MICROBIAL SPOILAGE

DEFINITION:

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 products.

As micro-organisms occur everywhere around us, there is always a risk of microbial spoilage.

SPOILED PRODUCT:

A spoil product may be described as one that has been renderd unfit for use.
A spoiled pharmaceutical product is becoming objectionable or perhaps evenly therapeutically inactive.

PHARMACEUTICAL SPOILAGE:

Microbial spoilage includes the microbial contamination of pharmaceutical products with the microbes which lead to spoilage of product affecting the drug safety and quality and is not intended for use. It decreases the stability and efficacy of the product.

TYPES OF MICROBIAL SPOILAGE:

Physiochemical

- VIABLE GROWTH
- GAS PRODUCTION
- PHYSICAL SPOILAGE
- OLFACTORY
- COLORATION

Chemical

- HYDROLYSIS
- ACETYLATION
- DEPOLYMERIZATION
- DEGRADATION/ METABOLISATION

Biological

- RELEASE OF TOXINS
- MICROBIAL METABOLITES

PHYSIOCHEMICAL SPOILAGE:

There are some chemical changes are caused by microbial species. Due to these changes the physical properties are also gets altered or deteriorate.

Viable growth:

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. **(Physical prosperity alteration)**.This microbial growth will alter the chemical nature and unfit for use.

Example: layer of mould on syrup, creams and ointments.

Coloration:

Color change occurs due to the alteration in components of chemical nature. These changes are due to:

- Change in pH of the formulation.
- Redox reaction in the product.
- Production of some other metabolites by microorganisms.

Examples

Pseudomonas species micro-organism metabolize wide range of compounds that cause coloration of blue-green, brown.

Gas Production:

Some micro-organisms contaminants in pharmaceutical formulations produces gases by their metabolic activities and form gas bubbles and broth/foam over the formulation. Formulations containing carbohydrates or starchy material are more susceptible for gas production.

Example:

Production of CO_2 in syrups caused by osmo-tolerant **mould** and **yeasts**. **Klebsiella** produces gas in creams and ointments containing vitamins and proteins.

Physical Spoilage:

These are some changes in physical appearance caused by microbial cell activities.

Example

In emulsions, some microbial cells cause hydrolysis of oil phase, cause change in oil-water equilibrium and make emulsion unstable.

Olfactory spoilage:

Spoilage by some organisms and moulds generates unpleasant smell from the product. This kind of spoilage mainly caused by microbial cells that produces sulphur containing gases[SO_2, H_2S] and fishy smell due to formation of fatty acids along with odor generates by amines and alcohol production.

Example

Contamination in syrup of Tolu by penicillin spp. Produces toluene like smell.

CHEMICAL SPOILAGE:

There are various types of chemical spoilage in pharmaceutical compound, these occurs due to various types of chemical reactions, mediated by contaminating microorganisms.

Hydrolysis:

Some bacterial cells contains enzymes that hydrolyze pharmaceuticals.

Example :

- Atropine hydrolyzed by Pseudomonas bacteria.
- Aspirin hydrolyzed by esterase producing bacteria.

Acetylation:

Some micro-organisms cellular enzymes cause acetylation of drugs resulting in loss of activity.

Example

Chloramphenicol acetylation caused by staphylococci and streptococci gram +ve bacteria by the enzyme chloramphenicol acetyl-transferase.

De-Polymerization:

It is a process in which the polymers are degraded to their monomers. A lot of polymers are used in formulation of many type of pharmaceutical preparations as diluents, binders, thickening, suspending agents, etc.

Example:

- Starch- depolymerize by bacterial amylase.
- Pectin- depolymerize by bacterial pectinase .
- Dextran- depolymerize by bacterial dextranase.

Degradation:

Due to the microbial contamination, the active therapeutic agents or the formulation ingredients can be degraded or metabolized.

Examples:

- Penicillin: degrade by batalactamase containing bacterial cells
- Prednisone degraded by aspergillus species bacteria.

BIOLOGICAL SPOILAGE

Due to the metabolic activity of microorganism in formulation, they produce many toxin or metabolites that contaminate the pharmaceutical product. which refers to biological spoilage.

- Mainly two types of chemicals released by the micro-organisms.

1. Microbial toxins **2. Microbial metabolites**

Microbial toxins:

Several microorganisms produce toxic molecules that may cause spoilage of pharmaceutical formulations. Such as endotoxins produced by some gram –ve bacteria like E.coli.

Example
Exotoxins by *Clostridium botulinum*.

Microbial metabolites:

Bacterial metabolites are the biosynthetic products from microbial cells. Bacterial cells produces various metabolites which cause product spoilage because these metabolites are toxic to humans.

Example

- Different amines and organic acids from bacterial cells.
- Fungi and moulds are more specifically grow on the formulations having Talc, kaolin, and starch.

HOW PRODUCT WILL Be CONTAMINATED:

The product gets contaminate due to various reasons like;

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

FACTORS THAT INFLUNACES THE GROWTH OF MICROORGANISM:

By understanding the influence of environmental parameters on microorganisms, it may be possible to manipulate formulations to create conditions which are as unfavorable as possible for growth and spoilage, within the limitations of patient acceptability and therapeutic efficacy.

The factors that affects the spoilage:

- NUTRITIONAL FACTOR
- MOISTURE CONTENT
- REDOX POTENTIAL
- STORAGE TEMPERATURE
- pH
- PACKAGING DESIGN

Nutritional factor:

- Presence of nutritional material enables or favors microorganisms to utilize these nutritional material as energy source and proliferate over pharmaceutical products.
- In any formulation the presence of vegetable/herbal extract or animal tissue or tissue extract provides nutritional support to microbial cells.
- De mineralized water [prepared by ion exchange method] also contain some nutritional material which support the growth of *Pseudomonas bacteria*.
- More complex formulations are more supportive for microbial growth.

Moisture Contents (water activity):

- Microorganisms readily need water or moisture to grow. The presence of water in any formulation supports microbial growth. Greater the solute concentration, lesser the activity.
- When solutes dissolved in water they form hydrogen and other bonds with water and form complexes water, the free or unbounded water molecules are termed as uncomplexed water and this un-complexed water supports microbial growth.
- Condensed water film sometime accumulates over some dry pharmaceutical formulation like tablets or oils due to the storage in humid atmosphere, supports fungal growth.
- Moisture films over viscous syrups can also increase water activity and support growth of fungi and yeast.

Redox Potential

- The oxidation-reduction or the Redox potential is defined as;

The ratio of the total oxidizing [electron accepting] power to the total reduction [electron donating power of substance], or in more easy way it's 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 major factor for energy production. If the oxygen is present in any formulation, then this condition favours microbial growth, if any product gets infected by microbial cell.

Packaging Material and Design:

- Packaging can have major influence on microbial contamination and spoilage of pharmaceuticals
- Multi dose containers 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 formulations of 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 affected by microbial cell between the temperature range of -20℃ – 60℃.
- Below -20℃ almost no microbial contamination is observed and the same as above 60℃ , microbial growth 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 which can produce and release pyrogens to the water.

PH of Pharmaceutical Formulations:

- 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 favours growth of moulds but inhibit bacterial growth.
- Some preparations having pH of 3-4 favours growth of moulds and yeast, i.e. fruit juice flavoured syrups.
- Basic pH formulations inhibit bacterial growth, i.e. magnesium and aluminium hydroxide gel, etc.
- Some preparations of neutral pH like mouthwashes, distilled water, are contaminated by bacterial cells i.e. pseudomonas .

TYPE OF SUSCEPTIBLE PHARMACEUTICAL PRODUCTS TO MICROBIAL SPOILAGE:

Liquids:

- **Simple aqueous solutions :**

  Some moulds will grow on such unlikely media as strong solutions of copper or Sulfuric acid and simple solutions of inorganic compounds will support the growth of many sorts of microbes.

- **Suspensions:**

  Aqueous suspensions of inorganic material for pharmaceutical use frequently support microbial growth, particularly as added preservatives tend to be absorbed and inactivated by suspended matter.

- **Emulsions :**

  O/W emulsions are particularly susceptible to spoilage as water in the continuous phase allows the contaminants to spread throughout the product. Spoilage in emulsions can be manifest by changes in rheological properties, including separation or breaking down, discoloration, changes in odor and taste and signs of visible growth also occur.

- **Creams and lotions:**

  Moulds growth is one of the most common causes of spoilage of creams of all types and can occur in products as varied as antifungal, calamine, baby and hair creams and a number of other cosmetic formulation including moisture and cleansing creams.

- **Syrups:**

  The sugar content of syrup inhibits the growth of many microorganisms by virtue of its high osmotic pressure but osmo tolerant moulds and yeasts are source of trouble.

- **Tinctures and elixirs:**

  In general, these formulations don't allow microbial survival because of their high concentrations of alcohol.

Tablets:

- **Raw material:**

  Solid raw materials may serve as source of contaminants which will later spoil a formulated product. Solids can be contaminated with E.coli and staphylococci. Spoilage of solid raw material itself is largely due to mould growth on the surface due to improper storage**.**

- **Tablets:**

  Tablets contain many excepients like lactose that is highly susceptible to mould and fungal growth.

ACCEPTANCE CRITERIA FOR MICROBIOLOGICAL QUALITY OF NON-STERILE DOSAGE FORM.

When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:

- 10^1 CFU: maximum acceptable count = 20,
- 10^2 CFU: maximum acceptable count = 200,
- 10^3 CFU: maximum acceptable count = 2000, and so forth.

Table. Recommended acceptance criteria for microbiological quality of non-sterile dosage forms

Route of administration	Total aerobic microbial count (CFU/g or CFU/ mL)	Total combined yeasts/moulds count (CFU/g or CFU/ mL)	Specified microorganism
Non-aqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli* (1 g or 1 mL)
Aqueous preparations for oral use	10^2	10^1	Absence of *Escherichia coli* (1 g or 1 mL)
Rectal use	10^3	10^2	–
Oromucosal use Gingival use Cutaneous use Nasal use Auricular use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Vaginal use	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of *Staphylococcus aureus* (1

PHARMACEUTICAL PRESERVATIVES

A chemical substance used to preserve organic substance from decay or spoilage by preventing microbial attack

PHARMACEUTICAL PRESERVATIVES are the chemical substances used to improve or amplify shelf life of drugs by preventing degradation of active and excipients, by reducing microbial production.

Pharmaceutical Products are preserve by addition of many types of antimicrobial agents (alone or in combination)

NEED FOR PRESERVATIVES:

- To protect our drug from microbial attack
- To enhance activity and efficacy of drug
- To increase shelf life of our product
- To stabilize our product

IDEAL PRESERVATIVE:

- Effective
- Stable Non toxic and non reactive
- Highly soluble
- Cost effective
- Compatible with drug components
- Odorless
- Tasteless

PRESERVATIVES:

It must decrease the percentage of the microbes and prevent any re-growth They can be:

- Microbiostatic
- Microbiocidal

CLASSIFICATION OF PRESERVATIVES:

A. MECHANISM OF ACTION

I. **Anti oxidants** that inhibits oxidation, are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged.

Examples:
BHA (butylatedhydroxyanisole)
BHT (Butylatedhydroxytoulene)
Vit-C
Vit-E

II. **Antimicrobial Agents** have biocidal properties that inhibit the growth of bacteria, yeast, and molds.

Examples:
Parabens
Sorbates
Benzoic acid
Methyl parabens

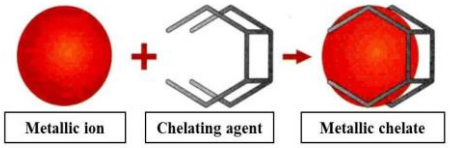
PREPARATIONS REQUIRED ANTIMICROBIAL PRESERVATIVES:

Preparations which contain water are at risk of microbial spoilage such as:

- Solutions
- Suspensions
- Emulsions
- Topical preparation e.g. creams
- Injectable
- Eye drops etc

III. **Chelating Agents** act as preservatives and protect product by forming complex with metals it preventing its deterioration

Examples:
EDTA
Citric acid etc



B. SOURCE

1. Naturally obtained

Lemon
Citirc acid
Vitamin-C
Castor Oli

2. Synthetically prepared

Salts of benzoates
Nitrates
Nitrites

➢Commonly used preservatives :

➢ Chlorhexidine gluconate :

Chlorhexidine came into use in 1950s. It is on the WHO list of essential medicines, the safest and the most effective medicines needed in health system. It is available over the counter.

Mechanism :

At physiological PH, its salts dissociate and release positively charged cation. The bactericidal effect is a result of the binding of this cationic molecule to negatively charged particle.

At low concentration, it act as bacteriostatic and at high concentration, it acts as bactericidal.

Uses:

It is used as disinfectant, antiseptic and as a preservative in many topical and dental formulate, ions. In dental, it is used in many mouthwashes. Using this as supplement to everyday oral hygiene for 4-6 weeks leads to moderate reduction in gingivitis.

As topical, Nepal was first country to use this in cream to treat umbilical cord infections of new borns.

Side effects:

It is ototoxic, and can lead to deafness, skin irritation, teeth discoloration. FDA limits its use to maximum of 6 months.

➢ Benzalkonium chloride:

It is frequently used preservative in ophthalmic and topical preparations. Its concentration ranges from 0.004 to 0.01%. Higher concentration can be toxic and cause irreversible damage to corneal endothelium.

Although historically, benzalkonium chloride as used in ophthalmic, its ocular toxicity and irritant properties, have led pharmaceutical companies to increase the production of preservative free preparation or to replace this with preservative which are less harmful.

➢ Sodium benzoate :

It is used in pharmaceuticals in concentration of 0.02 – 0.5% in oral medicines, 0.5% in parenteral and 0.1 – 0.5% in cosmetics.

Common side effects include irritation, restlessness and agitation. If combined with citric acid, it possess carcinogenic effect

PERFORMANCE REQUIREMENTS:

- **Antimicrobial Activity**

Active against Microbes at Low concentration

- **Aqueous Solubility**

Should be soluble to reach minimum inhibitory concentration

- **Stability Properties**

Stable during and at the end of manufacturing

- **Partioning Behaviour**

Remain in continuous phase in multiphase product

- **Organoleptic properties**

Odor and acceptable taste during administration of product

RELATIONSHIP BETWEEN:

Concentration

- Change in conc. will change the efficacy
- Performs best on lower concentration

e.g.: Phenol Chlorhexidine

Temperature

- Activity changes with temperature

e.g: Phenol Ethanol

DEGRADATION OF PRESERVATIVES:

- Temperature during processing
- Inappropriate pH
- Exposure to light

PRESERVATIVE CONCENTRATION FOR LIQUID ORAL PREPARATION:

Name	Recommended Concentration
Benzoic Acid	0.1 to 0.2%
Sorbic acid	0.1 to 0.2%
Methyl Paraben	0.25%
Propyl Paraben	0.5 to 0.25%

RECOMMENDED PRESERVATIVE CONCENTRATION

Name	Recommended Concentration
Benzyl alcohol	0.5 to 10%
Benzalalkonium chloride	0.01%
Methyl Paraben	0.01 to 0.5%
Phenol	0.065 to 0.02%

SIDE EFFECTS:

While choosing preservative for drug product consideration should be made about:

- Concentration
- Toxicity
- Selectivity
- Interaction with formulation etc

ANALYSIS OF PRESERVATIVES IN PRODUCTS:

- High performance liquid chromatography
- Capillary electrophoresis (CE)
- Gas chromatographic methods
- Thin layer chromatographic methods
- Flow injection analysis
- Titrimetric/Titration Method
- Flourimetric Methods
- Atomic Absorption Spectroscopic (AAS) Method

REFERNECES

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