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MICROBIAL SPOILAGE

DEFINITION

The action or process of spoiling, especially the deterioration of food and other perishable goods.

Any change which renders a product unacceptable for humanconsumption.

Complex event in which a combination of microbial andbiochemical activities may interact.

One of the major reason that led to preservation.

MICROBIAL SPOILAGE: This refers to damage to food,pharmaceutical products that is caused by microorganisms (bacteria, moulds and yeasts). Microorganisms can grow in almost all kinds of food products. As micro-organisms occureverywhere around us, there is always a risk of **microbial spoilage**.

TYPES OF SPOILAGE

- MICROBIAL SPOILAGE
- NON-MICROBIAL SPOILAGE
- Based on rate of spoilage: There are three types-
  - Highly perishable: Meat, Fish, Egg, Milk, Most fruits and Vegetables.
  - Semi perishable: Potatoes, Some Apple Varieties, Nut meats.
  - Non-perishable or stable: Sugar, Flour, Dry Beans.
- PHARMACEUTICAL SPOILAGE

PHARMACEUTICAL SPOILAGE

Spoilage of pharmaceutical products and drugs are referred as the changes in the physical and chemical properties in such a way that the formulation or therapeutic agent gets deteriorated and is not suitable for use.

Pharmaceutical product is a combination of two things:

1. Active drug
2. Formulation additives

If there is any change in their efficacy, action, etc. it is saidto be spoiled product which cannot be used.

PHARMACEUTICAL SPOILAGE

PHYSICAL	CHEMICAL	MICROBIAL
Deterioration of pharmaceuticals due to physical factors like, heat, temperature, evaporation, etc.	Deterioration of pharmaceuticals due to chemical reactions like oxidation, reduction, hydrolysis, photolysis, ionization, etc.	Deterioration of pharmaceuticals due to contamination of any microbial cell like bacteria, fungi, moulds, etc.

MICROBIAL SPOILAGE OF PHARMACEUTICALS

- Microbial spoilage includes the microbial contamination of pharmaceutical products with the microbes which leadto spoilage of product affecting the drug safety and quality and is not intended for use.
- Shortly, microbial spoilage of pharmaceutical products isthe deterioration of pharmaceuticals with contaminant microbes.
- It decreases the stability and intensity of the product.

TYPES OF MICROBIAL SPOILAGE

PHYSIO-CHEMICAL	CHEMICAL	BIOLOGICAL
➢ VIABLE GROWTH ➢ GAS PRODUCTION ➢ PHYSICAL SPOILAGE ➢ OLFACTORY ➢ COLORATION	➢ HYDROLYSIS ➢ ACETYLATION ➢ DEPOLYMERIZATION ➢ DEGRADATION/ METABOLISATION	➢ RELEASE OF TOXINS ➢ MICROBIAL METABOLITES

PHYSIO-CHEMICAL

In this kind of spoilage, there are some chemical changesare caused by microbial species and due to these changesthe physical properties are also gets altered or deteriorate,thus it is called the physico-chemical spoilage.

1. **VIABLE GROWTH:** In this kind of spoilage, microbial cells form a visible layer over the surface of pharmaceutical formulations. This layer or the presence of microbial cell can be clearly seen by naked eyes.

   Example: layer of moulds over syrups or sugar containingproducts, creams, ointments.

2. **COLORATION:** Color change occurs due to the alteration in components of chemical nature, and these changes are caused by change in pH of the formulation, redoxof the product, production of some other metabolites by microorganisms.

   Example: Pseudomonas species microorganism metabolizes wide range of metabolites that cause coloration of blue-green,brown.

   Surface decoloration of tablets containing biological productsby some moulds.

3. **GAS PRODUCTION:** Some microorganism contaminants in pharmaceutical formulations

produce gases by their metabolic activities and form gas bubbles and foam over the formulation.

Formulations containing carbohydrates or starchy material aremore susceptible for gas production.

Example: production of CO2 in syrups caused by osmo-tolerant mould and yeasts.

Klebsiella produces gas in creams and ointments containingvitamins and proteins.

Desulphovibrio oxidise simple organic compounds andproduces hydrogen sulphide in suspensions.

4. **PHYSICAL SPOILAGE:** These are some changes inphysical appearance caused by microbial cell activities.

   Example: In emulsions, some microbial cells cause hydrolysisof oil phase, cause change in oil-water equillibrium and make emulsion unstable. Hydrolysis of oils also affects pH of formulation and alters the stability of emulsions.

   In syrups, microbial cells metabolise sugar molecules, due to which the concentration of syrup changes and product becomes unstable.

5. **Olfactory spoilage:** Spoilage by some organisms and moulds generates unpleasant smell from the product. Thiskind of spoilage is mainly caused by microbial cells that produce sulphur containing gases [SO2, H2S] and fishy smell due to formation of fatty acids along with odour generated by amines and alcohol production.

Example: Contamination in syrup of Tolu by penicillin species produces toluene like smell.

Actinomycetes produce smell of geosmin in water phaseused for formulations.

CHEMICAL SPOILAGE

There are various types of chemical spoilage in pharmaceutical compounds. This occurs due to various types of chemical reactions mediated by contaminatingmicroorganisms.

1. **HYDROLYSIS:** Some bacterial cells contain enzymesthat catalyse hydrolysis of pharmaceuticals.

   Example: Atropine hydrolysed by Pseudomonas bacteria.

   Aspirin hydrolysed by esterase producing bacteria. Gelatin hydrolysed by Bacillus and Clostridium species.

2. **ACETYLATION:** Some microorganisms' cellular enzymes

   cause acetylation of drugs and cause loss of activity.

   Example: Chloramphenicol acetylation caused by staphylococci and streptococci gram positive bacteria by the enzyme chloramphenicol acetyl-transferase.

3. **DEPOLYMERISATION:** It is a process in which the polymers are degraded to their

monomers. A lots of polymersare used in formulation of many type of pharmaceutical preparations as diluents, binders, thickening, suspending agents, etc.

Example: Starch- depolymerized by bacterial amylasePectin- depolymerized by bacterial pectinase

Dextran- depolymerized by bacterial dextranase

Cellulase- depolymerized by bacterial cellulase.

4. **DEGRADATION:** Due to the microbial contamination, theactive therapeutic agents or the formulation ingredients can bedegraded or metabolized.

   Examples: Penicillin degraded by beta lactamase containingbacterial cells

   Fatty acids metabolized by some moulds

   Prednisone degraded by aspergillus species

BIOLOGICAL SPOILAGE

Some bacterial cells contaminate the pharmaceuticals and utilize the various compounds present in that formulation toperform their metabolic activities. Due to these metabolic activities, the microbial cells produce certain chemicals, which they release in

pharmaceutical preparations. This is called biological spoilage.

Mainly two types of chemicals are released by themicroorganisms:

1.Microbial toxins

2.Microbial metabolites

MICROBIAL TOXINS: Several microorganisms produce toxic molecules that may cause spoilage of pharmaceutical fomulations. Such as endotoxins produced by some gram –vebacteria like Escherichia coli, exotoxins by Clostridium botulinum.

MICROBIAL METABOLITES: Bacterial metabolites are the biosynthetic products from microbial cells. Bacterial cellsproduce various metabolites, which cause product spoilage because these metabolites are toxic to humans.

Examples are different amines and organic acids frombacterial cells, metabolites

from fungi and moulds.

Fungi and moulds more specifically grow on theformulations having Talc, kaolin, and starch.

REASON OF CONTAMINATION

The product gets contaminated due to various reasons like

- **Accidental exposure to the environment**
- **Improper storage conditions**
- **Inadequate preparation of formulation**
- **Improper sterilization**

FACTORS AFFECTING SPOILAGEOF PHARMACEUTICAL PRODUCTS

- By understanding the influence of environmental parameters on

microorganisms, it may be possible to manipulate formulations to createconditions, which are unfavorable for growth and spoilage within the limitations of patient acceptability and therapeutic efficacy.

- The factors that affect spoilage:

➢ **NUTRITIONAL FACTOR**

➢ **MOISTURE CONTENT**

➢ **REDOX POTENTIAL**

➢ **STORAGE TEMPERATURE**

➢ **pH**

➢ **PACKAGING DESIGN**

NUTRITIONAL FACTOR

- Presence of nutritional materials enable or favour microorganismsto utilize these nutritional materials as energy source and proliferateover pharmaceutical products.
- In any formulation, the presence of vegetable/herbal extract oranimal tissue or tissue extract provides nutritional support to microbial cells.
- Demineralized water (prepared by ion exchange method) alsocontains some nutritional materials, which support the growth of*Pseudomonas* bacteria.
- More complex formulations are more supportive for microbial growth.

MOISTURE CONTENT [Water Activity]

- Microorganisms need water or moisture to grow. Thepresence of water in any formulation supports microbial growth. Greater the solute concentration, lesser the activity.
- When solutes are dissolved in water, they form hydrogen and other bonds with water and form complexed water. The free or unbound water molecule is termed as uncomplexed water and this uncomplexed water supports microbial growth.
- Condensed water film sometime accumulates over some drypharmaceutical formulations like tablets or oils due to storage in humid atmosphere, supports fungal, i.e. *A.glaucus* and yeast growth.
- Moisture films over viscous syrups can also increase water activity and support growth of fungi and yeast.
- Some halophilic bacteria grow in high salt conditions.

MICROORGANISM	WATER ACTIVITY
CLOSTRIDIUM BOTULINIUM	0.94
ESCHERICHIA COLI	0.93
SALMONELLA SPP.	0.94
SHIGELLA SPP.	0.96

REDOX POTENTIAL

- The oxidation-reduction or the Redox potential is defined asthe ratio of the total oxidizing [electron accepting] power to the total reduction [electron donating power of substance], orin more easy way, it is a property of any chemical to give or accept electrons.
- Microbial growth in any environment is influenced by its oxidation-reduction balance, i.e. redox reaction. Electron transfer is a major factor for energy production. If oxygen is present in any formulation, then this condition favours microbial growth.

PACKAGING MATERIAL AND DESIGN

- Packaging can have major influence on microbial contamination and spoilage of pharmaceuticals. Multidosecontainers are more affected by microbial contamination because they are again and again exposed to environment when the drug is withdrawn from container.
- Wide opening mouth containers that contain formulationsof vegetable oils, protein, vitamin, animal extracts are readily contaminated as they provide large surface area exposure to the environment.

STORAGE TEMPERATURE

- The pharmaceutical formulation can be affected by microbial cells between the temperature range of -20℃ – 60℃.
- Below -20℃, almost no microbial contamination is observed and above 60℃, microbial growth is suppressed. The reason for that is inactivation and denaturation of cellular enzymes, which are responsible for cell metabolic activities.
- Water for injection is stored at 80℃ before preparation to minimize bacterial growth and to prevent bacterial activity toproduce and release pyrogens to the water.

pH OF PHARMACEUTICAL FORMULATIONS

- pH is represented as the potential of hydrogen ions. Some bacterial cells are grown better in acidic medium and some are in basic medium, this happens because in their favourable pH, the enzymes of bacterial cells are more active to perform their metabolic activities.
- Some preparations of pH around 5-6 favour growth of moulds, but inhibit bacterial growth.
- Some preparations having pH of 3-4 favour growth of moulds and yeast, i.e. fruit juice flavoured syrups.
- Some preparations of neutral pH like mouthwashes, distilled water, antacid preparations are contaminated bybacterial cells, i.e. Pseudomonas spp.
- Basic pH formulations inhibit bacterial growth, i.e. magnesium and aluminium hydroxide gel, etc.
- Sometime, primary fungal growth occurs in product. These fungal cells metabolize the chemical and produce acids and raise the pH offormulation and favour secondary bacterial growth.