solid dosage forms.
- ✓ Parenteral products are produced sterile and must maintain their sterility throughout their shelf life.

Therapeutic Stability Analysis:

Therapeutic stability is an event that can occur due to extreme changes in chemical, physical and microbiological stability.

For Example:

- ✓ Depending on the storage conditions of an active agent which exhibits polymorphism, the dissolution characteristics and thus its absorption can also change when the crystal shape changes.
- ✓ In suppositories, it can be observed that the melting properties of the semi-solid excipient does not dissolve in body temperature and the therapeutic effect changes.

Toxicological Stability Investigations:

Toxicological stability is a rarely observed event.

- This type of stability problem is observed if the substance is not sufficiently stable and the decomposition products are more toxic than the active substance or if the degradation products of the auxiliary substances are toxic.