Selection of containers and closures

- Selection of Containers & Closures should be such that it should ensure that the products must remain its purity, potency & quality during intimate contact with the container throughout its shelf life.
- **Glass:-** Glass is employed as the container material of choice for most SVIs. It is composed, principally, of silicon dioxide, with varying amounts of other oxides, such as sodium, potassium, calcium, magnesium, aluminum, boron, and iron. Glass is preferred for clarity reasons
- Types:- The USP provides a classification of glass:

✓ Type I, a borosilicate glass;

✓ Type II, a soda-lime treated glass;

✓ Type III, a soda-lime glass; and

✓ NP, a soda-lime glass not suitable for containers for parenteral.

- **Plastic:-**Plastic packaging has always been important for ophthalmic drug dosage forms and is gaining in popularity for injectable dosage forms. Plastic bottles for large volume injectable (LVIs) have been used for many years. Plastic vials for SVIs may be a wave of the future plastic packing offers such advantages of cost savings elimination of the problems caused by breakage of glass and increase convenience of use. Plastics are light weight, less fragile & easy to handle but not clear as that of glass.
- **Rubber:-** Rubber formulations are used as rubber closures, rubber plungers and other applications. The most common rubber polymers used in SVIs closures are natural and butyl rubber. Butyl rubber has great advantages over natural rubber in that butyl rubber requires fewer additives, has low water vapor permeation properties and has good characteristics with respect to gaseous permeation reactivity with the active ingredient. Rubber permits the entry of hypodermic needle into injection vials & also provide resealing of the vial after needle is withdrawn

Filling and Sealing of Ampoules

- Ampoules are thin-walled glass containers, which after filling, are sealed by either tip sealing or pull sealing. The contents are withdrawn after rupture of the glass, or a single occasion only. These are great packaging for a variety of drugs. The filed – in product is in contact with glass only and the packaging is 100% tamper proof. The break system OPC(one –point cut) or the color break ring offer consistent breaking force. There are wide variety of ampoule types from 0.5 to 50ml volume.
- Here, the measured amounts of liquid deliver from the small orifice into the ampoule by filling machine.
- The size of the delivery tube is governed by opening in the container to be used, the viscosity and density of the liquid and the speed of delivery desired.
- The tube must free enter the neck of the container and deliver the liquid deep enough to permit air to escape without sweeping the entering liquid into the neck or out of the container.
- Filling machine parts should be constructed of non-reactive materials such as borosilicate glass or stainless steel.
- The solutions are usually filled in the bottle by gravity, pressure or vacuum filling device.
- Emulsion and suspension required specially designed filling equipment because of their high viscosity.
- Powders such as antibiotics, are more difficult to subdivide accurately and precisely into Individual dose containers than are liquid.
- Container should be sealed in the aseptic area in immediately adjacent to the filling machine.
- It is obvious that a sterile container that has been opened can no longer be considered tobe sterile. Therefore, temperature proof sealing is essential.
- Ampoules may be closed by melting a portion of the glass of neck to either form tip-seals or pull seals.
- Tip-seals are made by melting sufficient glass at the tip of the ampoule neck to form a bead of glass and close the opening. This is performed in a high temperature gas oxygen flame.
- Pull-seals are made by heating the neck of a rotating ampoule below the tip, then pulling the tip away to form a small, twisted capillary just prior to being melted closed. Pull sealing process is slower one, but the sealing done by this is more secure than that of tip sealing.
- Excessive heating of air and gasses in the neck causes expansion against the soft glass with the formation of fragile bubbles at the point of seal.

Filling and Sealing of Vials and Infusion bottle

- The solutions, which sterilized through filtration, are to be filled under the aseptic conditions. During the filling of product to the containers, should be for the prevention of contamination, especially the product is sterilized by the filtration and will not be sterilized in to the final container. The second one is called as aseptic fill. A liquid is more easily exposed uniformly into the container having the narrow mouth than is used for solid. Liquids which are mobile are easier to transfer and subdivide than viscous or sticky fluids, since these require heavy-duty machinery for the rapid production filling. The filling of liquids into containers with high accuracy involves the following methods

i) Volumetric filling

ii) Time/pressure filling

- By closing the opening using the rubber closure (stopper) the glass or the plastic vials are sealed properly. This should be done by after filling with care, to prevent the contamination of the contents inside.
- By using the aluminum caps the rubber stoppers are held in appropriate place.
- Rubber closures that uses for the intravenous administration have a permanent hole through the closure.
- A 500ml of infusion bottle is considered suitable for preparation of parenteral solutions. It is assumed that the bottle has been stored with a double cap protecting the mouth. The outer cap is discarded and the inner cap is removed. After ensuring that the bottle neck is not chipped, the solution is poured in and immediately the inner cap is replaced.

Quality Control Tests of Parenteral Products

- **Sterility test:** It is a method carried out to detect confirm absence of any viable form of microbes in product. The method used for sterility tests are

a. Direct transfer method

b. Membrane filtration method.

a. Direct transfer method: Open each sample container and with draw the require amount of the sample. Inject one-half of sample in a test tube containing fluid Thioglycolate Medium (FTM). Inject another half in the test tube containing Soyabean-casein digest Medium(SCM). Volume of the medium must be sufficient to promote and expedite microbial growth. Adequate mixing between the sample inoculums and the culture medium must take place to maximize interaction and facilitate microbial growth. If the product to be tested contains any anti-microbial agent, using suitable reagent it should be neutralized before the test.

b. Membrane filtration method (MF): This method is employed in the following cases:

1. Oil & oily preparations

2. Alcoholic preparations

3. For preparations miscible with or soluble in aqueous or oily solvents.

The steps involved in MF sterility test method are

i). The filter unit must be properly assembled and sterilized prior to use.

Ii). The contents are transferred to the filter assembly under strict aseptic conditions.

iii) The membrane is removed aseptically.

iv). Membrane is cut in half.

iv) One half is place in suitable volume of FTM and another in an equal volume of SCM.

Interpretation of results:

i). If there is no visible evidence of microbial growth, it may be interpreted that the sample is without intrinsic contamination and the product complies the test for sterility.

ii). If microbial growth is found, the product does not complies the test for sterility and the sterility test may be repeated.

- **Clarity test (particulate matter evaluation):-** Particulate matter in parenteral solutions has been recognized as an acceptable. Since the user could be expected to conclude that the presence of visible dirt would suggest that, the product is of inferior quality.

a). In visual method, the entire product should be inspected by human inspectors under good light baffled against reflection into the eye and against black and white background. Dark background detects light particles and light background detects dark particles. Any container with visible particle if seen is discarded.

b) Some other methods of clarity testing can be listed as Filtration method, Light scattering method, Light absorption, Light blockage methods, etc...

- Once the particles are detected, then they are identified by various methods like microscopy,Xray powder diffraction, mass microscopy, micro-chemical tests, polarized light microscopy and scanning electron microscopy.

Leakers test:- Leaker test for ampoules is intended to detect incompletely sealed ampoules so that they can be discarded in order to maintain sterile condition of the medicines. Open capillaries or cracks at the point of seal result in LEAKERS.

- The leaker test is performed by immersing the ampoules in a dye solution, such as 1% methylene blue, and applying at least 25 inches of vaccum for a minimum of 15 mins.
- Another means of testing for leakers is a high frequency spark test system, which detect presence of pinholes in ampoules.
- Bottles and vials are not subjected to such a vaccum test because of the flexibility of the rubber closure.

Pyrogen test:- Pyrogens are the metabolic products of microbes. Most bacteria, moulds and viruses produce Pyrogen. Most potent pyrogenic substance called endotoxins are produced by gram negative bacteria .Pyrogens when injected into a human, shows marked rise in the temperature , chills, body aches, cutaneous vasoconstriction and increased arterial blood pressure. The most likely source of pyrogens are water, contaminated solutes and containers.

- The test involves measurement of the rise in body temperature of rabbits following the IV injection of a sterile solution into ear vein of rabbit.
- Dose not exceeding 10 ml per kg injected intravenously within a period of not more than 10 mins.
- Selection of animals - healthy, adult, not less than 1.5kg.
- Equipment and material used in test - glassware, syringes, needles.
- Retaining boxes - comfortable for rabbits as possible.
- Thermometers - standardized position in rectum, precision of 0.1°C.

Preliminary Test (Sham Test): If animals are used for the first time in a pyrogen test or have not been used during the 2 previous weeks, condition them 1 to 3 days before testing the substance by injecting IV 10ml per kgpyrogen free saline solution warmed to about 38.5°c. Record the temperature of the animals, beginning at least 90 mins before injection and continuing for 3 hours after injection. Any animal showing a temperature variation of 0.6° or more must not be used in main test.

Main Test: The main test is carried out by using a group of 3 Rabbits. Dissolve the substance in,or dilute with, pyrogen free saline solution. Warm the liquid to approximately 38.5° before injection.Inject the solution under examination slowly into the marginal veins of the ear of each rabbit over a period not exceeding 4 mins. Record the temperature of each animal at half hourly intervals for 3 hours after injection. The difference between the initial temperature and the maximum temperature which is the highest temperature recorded for a rabbit is taken to be its response.

- Interpretation of Result:

a). The test is carried out on the first group of 3 rabbits; if necessary on further groups of 3 rabbits to a total of 4 groups, depending on the results obtained.

b). Intervals of passing or failing of products are on the basis of summed temperature response.

If the difference is negative, the result is counted as zero response.

No. of Rabbits	Individual Temp. Rise(°C)	Temp. Rise in group (°C)	Test
3 Rabbits	0.6	1.4	Passes
(If above not Passes)-: 3+5=8 Rabbits	0.6	3.7	Passes
If above Test not passes,the sample is said to Pyrogenic.			

Bacterial Endotoxin Test (BET) or Limulus Amoebocyte Lysate Test (LAL Test):-

- The bacterial endotoxin test (BET) is a test to detect or quantify endotoxins from gram negative bacteria using Amoebocyte lysate from the horse shoe crab (Limulus polyphemus or Tachypleustridentatus) .
- The endotoxins of gram-negative bacteria forms a firm gel within 60 mins in the presence of lysate of amebocytes of limulus polyphemus of horseshoe crab, when incubated at 37◦c. Hence, the test is only effective with gram-negative bacteria, which constitute the majority and the most potent of the pyrogens. The addition of a solution containing endotoxins to a solution of a lysate produces turbidity, precipitation or gelation of the mixture.