Parenteral Products (3)
The term sterilization for pharmaceutical preparations, means the **complete destruction** of all **living organisms** and their spores or their complete removal from the preparation.

Five sterilization processes are described in the USP:

a. STEAM
b. DRY-HEAT,
c. FILTRATION,
d. GAS,
e. IONIZING RADIATION.
For sterilization purposes, microorganisms can be categorized into three general categories:

A. **Easy to kill** with either dry or moist heat;

B. **Susceptible to moist heat**, but **resistant to dry heat** (Bacillus subtilis);

C. **Resistant to moist heat** but **susceptible to dry heat** (Clostridium sporogenes).
Sterilization of Parenterals

• Thermal methods: e.g. dry heat, moist heat.
• Non thermal: e.g. gas, ionizing radiation, filtration.

1. **Dry Heat Sterilization**: 
   **Used for:** thermostable materials as
   1 – glass containers
   2- oily vehicles.

   **Methods:** Heated at $160^0$ C for 2 hrs or $180^0$ C for 30 minutes in dry oven.

   **Mechanism:** Cause protein coagulation in micro organism through cell wall dehydration.
Lag time is required for material to reach sterilizing temp. It depends on:

1- Type of the oven.
2- Size of the oven.
3- Air circulation: In modern oven, fan (blower) may be used for good air distribution.

Disadvantages:
• Presence of lag time.
• Capacity of filling.
• Circulation of air should be adjusted.
• Many materials can not tolerate dry heat
2- Moist heat sterilization (steam) (autoclaving)

**Mechanism**: moist heat causes **coagulation** of proteins.
a. Application of pressure:

Because it is **not possible** to raise the temperature of the steam **above 100 C.** under atmospheric conditions, **pressure is employed** to achieve **higher temperature** (it should be recognized that the **temperature, not the pressure** is **destructive** to the microorganisms and that the **application of pressure** only for the purpose of **increasing the temperature** of the system).
b. **Time of application:**

Time is an important factor in the destruction of microorganisms by heat. The usual conditions (time/pressure/temperature), are as follow:

- **10 pounds pressure (115.5°C) for 30 minutes**
- **15 pounds pressure (121.5°C) for 20 minutes**
- **20 pounds pressure (126.5°C) for 15 minutes**

As can seen, the greater the pressure applied, the higher the temperature obtainable and the less the time required for sterilization.

The temperature at which most autoclaves are routinely operated is usually **121°C**.

**Uses:** sterilization of the final product with aqueous vehicles (LVP or SVP).
3-Gas sterilization:
• Old gases e.g. Formaldehyde, SO$_2$  
  1- highly reactive and irritant.  
  2- Difficult to remove residues.
• New gases e.g. ethylene oxide, $\beta$ – propiolactone. (non irritant)

• **Ethylene oxide**: alone it is highly flammable, so it is mixed with an inert gas e.g. CO$_2$.

• **Mechanism of action**: alkylation of various reactive groups (interference with the metabolism) ⇒ stops protein syntheses and cell wall formation.

• **Disadvantages**: may also alkylate drugs especially in the liquid form. So the use of ethylene oxide is restricted for powdered drug which are not affected.

• **Used for** plastic materials (e.g. syringe), dry powders which are not affected by gas (e.g. Aspirin powder), surgical rooms.
4- Radiation sterilization

Advantage:
1) Highly effective.
2) Treatment times is very short.
3) It is a cold process resulting in a temperature rise of only a few degrees and so is suitable for thermolabile materials.
4) Products are processed in the final container after packaging with no risk of subsequent contamination until used.

Disadvantage: hazard effect of radiation on workers

Used for: vitamins, hormones and antibiotics.

Types of radiation:
1. Ultraviolet radiation (UV)
2. X-rays
3. gamma rays (Using \( \gamma \)-emitter e.g. Cobalt-60 to get 2.5 megarads)
5- Sterilization by Filtration:

• Used for LVP.

• Suitable pore size is 0.22 μm.

Membrane filters have 2 types:

• Disposable filters: Polymeric cellulose and its derivative or Nylon.

• Reusable filters: glass or stainless steel.

It is more economic and strong but has a disadvantage of tedious cleaning process.
ADVANTAGES OF BACTERIAL FILTERS

a. Its ability to sterilize thermolabile materials
b. Inexpensive equipment required
c. The complete removal of living and dead M.O. as well as other particulate matter from the solution

DISADVANTAGES OF BACTERIAL FILTERS

a. The membrane is fragile thus it is essential to be sure that the membrane is not ruptured
b. Filtration of large volumes of liquids would require more time (particularly if the liquids were viscous)
-Test of filters:

1- Bubble point test:
1. A filter is wetted with \( \text{H}_2\text{O} \) or ethanol
2. Air pressure is applied and gradually increased till bubbles appear in the down solution
3. The pressure at this point is determined.

Bubble point pressure is inversely proportional to pore size. 
0.22 μm filter → gives Bubble point at 50 – 55 Psig (pound/square inch gauge).
If bubbles point pressure is less than \(< 50 \Rightarrow \text{defect in filter.} \)
2- Microbial Challenge test:

1. Bacterial suspension of *pseudomonas diminuta* is filtered through filter
2. Sterility test is carried out for the filterate.

*If no m.o*: good filter.
*If m.o*: defect in filter.
Indicator Tests For Indication Of The Efficiency Of The Sterilization Process

Physical indicator tests

1) The performance of steam sterilizers can be tested by observing the reading of temperature, pressure throughout the sterilization cycle.

2) In case of sterilization by radiation: A measurement of the amount of energy.

3) In case of sterilization by filtration: A bubble pressure test is used to determine the pore size of filters.
Chemical indicator tests

**Types that cannot indicate time of exposure:**

A) Klintex papers:

These are **paper strips or stickers** attach to each object to be sterilized.

The word **(sterile)** is written on the strip **(colorless)** but after exposure to the sterilizing agent as **steam** the word **(sterile)** will be cleared.
B) Klintex test tablets:

These contain 75% lactose, 24% starch and 1% magnesium trisilicate.

They are hard and white but after steam sterilization they become brown and gelatinous.
Types that indicate time of exposure:

Browne’s tubes:
Sealed glass tube which contains a red fluid (an ester and acid-base indicator) that changes to yellow, brown and finally green on heating.

The ester undergoes heat hydrolysis to form an acid + alcohol.

The acid will change the color of the indicator.
Biological indicator tests

Biological indicators consist of bacterial cultures which are usually used in the form of impregnated strips e.g. paper and metal foil and are placed in different sites in the sterilizer.

At the end of the process, the bacteria are transferred to a nutrient medium which is incubated and the presence or absence of growth is noted.
PARENTERAL QUALITY CONTROL

1. Volume of container.
2. Sterility testing.
3. Pyrogen testing.
4. Clarity.
5. Leakage.
a) Volume in container:

- **Number of containers tested depends on CONTAINER VOLUME as follows:**

<table>
<thead>
<tr>
<th>Volume of the container</th>
<th>Number of containers taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 10 ml injections</td>
<td>1 Container</td>
</tr>
<tr>
<td>3-10 ml injections</td>
<td>3 Containers</td>
</tr>
<tr>
<td>&lt; 3 ml injections</td>
<td>5 Containers</td>
</tr>
</tbody>
</table>

b) Test:

1) Oily injections

1. The containers are **WARMED** → to be easily transferred to a graduated cylinder.
2. Cooled before measuring the volume.

2) Aqueous injections

Carefully transfer the volume of the container to a graduated cylinder to be measured.
Results

1. The volume should **not be less** than the labeled volume.
2. A certain volume **excess** is allowed and it ranges from 2-**20%** according to the volume of the injection.
3. **As volume of the injection** $\uparrow \rightarrow$ **volume excess** $\downarrow$ e.g.

<table>
<thead>
<tr>
<th>Injection volume</th>
<th>Volume excess</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 ml injection</td>
<td>20%</td>
</tr>
<tr>
<td>50 ml injection</td>
<td>2%</td>
</tr>
</tbody>
</table>
b) Sterility Testing:

Two media are used:

- **Theoglycollate medium**: for **anaerobic** growth.
- **Soya bean – casein digest medium**: for **aerobic** growth.
- Sterility testing is carried by 2 methods – direct inoculation or Membrane filtration.

1) Direct transfer:

- A specified volume of the parenteral product is transferred to each of the **two test media** for aerobic and anaerobic growth.

- The media are **examined visually** for growth for 14 days, **growth** is indicated by **turbidity**.
2) Membrane Filtration:
Used for
1- Liquids containing antimicrobial agent.
2- Oily injection, ointments and creams.

- The liquid is filtered through **two membrane filters** which are then **washed** with **fluid A** (for liquids miscible with water). 1gm peptic digest of animal tissue in one liter of water at pH 7.1 and sterilized by steam.
- or **fluid D** (for liquids immiscible with water as it contains surfactant). Fluid A to which is added polysorbate 80 (SAA).
- **Transfer** the two filters to the **two test media** for aerobic and anaerobic growth.
- The media are examined for microbial growth (**turbidity**) for 7 days.
- For oily injections, ointments and creams, these are dissolved in **isopropyl myristate** and filtered through 2 membrane filters.
c) Pyrogen (Endotoxin) testing:

1. Pyrogens are the **metabolic** products of **microorganisms** they are phospholipids.

2. The **most dangerous** pyrogens are those produced by **Gm -ve bacilli**.

   - **It Cause** fever & may lead to **death** in large doses.
The endotoxin characteristics

- water-soluble
- Unaffected by the common bactericides
- Non-volatile

These are the reasons why pyrogens are difficult to be destroyed once produced in a product.

**Elimination of pyrogen:**
- Being organic compound, pyrogen can be destroyed with high heat by oxidation at 180°C for 3-4 hrs.
- This method is effective for metal container contaminated with pyrogen.
- It is not practical for solution...
Elimination of pyrogen:

- Pyrogen in solution are eliminated chemically by oxidation with peroxides, acid, and alkali.

- Physically, by reverse osmosis or adsorption of pyrogen in solution by asbestos and charcoal.

Tests for pyrogenic activity

1. Rabbit test
2. Bacterial endotoxins
1) Pyrogen testing

1. Three healthy rabbits are chosen.

2. Accurate thermometers are used to record their body temperature (control temp).

3. Test solutions are injected.

4. Rabbit temperatures are recorded at 30 min intervals between 1 and 3 h.
Results:

1. If no rabbit shows an individual temp. rise of 0.5 C or more above its control temperature, the product meets the requirements for the absence of pyrogens.

2. If any rabbit shows an individual temperature rise of 0.5 or more, repeat the test using five other rabbits.

3. If not more than three of the eight rabbits show individual rises in temperature of 0.5 or more and if the sum of the eight temperature rises does not exceed 3.3, the material under examination meets the requirements for the absence of pyrogens.
2) *Limulus Amebocyte Lysate Test*

- *It is called In-vitro pyrogen* or bacterial endotoxin test (BET).

- The test principle is based on the reaction of "Limulus-Amebocyte Lysate" (LAL) (an enzyme) with *pyrogens* forming Opaque gel

- The reaction accomplishes within **15-60** minutes.

- As the *concentration* of pyrogen increases, the gel becomes more *turbid* and *thick*. 
Advantage of LAL test

1. It is *in-vitro* and does not require animal handling, thus is more *convenient*
2. It is 10 times more *sensitive* than that of the *in-vivo* rabbit test
3. It is *economical*
4. It consume less *time*, i.e., 1 vs 3 hours required by *rabbits* test.
d) Clarity Test

- **Tests**
  The solution is examined visually in presence of a source of light against

  ![Black background](image1) ![White background](image2)

- Particles can be counted using Coulter counter device.
• The particulate matter may be capable of blocking the blood vessels.

• A person perfect vision under inspection conditions is able to detect particles of size range 40 – 50 μm.

• Pulmonary capillary are approximately 7 μm in diameter, thus particle of this size entrapped in vascular bed resulting in multiple pulmonary infarction.
Sources of particulate matter

1. Un-dissolved substances.
2. Vehicle.
3. Final container.
5. Environmental contaminants.
7. Personnel.
A) VISUAL INSPECTION

- The method is used for the evaluation of large volume parenterals.

- To increase the sensitivity of the method, the visual inspection of the sample container may be coupled with the application of vacuum to make leakage more readily observable.

- This method is simple and inexpensive.

- However, the method is insensitive and operator dependent.
B) DYE TESTS

- The test container is immersed in a bath of colored dye solution.
- Pressure is applied for some time.
- The dye test can be optimized by use of a surfactant in the dye solution to increase the capillary migration through the pores.
- The container is removed from the dye bath and washed.
- The container is then inspected for the presence of dye either visually or by means of UV spectroscopy.
- The test is inexpensive and is requires no special equipment.
- However, the test is slow.
- The test is used for ampoules and vials.
PARENTERAL SUSPENSIONS

- Usually contain 0.5 – 5% solids of particle size 5-10 μm.
- However, certain antibiotics may contain up to 30% solids e.g. Benzathine penicillin (penadure)

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. insoluble drugs.</td>
<td>1. Difficult formulation and manufacturing.</td>
</tr>
<tr>
<td>2. unstable drugs.</td>
<td>2. Patient discomfort (more pain).</td>
</tr>
<tr>
<td>3. sustained release (depot)</td>
<td>3. Not used as IV.</td>
</tr>
<tr>
<td>e.g. insulin.</td>
<td>4. Difficult dose uniformity.</td>
</tr>
</tbody>
</table>

Factors considered in suspension formulation:

- Syringeability (withdrawing suspension into syringe)
- Injectability (pressure for injection).

Both 1,2 depend on:

- Viscosity.
- Particle size.
## Types of Suspensions

<table>
<thead>
<tr>
<th></th>
<th>Flocculated</th>
<th>Non-flocculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floccules formation</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Rate of sedimentation</td>
<td>Rapid</td>
<td>Slow</td>
</tr>
<tr>
<td>Sediment</td>
<td>Large - porous</td>
<td>Small - compact</td>
</tr>
<tr>
<td>Re-dispersion</td>
<td>Yes</td>
<td>No (Caking)</td>
</tr>
</tbody>
</table>

*Diagram showing:*
- Flocculated suspension: Initial state (f = 1), state of suspension on storage after some time, deflocculated suspension.
- Non-flocculated suspension: Clear supernatant, cloudy supernatant, hard cake.
Sedimentation Behavior

- Velocity of sedimentation expressed by Stoke’s equation
  \[ V = \frac{d^2 (\rho_s - \rho_o) g}{18\eta} \]

Where,
- \( V \) = sedimentation velocity in (cm/sec)
- \( d \) = Diameter of particle (cm)
- \( \rho_s \) = density of dispersed phase (gm/cm\(^3\))
- \( \rho_o \) = density of dispersion media (gm/cm\(^3\))
- \( g \) = acceleration due to gravity (980 cm/sec\(^2\))
- \( \eta \) = viscosity of disperse medium (poise)
Factors Affecting Sedimentation

Particle size diameter (d)
\[ V \propto d^2 \]

Sedimentation velocity (v) is directly proportional to the diameter of particle.

Density difference between dispersed phase and dispersion media (\( \rho_s - \rho_o \))
\[ V \propto (\rho_s - \rho_o) \]

If density of the dispersed phase and dispersion medium are equal, the rate of settling becomes zero.
• Viscosity of dispersion medium (\( \eta \))
  \[ V \propto \frac{1}{\eta} \]

• Sedimentation velocity is inversely proportional to viscosity of dispersion medium.

• So increase in viscosity of medium, decreases settling, so the particles achieve good dispersion system.

• Greater increase in viscosity gives rise to problems like syringeability and injectability of suspension.
GRF: SUSPENSION cant be TERMINALLY sterilized?*****

• Sterilization by heating may cause dissolution of suspension and Upon cooling → precipitation with new particle size.

• Filtration cant be used → Size of filter 0.22 μm, suspension particle are retained on it.

So, sterile solid is prepared and sterile vehicle is mixed under aseptic conditions (Sterile re-crystallization)
Preparation of Suspensions by Sterile Re-crystallization:

1. Active ingredient in solution (Organic solvent)
2. Sterilizing filter (i.e. 0.22 micron)
3. Counter solvent
4. Sterilizing filter (i.e. 0.22 micron)
5. Removing organic solvent
6. Aseptic Mix/Mill/Fill
PARENTERAL EMULSIONS

• It is a two-phase system prepared by combining two immiscible liquids, one of which is dispersed uniformly throughout the other.
Classification of emulsions:

- Based on dispersed phase
  - Oil in Water (O/W): Oil droplets dispersed in water
  - Water in Oil (W/O): Water droplets dispersed in oil
## Instability of emulsions

<table>
<thead>
<tr>
<th>1) Creaming</th>
<th>2) Sedimentation</th>
<th>3) Coalescence (cracking)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The dispersed globules move <strong>UPWARD</strong> and form a thick layer at the surface of the emulsion</td>
<td>The dispersed globules move <strong>DOWNWARD</strong> and form a thick layer over the bottom of the container.</td>
<td>The dispersed globules coalesce together and <strong>TWO SEPARATE</strong> layers of the dispersed phase and continuous phase are formed.</td>
</tr>
<tr>
<td>Both are <strong>TEMPORARY</strong> phase → <strong>can be re-distributed</strong> by mild shaking or stirring to get a homogeneous product.</td>
<td></td>
<td><strong>DIFFICULT</strong> to re-disperse by shaking or stirring → so it is</td>
</tr>
</tbody>
</table>
Ingredients:
1- Oily phase  2- Emulsifiers  3- Aqueous phase

**Oily phase:**
- most commonly used oils derived from cotton seed, Soya bean, safflower seeds.

**Emulsifiers:**
- Natural lethicin (form egg yolk or soybean)
  - It is stable towards hydrolysis and oxidation.
  - Its concentration should not exceed 1% because it may cause side effects on long term use (hemolysis).
- Non ionic SAA: e.g. Pluronics.

**Aqueous phase:**
- Sterile WFI, additives required to adjust isotonicity as glycerin, sorbitol.
Advantages of emulsion (pharmaceutical application):
1. Parenteral nutrition.

2. Formulation of emulsion to contain specific drugs. e.g.:
   - To decrease Drug irritation e.g. diazepam.
   - To increase drug stability: physostigmine salicylate.
   - It gives depot action: e.g. vitamin B$_{12}$

3. It could be injected I.V (nanoemulsion).
Problems associated with the use of parenteral emulsions

• There is a limited list of surface-active agents that may be employed to stabilize parenteral emulsions (due to toxicity concerns).

• When administered intravenously it is essential that the droplet size is less than 1 μm to prevent blockage of blood flow within the capillaries.

• Emulsions are difficult to sterilized. Normal sterilization methods, e.g. heat and filtration, are not suitable.
D. DRY POWDERS

• Upon reconstitution with sterile vehicle:

• solution or suspension. (must be written on label)

[I] give solutions, e.g. thiopental Na

[2] give suspension, e.g. Ampicillin trihydrate

Preparation of dry powder:
1. Lyophilization.
2. Spray drying.
Factors considered during choice of container:

- inert.
- Impermeable.
- Protection of drug.
- Not easily broken.
- Easily used.
1- Glass
• **Advantages**: transparent (easily examination for content), economic.
• **Disadvantages** : fragile and heavy.

• Types:
  • **Type I**: Neutral or Borosilicate glass: it is expensive, harder, has high thermal and chemical resistance.
  • **Type II**: Soda glass with *surface treatment* to remove alkalinity.
  • **Type III**: Soda glass limited alkalinity.
  • **Type IV**: NP soda glass (non parenteral soda glass).

• *Alkalinity of type IV > III > II > I.*
## 2. Plastics

<table>
<thead>
<tr>
<th>used for:</th>
<th>Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Catheter.</td>
<td>2. polypropylene: catheter.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. flexible.</td>
<td>1. more permeable.</td>
</tr>
<tr>
<td>2. non breakable.</td>
<td>2. make adsorption.</td>
</tr>
</tbody>
</table>