Chapter 11
Sterile Products

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Abstract Sterile products include parenterals, surgical dressings, sutures, ligatures, and ophthalmic preparations. Parenteral products are meant for delivering the drug beside the intestine, and hence bioavailability of drugs may be enhanced. This chapter covers various types of parenterals, their preparation methods, and their advantages over other route of administration. Moreover, ophthalmic preparations have been also discussed in detail along with their different types and their methods of preparation. Moreover, various excipients used in formulation of parenterals and ophthalmic products have been also discussed, and their impact on products stability and efficacy have been also elaborated. Moreover, the chapter also enfolds a detailed description on key environmental parameters needed to be controlled for sterile pharmaceutical products.

Keywords Parenterals · Ophthalmic products · Ocular inserts · Environmental monitoring · Clean room

11.1 Introduction

Many pharmaceutical products such as injections, dressings, sutures etc., that come in contact with broken skin, bloodstream, or internal organs must be sterile in order to avoid the possibility of microbial infection. Pathogenic microorganisms are obviously primary threat to sterility; however, nonpathogenic microorganism if

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accidentally gains access to body cavities in a large number may pose danger to patients. Therefore, injections, dressings, sutures, ligatures, ophthalmic preparations, irrigation fluids, implants, etc. must be sterile [1].

11.2 Parenteral Dosage Forms

Dosage form purpose is to deliver drug to site of action, i.e., whether it is through oral route in the form of tablets, capsules, syrups, etc. or through parenteral route. The word parenteral is from two Greek words, *para* means beside and *enteron* means intestine. So, parenteral delivers the drug beside the intestine. In parenteral dosage form, we breech the barrier (i.e., the skin which is one of the tough barriers of human body) with the help of a needle [2]. Different routes of parenteral administration are summarized in Table 11.1.

Advantages Parenteral preparations are adventitious as they provide the rapid onset of action because drug is directly entering into the systemic circulation, and

Table 11.1 Common routes of parenteral administration [2]

Routes of administration	Important points
Intravenous route	Injection into the vein
	Quickest onset and avoids irritation of tissues
	Usually, for small volume parenterals (SVP) as well as large volume parenterals (LVP)
	Pharmacological response can hardly be reversed
	Requires skilled personnel
Intramuscular route	Injection to the gluteal muscle, thighs, deltoid (upper arm)
	Usually, for SVP up to 2 mL or some time up to 4 mL
	Can be used for sustained release (injectable depot)
Subcutaneous route	Injection into the subcutaneous layer (fat beneath the skin)
	Usually, for SVP up to 2 mL (e.g., vaccines, insulin, etc.)
	In case of emergencies when veins are not traceable LVP, 250–1000 mL can be administered (hypodermoclysis)
Intradermal route	Injection into dermal layer of the skin, for diagnostic purpose, e.g., test for allergies
Intra-arterial route	Injection into an accessible artery, for example, in drug addicts whose veins are difficult to be found
Intrathecal route	Injection into the cerebrospinal fluid in order to bypass blood-brain barrier
Intradural and extradural	Injection into the spinal cord, within the dural membrane (intradural) or outside the dural membrane (extradural), e.g., for spinal anesthesia
Intracardiac injection	Injection directly into the muscles of the heart, e.g., administration of epinephrine in emergency case of cardiac arrest when vessels are not easily accessible

secondly parenteral provides predictable effect. The bioavailability is almost complete or 100% because parenteral bypasses the first-pass effect. There is no or less absorption problems for parenteral because this route avoids GI track and moreover provides a reliable drug administration to very ill or unconscious patient [2].

Disadvantages This route involves irreversible drug administration and bears more risks as compared to others. This invasive route can cause fear, pain, tissue damage, or infection. Quite riskier mode of administration and preparation must be sterile. Without the presence of expert, parenteral route of drug administration becomes difficult [2].

11.2.1 Brief History of Parenterals

In the mid-nineteenth century, parenteral dosage forms were introduced. The first official injection was morphine, which came in British Pharmacopeia in 1867, and then cocaine injection in 1898. In national formulary the first injection was introduced in 1926, while the first monograph for injection appeared in USP in 1942. Now the current USP contains more than 500 injection monographs. Injectable dosage form is increasing rapidly. Most of the new products that come in market nowadays are in the form of injectables. It is because most of the new drugs have bioavailability problems, i.e., if we deliver the drug through oral route by means of any dosage form like tablet, capsule, syrups, etc., it has a very limited absorption from GIT. Therefore for enhancement of bioavailability, injectables are preferred [3].

11.2.2 Types of Parenterals

11.2.2.1 Parenteral Solutions

Most of the products are in solution form. Drug solutions suitable for parenteral administration are known as injections/injectables. Injectable solutions are prepared by dissolving drug (active ingredients) and excipients, maintaining a specific pH. After this solution is filtered through 0.22 μm membrane filter and finally autoclaved (if thermostable) [3].

Most of the solutions have similar viscosity and surface tension to that of water, while some solutions like streptomycin injections and ascorbic acid injections are quite viscous. Large and small volume parenterals containing no antimicrobial agents should be terminally sterilized. Volume of parenteral should be greater than the mentioned label because some of the content may retain within the container [4].

11.2.2.2 Parenteral Suspension

Formulation of parenteral suspensions is quite difficult. An injectable suspension contains various components like it has active ingredients that are suspended in antimicrobial preservative aqueous vehicle, surfactant, dispersing or suspending agent, and a buffer or a salt [4].

11.2.2.3 Parenteral Emulsion

Emulsions are dispersion of one immiscible liquid into another liquid with the help of emulsifying agents. This system is made by the addition of emulsifying agent. Examples of parenteral emulsions include:

- Oil in water emulsion (for sustained-release depot preparation given IM).
- Water in oil emulsion (allergen extract).
- Chylomicra: Lipid emulsion is the most widely used class of emulsion. Chylomicra are 0.5 to 1.0 μm spheres consisting of central core of triglycerides and the outer layer is made up of phospholipid. IV emulsion usually contains fats up to 10%; this ratio can be increased upto to 20%. The emulsions containing fats can be used as TPN for providing nutrients and calories [4].

11.2.3 Formulation Considerations for Parenterals

While designing formulation, we need to consider different factors. Excipients are one of the most important factors among them. General classes of excipients used in parenteral formulation are summarized in Table 11.2. Certain excipients are unique to each category of parenteral formulations. However, there is a general understanding for the main factors that need to be considered while designing formulation for parenteral products [5].

Table 11.2 Common excipients used in parenterals

Excipients	Examples
Vehicle	*Aqueous-based vehicle*: e.g., water for injection USP, sterile water for injection USP, bacteriostatic water for injection USP, etc.
	Nonaqueous vehicle: e.g., corn oil, cotton oil, peanut oil, sesame oil, etc.
	Co-solvents: e.g., glycerol, propylene glycol, ethanol, etc.
Buffers	Sodium citrate/citric acid and sodium acetate/acetic acid
Surfactants	*Nonionic surfactants (only)*: Tween®series, poloxamers, etc.
Preservatives	In multidose containers and non-terminally sterilized products, methyl and propyl para-hydroxybenzoic acid in low concentration of about 0.2% w/v, etc.
Antioxidants	Sodium bisulfite, sodium metabisulfite, ascorbyl palmitate, ascorbic acid, etc.
Tonicity modifiers	Sodium chloride, dextrose, mannitol, etc.

11.2.3.1 Type of Parenteral

Various types of preparations like solutions, suspensions, emulsions, etc. are there. Depending on type of preparation, we choose the suitable excipients. Selection of excipients depends upon various factors, i.e., the solubility of therapeutic agents, the route through which parenteral is being administered, amount of volume, and onset of action [5].

Solubility of Drug

Solubility is important because if drug is soluble in a vehicle or a solvent, it is easy to prepare a solution. If a drug is insoluble, we will consider suspension. Sometimes co-solvents are used in case when drug is insoluble in water, while considering the co-solvents, we need to focus on their toxicity because some of the co-solvents like ethanol are toxic if used in a higher amount [5]. Sometimes it happens that the drug is dissolved in a co-solvent system, but after some time the drug recrystallizes to its solid form. So, it should be confirmed that the drug will remain soluble in the used co-solvent system throughout its shelf life [5, 6].

Desired Routes of Administration

For IV route, mostly solutions are prepared using different excipients, while for subcutaneous or intramuscular route, we select the emulsions. But for emulsions, the size of droplet is kept below 1 μm because bigger droplet can irritate blood vessels same as the case for suspensions [5].

Volume Be Administered

Large volume parenterals up to 500 mL are administered intravenously, while the small volume parenterals can be administered through any route depending on type of preparation (either it is solution, suspension, or emulsion) [5].

Onset of Action

The most rapid action is produced by IV, compared to SC and IM. Aqueous solution has rapid onset as compared to oil-based solution or oil-based suspension or aqueous suspension. As for a suspension the drug must be dissolved first into solution form and then it will be active for pharmacological action [5].

11.2.3.2 Properties of Drug

For development of stable and safe parenteral products, the properties of drug need to be thoroughly investigated [5]. The physical and chemical properties of drug may affect the choice of formulation components for parenteral products. The important factor that affects the decision-making in formulation development process includes crystal characteristics/polymorphism, solubility, ionization constant (pKa), and particle size [7].

11.2.3.3 Properties of Vehicle

Vehicle is a medium which provides a medium to a system to carry a drug. There are three different types of vehicles: aqueous-based vehicles, oil-based vehicles, and hydroalcoholic vehicles [7].

Aqueous-Based Vehicle

Aqueous vehicles are used for freely soluble therapeutic agents like the drug which is highly soluble in water. Such drugs can be easily dissolved and form solutions in water [5]. For the drug with low aqueous solubility, co-solvents are used. Water is also used as an external phase for emulsions in which oil droplet is suspended in water by using various emulsifying agents. In USP, there are three different types of water mentioned used in pharmaceutical preparations, i.e., water for injection, sterile water for injection, and bacteriostatic water for injection [8].

Water for Injection USP Water for injection is non-sterile and used for large volume parenterals. After the product formation, these are terminally sterilized (i.e., when all the production processes are done and packed in plastic pouch-type container) by using autoclave or radiations. According to different pharmacopeias, water for injection has various limits regarding purity, appearance, amount of dissolved solids, pyrogens, and sterility. Pyrogens are substances that cause fever in a body. The most common pyrogens are endotoxins, which are lipopolysaccharides produced by microorganisms [7]. Pyrogens can be removed by heating at 250 °C for 30–45 min mostly or 180 °C for 3–4 h using autoclave. The most common method used to remove pyrogens from large volume parenterals is distillation or reverse osmosis. In the distillation process, water is simply boiled, all the solids and contaminants are left behind, and the steam moves to condenser where it converts back to pure water and used for parenteral preparations. In reverse osmosis, a semipermeable membrane is used which can segregate all the contaminates and dissolve solids in water to give pure water [8].

Sterile Water for Injection USP Sterile water for injection is sterile and used for reconstitution of drugs in powder form, e.g., ceftriaxone is in powder form packed

along with an ampule of water on which it is mentioned that "sterile water for injection" is used for the reconstitution of ceftriaxone to administer parenterally [8].

Bacteriostatic Water for Injection USP This contains antimicrobial agents and most commonly benzyl alcohol in 0.9% w/v proportion. It is mostly used in multi-dose containers where the chances of microbial growth increase due to instant pricking.

Nonaqueous Vehicle

Nonaqueous vehicle is used where the drug is insoluble in water so oil is used for the drug dissolution. Nonaqueous vehicle can be either solution or suspension or as a droplet dispersed in an aqueous medium. Most common nonaqueous vehicles are corn oil, cotton oil, peanut oil, and sesame oil (these oils are very stable against oxidation so less or no chances of rancidity). Mineral oils cannot be used in parenteral solutions. Two problems occur with oily solution or suspension because of their high viscosity, i.e., irritation to the muscles where administered and pain, and in some patients there is a chance of sensitivity (some patients are sensitive to certain oils). Sometimes therapeutic agents are actually used as vehicle, e.g., benzyl benzoate is used as a nonaqueous vehicle and itself it is a therapeutically active compound [9].

Co-solvents

To increase the solubility of drug sometimes co-solvents are used. The common co-solvents include glycerol, propylene glycol, and ethanol. Co-solvents should be used in such concentrations that can assure the solubility of a product throughout the shelf life [5].

11.2.3.4 Surfactants

Surface-active agents are amphiphilic compounds used for solubility enhancement or to stabilize the particles used in suspension. Surfactants are used in concentration above the CMC (critical micelle concentration) to increase the solubility. Amphiphilic polymers (surfactants) form micelle when meet with physiological medium. If concentration is higher than CMC, it forms micelles, but if concentration is lower than CMC, it does not form the micelle rather deposits on the surface of particles which are suspended in the suspensions [5]. Nonionic surfactants are more commonly used in parenteral formulations. Examples include polyoxyethylene sorbitan fatty acid esters like Tween series (Tween 20, Tween 40, etc.) in concentration of 0.1–0.5% poly(oxyethylene)-poly(oxypropylene) like poloxamers in concentration of 0.01–5% [5].

The type of surfactant used in formulation depends on the hydrophilicity and lipophilicity of the surfactant. The surfactants are amphiphilic compounds having both hydro- and lipophilic nature. Each surfactant has some value of hydrophilic-lipophilic balance, known as HLB. If HLB value is more than 10, this denotes surface-active agent is hydrophilic in nature, and these will be used for hydrophilic vehicles or hydrophilic mediums, while if HLB value is less than 10, it means the surface-active agents will be used for lipophilic medium. For solubilization, higher concentration, i.e., more than CMC, is required [10].

Sometimes combinations of surfactants are used, and these types of delivery systems are known as self-emulsifying drug delivery systems (SEDDs). Examples include amphotericin B as a complex with sodium deoxycholate (Fungizone) and sodium cholesteryl sulfate (Amphocil). When the powder is constituted with water, it forms a colloidal solution before intravenous infusion [11].

11.2.3.5 Buffers

Buffers are combination of a weak acid and its salt or a weak base and its salt. Buffers are required because pH is required to be maintained, not only for biocompatibility but also for the stability of the drugs. Some of the drugs might degrade at a certain pH; so the pH should be maintained such that it is biocompatible and to ensure stability throughout shelf-life [5]. Thus, buffers enhance the chemical stability. Examples of buffers used for parenterals include acetic acid/sodium acetate and citric acid/sodium acetate [9].

11.2.3.6 Preservatives

Preservatives are most commonly used in multiple dose preparations, since each time a dose is taken out, therefore chances of contamination are there. The product is not terminally sterilized. Examples are esters of para-hydroxybenzoic acid like methyl and propyl para-hydroxybenzoic acid in low concentration of about 0.2% w/v [5].

11.2.3.7 Osmolarity Adjustment

Osmosis is the movement of water through a semipermeable membrane. Movement of water takes place as long as concentration gradient is present. When the equilibrium is achieved, the molecules of water at both sides are the same and no further movement takes place. This movement can be stopped by applying pressure; the minimum pressure required to stop the movement across the membrane is termed as osmotic pressure. If we have high concentration of solute, then we need high pressure to stop the movement of water which means the osmotic pressure of solution is higher. If the concentration of solute is low, less amount of pressure is required to stop the movement of water; thus the solution will have low osmotic pressure. This all depends upon the concentration of solute in a solution [5].

A solution will be considered isotonic if it has the same osmotic pressure to that of the blood. So ideally the solution administered parenterally should be isotonic. If the solution is hypertonic, then there will be movement of water from the blood to the product, and if the solution is hypotonic, then there will be movement of water from the product to the blood. Therefore, it is important that the solution to be administered must be isotonic [2] (Table 11.2).

11.2.4 Manufacturing of Parenterals

11.2.4.1 Manufacturing of Injectable Solution

By using a suitable vehicle drug, excipients are dissolved. Water for injection is the most commonly used vehicle for preparation of injectable solutions. Injectable products are most common and can be administered by any route [5].

Injectable solutions are manufactured by dissolving the drug and excipients. Then the pH of solution is adjusted because after addition of some excipients the pH of the solution can be changed. To maintain a constant pH, some buffers are added. The isotonic solution is now filtered through 0.22 μm membranes. These membranes have small pores. The sterile solution is then filled aseptically for thermolabile substances and autoclaved for thermostable materials. For multiuse containers, antimicrobials are added. The total filled volumes of fluid into a parenteral container are greater than labeled volume [12].

11.2.4.2 Manufacturing of Injectable Suspension

Suspensions are the products in which drug molecules are dispersed as a small particles (the drug does not dissolve as in solution form).

Aqueous vehicle is prepared containing all the excipients. Then active ingredients are suspended as a particle. Two methods are used for the preparation of injectable suspensions [12].

Incorporation of Sterile Powder in Sterile Vehicle

This is the most widely used method for preparation of injectable suspensions. In this method sterile powders are mixed and dispersed in sterile vehicle. Examples include Penicillin G Procaine injectable suspension USP sterile vehicle and all the soluble excipients (including lecithin, sodium citrate, povidone). All these are dissolved in vehicle and passed through 0.22 μm membrane. The solution is then transferred to pre-sterilized mixing filling tanks, and then the powder which is made sterile by freeze-drying and spray-drying, is added into already prepared vehicle in a sterile manner [5].

Sterile In Situ Crystallization and Mixing with Sterile Vehicle

This is another method for preparation of injectable suspensions. In this method sterile in situ crystals are taken and then these crystals are mixed with sterile vehicle. Example is sterile testosterone injectable suspension USP. First, we prepare a vehicle which is sterile and filtered through 0.22 μm membrane. Testosterone is dissolved in acetone separately and then it is filtered through 0.22 μm membrane. The testosterone sterile solution is added to sterile vehicle (already prepared). The drug is insoluble in the vehicle, so it crystallizes. The suspension is brought to desired volume and filled in normal prescribed manner [13].

For injectable suspensions, flow property is very important factor. Like if we observe the buffer oral suspension, it is quite viscous and such type of consistency is not acceptable for injectables. The flow property is usually characterized in terms of syringe ability and injectability. Syringe ability and injectability is defined in terms of withdrawal from container into syringe, and there will be no clogging and foaming and accurate dose measurement will be possible. Like if we have a container of 5 ml, once we draw the dose, it comes out 4.5 ml or 4.8 ml and it causes dose variations [13].

11.2.4.3 Manufacturing of Injectable Emulsions

Two types of emulsions are commonly prepared: oil in water and water in oil emulsion. Example of W/O emulsion is preparation of allergenic extracts like vaccines. In W/O emulsion external medium is oil while the droplets which are dispersed are water [13]. This emulsion is administered subcutaneously; depot preparations are administered in the muscles in bolus where it releases slowly to surroundings and then distributed throughout the body. Emulsifiers are used less commonly because of problem encountered like autoclaving (some of the emulsifier cannot be autoclaved), and some of the emulsifiers are toxic and can produce unwanted physiological effects; hence these properties limit the use of emulsifier [14].

The most common emulsion administered intravenously is chylomicron which is lipid emulsion and in use for the last 25 years. It has a very fine droplet size of 0.5–1 μm, the central core of droplet is made of triglyceride, and outer core is of phospholipid. It can administer about 20% of fats. These kinds of emulsions are usually used for patients who require nutrients in sufficient amount and cannot take it orally. It provides a lot of fatty acid and calories during TPN [14].

11.2.5 Sterilization of Parenteral Formulations

Sterilization may be defined as “the absence of living microorganisms (either through the destruction of all living microorganisms or by their removal) in pharmaceutical preparations” [15]. Five different methods are used for sterilization of pharmaceutical raw materials and pharmaceutical preparations. Different methods of sterilization are summarized in Table 11.3.

Table 11.3 Different methods of sterilization

Moist heat sterilization	
Procedure	In this method, pharmaceutical products are exposed to specific temperature for specific time, which results in efficient sterilization. For example, at 103.4 kPa pressure (i.e., 15 pounds per square inch) and 121 °C temperature, sterilization is achieved in 20 min, whereas at 68.91 kPa pressure (10 pounds per square inch), sterilization is achieved in 30 min.
Mechanism	Denaturation/coagulation of microbial proteins.
Applications	This method is used for sterilization of such materials which are both thermostable (within the conditions of the sterilization cycle) and through which moisture can perfuse.
Dry heat sterilization	
Procedure	It is performed at higher temperature and longer time as compared to moist heat sterilization, e.g., exposure at 170 °C for 1 h or 160 °C for 2 h or 140 °C for 4 h.
Mechanism	Exposure to higher temp for longer time, microorganisms are destroyed due to cellular dehydration and then pyrolysis/oxidation.
Applications	This method is employed for sterilization of such thermostable materials/products that cannot be readily sterilized by moist heat, e.g., heat-stable drugs/excipients, nonaqueous vehicles, such as oils, glycerin, propylene glycol, and glassware (e.g., bottles).
Filtration sterilization	
Procedure	This method involves the removal of microorganisms from solutions through sterilizing filters having pore diameter of 0.22 μm.
Mechanism	Very small pore diameter (0.22 μm) is sufficient for entrapment of bacteria and fungi. After using the filters (which contain the entrapped/retained microorganisms), these are then safely discarded.
Applications	This method is used for sterilization of thermolabile therapeutic solutions.
Radiation sterilization	
Procedure	In this method raw materials/products are exposed to a defined dose of ionizing radiation. Usually, gamma radiations in the range of 25–40 k Gy are employed for sterilization.
Mechanism	Microorganisms are destroyed due to exposure to gamma radiations.
Applications	This method is used for sterilization of therapeutic agents/excipients or parenteral formulations that are manufactured and packaged under aseptic conditions but are neither terminally sterilized nor sterilized by filtration.
Gas sterilization	
Procedure	In this method, materials/products are exposed to mixtures of ethylene oxide or propylene oxide along with an inert gas, e.g., carbon dioxide. This is done within a specially designed apparatus.
	Sterilization efficiency may be enhanced in the presence of moisture (up to 60%) and elevated temperature (55 °C).
Mechanism	The reactive gases destroy microorganisms by chemical reaction with cell proteins and DNA.
Applications	It is used for sterilization of medical devices and surgical accessories, e.g., packaged catheters and blankets. Therapeutic agents/excipients may also be sterilized by this technique.

11.3 Sterile Ophthalmic Preparations

Development of therapeutics for treatment of ocular disease is a challenging task, because ocular tissue is one of the most sensitive tissues of the human body. Moreover, many challenges need to be circumvented for improved bioavailability of drug through ocular route. For instance, frequent blinking, rapid tear turnover, and nasolacrimal drainage are some of the challenges that limit the bioavailability of topically applied ophthalmic products.

It is important to understand the anatomy of the eye, in order to know where the drug is applied in ocular tissue [16] (Fig. 11.1).

11.3.1 Anatomy of the Eye

Eye is a ball suspended in the ocular orbit and is composed of multiple tissues that coordinate to focus, transmit, and detect incoming light. The central path is transparent, the aqueous humor. The light travels through it and reaches the retina, that with the help of photoreceptors is converted into image [17].

Cornea: The outermost layer of the eye is cornea, which is a transparent membrane on the surface of the eye. This is the first and most important barrier of the eye. Cornea consists of three layers: the epithelium, stroma, and endothelium. These layers vary in their barrier properties. Cornea has no blood vessels so the nutrients are taken from the surrounding aqueous humor.

Sclera: Another tough part of the eye is sclera which is like a fibroblastic capsule, which encloses the eye and provides support as well as protection to the interior structure. Sclera is connected to the cornea through limbus [16].

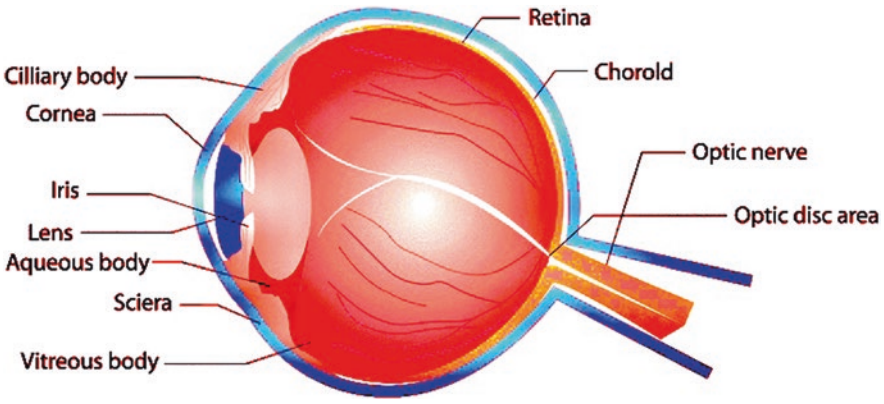

Fig. 11.1 Anatomy of the eye

The limbus: It is rich in blood vessels. Most of the absorption from topically applied ophthalmic products occur from this part of the eye.
The interior side of limbal area has a small opening, called canal of Schlemm, which helps to regulate intraocular pressure [17].
Iris: Next, there is *iris*, which is a ring of muscular tissue present in front of the lens, and it segregates the anterior and posterior chamber. It controls the entry of light into the back of the eye through the pupil.
Lens: It is a flattened sphere that is connected to the ciliary body by a fiber-like strand.
Vitreous humor: It is a transparent gelatinous material and has no turnover. It is in direct contact with the retina.
Retina: It is bilayer and a highly metabolically active tissue that is responsible for transforming light into an electrical signal that is converted to image by the brain [17].

11.3.2 Topical Ophthalmic Products

Topical ophthalmic products are applied to the surface of eye, the cornea. The common topical ophthalmic products are eye drops, ointments, and gels. They are typically used for treatment of diseases of anterior segments, for example, the different corneal layers, conjunctiva, sclera, iris, ciliary body, etc. [17]

11.3.2.1 Formulation Considerations

The bioavailability from topical products is affected by many factors, such as continued turnover of tears, nasolacrimal drainage, etc. The absorption from corneal surface can be increased by:

– Increasing the residence time of drug on the corneal surface
– By enhancing the permission of the drug through membrane

The residence time of drug in the eye can be prolonged by increasing the viscosity. However, viscosity higher than 70cps can cause blurring of vision and discomfort [17].

Drug permeation through ocular barrier can be increased by using lipophilic prodrug. For example, epinephrine has limited permeation through the membrane; however, its lipophilic prodrug, dipivefrin, can easily pass the corneal epithelium that is hydrolyzed to the parent epinephrine, after crossing the membrane. Moreover, permeability can also be increased by the use of penetration enhancers.

The osmolarity of the product is also a critical parameter to be maintained. Ideally, ophthalmic product should be isotonic, i.e., 300 milli osmols/liter). Very high hypertonic solutions can cause discomfort and induce irritation and lacrimation; hence the drug will be drained off.

The optimum pH value for ophthalmic products is 6–8. Very acidic and basic products can cause irritation and lacrimation. The increase in tear production will drain the drug off the corneal surface [18].

11.3.2.2 Classes of Topical Ophthalmic Product

The common classes of topical ophthalmic products are eye drops, eye ointment, gels, and gel-forming solutions.

Eye Drops

One of the most common topical ophthalmic products is eye drops. It is applied to the cul-de-sac. Usually more than one drop is instilled into the eye. The commercial ophthalmic droppers usually deliver 30–50 μL of fluid depending on fluid viscosity. It should be noted that in normal conditions eye can accommodate 10–15 μL of fluid. Typically, two to three drops are administered; the excess amount is drained off from the eye. Therefore, frequent administration is required. Eye drops can be in the form of solutions, suspensions, or emulsions [18].

Solution

Solutions provide uniformity of dose, improved bioavailability, and easy production process. Most of the drugs intended for ophthalmic administration are water soluble; therefore, it is easier to formulate in the form of solution. However, in case of water-insoluble drugs, salt formation is a common approach. The most common salts are hydrochloride, sulfate, nitrate, and phosphate. It is important to note that salt formation may change the physicochemical properties of drugs. For instance, different salts of the same drug pose different levels of irritation, as shown in Table 11.4.

Gel-Forming Solutions

These solutions tend to form gel after coming in contact with eye. Gel formation increases the residence time of drug on the absorption surface which in turn increases absorption. For example, timolol ophthalmic solution is applied twice a day for management of glaucoma, while the gel-forming solutions require only once a day administration. There are different mechanisms of gelation [19].

Table 11.4 Buffer capacity and ocular discomfort with different salts of epinephrine

Salt	Ocular discomfort level	Buffer capacity
Epinephrine bitartrate	Moderate to severe stinging	High
Epinephrine hydrochloride	Mild to moderate stinging	Medium
Epinephrine borate (decreased stability)	Occasional mild stinging	Low

Thermosensitive gelation: The product is solution at ambient conditions and forms gel at ocular temperature.

pH-sensitive gelation: Some solutions undergo gelation at ocular pH (i.e., 7.0 to 7.3).

Ion-sensitive gelation: Ocular fluid has a certain ionic strength. Some of the polymers form gels in the presence of certain ions or enzymes, such as lysozymes. For example, Timoptic-XE® uses gellan gum, which forms gel in response to higher ionic strength of tear fluid (US patent 4861760).

Another product uses xanthan gum, which forms gel in response to lysozyme (US patent 6174524) [19].

Suspensions

Suspension is recommended when the drugs are not soluble. The drug particle is dispersed in suitable vehicle. The particle size in ophthalmic suspensions is typically less than 10 μm, since bigger particle can produce irritation. Ophthalmic suspension should have good flowability and be easily resuspended upon shaking [19].

Ophthalmic suspensions are also used to increase the compatibility of the drug. Some drugs might have irritating property in solution form but nonirritating when administered as suspension. For example, betaxolol (beta-blocker) used in glaucoma produced a serious discomfort. Many solution-based formulations of betaxolol (β-blocker) were tried but showed limited success. Insoluble form of betaxolol (Betoptic®S), using 20 μm particles of high molecular weight polyanionic polymer carbomer and a sulfonic acid cation exchange resin, improved efficacy and ocular tolerance (US patent 4911920) [20].

Powders for Reconstitution

The drug that is unstable in solution or suspension form is formulated as powder that needs to be reconstituted before administration. These powders are usually manufactured by lyophilization (freeze-drying). The solutions are added into individual glass vials that are freeze-dried to form dry powder. Lyophilization requires bulking or stabilizing agents like mannitol and potassium acetate. A separate sterile vehicle should be dispensed along with powder for reconstitution [18].

Ocular Inserts

Ocular insert is a sterile preparation covered in a thin multilayered solid or semisolid device designed to be placed in cul-de-sac to be in contact with the eye for longer period to provide a constant bioavailability. It prolongs the contact with corneal surface; hence the therapeutic activity of drug can be improved. To achieve this

viscosity, enhancing agents are added to eye drops to increase the retention time of drug at eye surface, but these dosage forms only give sustained drug eye contact for specific period and do not yield a constant bioavailability [21]. Apart from that, several other approaches have been adopted for increasing corneal residence time of drug, such as microparticles, gels, gel-forming solutions, micelles, liposomes, nanoemulsions, NPs, ocular inserts, etc. [22]

Among these approaches ocular inserts have gained special interest for prolonging ocular residence time of drugs and reliably providing controlled release without any irritation to patient [23].

Soluble Ocular Inserts These are also called erodible ocular inserts. Polymers are being added in ophthalmic solutions to increase the viscosity and retention time. These are usually made of cellulose and water-soluble polymers, which can be sterilized by gamma radiations [23]. These are inserted into cul-de-sac of the eye for the improvement of absorption, enhanced bioavailability, and therapeutic effect. Since these are made with water-soluble polymers, so with the passage of time, these slowly dissolve and erode releasing their drug content. The potential advantage of erodible ocular inserts is not to remove at the end of useful dosing interval [23, 24].

Insoluble Ocular Inserts These are also called non-erodible ocular inserts. The first controlled topical dosage form was designed in 1975 by Alza Corporation. The insoluble inserts have been divided into three different groups. These include diffusion systems, osmotic systems, and hydrophilic contact lenses. The first two classes contain a reservoir, which is in contact with the inner surface of the retina. The reservoir contains a liquid, a gel, a colloid, a semisolid, a solid matrix, or a carrier-containing drug homogeneously or heterogeneously dispersed or dissolved therein [25].

11.4 Environmental Control for Sterile Manufacturing

Manufacturing of sterile pharmaceuticals and medical devices requires *clean room*, which is a controlled environment where the limit for the number of specified size is properly maintained [26]. The construction of the rooms for sterile manufacturing needs a proper design which minimizes generation, introduction, and retention of the particles [27]. Furthermore, other key parameters which include temperature, humidity, air flow filtration/velocity, and pressure are the trigger factors to be controlled for ensuring production of the aseptic products [28].

For parenteral drug manufacturing, environmental control is the major challenge to be addressed, because quality of the final products is directly influenced by the processing environment. The objective of environmental control in a sterile facility is to minimize the presence of all contaminants (both viable and nonviable) [29].

Microorganisms and dust particles suspended in the air are most likely to gain access to the product; therefore, an adequate environmental control program is

needed. The following are the most imperative facilities that are required for ensuring and controlling the aseptic environment for the sterile products [30]:

1. Heating, ventilation, and air conditioning (HVAC) system
2. Personnel contamination control systems
3. Cleaning and disinfection of the area
4. Environmental monitoring systems

11.4.1 Heating, Ventilation, and Air Conditioning (HVAC) System

HVAC systems are considered the most imperative component of the environmental control systems design [31]. The main objective of HVAC system is to ensure the required standard environmental conditions for manufacturing of the pharmaceutical products [32].

Several components of an HVAC system can be segregated as follows:

– Blowers and fans for air generation.
– Cooling, heating, humidifying, and dehumidifying system for air conditioning.
– Metal ducts for air distribution networks.
– Air filtration equipments, depending upon the type of manufacturing. For sterile manufacturing, HEPA filters are installed.

For designing an effective HVAC system, some of the operational parameters need to be defined, since these parameters are considered critical for aseptic processing.

11.4.1.1 Temperature and Humidity Control

Temperature control is required to offer a comfortable working environment for operator. Usually 19–23 °C is considered acceptable [26].

Within manufacturing facility, some of the areas, like those where autoclaves, dry heat sterilization tunnels, and ovens are located, provide high heat loads on the systems. If not controlled, these loads can result in an environment which may not only cause discomfort for the working personnel but also increase the contamination chances due to high perspiration of operators [33].

Humidity control is also being considered the key factor for quality manufacturing of the pharmaceutical products. However, this also depends on the product requirement. Comfort levels are in the range of RH 45–55%, whereas manufacturing process requirement can vary widely. Some products (freeze-dried products) are manufactured in controlled environment with a relative humidity range of only 15–30%. Normal humidity levels can be easily achieved with air conditioning

systems. Humidity levels lower than the normal can be achieved by air dryers in the air supply system, which work on the adsorption principle.

11.4.1.2 Pressure Differential Control

Pressure gradient is a mean to prevent cross-contamination between environments. Pressure gradients are established so as to provide critical environments with higher pressures than those that are less critical for the process. To increase the pressure of any environment, sufficient air must be added to ensure an overflow to the adjacent environment.

Differential pressure among various rooms or environments will be relative, i.e., one room will be measured relative to another.

11.4.1.3 Airborne Contamination Control

The HVAC system should provide control over the airborne viable and nonviable particles so as to satisfy the level of control specified for the environment. To prevent airborne contaminants from entering the clean or aseptic environment, all air supplied for the environment must be filtered [34].

One of the primary functions of the filtration system is to provide sufficient level of cleanliness by recirculating the air contained within the environment through various filters, mainly HEPA filters, thus providing a polishing effect [35]. This capacity is commonly defined by the number of a*ir changes* per hour or by the recirculation ratio of the system.

Air Changes Rate (ACR)

The frequency of air turnover in the clean room. It is the measure of how quickly the air in an interior space is replaced by fresh air:

$$\text{Air change rate} = \frac{\text{Volume of air flow per hour}}{\text{Volume of room}} \tag{11.1}$$

Air is distributed in such manner that it flows into the maximum security room at the greatest volume flow rate, hence producing positive pressure, which is successively reduced so that the air flows from the maximum security area to the other less critical areas for return to the filtration system.

The level and type of filtration needed depend on the level of cleanliness required. As a universal method for cleanliness classification, several industries have adopted a standard. This standard classifies cleanliness according to the number of particles, 0.5 micron or larger per m^3 of air as shown in Table 11.5.

Table 11.5 Limits for airborne particle in different classes of clean room [38]

	Maximum number of particles allowed per m^3 equal or greater than the tabulated size			
	at *rest state*[a]		in *operation state*[b]	
Grade	0.5 μm	5 μm	0.5 μm	5 μm
A	3520	20	3520	20
B	3520	29	352,000	2900
C	352,000	2900	3,520,000	29,000
D	3,520,000	29,000	Not defined	Not defined

[a]*at rest state* means the condition where the installation is complete and is operating in such manner agreed upon by the customer and supplier, but in the absence of any personnel
[b]*in operation state* means the condition where the installation is functioning in specific operating mode and in the presence of specific number of personnel

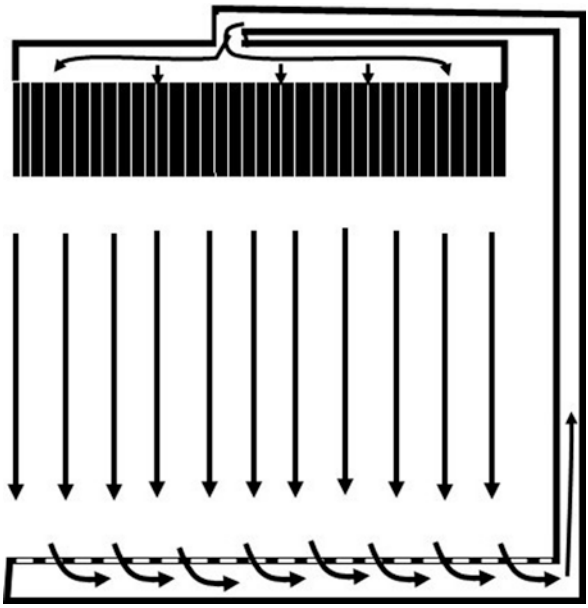

Fig. 11.2 Vertical laminar flow clean room

Laminar airflow (LAF) or unidirectional airflow has greatly improved the potential for environmental control of aseptic areas. Currently it is the only mean for achieving class 100 rooms. A laminar flow (unidirectional airflow) room is one in which air is introduced evenly from high efficiency particulate air (HEPA) (Fig. 11.2) filter panels and returned into the opposite surface of the room. The air moves in parallel flow lines with uniform velocity and minimum eddies. The air velocity across the room is held in the range of 90 ± 20 ft/min. Contamination is prevented because it is swept away with the airflow.

LAF rooms may have vertical flow, i.e., when the airstream is perpendicular to the floor, or horizontal flow, the air travels parallel to the floor. Vertical flow room

offers a continuous clean air shower. Contamination generated in the area will be swept down and exhausted through a perforated floor. This type of room minimizes cross-contamination from one side of the room to the other in any direction. In a horizontal flow room, contamination generated downstream of the filter face will be exhausted across the room at the opposite wall. This type of room will minimize cross-contamination of operations taking place perpendicular to the airflow, but in operations taking place parallel to the flow, downstream contamination can occur, which is one of the limitations on the use of this type of room.

Advantages of LAF system: Unidirectional airflow possesses characteristics that make it one of the best contamination control systems. The following are some of the practical and technical advantages of LAF system:

- Particles less than 15 micron are swept at the same speed as the air; thereby particles of the largest group of airborne contaminants are removed.
- Air is filtered through HEPA-filtered air supplied, which provides the lowest contamination level possible.
- Particle removal efficiency of HEPA filter is 99.97% for particles 0.3 micron and larger. Thus, most bacteria are above 0.5 micron size, thus restrained.
- Due to the high recirculation ratio, unidirectional airflow systems provide the best cleaning and recovering capabilities.
- These are the only mean to achieve class 100 environments.
- Since the flow is unidirectional, the particles are not spread.

HEPA filter is a filter assembly that removes at least 99.97% of airborne particles with 0.3 micron in diameter [33]. The filter medium is composed of a folded mat of randomly arranged fibers typically composed of fiberglass. Every fold is spaced by grooved aluminum or paper separator. The particles in HEPA filter are trapped through a combination of straining, impingement, and entanglement (discussed in Sect. 4.3).

11.4.1.4 Personnel Contamination Control Systems

Excellent environmental control can be achieved only if the movements of personnel and supplies from one area to another are decreased [34]. It should be kept in mind that the access by personnel to the aseptic corridor and aseptic compounding and filling rooms is only through an *airlock.* For processing the aseptic products, the rooms with automatic lock door system are highly imperative and required. These will be very helpful to maintain the clean environment of the adjacent processing rooms. Furthermore, this system will prevent and control entrance of the microbes and particles from the non-sterile declared zones.

Personnel should be allowed to enter aseptic area only after following strictly the prescribed procedure for removing their street clothing, washing their hands, and wearing gowns, hats, shoes, face masks, gloves, and other prescribed outfits [29, 32]. After entering the area, they should not be allowed to move in and out of the

area without re-gowning. Personnel working in this area should receive regular training on maintenance of discipline in clean rooms.

The lowest numbers of personnel required should be present in clean rooms. There should be minimum movement in the area, since a study showed that when we sit in working condition, human generate 0.5 million particles/min [36] of 0.3 μm and above. Similarly, one million particles/min and 5million particles/min are generated while standing and walking, respectively. Hence *penguin movement* is the suggestive walking style.

11.4.1.5 Cleaning and Disinfection of the Area

The cleaning equipment (e.g., mops) selected should be effective in cleaning and should not produce lint. The ceiling, walls, and other structural surfaces must be cleaned periodically [32].

All equipment and surrounding working area must be cleaned thoroughly at the end of the working day. No residue of the previous process should be present. After thorough cleaning, surfaces should be disinfected by either spraying or wiping and effective disinfectant on all surfaces. Irradiation from UV lamps is also sometimes used to reduce the viable microorganisms present in the area. UV lamps should be kept clean and checked periodically for its irradiation efficiency.

11.4.1.6 Environmental Monitoring Systems

The assessment of the level of environmental control can be performed by measuring total particle counts (viable and nonviable) in air sample, for example, the optical particle counter analyzer, which works on the principle of light scattering which is very useful to identify the number of particles in air sample [33, 37]. This device generates a plot, where the number of particles is displayed along with their respective particle sizes in the range of 0.3 μm up to 25 μm. The recommended particle size for the clean room, either equal to 0.5 μm or >0.5 μm, needs to be measured for quality compliance.

Viable counts are made using one or more of several methods, such as settling plates, contact plates, slit-to-agar samplers, or centrifugal samplers.

Settling plate sampling: In this approach, the uncovered petri dishes with the agar medium are placed on different spots for a specific period of time, where the microbe-bearing particles are more likely to be deposited on plate surface. It is a direct method of assessing the number of microorganisms depositing onto the product or surface in a given time.

Contact plate sampling: In this method, the agar plates are placed on the sampling spots for approximately 10 seconds in such a way that will interact with the surface evenly using a constant force. This will expose the maximum parts of the spots, where the microbes need to be collected from. Contact plate sampling is

helpful for assessing the microorganisms on the surfaces of the building/machines where these organisms have either directly been deposited from the environment or from the contact by the workers and supervisors.

Slit-to-agar samplers: In this method, the air is introduced into the agar plates through a narrow slit, which whirls around the central axis and spreads across the entire surface. It is ideally required to cover the entire surface within 1 h, so the speed of the injected air will be fixed accordingly. This method is very handy to determine level of contamination at different periods of time during the ongoing activities.

Centrifugal samplers: This method utilizes an impeller device in the head of the air sampler to draw air. The impeller is then rotated to produce a centrifugal force which causes particles and microorganisms to impact onto an agar strip that is fixed around the border of the sampling head.

The results need incubation for 48 h at a specific temperature. In this time, the microorganisms multiply forming clear colonies. The number of microbes present in the sample is measured in terms of colony-forming units (CFU).

11.4.2 Classification of Clean Room

Sterile manufacturing involves different sections, such as compounding, filling, and packaging section. Each section requires different standards for environmental cleanliness.

Clean rooms are classified on the basis of cleanliness level in terms of viable particle and nonviable particles. Previously, the most universally applied classification was based on Federal Standard 209 of the US FDA. This standard was first published in 1963 in the USA. It was revised in 1966 (209A), 1973 (209B), 1987 (C), 1988 (D), and 1992 (E). However, FS 209E was officially cancelled by the US government on November 29, 2001 and was replaced with ISO 14644-1. The main limits are detailed in Tables 11.5 and 11.6.

Based on the required standard of cleanliness, clean room may be divided into critical area and supporting area. *Critical area* is the area for sterile manufacturing, where manufacturing containers, formulation ingredients, primary packaging material, and closures are exposed to environment. Critical area must be designed in such a way that it ensures sterility of the final products [39], while the *supporting area* is the area adjacent to the critical area.

For aseptically produced products, it is very imperative to control environmental cleanliness to the highest standard. However, *terminally sterilized products* require less stringent control compared to aseptic manufacturing (Table 11.7).

Aseptically prepared products: These are the products which are prepared within aseptic environment and are packed by closed system of **aseptic** transfer. It does not undergo sterilization step after packing.

Table 11.6 Limits for viable count in different classes of clean room [38]

Grade	At *rest state*[a]		In *operation state*[b]	
	Air sample CFU/m^3	Settle plates (diameter 90 mm) (CFU/4 h)	Contact plates (diameter 55 mm) (CFU/plate)	Glove print (5 fingers) (CFU/glove)
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	–
D	200	100	50	–

Table 11.7 Clean room requirement for aseptically prepared products and terminally sterilized products [40]

Sterile manufacturing	Clean room requirement	Pressure differential
Terminally sterilized		
Solution preparation	Class D	+15 pa next to outside
Component preparation	Class D	+15 pa next to outside
Filling (usually)	Class B[a]/C[b]	+15 pa adjacent area
Filling (risk of contamination)	Class A[a]/C[b]	+15 pa adjacent area
Aseptically prepared		
Solution preparation (needs filtration)	Class C	+15 pa next to outside
Product preparation	Class A[a]/B[b]	+15 pa adjacent area
Filling	Class A[a]/B[b]	+15 pa adjacent area

[a]Critical area
[b]Surrounding area

Terminally sterilized products: These are the products which are sterilized in the final container.

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