

## UNIT $\Rightarrow$ 5

### # Types of 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 activity, action, efficacy, or stability is affected and that formulation is not suitable for use.
- There are several factors that cause spoilage of pharmaceuticals:

#### Physical

#### chemical

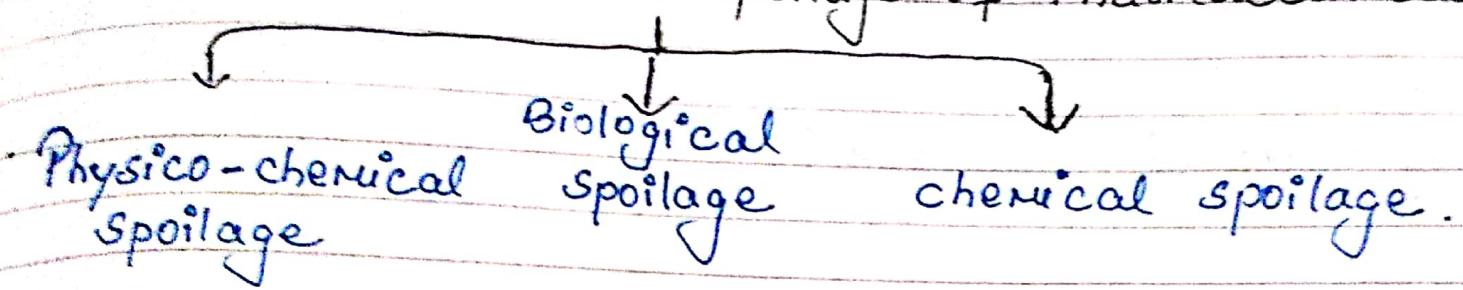
#### Microbial

- Spoilage due to physical factors like heat, temp. evaporation etc.
- Spoilage due to chemical sk<sup>n</sup> like oxidation, reduction, ionizat<sup>n</sup>, photolysis.
- Spoilage due to contamination of any microbial cell i.e., fungi, bacteria, moulds etc.

### # Microbial spoilage :

- Microbial spoilage include the contamination of pharmaceutical products with the microbes which lead to spoilage of the product affecting drug safety and quality, and not intended for use.

## \* Types of Microbial spoilage of Pharmaceuticals



### A) Physico-chemical spoilage :

- In this kind of spoilage there are some chemical changes are caused by microbial cells and due to these changes the physical properties are also gets altered, thus is called physico-chemical spoilage.

#### i) viable growth :

- In this kind of spoilage microbial cell forms a visible layer over the surface of pharmaceutical formulations. This layer on the presence of microbial cell can be clearly seen by naked eyes.

#### ii) coloration :

- colour changes occur due to alteration of component chemical nature of pharmaceutical prepn/formulation and these are caused by change in pH of the formulation.

#### iii) Gas production :

- some microorganisms contaminants in pharmaceutical formulation produces gases by

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

### b) chemical spoilage :

- In this kind of spoilage occurs due to various types of chemical reactions mediated by contaminating microorganisms.

#### i) Hydrolysis :

- Some bacterial cells contain enzymes that catalyse hydrolysis of pharmaceuticals.

#### ii) Acetylation :

- Some microorganism have cellular enzymes cause acetylation of drugs cause loss of activity.

#### iii) Degradation :

- Due to the microbial contamination, the formulation ingredients can be degraded or metabolised.

### c) Biological spoilage :

- Some bacterial cells contaminated the pharmaceutical products & that microbial cell perform their metabolic activity & produces certain chemicals & these chemicals cause spoilage of the product.

• The chemicals release by the microorganism  
are Microbial toxins & microbial metabolite

# factors affecting microbial spoilage  
of pharmaceutical product

# **FACTORS AFFECTING MICROBIAL SPOILAGE OF PHARMACEUTICALS**

**Microbial spoilage means contamination of pharmaceutical formulations, and due to this there are some changes in physical and chemical properties of product.**

The product gets contaminated due to various reasons like

**Accidentally exposure to the environment**

**Improper storage conditions**

**Inadequate preparation or formulation**

**Improper sterilization**

### **Nutritional factors:**

Presence of nutritional material enable or favours 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.

Demineralized water (prepare by ion exchange method) also contain some nutritional material which support the growth of *Pseudomonas* bacteria.

More complex formulation (a formulation that contain wide variety of substances) are more supportive for microbial growth.

### **Moisture Content (Water activity):**

Microorganism readily need water or moisture to grow. The presence of uncomplexed water in any formulation supports microbial growth. Greater the salure concentration, lower the water activity.

When solutes dissolved in water they form hydrogen and other bonds with water and form complexed water, the free or unbonded water molecules are termed as uncomplexed water, and this uncomplexed water supports microbial growth. Water activity is a measurement of amount of uncomplexed water. If water activity is high, then the uncomplexed water content is also high

Condensed water film sometime accumulate over some dry pharmaceutical formulations like tablets or oils due to the storage in humid atmosphere, supports fungal i.e. *A. glaucus* and yeast growth.

### **Redox potential :**

The oxidization-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 substances.), 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 reactions. Electron transfer is major factor for energy production. If the oxygen is present in any formulation, or if there is any compound that have high redox potential, then this condition favours microbial growth, if any product gets infected by microbial cell.

## **Packaging material and packaging 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 formulations can affected by microbial cell between the temperature range of -20<sup>0</sup>C - 60<sup>0</sup>C. below -20<sup>0</sup>C almost no microbial contamination is observed and the same as above 60<sup>0</sup>C, microbial growth supressed. The reason for that is inactivation and denaturation of cellular enzymes, which responsible for cell metabolic activities.

## **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 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

some preparations of neutral pH like mouthwashes, distilled water, antacid preparations are contaminated by bacterial 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 any product, these fungal cell metabolize the chemicals and Produce acids and raise the pH of formulations and favours secondary bacterial growth.

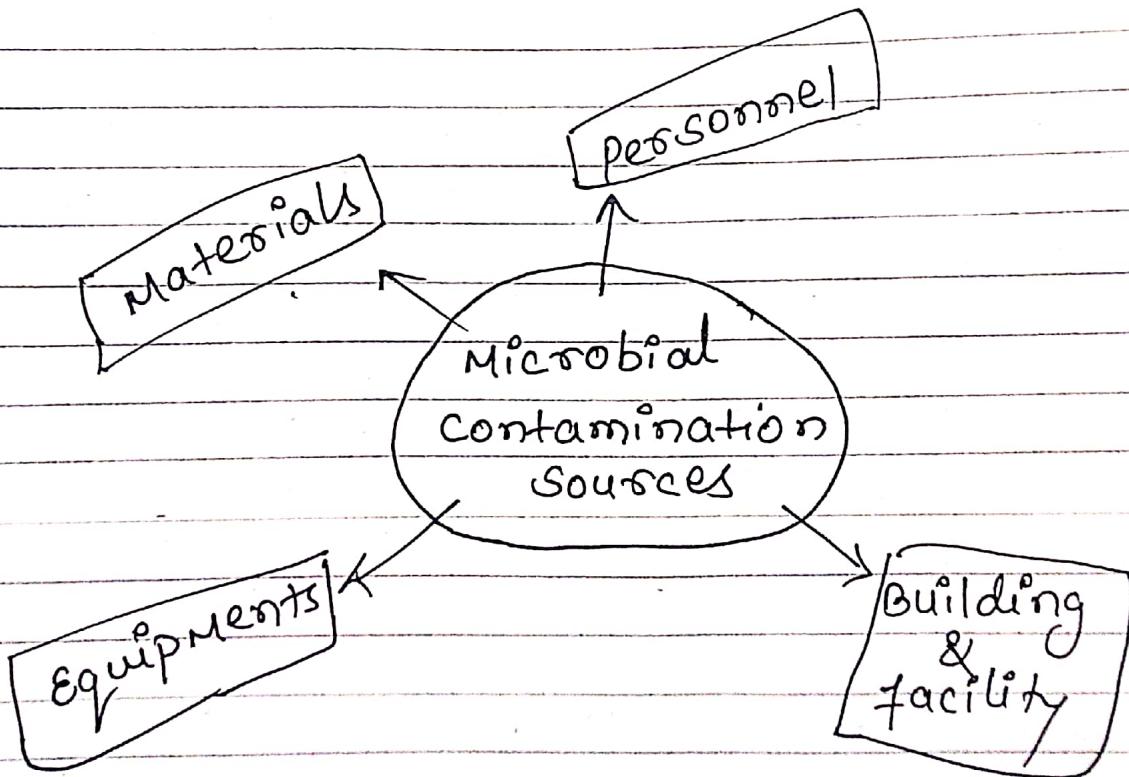
# MICROBIOLOGY

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# Sources and types of microbial contaminants

\* sources of contamination -

- Contaminants can gain entry into a production process stream from several sources such as personnel, poor facility design, Incoming ventilation air, machinery and other equipments for production, Raw material and semi-finished material, packaging material.



## → personnel :-

- personnel who works to prepare formulations in Aseptic area, they have more chances to contaminate the pharmaceutical preparation.

## • The main reasons for contamination from the personnel :-

- Lack of training.

- Direct contact b/w the many personnel hands during starting material, primary packaging material & intermediate or bulk product.

- personnel doesn't proper cleanliness
- Improper personnel cleanliness.

## → Building and facility -

- The buildings and manufacturing facilities may also contribute to the contamination.

## • The main reasons of contamination due to facility issues include -

- Rough floors, walls and ceiling.

- Lack of air filtration system.

- Inadequate washing, cleaning, toilet and locker facilities.

## → Equipments :

- The equipments and utensils used in processing, holding, transferring and packaging are the common source of pharmaceutical contamination.
- The main reasons for contamination from the equipments include:
  - Improper cleaning and sanitization.
  - use of defective equipments
  - Improper calibration of equipments.

## → Materials :

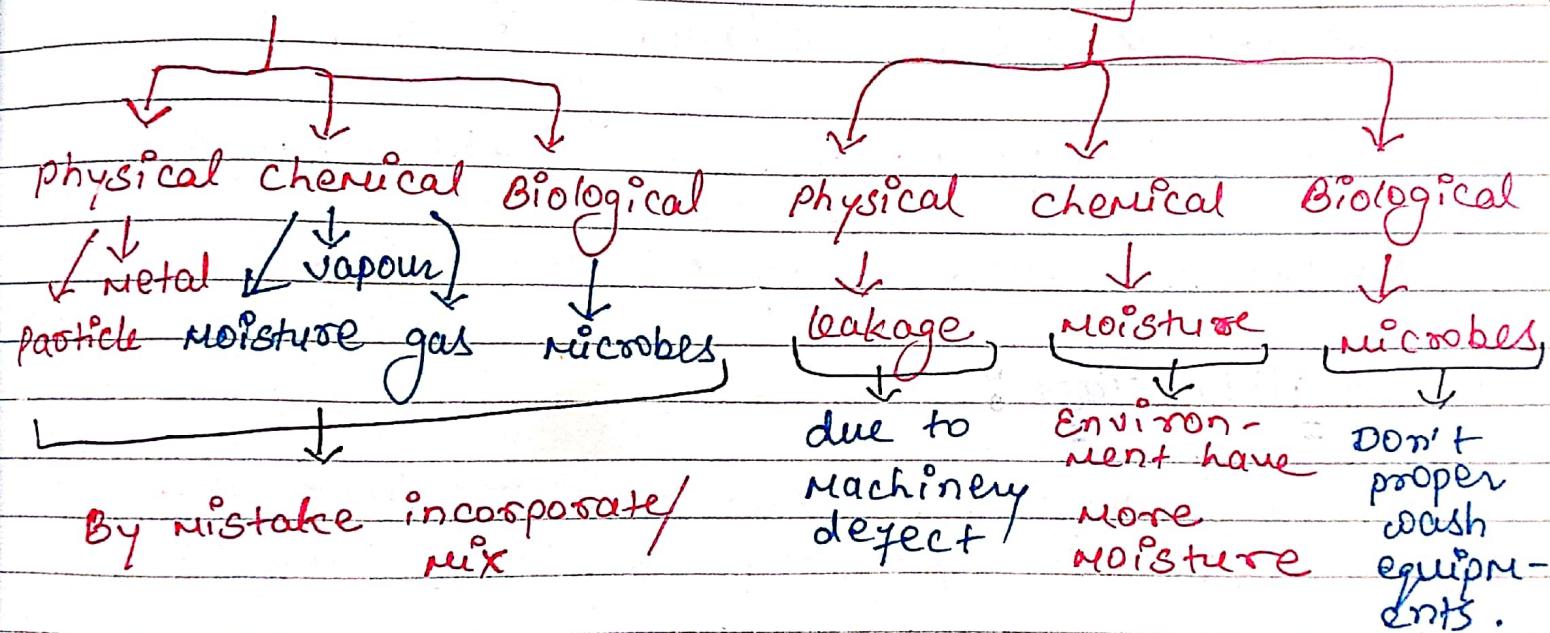
- The raw materials used for production can be potential source of contamination.
- The main reasons for contamination from the raw material include:
  - Degradation from exposure to excessive environmental conditions such as heat, cold, sunlight, moisture etc.
  - storage and handling mistakes causing mix-ups .

## \* Types of microbial contamination

### Microbial contamination

↓  
Direct contaminat  
[If Raw material are not good or using wrong process/procedure during pharmaceutical preparation]

↓  
Indirect contaminat  
[If we use uncalibrated equipments or due to environment our pharmaceutical preparation got contaminated]



By mistake incorporate/mix

## # Assessment of Microbial contamination and Spoilage :

→ physical and chemical changes :

- It is the changes of different pharmaceutical formulations indicate microbial contamination and spoilage.
- change in viscosity, pH, emulsion stability and loss of surface activity of formulation indicates microbial spoilage.
- Measurement of oxygen consumption of the product can indicate the degree of oxidative attack and microbial growth.

→ Sterility Test :

- Testing which confirms that products are free from the presence of viable microorganism.
- claim to be sterile or free from viable microorganism.
- Test is conducted by competent and experienced personnel in an adequately clean room with laminar flow cabinet facilities.
- All injectables and ophthalmic preparations are sterile hence, these preparation are tested by the sterility test.

## # Preservation of Pharmaceutical products using Antimicrobial Agents

- pharmaceutical preparation or formulations are the prepared or finished dosage forms that can be administered through various routes.
- pharmaceuticals also contain the API (Active pharmaceutical Ingredients), these are the chemical ingredients used to prepare pharmaceutical dosage forms.
- Antimicrobial agents are the compounds that has properties to kill the microbial cell or inhibit the growth of microbial cells.
- Pharmaceutical products are preserved by addition of many types of antimicrobial agents (alone or in combination)
- Antimicrobial agents being added in any pharmaceutical preparation to prevent the drug degradation and improve its stability, shelf life and efficacy.

- In any ideal antimicrobial agent should have following properties:
  - Maintain its activity throughout the shelf life of product.
  - Not react with the active therapeutic (ingredients) compounds.
  - Exert wide spectrum antimicrobial activity at low concentration.
  - Not react with the containers or packaging material.

### formulation

#### Reason to add Antimicrobial agent

### Emulsion

usually antimicrobial agents concentrated in aqueous phase, since this is the phase where bacterial cell can grow.

### Ophthalmic

It is prepared in multi-dose container, when we use these prep" it expose in air & got contaminated.

### Ointments

This prep" are present in semi-solid form which is ~~used~~ contaminated by using our finger.

### parenteral

Due to repeatedly use this prep" got contaminated.

That's why we use Antimicrobial Agents.

formulation

Antimicrobial agent

oral  
preparation

- Methyl, ethyl, propyl, parabane and their combination.
- Sodium benzoate.
- Sorbic acid, potassium sorbate.

Topical  
preparation  
[for skin]

- Benzalkonium chloride.
- Benzethonium chloride.
- Methyl, ethyl, propyl parabane and their combination.
- Chlorocresol.

Parenteral

- Benzyl alcohol.
- chlorobutanol.
- methyl, ethyl, propyl parabane and their combination.
- Benzoic acid, sorbic acid.

ophthalmic

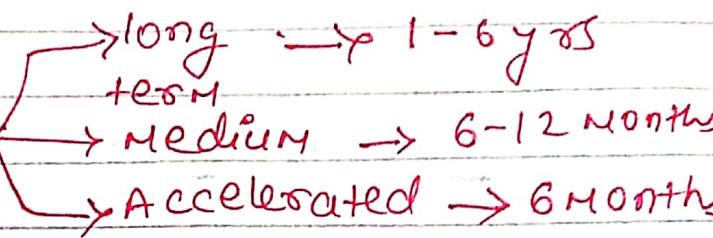
- Benzalconium chloride.
- Benzoic acid, sodium benzoate
- Oxydurea
- chlorhexidine.

## Evaluation of Microbial Stability of Formulations

- Drugs or APIs (Active Pharmaceutical Ingredients) are degraded by many factors like temp., light, pH, humidity, air etc. Due to many of these factors, drug concentration which is degraded during storage ~~can~~ affect drug stability.  
*so* the drugs stability studies are used to evaluate the information about
  - How much amount or percentage of drug is degraded at specific time period.
- Normally drug compounds or APIs are placed / in shelves under normal storage conditions for longer time duration and at various time intervals, then the drug or chemical is evaluated for study. But these kind of stability evaluation methods are much time consuming and done in longer time durations.

## \* Stability

= On the basis of duration



- long term → many types of alcohols.
- Medium → normal pharmaceutical products
- Accelerated → normally pharmaceutical products
  - ↳ mostly used.
    - ↳ It saves time, the drugs or chemicals are placed in stress full condition like high temp, pH, light intensities and thereafter drug stability is evaluated.

## • Stabilities

Parameters for evaluation.

### physical

colours, odours, taste, particle size, melting point.

### chemical

conc. of active compound,  
degradation, drug excipient  
degradation of preservatives

### microbiological

degradation of product or formulation due to microbial contamination.

## # Growth of Animal cell in culture :

### → Animal cell culture -

- Animal cell culture may be defined as the cultivation of any animal cells, out from the body (In-vitro) by providing the cells with a suitable environment and required nutrients.

### → Types of cell culture :

- Cell culture is classified into three

#### i) Primary cell culture

- Adherent cell culture
- Suspension cell culture

#### ii) Secondary cell culture

#### iii) Cell line

- finite cell line
- continuous cell line

### → Equipments used in animal cell culture :

- Incubator
- Sterilizer
- Microscope
- Washing-up equipments
- Sterilizing & Drying oven
- Water Purification
- Cell freezing
- Centrifuge
- Automatic pipette

Note : It is more difficult to culture the cells of animal as compare to the microorganisms because the animal cells require more nutrients and typically they grow only when they are attached to a specially coated surface.

→ few points that must be kept in mind, in order to see proper growth of the animal cell in culture, are listed below

- It requires rich media.
- Most of the animal cell grow only on the special solid surface.
- Primary cells have limited growth.
- Culture cell can be induced to fuse into heterokaryons.
- Knowledge regarding transformed cell.

1) Rich media are required for culture of animal cell.

- In order to cultivate animal cell, we require a culture media that must be rich in nutrients that are required by the cells of an animal to show their proper growth.

- The media must comprise of :
  - a) Nine essential amino acids.
  - b) Vitamins and peptide or protein growth factors, generally provided by serum.
- 2) \* Most animal cells grow only on special solid surface :
- Most of the cultured animal cells require a surface to grow.
- There are many cells that have the capability to adhere and grow on glass or on specially treated plastics with -vely charged groups on the surface.
- Actually, the cultured cells secrete collagen and other matrix components, which binds to the culture surface & function as a bridge between culture surface & cells.

- 3) \* Primary cells have limited growth
- The primary cells have limited growth potential in culture and may give rise to a cell strain.

Note : cell strain is a population of cultured cells of animal origin, that has a finite lifespan in contrast to a cell line.

- A cell line is a population of cultured cells of animal origin that has undergone a change allowing the cells to grow indefinitely in contrast to a cell strain.

- cell strain = finite lifespan
- cell line = infinite lifespan and growth.

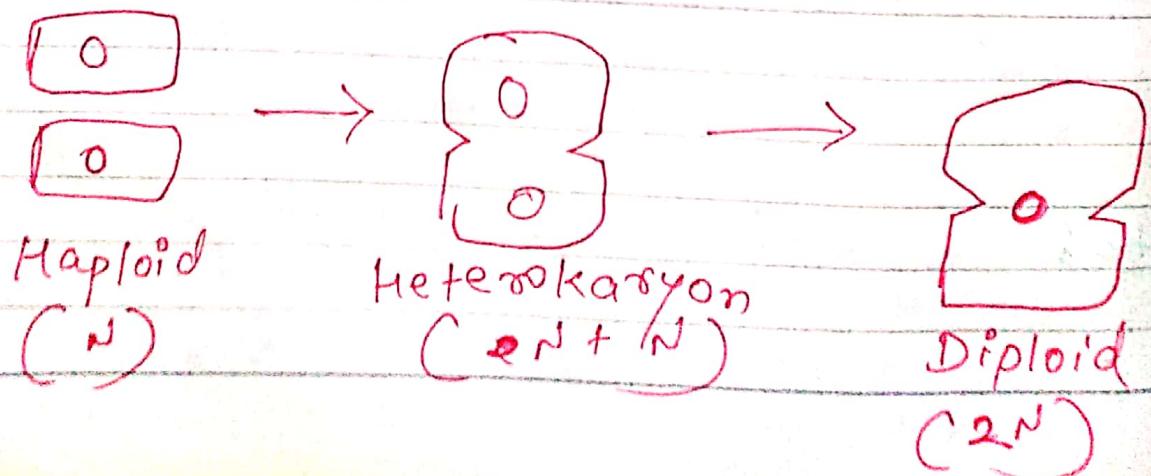
J  
attack chemical  
to show.

- 4) \* culture cell can be induced to fuse into heterokaryones.

- The cultured cell can be induced to fuse into heterokaryons (hybrids) by treatment with certain substances or polyethylene glycol.

Heterokaryons b/w cells of different species tend to lose the chromosome of one species as they divide.

- Note: Heterokaryon is a multinucleate cell that contains genetically different nuclei.



\* knowledge regarding transformed cells

- The transformed cells that are derived from tumours or arise spontaneously from primary rodent cell group indefinitely in cultures

Or

If we extract a cell from tumour or from rodent these are the transformed cell grow indefinitely in culture.

- The transformed cells derived from a single parent cell are called cell lines.

## Application of cell culture in pharmaceutical industry & Research :

- cell culture is used as a model system in research.
- cell culture for toxicity testing in pharmaceutical industry/ & research.
- cell culture is used for cancer research.
- cell culture is used in the field of virology.
- cell culture as cell-based manufacturing.
- cell culture for genetic counselling.
- cell culture is used in genetic engineering.
- cell culture used in gene therapy.

1) cell culture is used as a model system in Research.

- cell culture is considered as a good model system that helps us to study following things in the field of research :
  - A good model System to study basic cell biology & biochemistry.

- A good model system to study the interaction b/w the disease-causing agents & cells.
- A good model system to study the effect of drug substances on cells.

2) cell culture is used for cancer research:

- we can culture the normal as well as cancer cells, by this we can closely study the difference between the normal and cancer cells.
- we can convert any normal into a cancer cell by using the chemical, radiation and viruses and thereby we can study the mechanism that causes the change.
- we can use the cultured cancer cell as a test system through which we can determine the drug as well as method by which we can selectively destroy any cancer cell

3) cell culture is used in the field of virology

- cell culture is still considered as one of the method through which we can cultivate viruses in order to use them in vaccine production. cell cultures are widely used in the detection and isolation of viruses and it also provides a detail that how do the viruses grow & infect organism.

#### 4) cell culture as cell-based manufacturing

- Large scales production of viruses for vaccine production like polio, rabies, chicken pox, etc.
- Large scales production of cells that have been genetically engineered to produce proteins that have / been medicinal or commercial value. Eg ⇒ antibodies, insulin, hormones.

#### 5) cell culture for toxicity testing in pharmaceutical industry and research

- The cell culture are widely used either all alone or sometime in conjugation with animal tests in order to study the effects of new drugs, new cosmetic & new chemical on the survival and growth of the cell.