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A novel proposition of facilities required for sterile pharmaceutical preparation

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Abstract
Sterile facility is required for Realizing the crucial importance of quality, safety and efficacy of sterile pharmaceutical preparations such as eye drops, intravenous admixtures, parenteral nutrition and cytotoxic drug reconstitutions (CDR) in hospitals. There is an urgent need for better clean room facilities, water supply system and sterilization facilities in new as well as existing hospitals. To assist those in the planning and development of such facilities, the FDA, WHO, ISO and Good Manufacturing Practices has established the "Guides to the Development of Sterile Pharmaceutical Preparation Facilities for Healthcare Establishments".
This document addresses several important aspects including policies, design, layout and specifi cations, management and quality control as well as storage, distribution and ancillary areas. It also provides recommendations for the layout of CDR and non-CDR preparation facilities and also lays down the specific requirements during the construction process of such facilities. To ensure quality, safety and efficacy of products and also protect personnel, the document is intended to promote awareness amongst healthcare planners and developers of the stringent regulatory requirements for such facilities. It is our fervent hope that relevant stakeholders involved will find this guide useful and applicable. Finally, I would like to honor and thank each and every one of you that have played important role and made remarkable contributions towards the success of the project of this guideline.

Keywords: Preparation facilities, clean room, hvac system, water treatment plant, filtration

Introduction
"Sterile pharmaceutical" means any dosage form devoid of viable microorganisms, including but not limited to parenteral, injectable and ophthalmic.
One of the most critical operations in pharmaceutical manufacturing is the processing of sterile product. The productions of sterile products, specifically the ones that cannot be terminally sterilized [1], involve complex and demanding processes to prevent the products' contamination and require a great amount of resources.

What is sterile?
Sterile simply means there are no microorganisms that can cause infection in the patient.

Why sterile facility is required for pharmaceutical preparation?
Unlike products that are terminally sterilized (the preferred method by major regulatory agencies) a Sterile operation maintains acceptable sterility at critical steps of the manufacturing process (when sterile filtration or other means are not possible) and filling operations (when terminal sterilization is not an option).
When the product can be terminally sterilized (autoclaving the most common method), Aseptic processing is not necessary. Aseptic processing is common for parenteral (injectable drugs.)Whether produced in an Aseptic manner or terminally sterilized, parenteral must be sterile in their final form to avoid problems for the patient.
Products that are not sterile may contain pyrogens "An agent capable of inducing an increase in body temperature; usually refers to fever caused by bacterial [2] endotoxins."
An Endotoxin is "Cell wall debris (lip polysaccharide) from Gram-negative bacteria."
These may include bacteria such as *E. coli*, Salmanella, Shigella, Haemophilus, Pseudomonas, and Neisseria as well as other pathogens.

Whereas drugs such as OSD's (Oral Solid Dosage) do not require sterility since the body's natural defense mechanisms engage after ingestion, parenteral are injected intramuscularly (I.M.) or intravenously (I.V.) and bypass the defense mechanisms [3]. A simple example of this is normal drinking water. If you drink safe water, there is no ill effect. But if you were to inject the same water with a syringe, you could get extremely sick.

What is sterile production?

Pharmaceutical manufacturing is the processing of sterile products. The productions of sterile products, specifically the ones that cannot be terminally sterilized, involve complex and demanding processes to prevent the products' contamination and require a great amount of resources.

What is sterile pharmaceutical product?

Sterile pharmaceutical products must, by definition, be free of microorganisms [4], and it is important to understand that this is an absolute requirement. Thus, the presence of one single surviving microbial cell is sufficient to render the product non-sterile**.**

Types of sterile pharmaceutical products

- Aqueous intravenous solution
- Oily solutions
- Aqueous suspension
- Oily suspension
- Freeze dried (lyophilized) powder
- Contact lens solutions-
- Wetting agents,
- Cleaning solutions,
- Soaking solution
- Surgical dressings.
- Implants.
- Absorbable hemostats-oxidized cellulose,
- Absorbable gelatin foam,
- Human fibrin foam,
- Calciumalginate.
- Surgical ligatures and sutures- catg

Sterile Preparation Facilities

Preparation of sterile pharmaceutical products in hospitals involves the activities such as preparation of Eye Drops, Intravenous Admixtures, Parenteral Nutrition's and Cytotoxic Drug Reconstitutions [5]. For the purpose of these guidelines, the sterile preparation facilities are classified into Cytotoxic Drug Reconstitution (CDR) and Non-Cytotoxic Drug Reconstitution (Non-CDR).

List of Equipment Required

1. CDR Room

a. Cytotoxic Drugs Reconstitution (CDR) cabinets / isolator 4ft. or 6 ft. with stainless steel stand
b. Trolley (stainless steel)
c. Intercom system
d. Non-wheeled stainless steel stool with adjustable height (for safety reasons)
e. Roller mixer

2. Component room

a. Rack/shelves for keeping of sterile bags, syringes, needles, filters, etc. (stainless steel)
b. Phenolic bench top with stainless steel drawers
c. Trolley (stainless steel)
d. Sink (stainless steel)
e. Intercom system

3. Personnel Gowning Room

a. Garment cabinet or five-tier lockers for sterile gloves, head caps, mask
b. Wall mounted six foot long mirror
c. Cross over bench

4. Personnel Changing Room

a. Sink with elbow tap (stainless steel)
b. Cabinet (to hang street clothes)
c. Wall mounted six foot long mirror
d. Liquid soap dispenser (foot-operated)
e. Electrical hand dryer
f. Cross over bench

5. Storage, Receiving and Distribution Room

a. Pharmaceutical refrigerator, twin doors connected to an essential power supply
b. Trolleys (stainless steel)
c. Computers and printers including "Uninterrupted Power Supply"
d. Display for temperature, relative humidity and pressure for the clean rooms
e. Tables and chairs
f. Telephone
g. Intercom System
h. Filing cabinets
i. Pneumatic tube terminal (optional)

PN / IV ADMIXTURE / EYE DROP (NON-CDR)

A. Facility Requirement

1) Appropriate clean room facilities shall be provided for Non-Cytotoxic Drugs Reconstitution (Non-CDR) activities such as the preparation of Parenteral Nutrition Solutions, Intravenous Admixtures and Eye Drops
2) The facility shall have personnel changing rooms (for changing and gowning), a component room, a preparation room and an area for storage, receiving and distribution activities
3) Flooring shall be a continuous, non-cracking material that is mechanically and chemically robust. Preferably, floors shall be overlaid with wide sheet vinyl flooring with heat-welded seams and coving to the sidewall
4) Walls and ceilings shall be free from cracks, built with a smooth, non-shedding, cleanable finish that is impervious to water, cleaning and sanitizing solution. To avoid condensation problem, sandwich panel wall system (e.g. Polyurethane panel) shall be used
5) Bare wood, ledges and other unsealed surfaces shall be avoided in clean rooms. Glass window is required for the preparation room and it shall be of flushed double glazed type
6) There shall be two parts of personnel changing room. The second or final part of the personnel changing room leading into the preparation room shall be of the same grade as the latter
7) A sink for hand wash can be fitted in the first or earlier part of the changing room. The preparation room shall not contain any sink or floor drains

8) Taps shall be elbow, foot or beam-operated. Surface of materials, including bench tops, shall have minimum joints and seams; be non-shedding and easy to clean
9) All doors for clean rooms shall be fitted with inter-locking system so that only one door can be opened at a time to ensure the pressure cascade is not compromised. All airlock doors shall be provided with self-closers
10) Doors and windows shall have a hard, smooth, impervious finish and close tightly and also fit flush with surrounding walls. The size of all doors shall be sufficient for the equipment to be brought into
11) A positive pressure unidirectional airflow cabinet or isolator shall be used for Parenteral Nutrition and Eye Drop. For IV Admixture preparations, a negative pressure unidirectional cabinet or isolator shall be used to ensure maximum personnel protection
12) The cabinet and isolator used shall be of a Grade A air quality for the protection of product. There shall be sufficient space underneath the cabinet for allowing cleaning process
13) The preparation room shall be of Grade B if a unidirectional airflow cabinet is used. If an isolator (positive isolator) is used, the room shall be of at least Grade D air quality
14) Since the preparation room shall not have a work bench, equipment installed (either cabinet or isolator) shall come with its own stands
15) Component room shall be of Grade C or D air quality and shall be entered by personnel via a personnel changing room of a similar grade
16) Utility cabinet, stainless steel sink with an appropriate depth and backsplash to avoid splashing and work bench shall be fitted in the component room
17) Buffer/staging room or a hatch shall be used for transferring materials (e.g. components, cleaning materials and equipment). If a one way flow of facility is not possible, the buffer/staging room or hatch can be used for transferring materials and products out as well
18) Adequate numbers of plug points shall be made available
19) Plug points connected to essential power supply shall be made available for pharmaceutical refrigerators. In case of power failure, Uninterrupted Power Supply (UPS) shall be provided for the unidirectional airflow cabinet/ isolator and HVAC system
20) For existing facilities, an accredited agent shall be appointed to test the performance of the facilities on a regular basis
21) Heating, Ventilation and Air-conditioning (HVAC) System

a. Humidity, temperature, pressurization and air filtration or air cleanliness shall be controlled in order to protect the products, personnel and the environments. Appropriate devices for measuring and monitoring the parameters shall be installed or made available. (E.g. pressure gauges thermo-hygrometers, etc.)
b. Due consideration shall be given to the placement of ceiling mounted HEPA filters to avoid creating of air currents inside the cabinet underneath. Diffusers shall not be used
c. Pre-filters (primary and secondary) of AHU and HEPA filters shall be changeable from outside the clean room
d. Temperature (not more than 22oC) and humidity (55 ± 5%) need to be controlled primarily for the stability of products and the comfort of personnel. Equipment installed shall not jeopardize the set temperature of the room
e. The air pressure shall be made higher in the cleaner grade of clean rooms. The air return grilles shall be at the low-level to sweep or purge the rooms

22) Environmental control is a critical factor in determining the successful operation of the manufacturing facility especially a clean room. Therefore, the design and construction, which related to a clean room shall include consideration for:

f. Building finishes and structure
g. Air filtration
h. Air change rate or flushing rate
i. Location of air terminals and directional airflow
j. Room pressure
k. Particulate loading (viable and non-viable)
l. Temperature (not more than 22 ^{o}C)
m. Relative humidity (55 ± 5%)
n. Pressure differentials (10 – 15 Pascal's)
o. Material flow
p. Personnel flow

List of Equipment Required

List of equipment for Non-CDR facilities (Intravenous Admixture [IV Ad], Parenteral Nutrition [PN] and Eye Drops)

1. Preparation Room

a. Positive Pressure Unidirectional (horizontal) Airflow cabinets / isolator 4 or 6 ft. with stainless steel stand for Parenteral Nutrition and Eye Drop
b. Negative Pressure Unidirectional cabinets / isolator 4 or 6 ft. with stainless steel stand for IV Admixture preparations
c. Trolley (stainless steel)
d. Intercom system
e. Stainless steel stool with wheels and adjustable height

2. Component Room

a. Rack/shelves for keeping of sterile bags, syringes, needles, filters, etc. (stainless steel)
b. Phenolic bench top with stainless steel drawers
c. Plug points
d. Trolley (stainless steel)
e. Sink (stainless steel)
f. Intercom system

3. Personnel Gowning Room

a. Garment cabinet or five-tier lockers for sterile gloves, head caps, mask
b. Wall mounted six foot long mirror
c. Cross over bench

4. Personnel Changing Room

a. Sink with elbow tap (stainless steel)
b. Wall mounted six foot long mirror
c. Cabinet (to hang street clothes)
d. Plug points
e. Liquid soap dispenser (foot-operated)
f. Electrical hand dryer
g. Cross over bench

5. Storage, Receiving and Distribution Room

a. Pharmaceutical refrigerator, twin doors connected to an essential power supply
b. Trolleys (stainless steel)

c. Computers and printers including "Uninterrupted Power Supply"
d. Display for temperature, relative humidity and pressure for the clean rooms
e. Table/chairs
f. Telephone
g. Intercom System
h. Filing cabinets
i. Pneumatic tube terminal (optional)

Clean Room

"A room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room and in which other relevant parameters, e.g. temperature, humidity, and pressure, are controlled as necessary"

Fig 1: clean room

According to Three Approved Guidelines

Minimizing the introduction, generation and retention of particles in a cleanroom are done in 3 ways

- Supplying the room with a large quantity of air filtered with high efficiency filters (HEPA or ULPA) to dilute and remove particles [6], bacteria and chemicals from within the room. The air is also used to pressurize the room and ensure that no contaminated air flows into the cleanroom
- The cleanroom itself must be built with materials that do not generate contaminants, particles, or outgas airborne chemical and must also be easy to clean.
- Cleanroom operators must wear garments that minimize dispersion of particles and micro-organisms generated by people such as hair, skin flakes, clothing fibers, etc. In fact, operator base contamination accounts for 70% to 80% of contamination [7].

Three main purposes are

1. The concentration of airborne particles is controlled.
2. Constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room
3. Other parameters (Temperature, Humidity, and Pressure) are controlled

Sources of Contamination

Possible sources of contamination are

1. Atmosphere
2. Operator
3. Raw materials
4. Equipment

1. Atmosphere

Atmosphere is invariably heavily contaminated with particles and microorganisms.

a) Contaminants in outside air →

Originate from soil and carry soil organisms including-
1. Bacteria spores (*Bacillus spp, Colostrum spp*)
2. Mould spores (Penicillium, Mucous, Aspergilius)
3. Yeasts
4. Micrococci

b. Contaminants in indoor air →

1. Originate from human body and clothing's-
2. Bacteria spores on human skin (Staphylococcus spp, Streptococcus spp)
3. These will also occur in droplets expelled out into the air from respiratory tract by talking, coughing, sneezing etc.

2. Operator (Most risky factor)

The skin, hair and clothing of the operator are potent sources of particulate and microbial contamination. Organisms found on the skin and transmitted on the skin particles are –

- Staphylococcus
- Diphtheroids
- Lipophillic yeasts
- Dermatophytic fungi

3. Raw materials

1. drugs which are obtained from natural sources

Example

- Plant source – saprophytic bacteria, yeast, molds;
- Animal source –pathogenic bacteria / spores.

2. Packaging materials and closures contaminate specially the parenteral solutions.
3. Pigments: Salmonella
4. Starches: Coliforms
5. Gums: Actinomyces
6. Water – prime source of particulate contamination

4. Equipment

During their preparation and processing

- they may generate dusts
- From atmosphere, particles and droplets may be regimented on to the internal and external surface of equipment.

Classification of clean room
According to ISO and Federal Standard

CLEANROOM CLASSIFACTIONS

Class	FED STD 209E Equivalent	Maximum concentration limits (particles/m³ of air) for particles equal to and larger than the sizes listed below					
		0.1 micron	0.2 micron	0.3 micron	0.5 micron	1 micron	5 micron
ISO 1		10	2				
ISO 2		100	24	10	4		
ISO 3	1	1,000	237	102	35	8	
ISO 4	10	10,000	2,370	1,020	352	83	
ISO 5	100	100,000	23,700	10,200	3,520	832	29
ISO 6	1,000	1,000,000	237,000	102,000	35,200	8,320	293
ISO 7	10,000				352,000	83,200	2,930
ISO 8	100,000				3,520,000	832,000	29,300
ISO 9					35,200,000	8,320,000	293,000

The class is directly related to the number of particles per cubic foot of air equal to or greater than 0.5 micron.

(1) Class 100,000

Particle count not to exceed a total of 100,000 particles per cubic foot of a size 0.5µ and larger or 700 particles per foot of size 5.0µ and larger.

(2) Class 10,000

Particle count not to exceed a total or 10,000 particles per cubic foot of a size 0.5µ and larger or 65-70 particles per cubic foot of a size 5.0µ and larger.

Fig 2: Class 10,000 Clean room

(3) Class 1,000

Particles count not to exceed a total of 1000 particles per cubic foot of a size 0.5µ and larger or 10 particles per cubic foot of a size 5.0µ and larger.

(4) Class 100

Particles count not to exceed a total of 100 particles per cubic foot of a size 0.5µ and larger.

Fig 3: Class 100 Clean room

According to EU GMP

Airborne classification in the EU GMP	Grade	Maximum permitted number of particles/m³ equal to or above			
		at rest		in operation	
		0.5 µm	5 µm	0.5 µm	5 µm
The local zone for high risk operations, e.g. filling zone, stopper bowls, open ampoules and vials, making aseptic connections.	A	3 500	1	3 500	1
For aseptic preparation and filling, this is the background environment for the grade A zone.	B	3 500	1	350 000	2 000
Clean areas for carrying out less critical stages in the manufacture of sterile products.	C	350 000	2 000	3 500 000	20 000
	D	3 500 000	20 000	not defined	not defined

For the manufacture of sterile medicinal products normally 4 grades can be distinguished.

GRADE "A": The local zone for high risk operations. eg. Filling zone, stopper bowls, open ampules and vials.

GRADE "B": In case of aseptic preparation and filling, the back ground environment for grade "A" zone.

GRADE "C" &"D": Clean areas for carrying out less critical stages in the manufacture of sterile produce.
The three functional zones may be separated by a physical barrier (e.g. a stopover bench or airlock)

Fig 4: Changing Room

- The following requirements should be defined:
  - number of people passing through the gowning procedure
  - the gowning procedure (i.e. what garments are to be taken off and put on)
  - The frequency of garment replacement.
- Consideration should be given to the following provisions:
  - Storage and disposal of garments;
  - Storage before use and disposal of consumable items
  - Storage of personal items;
  - Hand-washing and drying or other decontamination processes;
  - Display or posting of gowning sequence, with clear instructions;
  - Full-length mirrors to check effective fit.

Design: Air Flow Patterns

Air flow patterns

Clean room airflow patterns can be categorized as either unidirectional or non-unidirectional (or mixed).

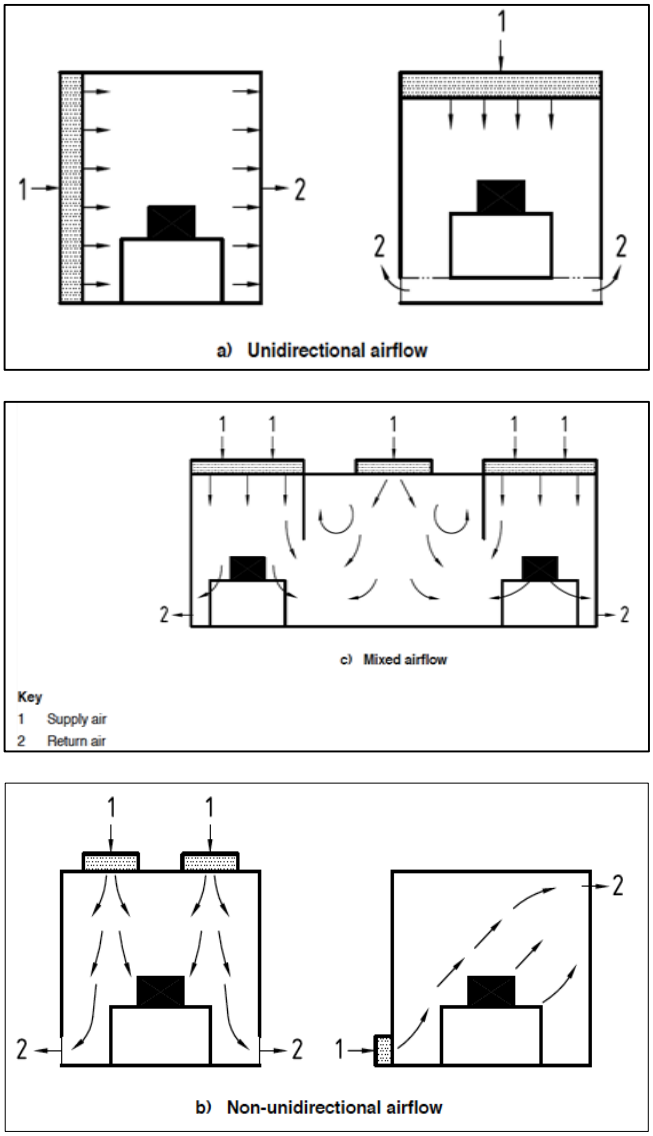


Fig 5: Air flow patterns

Personnel Practices and Procedures

a) Practices Related to Gowning

- All personnel entering the clean room must be familiar with the established gowning procedure
- Hands and fingernails must be scrubbed thoroughly with the disinfectant soap provided before entering the clean room.
- Hands should be dried with the hot air dryer. The use of paper or fabric towels in the clean area is forbidden.
- Skin lotions or lanolin base soaps should be provided for employees to tighten the skin and guard against epidermal scale.
- Eyeglasses must be washed and dried with lint-free tissue.
- Special procedures must be observed in utilizing air locks and air showers when present.
- Shoes must be covered with no shedding booties or changed to approved clean room footwear. Approved clean room garments must be worn.
- The hood must be tucked completely inside the uniform, and the uniform zippered securely to the neck.
- If any part of the clean room uniform becomes damaged, torn, or soiled during routine operations, the employee must return to the gowning area and replace the damaged part
- Normally, no dean room garment may be used a second time without being rewashed and desterilized.
- All hair is to be completely covered at all times.
- Personnel should avoid reaching under the hood or other parts of the garment with gloved hand.
- Coveralls are not to be unzipped in the clean room.
- No skin is to be exposed between the gloved hand and coveralls.
- If they become contaminated, gloves must be rinsed in the disinfectant solution provided.
- Cosmetics are not to be worn or applied in the clean room. This includes rouge, lipstick, facial powder, eye shadow and eyebrow pencil, mascara, eyeliner, false eyelashes, fingernail polish, hair spray, and the heavy use of an aerosol deodorant [8].
- No jewelry (i.e., large rings, necklaces, earrings, lockets, watches, bracelets) is to be worn in the clean room.
- Valuable items such as wallets may be carried into the clean room in the company supplied uniform pockets, provided that they are not removed inside the clean room.
- Personal items such as keys, coins, cigarettes, matches, pencils, handkerchiefs, - watches, tissues, and combs should not be carried into the clean

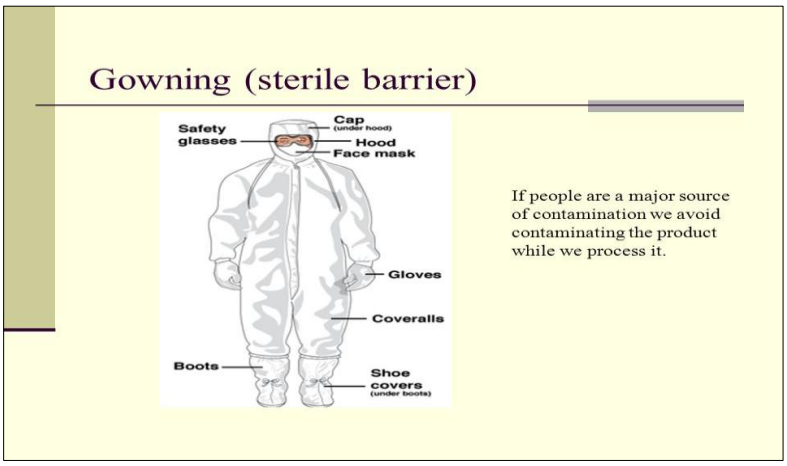


Fig 6: Personnel Gowning

Testing of clean and aseptic rooms

a) Commissioning Tests

British Standard 5295 lists the tests and procedures which should be used to commission a clean or aseptic room.

- **Final filter installation test:** This is done to demonstrate that the filter is not damaged and that the filter mounting frame does not leak at the gasket flange or the connection to the ducting.
- **Induction leak test:** This test demonstrates that particles cannot enter the room from leaks in construction joints or by back-streaming from openings.
- **Filter efficiency test:** Aerosol photometers and the generation and detection of DOP smoke or sodium chloride crystals are usually used for these tests.
- **Particulate contamination control test:** This is used to demonstrate that the number and size distribution of particles in the clean room air do not exceed the levels specified for the particular class of room. Microscopic techniques and direct reading light scattering photometers are used in this type of test.
- **Air pressure test:** This test determines the differential pressure between the clean area and adjacent areas. This is usually measured using a sensitive manometer.
- **Temperature and humidity tests:** Measurements are usually made with a sling psychrometer, repeat readings being taken after 30 seconds' whirling of the instrument until stable wet and dry bulb temperatures are obtained.
- **Air flow tests:** Air flow within ducts can be measured with a pilot tube and manometer. Air change rates can be demonstrated by tracer gas decay rate.
- **Noise level tests :** British Standard stipulates a maximum level of 65 dB
- **Lighting test:** The quality of the general illumination within the area and also at the work bench is measured using a portable photoelectric photometer. Recommended lighting levels at the work surface are <300 lux (28 ft. candles) for the British Standard and 100— 150 ft. candles (1076 – 1614 lux) for the US Standard.
- **Microbiological tests**
  - Settle plates
  - Agar contact plates and swabs
  - Process simulation tests
  - Sterility tests

b) Monitoring test

- Air pressure, temperature, and humidity measurements should be recorded continuously.
- Particulate contamination should be determined daily for aseptic rooms and weekly for clean rooms
- Tests for air flow velocity and uniformity should be carried out at 3monthly intervals.
- Tests for filter efficiency should be conducted yearly. Tests should also be repeated after repairs or maintenance
- Settle plates, agar contact plates and swabs, and sterility tests should be carried out during each work shift.
- It is recommended that process simulation tests should be conducted at 3 monthly intervals.

HVAC System

Heating Ventilation and Air Conditioning (HVAC) Systems

HVAC systems are an internal part of environmental control system design. The primary purpose of an HVAC system is to provide a specific set of environmental conditions required for the manufacturing process. To properly design HVAC system it is importantly to define the required operational parameters. The parameters discussed in the following sections are to be determined prior to designing an effective HVAC system [9].

Fig 7: HVAC System

An air conditioning system, or a standalone air conditioner, provides cooling, ventilation, and humidity control for all or part of an industry, house or building…

- Heating, ventilation and air conditioning (HVAC) constitutes up to 35 percent of energy used in manufacturing facilities. When the opportunity exists, energy conservation should be a factor in the original equipment selection and system design.
- The best HVAC design considers the interrelationship of building systems while addressing energy consumption, indoor air quality, and environmental benefit. HVAC systems can vary in design and complexity. Modifications can be added to the basic system to reach the desired HVAC operation.

Designing of HVAC in building should consider following

1. Air intake should be designed & situated to protect from sabotage.
2. Consider the need for filtration.
3. Units should be located in restricted access areas.

The ISO standards have been an outgrowth of these classes but have expanded the classifications to ISO 1 through 9 and widened the range of particulate sizes to micron through 5 microns. A rough comparison of the ISO and Federal Standard 209E is as follows:

ISO and Federal Standard 209E is as follows: ISO	Federal Standard 209E
1	---
2	---
3	1
4	10
5	100
6	1000
7	10000
8	100000
9	1000000

Air must also have low microbial levels. The above guidelines also recommend a maximum allowable level of colony-forming unit (CFU) per given volume of air. Particulate

filtration can eliminate the majority of microbial contamination. In areas with high background microbial levels (such as facilities surrounded by large amounts of farmland); however, other methods may also be employed such as carbon bed prefiltration.

Different components of HVAC and their functions

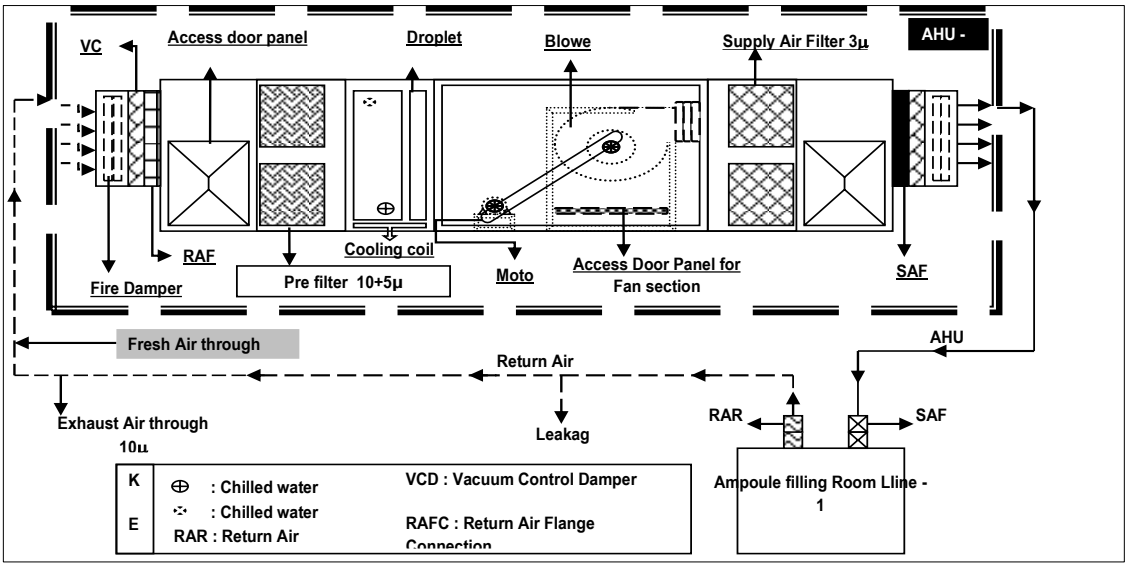


Fig 8: Components of HVAC

Types of filters used in HVAC

- Primary Panel filters, which are used mainly for lower filtration efficiency or as pre-filters
- Secondary filters, consisting of mini-pleated media or filter bags used for higher filtration efficiency.
- HEPA or tertiary filters, usually being the final filter in the system, providing the highest filtration efficiency.

HEPA Filter

A high efficiency particulate air or HEPA filter is a type of air filter that satisfies standards of United States Department of Energy (DOE).

Definition

A screen that filters out particles in the air by forcing them through microscopic pores. HEPA filters have different ratings for efficiency, which are generally posted on the filter itself. HEPA filter is so efficient that for every 10,000 particles that enter the filter within its filtering range, only 3 particles will get through.

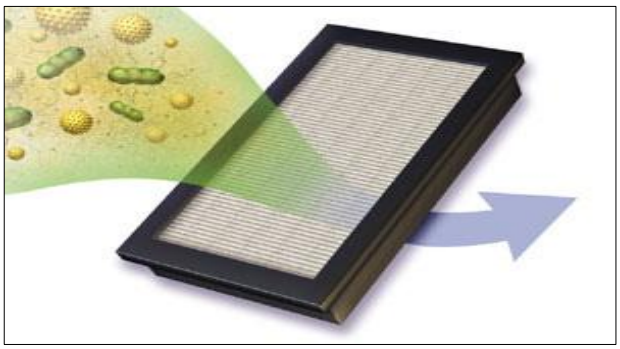


Fig 9: HEPA Filter

Optimum Parameters

Air filtration HEPA 99.97% efficiency and pressure relationship to adjacent areas positive. Optimal temperature 18 -250C and optimal humidity 15-20%.

Five classifications of HEPA filters exist

Type A HEPA filters: Also referred to as industrial filters. An efficiency performance of 99.97% retention of particulate matter 0.3 micrometers in size at an airflow of 85 L/minute.

Type B HEPA filters: Known as nuclear type is designed to handle nuclear containment. Filters are tested for pinhole leaks, as significant numbers of these leaks lead to an efficiency drop at slower air flows. The test checks for 99.97% retention of particulate matter 0.3 micrometers in size, but at 20% the normal airflow.

Type C HEPA filters: Called laminar flow filters due to their mostly exclusive use in biological laminar flow systems, filters are tested for particulate matter of larger sizes. Filter has an efficiency of 99.99%.

Type D HEPA filters: Knownas ultra-low penetration air.an efficiency rating of 99.999% retention of particulate matter 0.3micrometers in size at airflow of 85 L/minute.

Type E HEPA filters: Referred to as biological filters. These filters are created with a focus on stopping toxic, nuclear, chemical and biological threats.

Laminar Air Flow (LAF) System

High efficiency particle air filtration. “HEPA” filters + Lamination of Air flow. Laminar flow ensures a directional air flow for a distance of 140-200cm Combined by HEPA filters remove particles > 0.3 micron in an efficiency of 99.97% over the aseptic operating field in a uni-direction flow offering. Laminar airflow system should provide a homogenous air speed of 0.45 m/s ±2.0% at the working position.

Applications

Enhanced recovery of fastidious gram positive organism and filtration of enzyme solutions and diagnostic cytology.

Parameter of HVAC System

The parameters of HVAC include the following

- Temperature
- Relative Humidity
- Air Class
- Room to room Pressure Gradient
- Air Quality
- Sound level.
- Temperature: 20±5 ºC
- **Relative Humidity:** It is recommended to maintain RH within 50±5% in all manufacturing areas, unless there is any specific recommendation for any special operation. For example, for Effervescent product manufacturing it is recommended to maintain the relative humidity around 20%.
- **Air Class:** As per International standards; i.e. Federal standards, ISO standards; British standards etc.
- **Pressure Gradient:** It should be maintain relatively negative unless there is any special requirements. E.g. For sterile areas.
- **Air Quality:** It should be Dust and Odor free
- **Sound Level:** It should be maintained within 20 db

Role of HVAC in pharmaceutical industry

HVAC system plays an important role in product protection, personnel protection and environmental protection.

Product Protection

Contamination Control

Contaminants can originate from

- Environment (particles, micro-organisms, dust containing other products).
- Equipment (residues of other products, oil, particles, rust, gaskets, metal) and can be brought into the product by air movements. Contaminants are in fact the presence of anything in the manufactured product which should not be there.
- Contaminants can be products or substances other than the product manufactured (e.g. products resulting from air pollution), foreign products, particulate matter, micro-organisms, endotoxins, etc [10].

Cross-Contamination Protection

Cross-contamination can originate from

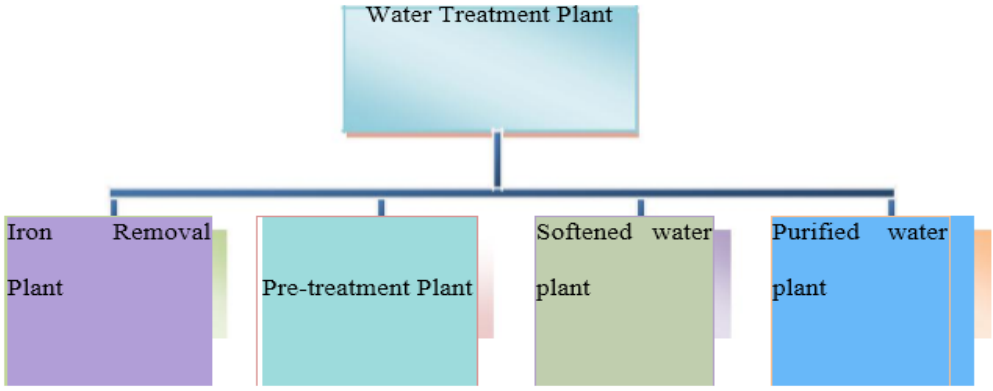
- poorly designed air handling systems and dust extraction systems,
- poorly operated and maintained air handling systems and dust extraction systems
- Inadequate procedures for personnel and equipment
- Insufficiently cleaned equipment Through all stages of processing, products should be protected from cross-contamination

Water Tretment System

Water is the most widely used raw material in the manufacture of Active Pharmaceutical Ingredients (API), intermediates, and finished dosage forms. As a raw material, high purity water is unique in that it is the only component that must be produced by the manufacturer, because it is not available from a vendor in a ready-to-use form. Water is utilized in the production of every type of pharmaceutical; in some products, such as parenteral, it is a critical component. It is, perhaps, the most important of all pharmaceutical utilities. In many pharmaceutical formulations, it is used as an excipient cleaning agent. Many API manufacturing and formulation facilities have United States Pharmacopoeia (USP) Purified Water (PW) systems while sterile manufacturing facilities have USP Water-for Injection (WFI) systems. The USP includes description and guidance for all types of water used in the processing of pharmaceuticals. Specific monographs in the USP include: PW, WFI, sterile water-for-injection, and bacteriostatic water-for-injection. Water used in the production of API, in many instances [11], may be potable water obtained from wells or other surface sources. These sources are considered acceptable provided water quality standards are established that are consistent with the compendia national primary drinking water standard of the U.S

Water Treatment Plant

In pharmaceutical Industry, raw water is treated in different stages to meet criteria specified for various applications. Process water should meet USP pacification for purified water. Besides soft water is used for boiler feed water [12] and generator cooling tower. Pre-treated water is used for drinking, sanitary, washing applications etc.



Iron Removal Plant

Bore whole water is passed through deep tube well to Iron removal plant. Iron is removed here with the help of sand filter. Alum is dosed to the raw water prior to entrance to the sand filter [13]. Required iron concentration is less than 0.1 ppm.

Sand filter

The sand in a pool sand filter (#20 silica sand; 45 - 55 mm) is specially graded to trap particles in the 20 - 100 micron range. As a sand filter collects dirt, its efficiency increases, trapping more dirt. ... Pool sand filters are known to be the lowest maintenance of the three types of pool filters.

How is sand used to purify water?
Safe drinking water can be reached with this type of sand filters. Thanks to mechanical and biological action in the sand layer, slow gravity sand filters remove bacteria as well as small particles from water, making it safe to drink. ... The sandfilter described below is designed for domestic use only.

What is the use of sand filter in water treatment?
Uses in Water Treatment. ... Slow sand filters produce high quality water without the use of chemical aids. Passing flocculated water through a rapid gravity sand filter strains out the floc and the particles trapped within it reducing numbers of bacteria and removing most of the solids.

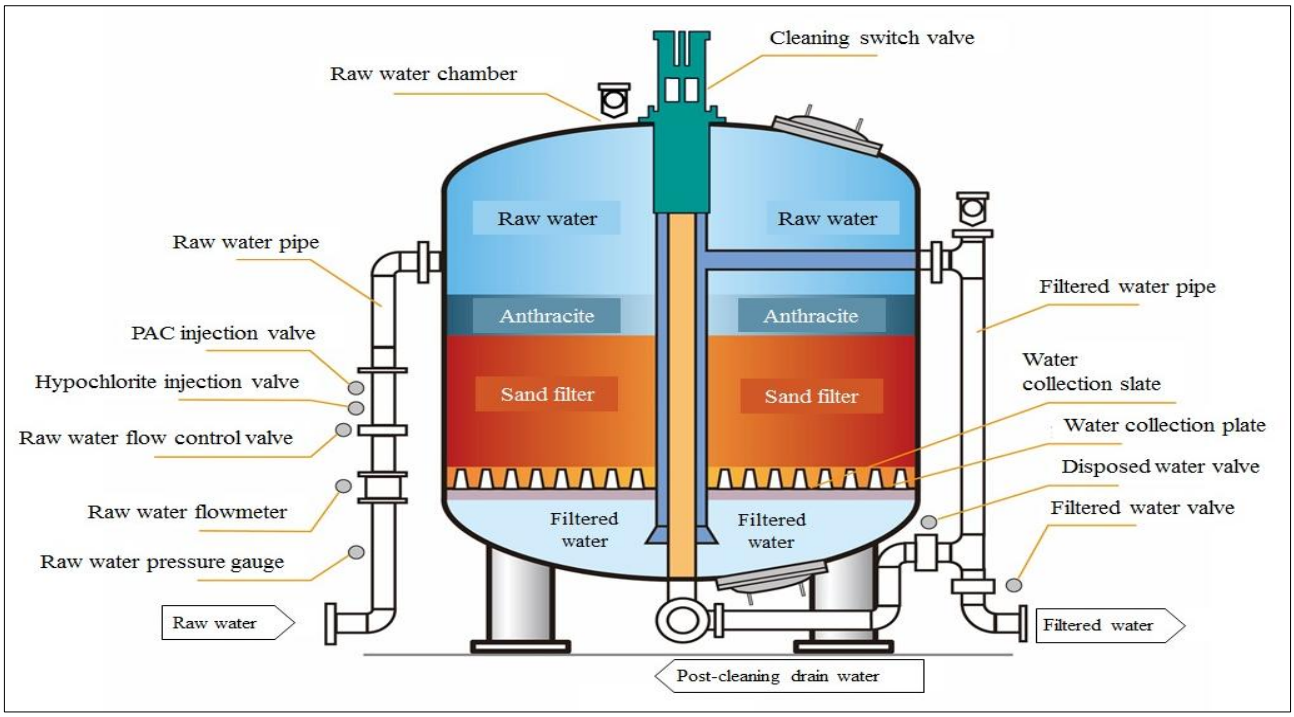


Fig 10: Sand Filter

What is Ion Exchange?
In the context of water purification, ion-exchange is a rapid and reversible process in which impurity ions present in the water are replaced by ions released by an ion-exchange resin. The ion exchange units are used to remove any charged substance from the water but are mainly used to remove hardness and nitrate from groundwater. Raw water is passed via two small polystyrene bead filled (ion exchange resins) beds. While the cations get exchanged with hydrogen ions in first bed [14], the anions are exchanged with hydroxyl ions, in the second one. The impurity ions are taken up by the resin, which must be periodically regenerated to restore it to the original ionic form

The following ions are widely found in raw waters

Cations	Anions
Calcium (Ca2+)	Chloride (Cl-)
Magnesium (Mg2+)	Bicarbonate (HCO3-)
Sodium (Na+)	Nitrate (NO3-)
Potassium (K+)	Carbonate (CO32

Pre-treatment Plant
This consists of tank, pumps, sand filters, activated carbon filters and dosing systems. Raw water pump takes water from the tank and force through the filtration media of the Omni filtration system. Flocculants such as alum is dosed to destabilize the colloidal particles and to give rise to insoluble compounds before entry to the filtration media. Omni filtration system consists of two filters installed in series and controlled by diaphragm valves. Water passes downwards through the filtering layers in the two units and flows out of system free of particulate material or undesirable elements. Sodium hypochlorite is dosed for oxidization as well as for minimizing microbiological contamination. Activated carbon filters remove color, odor and free chlorine.

Active carbon filter
Active carbon filters are most effective at removing chlorine, sediment, volatile organic compounds (VOCs), taste and odor from water. They are not effective at removing minerals, salts, and dissolved inorganic compounds.

How active carbon does filter work?
Decolorizing carbon, also called activated charcoal, is finely divided carbon often used to decolorize a solution. The small particles of decolorizing carbon provide a large surface area to which large colored molecules may become adsorbed.

What is the use of activated carbon in water treatment?
Activated carbon is commonly used to adsorb natural organic compounds, taste and odor compounds, and synthetic organic chemicals in drinking water treatment. Adsorption is both the physical and chemical process of accumulating a substance at the interface between liquid and solids phases.

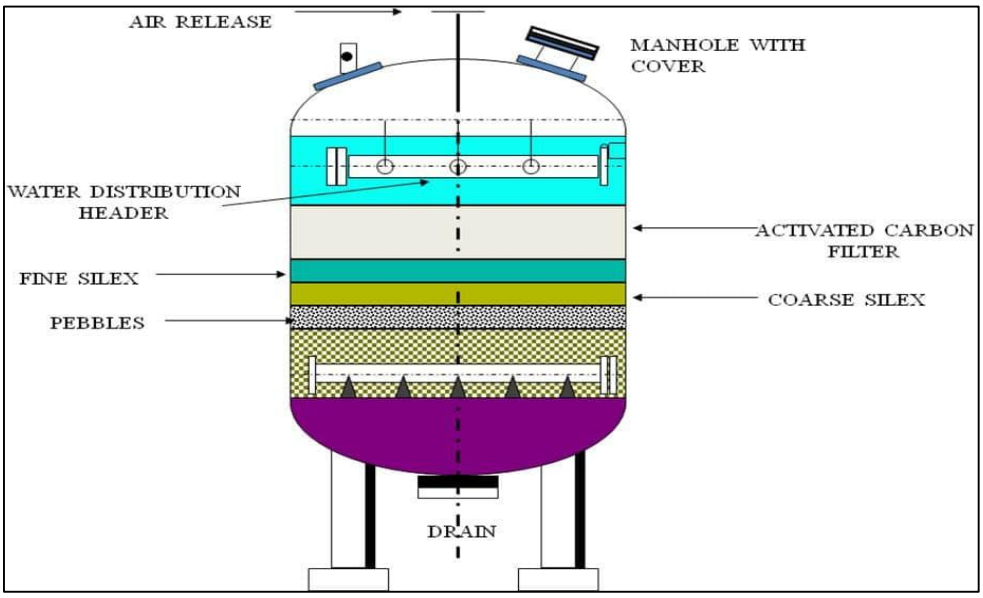


Fig 11: Activated carbon filter

Softened water plant

Water from the pretreatment plant is stored in a 8000 liter storage tank from where it is pumped through the treatment stages. Firstly water is pumped through a sand filter which removes suspended solids. Sodium Metabisulphate is dosed into the filtered water to neutralize any residual chlorine that could be harmful to the softener resin. Water is then passed through a duplex softener to remove most of the calcium and magnesium, ions. The softening system is operated in duty/standby mode. Regeneration of the water softener is initiated automatically after a preset volume of softened water has been produced. Then softened water is passed through a 10 micron cartridge filter to remove any resin particles. The softened water is split to a 12500 liter soft water tank and a 2000 liter softened water tank. The 2000 liter softened water tank provides a buffer for the soft water that is fed to the purified water plant. Water from this tank passes through a UV sterilizer to control microbiological contamination. An anti-scalent chemical compound is dosed into the softened water to remove any excess dissolved silica that could damage the reverse osmosis membranes. Caustic is dosed to increase the P^H of the softened water. This plant produces soft water with hardness less than 5 ppm.

Water Softeners can operate automatic, semi-automatic, or manual and is available in Simplex (single column) or Duplex systems (double column) for 24 hour continues soft water supply without any down time. In domestic applications a low level of hardness can be permitted. In industrial applications, particularly boilers, total hardness has to be almost totally removed.

Simplex Time Controlled Water Softener

it is applicable when water withdrawal is constant in time. The device starts regeneration cycle depending on the time elapsed. (Regeneration starts at 2 a.m.) The device is of intermittent operation, so it provides hard water during regeneration

Simplex Volume Controlled Water Softener

Regeneration starts depending on water consumption. Suggested in case of fluctuating water withdrawal. The device starts regeneration cycle depending on water quantity having passed through: it means the system is volume controlled. (Regeneration starts at 2 a.m.) The device is of intermittent operation, so it provides hard water during regeneration.

Duplex volume controlled water softener

The device is of continuous operation, so it is capable of providing soft water during regeneration, too. Suggested in case of fluctuating water withdrawal. The device starts regeneration cycle depending on water quantity having passed through: it means the system is volume controlled. (Regeneration starts automatically after the exhaustion of the resin column).

Information required to design a water softener system

- Total Hardness as CaCO3 present in the supply water
- Maximum flow rate required at point of use
- Quantity treated water expect to be use in 24-hours
- Operating hours of system per day
- Incoming water pressure
- Purpose of softened water

Fig 12: Duplex Volume Controlled Water Softener Cartridge Filtration

In many filtering applications, a choice between the use cartridge filter or bag filter has to be made. Both are sediment filters, that is to say they reduce the amount of sediment transported by the fluid trough filtration.

Cartridge filter can be surface or depth-type filters. Depth-type filters capture particles and contaminant through the total thickness of the medium, while in surface filters (that are usually made of thin materials like papers, woven wire, and cloth) particles are blocked on the surface of the filter.

Bag Filtration

Bag filters are in general frequently used for dust removal in industrial applications. The flow can be from the outside to the inside of the filter (that means the separation of particles happens on the external surface of the filter) or the other way around, depending on the application. The particles are normally captured on the internal surface of the bag filter. Bag filters are in general not designed for replacement when they are clogged and can be washed.

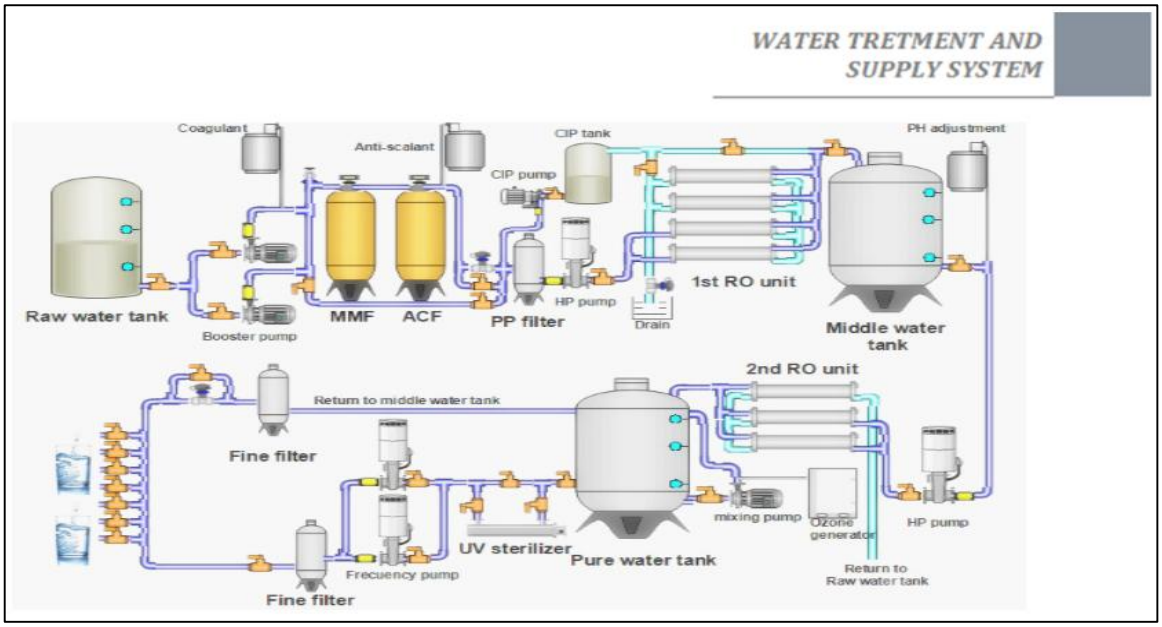


Fig 13: water treatment and supply process

Purified Water Plant

5 micron filter
↓
Duplex softener
↓
Activated carbon filter
↓
Buffer tank
↓
PUMP (Reverse osmosis)
↓
Pure water
↓
CDI
↓
Anode Cathode
↓
Feeding water system (<0.2 u filter)
↓
UV sterilizer
↓
Purified water tank (10,000 liter)
↓
1st& 2nd Heating and cooling exchanger Final UV sterilizer (254 nm Intensity)
↓
User points recycle to purified water tank
Flow 11.4 m3/hr

Reverse Osmosis

Reverse osmosis (RO) is a membrane-technology filtration method that removes many types of large molecules and ions from solutions by applying pressure to the solution when it is on one side of a selective membrane. The result is that the solute is retained on the pressurized side of the membrane and the pure solvent is allowed to pass to the other side.

In the normal osmosis process, the solvent naturally moves from an area of low solute concentration (High Water Potential), through a membrane, to an area of high solute concentration (Low Water Potential). The movement of a pure solvent to equalize solute concentrations on each side of a membrane generates osmotic pressure [13]. Applying an external pressure to reverse the natural flow of pure solvent, thus, is reverse osmosis. Reverse osmosis, however, involves a diffusive mechanism so that separation efficiency is dependent on solute concentration, pressure, and water flux rate. Reverse osmosis is most commonly known for its use in drinking water purification from seawater, removing the salt and other substances from the water molecules. Reverse osmosis is a process that industry uses to clean water, whether for industrial process applications or to convert brackish water, to clean up wastewater or to recover salts from industrial processes [14]. Reverse osmosis will not remove all contaminants from water as dissolved gases such as dissolved oxygen and carbon dioxide not being removed. But reverse osmosis can be very effective at removing other products such as trihalomethanes (THM's), some pesticides, solvents and other volatile organic compounds (VOC's) and this process removes over 70% of the following: Arsenic-3, Arsenic-4, Barium, Cadmium, Chromium-3, Chromium-6, Fluoride, Lead, Mercury, Nitrite, Selenium-4 and selenium-6,Silver.

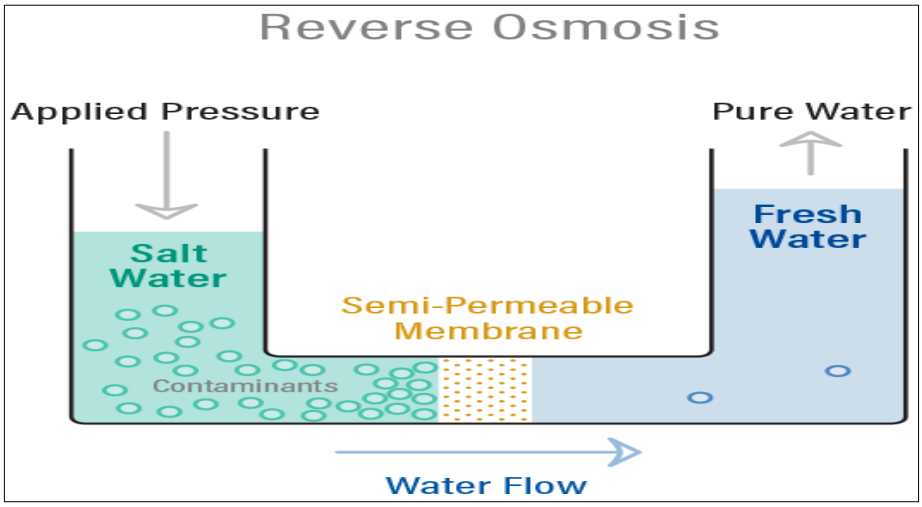


Fig 14: Reverse Osmosis

Osmosis Process

In the reverse osmosis process cellophane-like membranes separate purified water from contaminated water. RO is when a pressure is applied to the concentrated side of the membrane forcing purified water into the dilute side, the rejected impurities from the concentrated side being washed away in the reject water [15]. RO can also act as an ultra-filter removing particles such as some micro-organisms that may be too large to pass through the pores of the membrane.

Advantages of Reverse Osmosis

1. Nearly all contaminant ions and most dissolved non-ions are removed
2. Suitable for small systems with a high degree of seasonal fluctuation in water demand
3. Insensitive to flow and TDA levels [16].
4. Operates immediately without any minimum break-in period
5. Possible low effluent concentrations
6. Removes bacteria and particles
7. Simplicity and automation operation allows for less operator attention which makes them suitable for small system applications.

Limitations of Reverse Osmosis

1. High operating costs and capital
2. Potential problem with managing the wastewater brine solution
3. Pretreatment at high levels
4. Fouling of membranes

Electro dialysis

Electro dialysis is effective in removing fluoride and nitrate from water. This process also uses membranes but direct electrical currents are used to attract ions to one side of the treatment chamber [17]. This system includes a source of pressurized water, direct current power supply and a pair of selective membranes.

What is the Electro dialysis Process?

In this process, the membranes adjacent to the influent steam are charged either positively or negatively and this charge attracts counter-ions toward the membrane. These membranes are designed to allow the positive or the negative charged ions to pass through the membrane, where the ions move from the product water stream through a membrane to the two reject water streams.

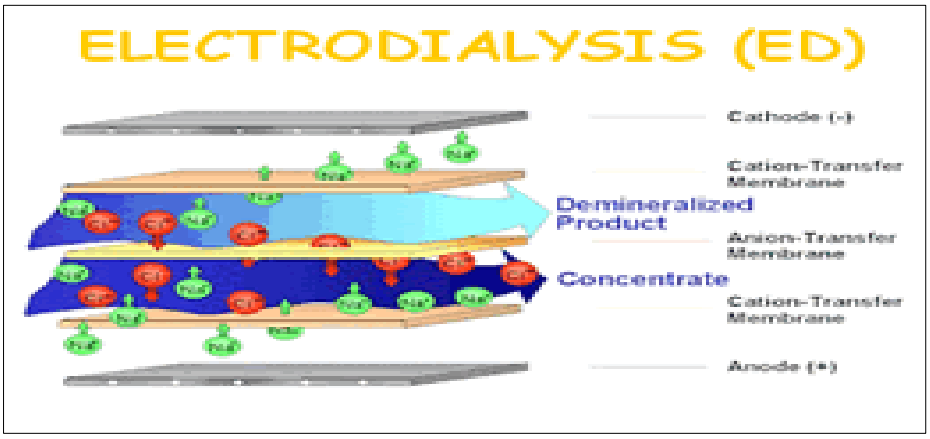


Fig 15: Electro Dialysis

Advantages of Electro dialysis

1. All the contaminant ions and many of the dissolved non-ions are removed
2. Insensitive to flow and TDS levels
3. Possible low effluent concentrations

Limitations of Electro dialysis

1. Operating costs and capital are high
2. Level of pretreatment required is high
3. Twenty to ninety percent of feed flow is rejected stream
4. Replacement of electrodes

Ultrafiltration

Ultrafiltration10 (UF) is a variety of membrane filtration in which hydrostatic pressure forces a liquid against a semipermeable membrane. Suspended solids and solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane [18]. This separation process is used in industry and research for purifying and concentrating macromolecular (103 - 106 Da) solutions, especially protein solutions. Ultrafiltration, like reverse osmosis, is a cross-flow separation process. Here liquid stream to be treated (feed) flows tangentially along the membrane surface, thereby producing two streams. The stream of liquid that comes through the membrane is called permeate [19]. The type and amount of species left in the permeate will depend on the characteristics of the membrane, the operating conditions, and the quality of feed. The other liquid stream is called concentrate and gets progressively concentrated in those species removed by the membrane.

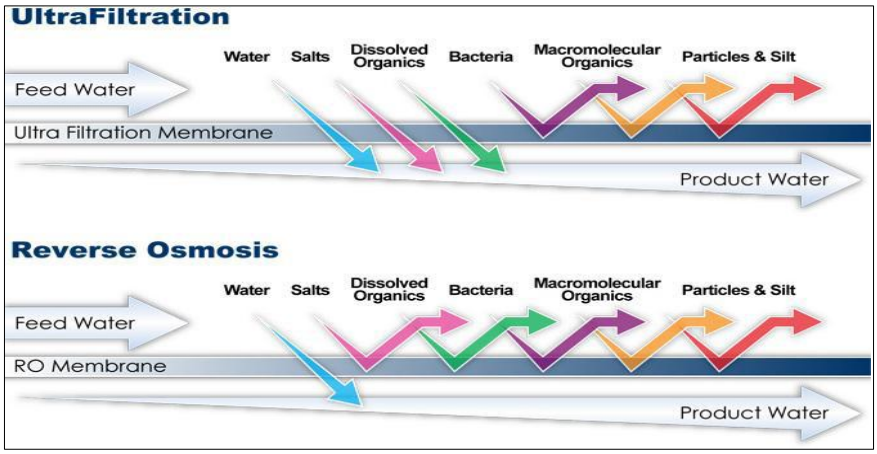


Fig 16: Ultra filtration process

Recovery

Recovery of an ultra-filtration system is defined as the percentage of the feed water that is converted into the permeate,

$$R = \frac{P}{F} \times 100$$

Where:
R =Recovery
P =Volume of permeate
F =Volume of Feed

Types of water supply in pharmaceutical preparation

Water Quality Specifications for Pharmaceutical Water

The USP and EP have adopted similar standards for the quality of Bulk Pharmaceutical Waters, as illustrated in Table. In addition to PW and WFI, the table also shows a grade called Highly Purified Water (HPW), as defined in EP and representing water meeting WFI specifications but produced by means other than distillation.

Table 1: Different standards of water

Parameter	Purified Water		Highly Purified Water		Water for Injection	
	USP	Ph Eur (bulk)	USP	Ph Eur (bulk)	USP	Ph Eur (bulk)
TOC (ppb C)	500	500	NA	500	500	500
Conductivity @ 20 °C	NA	≤ 4.3 µS/cm NA	NA	≤ 1.1 µS/cm	NA	≤ 1.1 µS/cm
Conductivity @ 25 °C	≤ 1.3 µS/cm	NA	NA	NA	≤ 1.3 µS/cm	NA
Nitrate (NO2)	NA	≤ 0.2 ppm	NA	≤ 0.2 ppm	NA	≤ 0.2 ppm
Heavy Metals (ppm as Pb)	NA	≤ 0.1 ppm	NA	NA	NA	NA
Aerobic Bacteria	≤ 100 CFU/ml	≤ 100 CFU/ml	NA	≤ 100 CFU/ml	≤ 100 CFU/ml	≤ 100 CFU/ml
Bacterial Endotoxins (EU/ml or IU/ml)	NA	NA	NA	≤ 0.25	≤ 0.25	≤ 0.25

N/A – Not an applicable requirement

Application to specific type of water to processes and dosage forms

Product licensing authorities specify the minimum grade of water for pharmaceutical use must be used during the manufacture of the different dosage forms or for different stages in washing, preparation, synthesis, manufacturing or formulation. The grade of water used should take into account the nature and intended use of the intermediate or finished product and the stage in the manufacturing process at which the water is used.

Quality of water for pharmaceutical use

Table 2: Quality of water for sterile medicinal products.

Sterile medicinal products	Minimum acceptable quality of water
parenteral	WFI
ophthalmic	Purified water
Hemofiltration solutions	WFI
Haemodiafiltration solution	WFI
Peritoneal dialysis solution	WFI
Irrigation solution	WFI
Nasal/ear preparations	Highly Purified water
Cutaneous preparations	Highly Purified water

Table 3: Quality of water for Non-sterile medicinal products

Quality of water for Non-sterile medicinal products. Non-sterile medicinal products	Minimum acceptable quality of water
Oral preparations	Purified
Nebuliser solutions	Purified*
Cutaneous preparations	Purified**
Nasal/ear preparations	Purified
Rectal/Vaginal preparation	Purified

*In certain disease states eg. Cystic fibrosis, medicinal products administered by nebulization are required to be sterile and non-pyrogenic. In such cases WFI or sterilized highly purified water should be used.
**For some products such as veterinary teat dips it may be acceptable to use potable water where justified and authorized taking account of the variability in chemical composition and microbiological quality.

Quality of Water used during the manufacture of active pharmaceutical ingredients (APIs).

Table 4: Quality of Water used during the manufacture of active pharmaceutical ingredients (APIs).

Type of manufacture	Product requirements	Minimum acceptable quality of water
Synthesis of all intermediates of APIs prior to final isolation and purification steps	No requirement for sterility or apyrogenicity in API or the pharmaceutical product in which it will be used.	Potable water*
Fermentation media	No requirement for sterility or apyrogenicity in API or the pharmaceutical product in which it will be used.	Potable water*
Extraction of herbals	No requirement for sterility or apyrogenicity in API or the pharmaceutical product in which it will be used.	Potable water**
Final isolation and purification	No requirement for sterility or apyrogenicity in API or the pharmaceutical product in which it will be used.	Potable water*
Final isolation and purification	API is not sterile, but is intended for use in a sterile, non-parenteral product	Purified water
Final isolation and purification	API is sterile and not intended for parenteral use	Purified water
Final isolation and purification	API is not sterile, but is intended for use in a sterile, parenteral product	Purified water with an endotoxin limit of 0.25EU/ml and control of specified organisms.
Final isolation and purification	API is sterile and a pyrogenic	Water for injection

*purified water should be used where there are technical requirements for greater chemical purity.
**the application would need to demonstrate that potential variations in the water quality, particularly with respect to mineral composition, would not influence the composition of the extract.

Quality of Water used during manufacture of medicinal products

Table 5: Quality of Water used during manufacture of medicinal products.

Manufacture	Minimum acceptable quality of water
Granulation	Purified*
Tablet coating	Purified
Used in formulation prior to non-sterile lyophilisation	Purified
Used in formulation prior to sterile lyophilisation	WFI

*For some veterinary premix products eg. Granulation concentrates it may be acceptable to use potable water where justified and authorized taking account of the variability in chemical composition and microbiological quality.

Quality of Water used for cleaning/rinsing.

Table 6: Quality of Water used for cleaning/rinsing.

Cleaning/rinsing of equipment, containers, closures	Product type	Minimum acceptable quality of water
Initial rinse	Intermediates and API	Potable water
Final rinse	API	Use same quality of water as used in the API manufacture
Initial rinse including clean in place (CIP) of equipment, containers and closures, if applicable	Pharmaceutical products-non sterile	Potable water
Final rinse including CIP of equipment, containers and closures, if applicable	Pharmaceutical products-non sterile	Purified water or use same quality of water as used in manufacture of medicinal product, if higher quality than purified water
Initial rinse* including CIP of equipment, containers and closures, if applicable	Sterile products	Purified water
Final rinse** including CIP of equipment, containers and closures, if applicable	Sterile non-parenteral products	Purified water or use same quality of water as used In manufacture of medicinal product, if higher quality than purified water
Final rinse** including CIP of equipment, containers and closures, if applicable	Sterile parenteral products	WFI***

*some containers, eg. Plastic containers for eye drops may not need an initial rinse; indeed this may be counter-productive since particulate counts
Could be increased as a result. In some cases e.g. blow-fill-seal processes rinsing cannot be applied; **If equipment is dried after rinsing with 70%
Alcohol, the alcohol should be diluted in water of the same quality as the water used for the final rinse; ***Where a subsequent depyrogenisation step

Is employed the use of highly purified water may be acceptable subject to suitable justification and validation data.

Microbiological test for water

The microbiological and chemical testing for Water used in pharmaceutical plant, Conductivity testing establishes a sample's ability to conduct electricity, which relates to the number of dissolved salts (ions) in the sample, high ion count lowers water purity and may indicate a processing problem. Total organic compounds (TOC) testing finds whether carbons in the sample are maintained below a mandated limit of 500 parts per billion (ppb), high is a reliable indicator of sample contamination. Bioburden testing establishes the number of microorganisms in a water sample; ensuring bacterial loads don't exceed mandated USP levels [20].

These criteria required testing for the following.

- The bacteria *Escherichia coli* (*E. coli*)
- The bacteria *Staphylococcus aureus* (*S. aureus*)
- The bacteria *Pseudomonas aeruginosa* (*Ps. aeruginosa*)
- The fungus *Aspergillus niger* (*A. niger*) Microbial test of water includes the estimation of the number of viable aerobic bacteria present in a given quality of water.

Procedure

- Transfer aseptically 1ml of the sample in each of two sterile petri dishes.
- Add to each dish approx. 20ml of sterile nutrient agar/ soyabean casein digest ager cover the petridishes and mix the sample with the agar by rotating the dishes 3 times both in clockwise and anti-clockwise directions.
- Allow the agar to solidify at room temperature.
- Invert the petridishes and incubate them at 37 °C for 48 hrs.
- After incubation, examine the plates for growth and count the number of colony forming units in each plate.
- The average of both the readings is the total microbial count per ml.

Qualification

PW, WFI systems are all considered to be direct impact, quality critical systems that should be qualified. The qualification should follow the validation convention of design review or design qualification

This guidance does not define the standard requirements for the conventional qualification stages DQ, IQ and OQ, but concentrates on the particular PQ approach that should be used for WPU systems to demonstrate their consistent and reliable performance. A three-phase approach should be used to satisfy the objective of proving the reliability and robustness of the system in service over an extended period [21]. Tests on the source water must be included within the validation programmed and continued as part of the routine monitoring. Test on the source water should meet the requirements for drinking-water and any internal specification.

Phase 1: Sample daily or continuously monitor the incoming feed-water to verify its quality. A test period of two weeks should be spent monitoring the system intensively. During this period, the system should operate continuously without failure or performance deviation. Usually water is not used for finished pharmaceutical product (FPP) manufacturing during this period.

The following activities should be included in the testing approach

- Undertake chemical and microbiological testing in accordance with a defined plan.
- Sample or continuously monitor the incoming feed-water daily to
- Verify its quality.
- Sample or continuously monitor after each step in the purification process.
- Sample or continuously monitor at each point of use and at other defined sample points.
- Develop appropriate operating ranges.
- Develop and finalize operating, cleaning, sanitizing and maintenance procedures.
- Demonstrate production and delivery of product water of the required quality and quantity.
- Use and refine the standard operating procedures (SOPs) for operation, maintenance, sanitization and troubleshooting.
- Verify provisional alert levels.
- Develop and refine test-failure procedure.

Phase 2: A further test period of two weeks should be spent carrying out further intensive monitoring while deploying all the refined SOPs after the satisfactory completion of phase 1. The sampling scheme should be generally the same as in phase 1. Use of the water for FPP manufacturing purposes during this phase may be acceptable, provided that both commissioning and phase 1 data demonstrate appropriate water quality and the practice is approved by QA. The approach should also:

– demonstrate consistent operation within established ranges;

– demonstrate consistent production and delivery of water of the required quantity and quality when the system is operated in accordance with the SOPs.

Phase 3: Phase 3 typically runs for one year after the satisfactory completion of phase 2. Water can be used for FFP manufacturing purposes during this phase which has the following objectives:

- to demonstrate reliable performance over an extended period;
- to ensure that seasonal variations are evaluated.
- the sample locations, sampling frequencies and tests should be reduced
- to the normal routine pattern based on established procedures proven during

Phases 1 and 2.

Continuous system monitoring

- After completion of phase 3 of the qualification programme for the WPU system, a system review should be undertaken. Following this review a routine monitoring plan should be established based on the results of phase 3.

Monitoring should include a combination of monitoring with online instruments (with appropriately qualified alarm systems) of parameters such as flow, pressure, temperature, conductivity and total organic carbon, and offline sample testing for physical, chemical and microbiological attributes. Offline samples should be taken from points of use or dedicated sample points where points of use cannot be

sampled. All water samples should be taken using the same methodology as detailed in production procedures. there should be a suitable flushing and drainage procedure in place.

- Tests should be carried out to ensure that the approved pharmacopoeia and company specification has been met. this may include the microbiological quality of water as appropriate.
  Monitoring data should be subject to trend analysis (trending should typically be within 2 sigma). Suitable alert and action levels should be established based on historical reported data.
- Any trend towards frequently exceeding alert limits should trigger a thorough

Investigation of the root cause, followed by appropriate corrective actions.

Maintenance of water systems

- PW systems should be maintained in accordance with a controlled, documented maintenance programme that takes into account the following:
- defined frequency for system elements;
- the calibration programme;
- SOPs for specific tasks;
- control of approved spares;
- issue of a clear maintenance plan and instructions;
- review and approval of systems for use upon completion of work;

Record and review of problems and faults during maintenance.

Sterilization Techniques

Sterilization refers to any process that eliminates, removes, kills, or deactivates all forms of life and other biological agents (such as fungi, bacteria, viruses, spore forms, unicellular eukaryotic organisms such as Plasmodium, etc.) present in a specified region, such as a surface, a volume of fluid, medication, or in a compound such as biological culture media [22]. Sterilization can be achieved through various means, including: heat, chemicals, irradiation, high pressure, and filtration. Sterilization is distinct from disinfection, sanitization, and pasteurization in that sterilization kills, deactivates, or eliminates all forms of life and other biological agents which are present.

Method of Sterilization

1. Physical Methods

- Incineration

Most common method of treating infectious waste toxic air emissions and the presence of heavy metals in ash has limited the use of incineration in most large cities

- **Moist heat (steam under pressure)**
- fastest and simplest physical method of sterilization used to sterilize bio hazardous trash and heat-stable objects; (e.g., autoclave)
- 121 degrees Celsius and 132 degrees Celsius most common
- media, liquids, instruments autoclaved 15 minutes at 121 deg Celsius
- infectious medical waste is often sterilized at 132 deg cel for 30-60 minutes for steam penetration through waste and displace trapped air inside autoclave bag

- **Dry heat**
- requires longer exposure 1.5-3 hrs and higher temps 160-180 deg cel
- sterilize glassware, oil, petrolatum, or powders

- **Filtration**
- method of choice for antibiotic solutions, toxic chemicals, radioisotopes, vaccines, and carbohydrates which are all heat sensitive
- using high-efficiency particulate air (HEPA) filters designed to remove organisms larger than 3.0 um from isolation rooms, op rooms, and biological safety cabinets (BSCs)

- **Ionizing (gamma) radiation**

Used for sterilizing disposables such as plastic syringes, catheters, or gloves before use.

2. Chemical Methods of Sterilization

Ethylene Oxide This highly reactive gas (C2H4O) is flammable, toxic, and a strong mucosal irritant. Ethylene oxide can be used for sterilization at low temperatures (20–60 8C). The gas has a high penetration capacity and can even get through some plastic foils. One drawback is that this gas cannot kill dried microorganisms and requires a relative humidity level of 40–90% in the sterilizing chamber. Ethylene oxide goes into solution in plastics, rubber, and similar materials, therefore sterilized [23] items must be allowed to stand for a longer period to ensure complete desorption.

Aldehydes: Formaldehyde (HCHO) is the most important aldehyde. It can be used in a special apparatus for gas sterilization. Its main use, however, is in disinfection. Formaldehyde is a water-soluble gas. Formalin is a 35% solution of this gas in water. Formaldehyde irritates mucosa; skin contact may result in inflammations or allergic eczemas. Formaldehyde is a broad-spectrum ger- micide for bacteria, fungi, and viruses. At higher concentrations, spores are killed as well. This substance is used to disinfect surfaces and objects in 0.5–5% solutions. In the past, it was commonly used in gaseous form to disinfect the air inside rooms (5 g/m3). The mechanism of action of formal-dehyde is based on protein denaturation. Another aldehyde used for disinfection purposes is glutaraldehyde.

Alcohols: The types of alcohol used in disinfection are ethanol (80%), propanol (60%), and isopropanol (70%). Alcohols are quite effective against bacteria and fungi, less so against viruses. They do not kill bacterial spores. Due to their rapid action and good skin penetration, the main areas of application of alcohols are surgical and hygienic disinfection of the skin and hands. One dis-advantage is that their effect is not long-lasting (no depot effect). Alcohols denature proteins.

Phenols: Lister was the first to use phenol (carbolic acid) in medical applications. Today, phenol derivatives substituted with organic groups and/or halo-gens (alkylated, arylated, and halogenated phenols), are widely used. One common feature of phenolic substances is their weak performance against spores and viruses. Phenols denature proteins. They bind to organic materials to a moderate degree only, making them suitable for disinfection of excreted materials.

Halogens: Chlorine, iodine, and derivatives of these halogens are suitable for use as disinfectants. Chlorine and iodine show a generalized microbicidal effect and also kill spores. Chlorine denatures proteins by binding to free amino groups; hypochlorous acid (HOCl), on the other hand, is produced in aqueous solutions, then disintegrates into HCl and 1/2 O2 and thus acts as a powerful oxidant. Chlorine is used to disinfect drinking water and swimming-pool water (up to 0.5mg/l). Calcium hypochlorite (chlorinated lime) can be used in nonspecific disinfection of excretions. Chloramines are organic chlorine compounds that split off chlorine in aqueous solutions. They are used in cleaning and washing pro-ducts and to disinfect excretions.Iodine has qualities similar to those of chlorine. The most important iodine preparations are the solutions of iodine and potassium iodide in alcohol (tincture of iodine) used to disinfect skin and small wounds. Iodophores are com plexus of iodine and surfactants (e.g., polyvinyl pyrrolidone). While iodophores are less irritant to the skin than pure iodine, they are also less effective as germicides.

Oxidants: This group includes ozone, hydrogen peroxide, potassium permanganate, and per acetic acid. Their relevant chemical activity is based on the splitting off of oxygen. Most are used as mild antiseptics to disinfect mucosa, skin, or wounds.

Surfactants: These substances (also known as surface-active agents, ten sides, or detergents) include anionic, cationic, amphoteric, and nonionic detergent compounds, of which the cationic and amphoteric types are the most effective (Fig. 17). The bactericidal effect of these substances is only moderate. They have no effect at all on tuberculosis bacteria (with the exception of amphotensides), spores, or no encapsulated viruses. Their efficacy is good against Gram-positive bacteria, but less so against Gram-negative rods. Their advantages include low toxicity levels, lack of odor, good skin tolerance [24], and a cleaning effect.

Types of sterilization and their uses

In the medical and pharmaceutical industries, sterilization is needed on an everyday basis in order to promote health and eliminate the risk of contamination. There are a number of different types of sterilization, and all require that the temperature, gases, humidity, and pressure levels used are accurately monitored to ensure validity and effectiveness. These types of sterilization include:

Steam: Steam sterilization, which was invented in 1880, is primarily used for glassware, surgical instruments, and medical waste.

EtO/EO: This method uses Ethylene Oxide gas to sterilize items that cannot withstand the high temperatures or humidity created using other methods. This is commonly used for electric components, plastics, and cardboard.

Depyrogenation/Dry Heat: Dry heat sterilization is used on products that may be degraded when exposed to steam or moisture, but which can withstand high temperatures. Metal instruments, needles, and petroleum products are often sterilized this way.

The Sterilization Process (Autoclaves)

Through history, humans have used fire to purify items. Heat generated through application of high temperatures acts by disrupting membranes and denaturing proteins and nucleic acids. Burning, however, is a bit excessive for everyday usage.

Fig 17: Autoclaves

Transmissible agents (such as spores, bacteria and viruses) can be eliminated through sterilizations. This is different from disinfection, where only organisms that can cause disease are removed.

Some of the methods used to achieve sterilization are:

- Autoclaves: Highly effective and inexpensive. Unsuitable for heat sensitive objects.
- Hot air ovens: Inefficient compared to autoclaves.
- Ethylene oxide: Suitable for heat sensitive items but leaves toxic residue on sterilized items.
- Low-temperature steam and formaldehyde: Effective for instruments with cavities or tubular openings.
- Sporicidal chemicals: Often used as disinfectants but can also sterilize instruments if used for prolonged periods.
- Irradiation: Gamma rays and accelerated electrons are excellent at sterilization.
- Gas plasma.

The preferred principle for sterilization is through heat, the autoclave being the most widely used method of achieving it.

In a dry air oven, it takes two hours at 160 °C to kill spores of the bacterium Clostridium botulinium (associated with canned food). Using saturated steam, the same spores are killed in just five minutes at 121 °C, proving that moist heat is more effective than dry heat.

Moist heat sterilization

Moist heat sterilization using autoclave is commonly used for the sterilization of bio hazardous trash, heat and moisture resistant materials such as aqueous preparation (culture media). This method is also used for the sterilization of surgical dressings and medical devices.

The most common type of steam sterilizer in the microbiology laboratory is the gravity displacement type. Other type of autoclave is Vacuum/Gravity Assi

Mode of Action/Principle of Moist Heat sterilization

Moist heat destroys microorganisms by the irreversible denaturation of enzymes and structural proteins. The temperature at which denaturation occurs varies inversely with the amount of water present. Sterilization in saturated

steam thus requires precise control of time, temperature, and pressure.

Pressure serves as a means to obtain the high temperatures necessary to quickly kill microorganisms. Specific temperatures must be obtained to ensure the microbicidal activity. Minimum sterilization time should be measured from the moment when all the materials to be sterilized have reached the required temperature throughout.

The recommendations for sterilization in an autoclave are 15 minutes at 121 °C (200 kPa). The temperature should be used to control and monitor the process; the pressure is mainly used to obtain the required steam temperature.

Alternative conditions, with different combinations of time and temperature, are given below.

1 atm = 325 Pa

Table 7

Temperature (°C)	Approximate corresponding pressure (kPa)	Minimum sterilization time (min)
126-129	250 (~2.5 atm)	10
134-138	300 (~3.0 atm)	5

In certain cases (e.g. thermo labile substances), sterilization may be carried out at temperatures below 121 °C, provided that the chosen combination of time and temperature has been validated.

Biological Indicators

The effectiveness of steam sterilization is monitored with a biological indicator using an envelope containing spores of Geo*bacillus stearothermophilus* (formerly *Bacillus stearothermophilus*; e.g. ATCC 7953 or CIP 52.81) for which the D-value (i.e. 90% reduction of the microbial population) is 1.5-2.5 minutes at 121 °C, using about 10^6 spores per indicator *(this is based on a worst case scenario that an item may contain a population of 10^6 spores having same resistance as that of Bacillus stearothermophilus*). After sterilization is over the strip is removed and inoculated into tryptone soya broth and incubated at 56 °C for 5 days. No growth of *Geobacillus stearothermophilus* indicates proper sterilization.

Table 8: List of commonly used bilogical indicators (BIs)

Spores of Bacteria	D Value
Geobacillus stearothermophilus (most common)	1.5-2.5
Bacillus coagulans	0.3
Clostridium sporogenes	0.8-1.4
Bacillus atropheus	0.5

Positive spore test results are a relatively rare event and can be attributed to operator error, inadequate steam delivery, or equipment malfunction.

Advantages of Steam Sterilization Method

1. Nontoxic to patient, staff, environment
2. Cycle easy to control and monitor
3. Rapidly microbicidal
4. Least affected by organic/inorganic soils among sterilization processes listed
5. Rapid cycle time
6. Penetrates medical packing, device lumens

Disadvantages of Steam Sterilization Method

1. Deleterious for heat-sensitive instruments
2. Microsurgical instruments damaged by repeated exposure
3. May leave instruments wet, causing them to rust
4. Potential for burns.
5. Dry-Heat Sterilization:

Sterilizing by dry heat is accomplished by conduction. The heat is absorbed by the outside surface of the item, and then passes towards the center of the item, layer by layer. The entire item will eventually reach the temperature required for sterilization to take place.

Dry heat does most of the damage by oxidizing molecules. The essential cell constituents are destroyed and the organism dies. The temperature is maintained for almost an hour to kill the most difficult of the resistant spores.

The most common time-temperature relationships for sterilization with hot air sterilizers are

1. 170 °C (340°F) for 30 minutes,
2. 160 °C (320°F) for 60 minutes, and
3. 150 °C (300°F) for 150 minutes or longer depending up the volume.

Bacillus atrophaeus spores should be used to monitor the sterilization process for dry heat because they are more resistant to dry heat than the spores of *Geobacillus stearothermophilus*. The primary lethal process is considered to be oxidation of cell constituents.

There is two types of dry-heat sterilizers:

1. The static-air type and
2. The forced-air type.

Static-air type is referred to as the oven-type sterilizer as heating coils in the bottom of the unit cause the hot air to rise inside the chamber via gravity convection. This type of dry-heat sterilizer is much slower in heating, requires longer time to reach sterilizing temperature, and is less uniform in temperature control throughout the chamber than is the forced-air type.

Orced-air or mechanical convection sterilizer is equipped with a motor-driven blower that circulates heated air throughout the chamber at a high velocity, permitting a more rapid transfer of energy from the air to the instruments.

Advantages of dry heat sterilization

1. A dry heat cabinet is easy to install and has relatively low operating costs;
2. It penetrates materials
3. It is nontoxic and does not harm the environment;
4. And it is **noncorrosive** for metal and sharp instruments.

Disadvantages for dry heat sterilization

1. Time consuming method because of **slow rate of heat penetration** and microbial killing.
2. High temperatures are not suitable for most materials.

Radiation Filtration

There are 2 general types of radiation used for sterilization,

ionizing radiation and non-ionizing radiation. Ionizing radiation is the use of short wavelength, high-intensity radiation to destroy microorganisms. This radiation can come in the form of gamma or X-rays that react with DNA resulting in a damaged cell. Non-ionizing radiation uses longer wavelength and lower energy. As a result, non-ionizing radiation loses the ability to penetrate substances, and can only be used for sterilizing surfaces. The most common form of non-ionizing radiation is ultraviolet light, which is used in a variety of manners throughout industry.

One industrial application of non-ionizing radiation is the breakdown of ozone (O_3). By adding ozone to water, bacteria are unable to sustain life. Unfortunately, ozone also destroys process media. Therefore ozone must be broken down so water can be used for its designated purpose. Since ozone is very sensitive to ultraviolet light, pass the water stream under UV bulbs. This breaks the oxygen-oxygen bonds and results in safe process water. Here is a simple representation of the system.

Advantages

No degradation of media during sterilization, thus it can be used for thermally labile media

Leaves no chemical residue

Administration of precise dosage and uniform dosage distribution

Immediate availability of the media after sterilization

Disadvantages

This method is a more costly alternative to heat sterilization requires highly specialized equipment.

Filtration Sterilization

Filtration allows for the exclusion of organisms based upon size. There are many types of filtration techniques, but when sterilizing a system membrane filtration is used. Membrane filtration traps contaminants larger than the pore size on the surface of the membrane. If contaminants are smaller than the desired particle, decrease the membrane pore size and trap the product [25] while passing the contaminants through the membrane. For greater system flexibility, filters can be added in parallel or series. When adding a filter of same pore size, in parallel, throughput increases. If instead a filter of differing pore size was added in series, separation of multiple microorganisms is possible.

Advantages

1. Absolute sterilization - separates particles based on size
2. Used for heat sensitive media
3. Removal of multiple particle sizes
4. Allows for fairly high throughput

Disadvantages

1. Each filter has a specific nominal pore size
2. Unable to separate microorganisms that have the same size
3. May require a high differential pressure.

Pharmaceutical Sterilization: its importance and future

With the increased amount of biological products coming to the market, new practices to provide greater assurance on pharmaceutical sterilization processes are necessary [26]. At the same time, however, changes in the types of materials and products are forcing changes in typical sterilization methods [28], and the effect of the sterilization process on the materials must be taken into consideration as well [27]. This presentation will walk you through the various types of terminal sterilization and aseptic processing methods used for pharmaceutical products, along with opinions on new ways of thinking in order to provide greater assurance than some of the industry's current practices allow.

Conclusion

Due to the rapidly expanding sterile pharmaceutical preparation services, it is very timely and essential that the Pharmaceutical Services Division. This will ensure uniformity and conformity in all the sterile preparation facilities developed henceforth. The primary objective of this review is to assist those involved in planning, developing and upgrading of CDR and non-CDR preparation facilities. It also aims to benefit pharmacists and other personnel who are engaged in managing these facilities. The recommendations made in this review take into consideration the Pharmaceutical Services Division policies, working environment and the fulfillments of customers' needs in accordance with the current international standards such as Pharmaceutical Inspection Co-operation Scheme (PIC/S) Guides for Good Preparation Practice (GPP).

We believe that the contents of this review will be able to serve as a standard reference for all hospital pharmacists, healthcare planners and developers with regards to the design, space, layout requirements and equipment's for the development of a sterile preparation facility. As for the existing facilities, we strongly suggest to all pharmacists to look into the possibility of upgrading their respective facilities based on the recommendations made in these review.

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Conflict of Interest

The authors declare that they have no conflict of interest

References

1. Leon Lachman, Herbert A. Liebermann., Theory and Practice of Industrial Pharmacy, Third Edition; Varghese Publishing House; 1987, Bombay,619-680.
2. U.S. Food & Drug Administration (FDA).
3. www.cgmp.com [Last access 12 June 2017]
4. Stephen J. Davis., Clean room Pressurization Methodology Prime Consideration for Pharmaceutical Facilities., Pharmaceutical Technology, 2003, 54-58.
5. FDA, Guidance for Industry: Stehle Drug Products Produced by Aseptic Processing-Current Good Manufacturing Practice, 2004.
6. Pharma info.net. [Last access 12 June 2017]7. USP, Chapter 1116 "Microbiological Control and Monitoring Environments Used for the Manufacture of Healthcare Products," Pharm Forum, 33(3); 2007.
7. Ichiya Hayakawa, Shuji Fujii, Kwang Young Kim., Design Theory for a Laminar Flow-Type Clean Room and Image Processing on Remotely Detected Particulates. Aerosol Science and Technology, 1987, 47-56.
8. Kenneth E. Avis, Herbert A. Liaberman and Leon Lachman, Pharmaceutical Dosage form: Parenteral Medication; Second Edition., Replika Press Pvt Ltd. Volume 2; 2005, New York, 235-316.
9. United States Pharmacopeia 35, General Chapter

<1231>, UnitedStates Pharmacopoeias Convention, Inc. 12601 TwinbrookParkway, Rockville, MD (2012).
10. European Pharmacopoeia Edition 7 (EDQM.226. avenue de ColmarBP 907, F-67029 Strasbourg, France, 2011).
11. The International Pharmacopoeia. Geneva, World HealthOrganization;http://www.who.int/medicines/publications/pharmacopoeia/overview/en/index.html. [Last access 12 June 2017]
12. WHO Guidelines for drinking-water quality, 3rd edition. Geneva,World Health Organization, 2008. http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/index.html.
13. WHO guidelines on good manufacturing practices: validation. In:WHO Expert Committee on Specifications for PharmaceuticalPreparations. Fortieth report. Geneva, World Health Organization,2006, Annex 4 (WHO Technical Report Series, No. 937);http://www.who.int/medicines/publications/pharmprep/TRS_937.pdf#page=119. [Last access 12 June 2017]
14. Guide to inspections of high purity water systems. Maryland, USFood and Drug Administration, 1993.http://www.fda.gov/ICECI/InspectionGuides.
15. The International Pharmacopoeia. Geneva, World Health Organization; http://www.who.int/medicines/publications/pharmacopoeia/overview/en/index.html
16. WHO guidelines on good manufacturing practices: validation. In: WHO ExpertCommittee on Specifications for Pharmaceutical Preparations. Fortieth report. Geneva, World Health Organization, 2006, Annex 4 (WHO Technical Report Series, No. 937); http://www.who.int/medicines/publications/pharmprep/TRS_937.pdf#page=119.
17. WHO Guidelines for drinking-water quality, 3rd edition. Geneva, World HealthOrganization, 2008; http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/index.html.American Society of Mechanical Engineers. Bioprocessing Equipment Standard.ASME — BPE 2000. [Last access 12 June 2017]
18. GOOD DESIGN PRACTICES FOR GMP PHARMACEUTICAL FACILITIES BY:-ANDREW. A.SIGNORE & TERRY JACOBS VOL-146, PAGE NOS:- 50,90-104,345
19. GOOD MANUFACTURING PRACTICES FOR PHARMACEUTICALS BY:-JAMES SWARBRICK VOL-2, PAGE NOS:- 20,21,22,23,36,37
20. www.who.org[Last access 12 June 2017]
21. www.wikipedia.com[Last access 12 June 2017]
22. www.p2pays.org [Last access 12 June 2017]
23. www.foreigntradeexchange.com [Last access 12 June 2017]
24. www.industrialleaders.org [Last access 12 June 2017]
25. www.scrzibd.com[Last access 12 June 2017]
26. https://en.wikipedia.org/wiki/Sterilization_(microbiology). [Last access 12 June 2017]27. (http://www.eurotherm.com/sterilization) [Last access 12 June 2017]
27. (http://www.mddionline.com/sterilization-equipment) [Last access 12 June 2017]
28. https://microbeonline.com/moist-heat-sterilization-definition-principle-advantages-disadvantages