STUDY MATERIAL FOR B.Sc. MICOROBIOLOGY

PHARMACEUTICAL MICROBIOLOGY

SEMESTER – VI

KAMAP COLLEGE

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PREPARED BY

MICROBIOLOGY DEPARTMENT

INDEX

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PHARMACEUTICAL MICROBIOLOGY

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UNIT - I

Introduction to Pharmaceutical microbiology

INTRODUCTION

Pharmaceutical microbiology is a critical discipline focused on understanding and controlling microbial aspects within the pharmaceutical industry, encompassing everything from manufacturing environments to product integrity. Its scope includes the monitoring of cleanroom environments to ensure compliance with international standards such as ISO 14644-1 and EU GMP Annex The role of the microbiologist extends from early drug development through commercialization, ensuring patient safety by managing risk assessments and microbial contamination control strategies.

The modern era of the pharmaceutical industry—of isolation and purification of compounds, chemical synthesis, and computer-aided drug design—is considered to have begun in the 19th century, thousands of years after intuition and trial and error led humans to believe that plants, animals, and minerals contained medicinal properties. The unification of research in the 20th century in fields such as chemistry and physiology increased the understanding of basic drug-discovery processes. Identifying new drug targets, attaining regulatory approval from government agencies, and refining techniques in drug discovery and development are among the challenges that face the pharmaceutical industry today. The continual evolution and advancement of the pharmaceutical industry is fundamental in the control and elimination of disease around the world.

ECOLOGY OF MICROORGANISMS IN PHARMACEUTICAL INDUSTRY:

The microbiological quality of pharmaceutical products is influenced by the environment in which they are manufactured and the materials used in their formulation

In microbiology and pharmaceutical development, environmental monitoring (EM) is a process that determines the quality of a controlled environment via microbial data collection. Data comes from samples of air, surfaces, and personnel in a clean space. Environmental monitoring plays a critical role in developing sterile (ie: injection, infusions, ophthalmic solution) and non-sterile (ie: solutions, capsules, tablets, ointments) drug products.

Environmental monitoring evaluates the quality of air, surfaces, and personnel through testing and sample analysis. Meeting appropriate hygiene standards is essential to the safety and efficacy of a finished product.

Requirements For Environmental Monitoring

According to American Pharmaceutical Review, all EM programs should use a combination of the following methods:

- Settle plates (air testing)

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- Contact plates (surface and personal testing)
- Surface/personnel swabs
- Active air samples
- Rinse samples

Air Monitoring

The air quality of any lab or production area is critical, as microorganisms in the air increase the risk of ingredients and products becoming cross-contaminated. According to the CDC, biological contaminants in the air may include bacteria, fungi, viruses, and pollens.

Laser particle counters or active air samplers are used to retrieve air samples. These samples are incubated and analyzed for the presence of microorganisms. If there is microbial contamination in the air, an identification test determines the origin of the microorganism. This step of environmental monitoring is vital for manufacturers of pharmaceuticals, cosmetics, food, and beverages.

Personnel Monitoring

Even with hygiene practices and proper PPE (gowns, gloves, goggles, face shields, and N95 masks), humans are the most significant risk of source of contaminants to an aseptic environment.

Individuals can easily perform personnel testing on themselves in one of two ways: swabbing the forehead or elbows with a cotton swab dipped in tryptic soy broth (TSB) or direct contact with a tryptic soy agar (TSA) plate and the fingertips. Plates will then be covered and incubated for about 18 hours before checking if microbes are present.

Surface Monitoring

No matter how thoroughly a lab or cleanroom has been sanitized, microorganisms can quickly transfer from one surface to another by touching the surface with something that is contaminated.

When testing a surface for microbes, an agar plate is directly placed on a surface and then covered and incubated. Personnel should then spray down the surface with isopropyl alcohol for further disinfection.

REGULATIONS AND RULES

Aseptic processing is a manufacturing method that produces pharmaceutical products that are free from bacteria. Regulatory agencies like the FDA and the European Medicines Agency (EMA) require pharmaceutical manufacturers to have an environmental monitoring program in place to ensure that aseptic processing is possible.

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1. Atmospheric Ecology

The air in pharmaceutical facilities is a primary vector for contamination, carrying both free-living cells and microbes attached to dust particles

- Dominant Flora: The most common airborne isolates in cleanrooms are Gram-positive bacteria, particularly staphylococci and micrococci.
- Spore-forming bacteria of the genus Bacillus and various fungi (e.g., *Aspergillus, Penicillium*) are also frequent, typically originating from outside air and soil
- Ecological Drivers: Temperature and moisture are the primary drivers of indoor air microbiome composition (Pacchioni et al., 2018). Even in controlled Class C and D environments, localized "hotspots" can occur, with active air samples recorded as high as 67.33 ± 17 CFU/m(Eissa, 2025).

2. Water Systems Ecology

Pharmaceutical water provides a unique niche for oligotrophic (low-nutrient) microorganisms

Gram-Negative Dominance: Unlike the air, water systems are dominated by Gram-negative bacteria such as Pseudomonas, Burkholderia, and Ralstonia. These organisms are ecologically significant as potential sources of endotoxins (pyrogens) Biofilm Formation: Microbes in water systems often survive by forming biofilms on the internal surfaces of pipes and stagnant ducts, which can lead to persistent out-of-specification results

3. Personnel Ecology: Skin and Respiratory Flora

Humans are the single largest source of microbial contamination in cleanrooms through "bio-shedding".

- **Skin Flora:** Normal human skin microbiota, including Staphylococcus spp. and Micrococcus spp., accounts for over 70% of isolated cleanroom
- **rRNA sequencing** to trace specific strains, like Staphylococcus cohnii, directly from personnel to production areas
- **Respiratory Flora:** Talking, coughing, and breathing release bacteria-laden respiratory particles. Healthcare and industrial workers can carry unique nasopharyngeal flora, including pathogenic or drug-resistant bacteria, due to occupational exposure

4. Raw Materials and Packaging

- **Raw Materials:** Natural materials (e.g., starches, gums, animal-derived gelatin) carry a higher microbial risk than synthetic chemicals. Specific contaminants like Acholeplasma laidlawii can be introduced through these starting materials

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- **Packaging:** Primary packaging (glass, plastics, rubber) must be sterile-processed to ensure it does not introduce microbes into the drug product If packaging integrity is compromised or if containers are opened in non-sterile environments, the risk of fungal contamination (e.g., Candida albicans) increases significantly .

5. Building and Equipment

- **Surface Ecology:** Building materials (polymeric and metallic) can support the rapid development of environmental biofilms if not properly sanitized
- **Equipment:** Stagnant water in equipment ducts or residues from previous batches can harbor microbial clusters Equipment design, such as the use of antimicrobial surfaces, is an emerging strategy to mitigate these

DESIGN AND LAYOUT OF STERILE MANUFACTURING.

FACTORS RESPONSIBLE FOR THE PLANT LOCATION CHOICES:

There are 2 types industry,

A small scale industry

A large scale industry

Availability of raw material,

Nearness to the potential market,

Supply of labor,

Transport and communication facilities,

Suitability of land & climate,

Safety requirements

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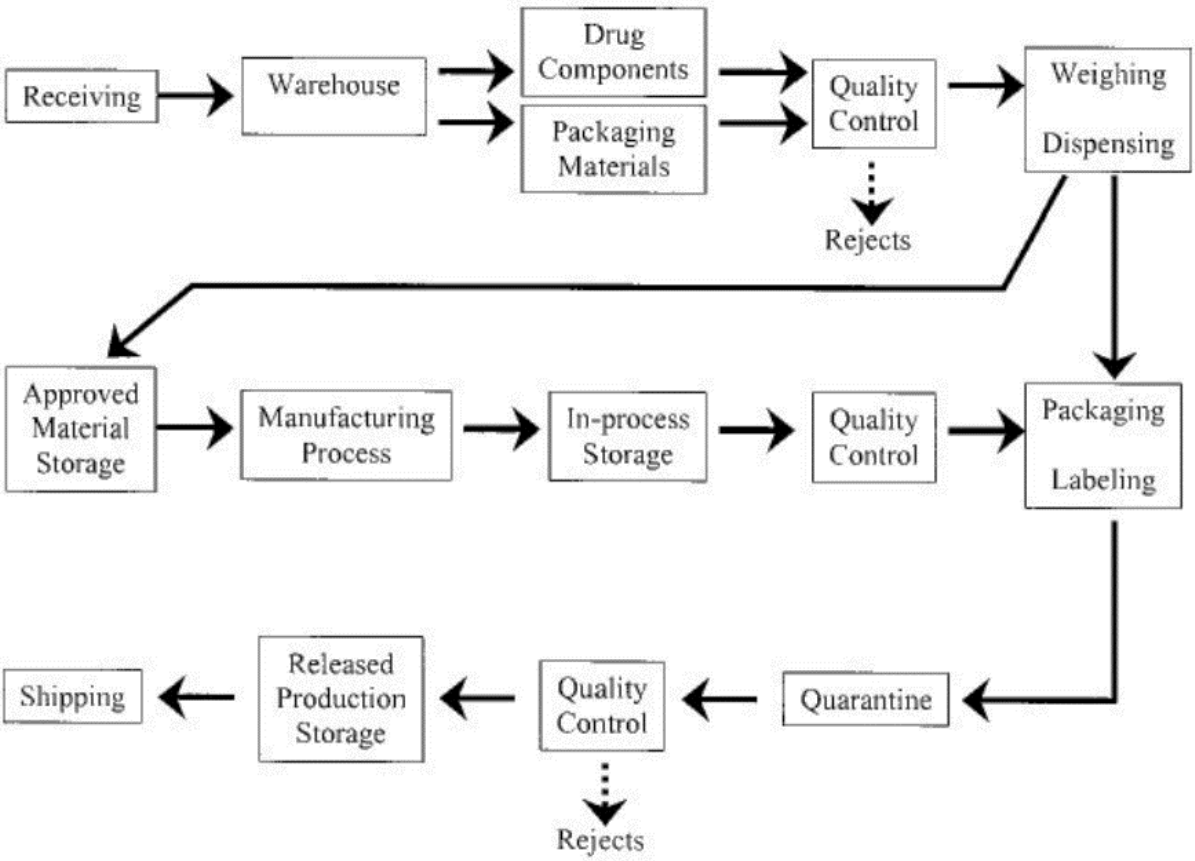

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Components of pharmaceutical plant layout

- Setup

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- Factory sites
- Factory building
- Warehouse
- Operation areas

Pharmaceutical Manufacturing Facility Design

In the world of pharmaceuticals, precision and quality are paramount. Pharmaceutical manufacturing facility design plays a pivotal role in ensuring that medications are produced efficiently, safely, and with the highest standards of quality.

This blog explores the key elements that go into designing a pharmaceutical manufacturing facility, highlighting the critical factors that contribute to its success.

1. Regulatory Compliance

The pharmaceutical industry is governed by a complex web of regulations and guidelines aimed at safeguarding public health. Regulatory bodies such as the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and many others set stringent standards for pharmaceutical manufacturing. These standards encompass every aspect of drug production, from raw material handling to final product distribution.

- **Understanding Regulatory Frameworks:** Pharmaceutical facility designers must have an in-depth understanding of the regulatory frameworks that apply to the regions where their products will be sold. This includes knowledge of Good Manufacturing Practices (GMP), Good Laboratory Practices (GLP), and other quality standards.
- **Documentation and Compliance:** Robust documentation and compliance systems are essential. Designing the facility to facilitate thorough documentation of processes, equipment validation, and adherence to safety protocols is crucial to meeting regulatory requirements.
- **Risk Assessment:** Facility designers also conduct comprehensive risk assessments to identify potential hazards and mitigate them. This may include implementing containment systems for hazardous materials, ensuring proper ventilation, and designing emergency response plans.

2. Cleanroom Design

Cleanrooms are the heart of pharmaceutical manufacturing facilities. They are designed to minimize the presence of airborne particles, contaminants, and microorganisms to ensure the sterility of pharmaceutical products. The cleanroom design involves several critical elements:

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- **Air Filtration:** Cleanrooms employ High Efficiency Particulate Air (HEPA) and Ultra-Low Penetration Air (ULPA) filters to remove particles from the air. The design must ensure proper air circulation and filtration.
- **Gowning Procedures:** Cleanroom personnel follow strict gowning procedures, including the use of specialized clothing, to prevent contamination. The facility design should include gowning areas with the necessary equipment and protocols.
- **Material Compatibility:** The materials used in cleanroom construction must be non-shedding, non-reactive, and easy to clean. Stainless steel and epoxy-coated surfaces are common choices.

3. Equipment Selection

Selecting the right equipment is a critical aspect of pharmaceutical facility design. The choice of equipment affects production efficiency, product quality, and compliance with regulatory standards. Key considerations include:

- **Scalability:** Equipment should be chosen with scalability in mind. As production volumes increase, the facility should be able to accommodate additional equipment or larger-scale machinery.
- **Automation:** Automation can significantly improve efficiency and reduce the risk of errors. Designers must plan for the integration of automated systems where appropriate.
- **Industry Standards:** Equipment must meet industry-specific standards, such as those outlined in the International Conference on Harmonisation (ICH) guidelines, to ensure product quality and compliance.

4. Material Flow and Layout

Efficient material flow and facility layout are essential for optimizing workflow and minimizing the risk of cross-contamination. Key considerations in this area include:

- **Process Flow:** Designers must carefully plan the flow of materials through the facility, from raw materials storage to finished product packaging. A well-thought-out process flow minimizes bottlenecks and delays.
- **Isolation:** Separating areas with different cleanliness requirements, such as raw materials storage and finished product packaging, is critical. Physical isolation prevents contamination.
- **Accessibility:** Accessibility for maintenance and cleaning is crucial. Equipment and workspaces should be designed to allow easy access for cleaning and maintenance personnel.

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- **Waste Management:** Efficient waste management systems are vital to handle both hazardous and non-hazardous waste generated during manufacturing. Compliance with disposal regulations is a priority.

5. HVAC Systems

Pharmaceutical manufacturing facilities rely on Heating, Ventilation, and Air Conditioning (HVAC) systems to maintain controlled environments. Proper HVAC design is essential for:

- **Temperature and Humidity Control:** Pharmaceuticals are sensitive to temperature and humidity fluctuations. HVAC systems must provide precise control to ensure product stability.
- **Air Filtration:** Air quality is critical in cleanrooms. HVAC systems incorporate HEPA and ULPA filters to remove particles and microorganisms from the air.
- **Pressure Differentials:** Controlling air pressure differentials between different areas of the facility is essential to prevent contaminants from entering cleanrooms or other critical zones.
- **Energy Efficiency:** Sustainable HVAC design can reduce energy consumption and environmental impact. Energy-efficient systems are increasingly important in pharmaceutical facility design.

6. Quality Control Laboratories

Quality control is integral to pharmaceutical manufacturing. Dedicated laboratories within the facility are responsible for testing and analyzing products to ensure their integrity. Key elements of these laboratories include:

- **Instrumentation:** High-precision instruments and equipment are used for testing, including spectrophotometers, chromatographs, and microbiological testing devices.
- **Testing Procedures:** Rigorous testing procedures are established to verify the quality and safety of pharmaceutical products. Documentation of these procedures is crucial for regulatory compliance.
- **Data Management:** Laboratories must have robust data management systems to track and store test results. This ensures traceability and facilitates regulatory audits.

7. Sustainability Initiatives

Pharmaceutical manufacturers are increasingly adopting sustainable practices to reduce their environmental impact. Sustainability initiatives in facility design may include:

- **Energy-Efficient Lighting:** The use of LED lighting and motion sensors to reduce energy consumption.

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- **Waste Reduction:** Implementing waste reduction measures, including recycling and waste-to-energy technologies.
- **Green Building Certifications:** Pursuing green building certifications such as LEED (Leadership in Energy and Environmental Design) to demonstrate commitment to sustainability.

8. Flexibility and Scalability

Pharmaceutical facility design must consider future growth and changes in production requirements. Flexibility and scalability are crucial to adapt to evolving pharmaceutical needs. Key considerations include:

- **Modular Design:** Facilities can be designed with modular components that allow for easy expansion or reconfiguration.
- **Adaptable Infrastructure:** Infrastructure should be adaptable to accommodate changes in equipment or production processes.
- **Regulatory Changes:** Design should also account for potential changes in regulatory requirements that may impact production methods or facility operations.

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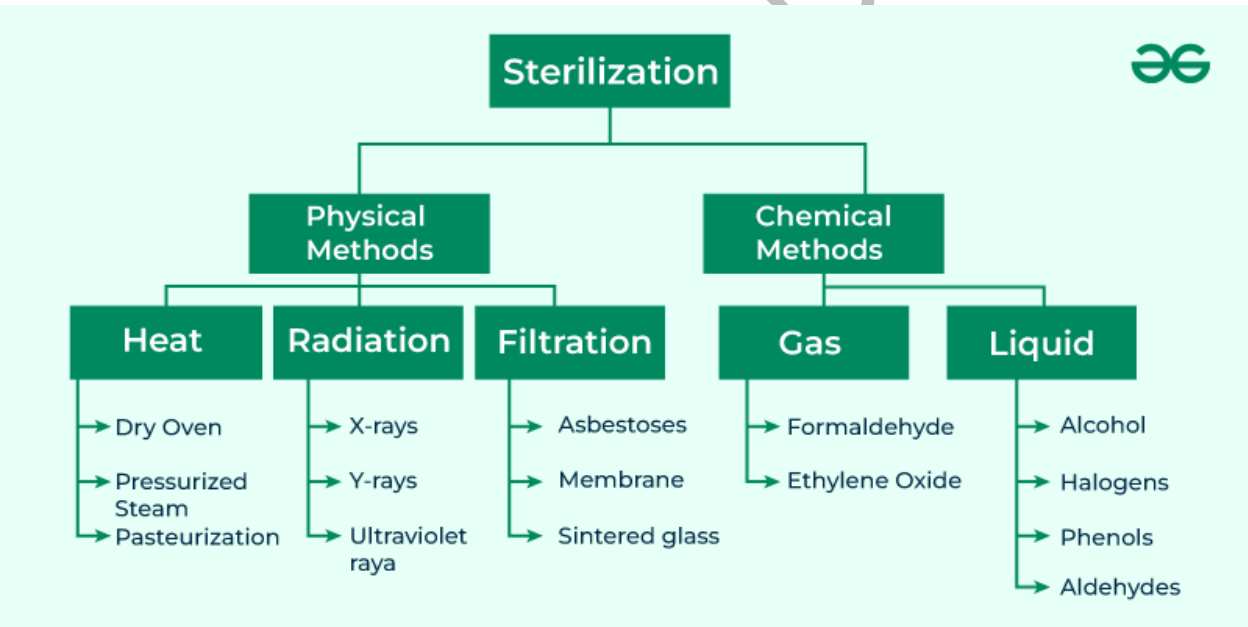
UNIT – II

Microbial contamination and spoilage of pharmaceutical products

Sterilization is an effective method, or a process used to kill all the vegetative spores and disease-causing microorganisms. Sterilization deactivates microorganisms including bacteria, spores, fungi, and unicellular and other biological agents that are present in food products or on any certain surface of the object. Sterilization can be achieved by various methods and sources including heat, chemicals, radiation, gases, and filtration. In this article, we will study sterilization, classification of sterilization, application and methods of sterilization in detail.

Sterilization

Sterilization is defined as the process of destruction or elimination of all pathogenic microorganisms and organisms capable of giving rise to infection such as all spores, bacteria, fungi, etc., all disease-causing. It can be achieved by both physical, chemical, and other effective methods that inhibit growth or are free from food products, fluids, objects, solid materials, food packaging materials, raw materials, and other different products.



Importance of Sterilization

Imprtance of sterilization are given below:

- Sterilization is used to prevent the transmission of certain pathogenic disease-causing microorganisms into the body.
- It helps in sterile products to prevent contamination.
- Sterilization is an important process in research development laboratories.

- It prevents the contamination of instruments and areas in the pharmaceutical industry.
- It is used in the preparation process of cultures and other microbiology experiments.
- The sterilization process is used in the food industries such as canning, and high-pressure methods.
- Sterilization is used for the preparation of sterile dosage forms and sterility testing.

Methods of Sterilization

In order to completely eradicate microorganisms, sterilization techniques involve classification of sterilization which includes physical and chemical methods such as autoclaving (pressurised steam), chemical sterilization using ethylene oxide or hydrogen peroxide, radiation sterilisation using gamma rays or electron beams, dry heat (hot air), filtration using small-pore filters, and plasma sterilization are all necessary (low-temperature plasma). Depending on the material sensitivity and particular sterilizing requirements, each technique is used.

Physical Sterilization

Physical sterilization is method of sterilization to destroy the microorganism by using physical methods like dry and wet heat, filteration and radiation. In these method wet or moist heat considerd to be the most effective method to sterilize the glasswares.

By Using Heat

The most effective and reliable method of the sterilization process. There are two methods by using heat-dry heat and moist heat.

Dry heat

- Flaming In this method heating instruments over the fire until they become hot in red. Instruments that are used such as point of forceps, Spatulas, inoculating loops, and Wires.
- Incineration It is a process that involves the combustion of organic substances contained in waste materials. Items such as contaminated cloth, animal carcasses, and pathological material. PVC and polythene can be dealt with.
- Hot air oven Hot air ovens are the electrical devices used for sterilization. The oven uses dry heat to sterilize items. Generally, they can be set at minimum to maximum temperature from 50˚C to 300˚C. The thermostat is present to control the temperature. This is the most widely used method of sterilization by dry heat.

Moist Heat

Moist heat can be categorized into 3 groups:

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- Temperature below 100 C Pasteurization of milk: This method is done by holding period at 63˚C for 30 minutes or 72˚C for 15-20 minutes followed by cooling. This method is not suitable for killing all spores.
- Temperature at 100 C Steam at atmospheric pressure is used to sterilize culture media. This is an inexpensive method. The principle of this method is first exposure kills vegetative bacteria and next exposure will kill vegetative bacteria that mature from the spore. It is intermittent sterilization by holding at a temperature of 100˚C for 20 minutes on three consecutive days.
- Temperature above 100 C An autoclave or steam sterilizer is an instrument that uses steam to sterilize equipment and other objects. This implies that all microscopic organisms, infections, parasites, and spores are inactivated. In any case, prions may not be annihilated via autoclaving at the regular 134˚C for 3 minutes or 121˚C for 15 minutes. It is suitable for the Items such as dressings, instruments, laboratory ware, media, and medical products.

Filtration

Filtration assists with eliminating microorganisms from heat-labile fluids such as sera and solutions of antibiotics. Its working principle as viruses go through the normal filter channels, filtration can be used to obtain bacteria-free filtrates of clinical samples for virus isolation.

Types of Filters

- Candle filters These filters are used for the purification of water for industrial and drinking purposes. These are made under various grades of porosity.
- Asbestos filters are disposable and single usage. They tend to alkalinize filtered fluids. Their usage is less because of their carcinogenic property.
- Sintered glass filters These filters have low absorptive properties. They are brittle and costly.
- Membrane filters These filters are made of cellulose esters or other polymers. They are usually used for water purification and analysis, sterility testing, and preparation of solutions.

Radiation

There are 2 types of radiation: Ionizing radiation & non-ionizing radiation

- Non-ionizing radiation In the non-ionizing method infrared is used for rapid mass sterilization of prepacked items such as syringes, and catheters. UV is used for disinfecting enclosed areas such as halls, operation theatres, and labs.

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- Ionizing radiation In ionizing radiation, gamma rays and x-rays are used for sterilizing plastics, syringes, swabs, catheters, animal feeds, cardboard, oils, and metal foils.

Chemical Sterilization

Chemical sterilization is the method of sterilization to make surface free from microorganisms using certain chemicals like, chlorine, formaldehyde and ethanol. These chemical can interfere with the machinery of the microoganisms to terminate them.

Using chemicals

- **Chemical agents:** The action of chemical agents are protein coagulation and Disruption of cell membrane resulting in exposure, damage, and loss of contents.
- **Chemical Alcohols:** Commonly used are Ethyl alcohol, and Isopropyl alcohol and must be utilized at concentrations of 60-90%. Isopropyl alcohol is used in the sanitization of the clinical thermometer. Methyl alcohol is viable against contagious spores, treating cabinets, and incubators. It is toxic and inflammable.
- **Aldehyde Formaldehyde:** It contains bactericidal and sporicidal and it has a great effect on viruses. It is used to preserve anatomical specimens and destroy anthrax spores on hair and wool
- **Phenols:** These are acquired from the distillation of coal tar between 170˚ to 270˚C. The deadly impacts include- it can cause cell membrane damage, releasing cell contents, and causing lysis.

Gases

Types of gases used for sterilization are:

- Ethylene oxide works because of its alkylating of the amino, carboxyl, hydroxyl, and sulfhydryl groups in protein molecules and furthermore on DNA and RNA. It very well may be utilized on instruments such as heart-lung machines, respirators, dental equipment, books, and clothing.
- Formaldehyde gas This is generally utilized for fumigation of operational theatres and other rooms in clinics. Formaldehyde is formed by the addition of 150g of KMnO4 to 280ml of formalin for each 1000cu.ft of room volume, in the wake of shutting the windows and different outlets. After fumigation, the doors ought to be sealed and left unopened for 48 hours.
- Beta propiolactone (BPL) is a result of ketene and formaldehyde with a boiling point of 163˚C. It has fat bactericidal activity yet is cancer-causing. It is equipped for killing all microorganisms and it is exceptionally dynamic against infections.

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Applications of Sterilization

In the Medical Industry and Surgeries

Normally all the surgical instruments and medications that are used for the treatments undergo contact with the aseptic part of the body. Therefore, they must be sterile. Examples of such instruments are scalpels, hypodermic needles, and artificial pacemakers.

Preparation of injectable medications and intravenous solutions for fluid replacement therapy requires sterility as well as all-around planned compartments to forestall sections of unusual agents after initial product sterilization.

Most clinical and surgical devices used in medical facilities are made of materials that can go under steam sterilization. Ethylene oxide gas has been used since the 1950s for heat- and moisture-sensitive medical devices. In the last few years, a number of various new, low-temperature sterilization systems have been developed and are being utilized to sterilize medical instruments.

Research and Laboratories

Many research facilities, laboratories, and their instruments are mainly dependent on the effective sterilization process.

Spoilage: Spoilage is chemical and physicochemical degradation of pharmaceutical products rendering it unsuitable for use. Spoilage is not desirable in pharmaceutical industry because deterioration of drugs and excipient occurs, as a result product may lose its quality and it may become ineffective. Entire batch may be required to be discarded. This may cost huge lose to the manufacturer. Moreover it may attract litigation from the consumers which may cause huge financial loss to the Company. Damaged products may damage reputation of the manufacturing company which may attract further financial loss. Microbial spoilage may present potential health hazards to the consumers like toxicity, infection or even death. Toxic metabolites may be produced due to microbial growth which may cause health hazards. Microbes may deteriorate drugs and thereby reduce potency of the medicament. There could be change in the appearance of the product like decolourisation, phase separation, and odour formation ete.

Following types of microbial spoilage occurs in pharmaceutical products.

1. Chemical spoilage.

2. Physicochemical spoilage

3. Biological spoilage.

Chemical Spoilage - Chemical spoilage means deterioration of chemical nature of drugs and excipients. Molecular structure of the ingredients may change. Thischange may affect physicochemical properties of the preparation. Potency of the drug may decrease. Further microbial growth may occur if chemical degradation of preservative occurs. Similarly chemical

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degradation of surfactants, organic polymers ete may damage specialised micro-environment for which they are used. Along with microbial contamination some - chemicals may also cause chemical spoilage like pesticide, disinfectants, bleaching agent, sanitizer etc. may add to chemical spoilage.

Physicochemical spoilage - This type of spoilage may change physico-chemical properties of product. Following types of changes may occur.

i. **Viable growth** - Viable growth of microbes may occur inside the container. These growth may be visible in the form of floating layers, turbidity, hemps, ete. Contamination of products by fungus species like Aspergillus sp. may cause this type spoilage. Some bacterial species may also show viable growth.

ii. **Gas production** -Some microbes may produce gas inside the containers. These gases may be visible in the form of bubbles, floccules etc. Contamination of products with bacteria like E. coli may produce gas if it contains sugars.

iii. **Colouration / Decolouration** - Some microbes may decolourise formulation or it may produce unique colour which is different from the normal colour of the product

iv. **Odour formation** - Microbial growth in the finished product may produce bad odour or characteristic odour. It may produce a characteristic rotten smell.

v. **Taste change** - Microbial spoilage may change the taste of the oral formulations. It may impart bitter or obnoxious taste to the oral formulation.

Biological Spoilage - Spoilage of pharmaceuticals may produce some undesirable and dangerous molecule which has undesirable biological effects. Some microbes may produce toxins, pyrogens or other harmful metabolites. These biomolecules may be present in the product from the very beginning. Spoilage may occur, although no microbial contamination was there. Factors affecting the microbial

Spoilage of the Pharmacuetical products.

There are so many factors which affects Microbial spoilage. Some of these factors reduce rate of spoilage where as some factors increases the rate of spoilage. These factors are related to nutritional requirement of micro-organisms, environment and nature of micro-organism. There factors must be studied to minimise the impact of spoilage.

Following factors affect the microbial spoilage of Pharmaceutical products.

i) Number of contaminating micro-organism - There may not be sufficient spoilage if number of contaminants are less. This low level it contaminants may not able to multiply due to design of the formulation. However during long storage these microbes may cause damage. If initial contamination is high then it may present an undesirable challenge. Products will be damaged quickly due to presence of high numbers of microbes. High level of contamination occurs due to lapses in manufacturing process or due to high loads of contaminants in raw materials.

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ii) Type of micro-organism -Some microorganisms are more aggressive than others. They can quickly multiply and spoilage is much faster. However, aggressive microbes may not multiply initially due to presence of preservative and other substances. Contamination of product with natural communities of non-aggressive micro-organism can facilitate growth of other aggressive contaminants.

iii) Presence of nutrients - In product Micro-organism can utilise formulation components as nutrients and utilise these components for biosynthesis and growth. Many formulations may contain crude animal, vegetable and microbial products which creates a conducive environment for microbial growth and subsequent spoilage. Additives like sugar, amino acid, polyhydric alcohol may act as microbial nutrients. Primary contaminants may produce metabolites which could be used by aggressive microbes as nutrient. Demineralised water which is prepare by ion-exchange resins may contain nutrients.

iv) Presence of water - Presence of water in formulation may promote microbial growth and subsequent spoilage. Uncomplexed water or free excess water promotes this spoilage. Presence of free water may be measured by water activity. It is a ratio between 'vapour pressure of formulation and vapour pressure of water in similar condition'. If water activity is 1 then it is conducive for microbial growth. Chances of microbial growth decreases with decrease in value of water activity. A value less than 0.88 is considered safe to prevent spoilage. However, some microbes can grow in extremely low water value condition. - Aspergillus glaucus can grow at a water activity value of 0.61.

v) Oxidation-reduction potential- Contaminating micro-organism may requires terminal electron accepter to facilitate functioning respiratory pathway. Presence of dissolved oxygen increases redox potential of the product thereby promote microbial growth and spoilage.

vi) Temperature -Storage temperature is a great controller of microbial growth and spoilage. However, spoilage may occur over a range -20oC to 60oC. Microbial growth and spoilage is less in low temperature and high temperature. Storing products at a cool place (8 oC to 12oC) may cause negligible spoilage. High temperature can also prevent spoilage. Water for injection is stored above 80oC before filling and sealing. So extreme temperature can minimise spoilage.

vii) **pH**- Microbes prefer neutral pH for their growth. However some microbes may prefer slightly acidic pH. Extremely acidic or alkaline pH of the formulation may prevent microbial growth and subsequent spoilage. Some communities of microbes can survive in extreme pH and change the pH, there by support growth of other micro-organism.

viii) Containers and packaging - Chances of contamination in single dose ampoules and vials are negligible. However multidose containers be contaminated by users themselves. Change in designs of these containers may minimize contamination and spoilage. Wide mouthed containers for creams and ointments are replaced by narrow mouthed tubes with screw cap closures. Multidose injections are stored in containers which has self sealing rubber wads. Containers are

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sealed such a way so that it can prevent entry of water and oxygen to minimise water activity and redox potential.

ix) Presence of protection materials - Some materials in formulation may protect microbes during sterilisation process. Polymers like gelatin, starch ete may increase microbial resistance to heat. Microbes may get adsorbed in particles and become more resistant to heat.

Sterile injectables are medications and other therapeutic products that are administered directly into the bloodstream or tissues of the body.

Unlike non-sterile drugs – like pills, gels, or aerosols – sterile injectables are bypassing the body's natural defenses. Delivering a product this way can be highly effective, but also increases the risk that any product contamination may pose to the patient.

Sterile injectables must therefore be free of microorganisms, particulates, or other contaminants that could harm a patient. Protecting that sterility requires a specialized manufacturing process that's far more technically complex than many other medical products.

The different types of injectables can be distinguished by their application:

- Intramuscular (IM) injections: Delivered into a muscle
- Subcutaneous injections: Delivered under the skin
- Intravenous injections (IV): Delivered directly into a vein
- Intrathecal injections: Delivered into the spinal canal
- Intraperitoneal injections: Delivered into the peritoneal cavity, the space that surrounds the abdominal organs

MANUFACTURING

There are two primary methods for manufacturing sterile injectables: terminal sterilization and aseptic fill-finish.

Terminal sterilization:

This approach relies on carefully controlled environments and procedures to reduce the risk of contamination during the manufacturing process, but actual sterilization occurs as a final step before the product is released. Typically, the product and container are sterilized by steam and/or gamma radiation.

The process is relatively faster than other options and more cost-efficient, as it requires less specialized knowledge, equipment and processes – but it can only be used with products and materials that can withstand temperatures up to 121 degrees Celsius or radiation exposure.

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Aseptic fill-finish:

This highly specialized process is used to produce the many sterile injectable products that cannot tolerate terminal sterilization. This means that full sterility must be maintained across all materials and packaging, process steps, and outputs throughout the entire manufacturing process.

That is critical because there is no final step to sterilize the drugs in their containers before shipping. Aseptic fill-finish requires advanced equipment and a deep understanding of the complex processes, testing methods, and regulatory standards this method requires.

To produce sterile injectables, there are five key components that must be carefully controlled from start to finish:

- **Sterile raw materials:** All components used to formulate the drug product must undergo rigorous testing to confirm their purity and potency.
- **Sterile compounding:** The process for generating the active pharmaceutical ingredient (API) must be carefully defined and completely sterile.
- **Sterile containers and closures:** The product itself must be placed in contaminant-free injection systems with sterile closures.
- **Sterile filling**: The product must be placed in its container in a cleanroom environment that excludes all potential contaminants, using a detailed process that also helps avoid contamination.
- **Sterile packaging:** If a product will be placed in some form of packaging (e.g., carton, blister, delivery device, etc.), this step of the manufacturing process must also be designed to prevent the filled container or its contents from being compromised.

NON-INJECTABLE STERILE FLUIDS

There are many other types of solution in a sterile form, for use particularly in hospitals.

A) Non-Injectable Water

This is sterile water, not necessarily of injectable water standards, which is used widely during surgical procedures for wound irrigation, moistening of tissues, washing of surgeons's gloves and instruments during use and, when warmed, as a haemostat. Isotonic saline may also be used. Topical water (as it is often called) is prepared in 500 ml and 1 l polyethylene or polypropylene containers with a wide neck and tear-off cap to allow for ease of pouring.

B) Urological (Bladder) Irrigation Solutions

These are used for rinsing of the urinary tract to aid tissue integrity and cleanliness during or after surgery. Either water or glycine solution is used, the latter eliminating the risk of intravascular haemolysis when electrosurgical instruments are used. These are sterile solutions produced in collapsible or semirigid plastic containers of up to 3 L capacity.

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C) Peritoneal Dialysis And Haemodialysis Solutions

Peritoneal dialysis solutions are admitted into the peritoneal cavity as a means of removing accumulated waste or toxic products following renal failure or poisoning. They contain electrolytes and glucose (1.4–7% w/v) to provide a solution equivalent to potassium-free extracellular fluid; lactate or acetate is added as a source of bicarbonate ions. Slightly hypertonic solutions are usually employed to avoid increasing the water content of the intravascular compartment. A more hypertonic solution containing a higher glucose concentration is used to achieve a more rapid removal of water. In fact, the peritoneal cavity behaves as if it were separated from the body organs by a semipermeable membrane. Warm peritoneal solution (up to 5 L) is perfused into the cavity for 30–90 minutes and then drained out completely. This procedure can then be repeated as often as required. As the procedure requires larger volumes, these fluids are commonly packed in 2.5 L containers. It is not uncommon to add drugs (for instance potassium chloride or heparin) to the fluid prior to use.

Haemodialysis is the process of circulating a patient's blood through a machine via tubing composed of a semipermeable material such that waste products permeate into the dialysing fluid and the blood then returns to the patient. Haemodialysis solutions need not be sterile but must be free from heavy bacterial contamination.

D) Inhaler Solutions

In cases of severe asthmatic attacks, bronchodilators and steroids for direct delivery to the lungs may be needed in large doses. This is achieved by direct inhalation via a nebulizer device; this converts a liquid into a mist or fine spray. The drug is diluted in small volumes of Water for Injections before loading into the reservoir of the machine. This vehicle must be sterile and preservative-free and is therefore prepared as a terminally sterilized unit dose in polyethylene nebules.

OPTHALMIC PREPARATIONS

Eye Drops

Some typical excipients for eye drops are given in Table 22.1. Eye drops are presented for use in (1) sterile single-dose plastic sachets (often termed Minims) containing 0.3–0.5 ml of liquid, (2) multiple-dose amber fluted eye dropper bottles including the rubber teat as part of the closed container or supplied separately, or (3) plastic bottles with integral dropper. A breakable seal indicates that the dropper or cap has not been removed prior to initial use. Although a standard design of bottle is used in hospitals, many proprietary products are manufactured in plastic bottles designed to improve safety and care of use. The maximum volume in each container is limited to 10 ml. Because of the likelihood of microbial contamination of eye dropper bottles during use (arising from repeated opening or contact of the dropper with infected eye tissue or the hands of the patient), it is essential to protect the product with a preservative (Matthews & Skinner, 2006). Eye drops for surgical theatre use should be supplied in single-dose containers.

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Examples of preservatives are phenylmercuric nitrate or acetate (0.002% w/v), chlorhexidine acetate (0.01% w/v), thiomersal (0.01% w/v) and benzalkonium chloride (0.01% w/v). Chlorocresol is too toxic to the corneal epithelium, but 8-hydroxyquinoline and thiomersal may be used in specific instances. The principal consideration in relation to antimicrobial properties is the activity of the bactericide against Pseudomonas aeruginosa, a major source of serious nosocomial eye infections. There is some concern over the toxicity of mercurials, and their use is becoming less common. Although benzalkonium chloride is probably the most active of the recommended preservatives, it cannot always be used because of its incompatibility with many compounds commonly used to treat eye diseases, nor should it be used to preserve eye drops containing anaesthetics. As benzalkonium chloride reacts with natural rubbers, silicone or butyl rubber teats should be substituted and products should not be stored for more than 3 months after manufacture because silicone rubber is permeable to water vapour. As with all rubber components, the rubber teat should be pre-equilibrated with the preservative before use. Thermostable eye drops and lotions are sterilized at 121°C for 15 minutes. For thermolabile drugs, filtration sterilization followed by aseptic filling into sterile containers is necessary. Eye drops in plastic bottles are prepared aseptically.

In order to lessen the risk of eye drops becoming heavily contaminated, either by repeated inoculation or by the growth of resistant organisms in the solution, use is restricted to 1 month after the container is first opened. This is usually reduced to 7 days for hospital ward use on one eye of a single patient. The period is shorter in the hospital environment because of the greater danger of contamination by potential pathogens, particularly pseudomonads.

B) Eye Lotions

Eye lotions are isotonic solutions used for washing or bathing the eyes. They are sterilized by autoclaving in relatively large-volume containers (100 ml or greater) of coloured fluted glass with a rubber closure and screw-cap, or packed in plastic containers with a screw-cap or tear-off seal. They may contain a preservative if intended for intermittent domiciliary use for up to 7 days. If intended for first aid or similar purposes, however, no bactericide is included and any remaining solution is discarded after 24 hours.

C) Eye Ointments

Eye ointments are prepared in a semisolid base—e.g. Simple Eye Ointment BP, which consists of yellow soft paraffin (8 parts), liquid paraffin (1 part) and wool fat (1 part). The base is filtered when molten to remove particles and sterilized at 160°C for 2 hours. The drug is incorporated prior to sterilization if heat-stable, or added aseptically to the sterile base. Finally the product is aseptically packed in clear sterile aluminium or plastic tubes. As the product contains virtually no water, the danger of bacteria proliferating in the ointment is negligible.

D) Contact Lens Solutions

Most contact lenses are worn for optical reasons as an alternative to spectacles. Contact lenses are of two types: hard lenses, which are hydrophobic, and soft lenses, which may be either

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hydrophilic or hydrophobic. The surfaces of lenses must be wetted before use and wetting solutions are used for this purpose. Hard, and more especially, soft lenses become heavily contaminated with protein material during use and therefore must be cleaned before disinfection. Contact lenses are potential sources of eye infection and, consequently, microorganisms should be removed before the lens is again inserted into the eye. Lenses must also be clean and easily wet table by lachrymal secretions. Contact lens solutions are thus sterile solutions of the various types described below. Apart from achieving their stated functions, either singly or in combination, all solutions must be non-irritating or must protect against microbial contamination during use and storage.

i) Wetting solutions

These are used to hydrate the surfaces of hard lenses after disinfection. As they must also cope with chance contamination, they must contain a preservative as well as a wetting agent. They may be isotonic with lachrymal secretions and be formulated to a pH of about 7.2 for compatibility with normal tears.

ii) Cleaning solutions

These are responsible for the removal of ocular debris and protein deposits, and contain a cleaning agent that consists of a surfactant and/or an enzyme product. As they must also cope with chance contamination, they contain a preservative, are isotonic and have a pH of about 7.2.

iii) Soaking solutions

These are solutions for disinfection of lenses but also maintain the lenses in a hydrated state. The antimicrobial agents used for disinfecting hard lenses are those used in eye drops (benzalkonium, chlorhexidine, phenylmercuric acetate or nitrate, thiomersal and chlorbutol). Ethylenediamine tetraacetic acid (EDTA) is usually present as a synergist. Benzalkonium chloride and chlorbutol are strongly bound to hydrophilic soft contact lenses and therefore cannot be used in storage solutions for these; chlorhexidine and thiomersal are usually employed. It must be added that the concentrations of all preservatives used in contact lens solutions are lower than those employed in eye drops, to minimize irritancy. Hydrogen peroxide is becoming commonly used but must be inactivated before the lenses are inserted onto the eyes. Finally, heat may be utilized as an alternative method to disinfect soft contact lenses, especially the hydrophilic types. Lenses are boiled in isotonic saline.

IMPLANTS

Implants are small, sterile cylinders of drug, inserted beneath the skin or into muscle tissue to provide slow absorption and prolonged action therapy. This isprincipally based on the fact that su ch drugs, invariably hormones, are almost insoluble in water and yet theimplant provides a rate of dissolution sufficient for a therapeutic effect. Implants are manufactured from the pure drug mad e into tablet form by compression orfusion. No other ingredient can be included because this may be insoluble or toxic, or, most importantly, may influence the rate of drug release. Copol

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ymers such as polylactic acid/polyglycolic acid may be used as the implant matrix to provide a controlled rate of drug delivery.

Compression of sterile drugs must be conducted under aseptic conditions using sterile machine pa rts and materials. After manufacture, the outer surface of theimplant is sterilized by immersion in 0.002% w/v phenylmercuric nitrate at 75 °C for 12 hours.

After thesurface has been dried, each implant is placed aseptically intosterile glass vial with a cott on wool plug at both ends. This prevents damage and reduces the risk of glass specules, formed w hen the vial is opened, adhering to theimplant. This compression process is not ideal and fusion pr ocesses may be used provided that the drug is heatstable. The pure drug is melted at 50 °C above i ts melting temperature and poured into moulds. The interior of the implant will be automatically s terilized by thisprocess if the melting temperature is high enough. It isalso possible to dry heat ste rilize the implant after packaging provided that the melting temperature is above 160 °C. Clearly, it is easier to manufacture sterile implants by fusion as the process does not require presterilized ingredients or aseptic processing. The implant hardness is also very convenient.

ABSORBABLE HAEMOSTATS

The reduction of blood loss during or after surgical procedures where suturing or ligature is either impracticalor impossible can often be accomplished by the use ofsterile, absorbable haemostats. These consist of a softpad of solid material packed around and over the wound that can be left in situ and absorbed by body tissues over a period of time, usually up to 6 weeks. The principal mechanism of action of these is their ability toencourage platelet fracture because o f their fibrous or rough surfaces, and to act as a matrix for complete blood clotting. Four products commonly used are oxidized cellulose, absorbable gelatine sponge, and human fibrin foam and calcium alginate.

A) Oxidized Cellulose

This consists of cellulose material that has been partially oxidized. White gauze is the most commonform,although lint is also used. It can be absorbed by the bodyin 27 weeks, depending on the size. Its action is basedprincipally on a mechanical effect and it is used in the dry state. As it in activates thrombin, its activity cannot be enhanced by thrombin incorporation.

B) Absorbable Gelatin Foam

This insoluble foam is produced by whisking warm gelatin solution to form a uniform foam, which is thendried. It can be cut into suitable shapes, packed in metalor paper containers and sterilized b y dry heat (150 °C for 1 hour). Moist heat destroys the physical properties of the material. Immedi ately before use, it can be moistenedwith normal saline containing thrombin. It behaves as a mechanical haemostat, providing the framework on which blood clotting can occur.

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C) Human Fibrin Foam

This is a dry sponge of human fibrin prepared by clotting a foam of human fibrinogen solution with human thrombin. It is then freezedried, cut into shapesand sterilized by dry heat at 130 °C fo r 3 hours. Before use it is saturated with thrombin solution. Blood coagulation occurs in contact wi th the thrombin in the interstices of the foam.

D) Calcium Alginate

This is composed of the sodium and calcium salts of alginic acid formed into a powder of fibrous m aterial and sterilized by autoclaving. It aids clotting by forming a sodium–calcium alginate complex in contact with tissue fluids, acting principally as a mechanical haemostat. It is relatively slowly absorbed and some residues may occasionally remain in the tissues.

SURGICAL LIGATURES AND SUTURES

The use of strands of material to tie off blood or othervessels (ligature) and to stitch wounds (sutu re) is an essential part of surgery. Both absorbable and nonabsorbable materials are available for t his purpose.

A) Sterilized Surgical Catgut

This consists of absorbable strands of collagen derivedfrom mammalian tissue, particularly (despit e its name) the intestines of sheep. Because of its source, it is particularly prone to bacterial conta mination, and even anaerobic spores may be found in such material.

Sterilization istherefore a particularly difficult process. As collagen isconverted to gelatin when ex posed to moist heat, autoclaving cannot be used. The official method is to pack the 'plain' catgut s trands (up to 350 cm in length) on a metal spindle in a glass or other suitable container with a tubi ng fluid, the purpose of which is to maintain both flexibility and tensile strength after sterilization. Probably the most suitable method is to expose the material to gamma radiation. There is minima l loss of tensile strength and the container can be overwrapped before sterilization to provide a sterile container surface for opening aseptically.

The alternative method involves placing the coiled suture immersed in a tubing fluid (commonly 9 5% ethyl alcohol with or without 0.002% w/v phenylmercuric nitrate) and storing for sufficient time to ensures terilization. The outer surface of the vial must be sterilized before opening to avoid contamination of the suture when removed. Therefore the vial is immersed in 1% w/v for maldehydein ethanol before use. It cannot be heated. A nonofficialmethod of sterilization is to im merse the catgut in a no aqueous solvent (naphthalene or toluene) and heat at 160 °C for 2 hours. The catgut becomes hard and brittleduring the process, and is aseptically transferred to anaqueous tubing fluid to restore its flexibility and tensile strength.

Catgut is packed in single threads up to 350 cm in length of various thicknesses related to tensile strength in single use glass or plastic containers that cannot be resealed after use. Any remaining material should be discarded. Hardened catgut is prepared by treating st

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rands with certain agents to prolong resistance to digestion. If hardened with chromium compounds, the material is known as chromicized catgut.

B) Non-Absorbable Types

Sutures and ligatures are also made from many materials not absorbed by the body tissues. These consist of uniform strands of metal or organic material that will not cause any tissue reactions and are capable of being sterilized. Depending on the physical stability of each material, they are preferably sterilized by autoclaving or gamma radiation. They are packed in single-dose sachets, either dry or surrounded by a preserving fluid with or without a bactericide. The different materials are described in the British Pharmacopoeia (2010); they include linen (adversely affected by gamma rays), nylon (either monofilament or plaited), silk and polypropylene

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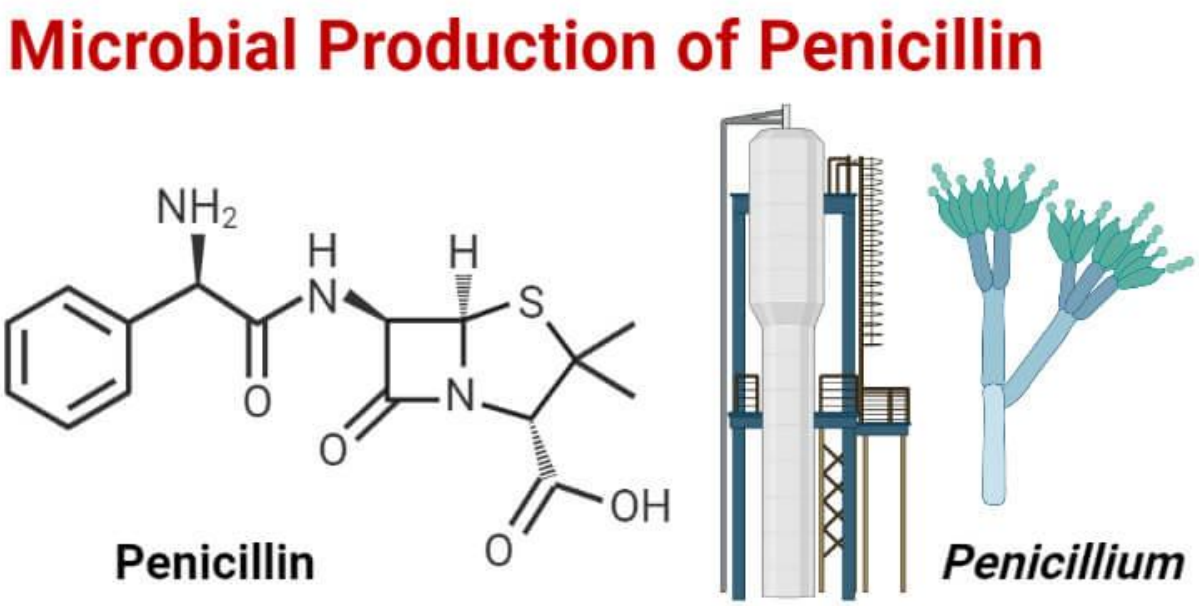
UNIT - III

PENICILLIN

Alexander Fleming in September 1928 accidentally discovered Penicillin. He found that the fungus, Penicillium notatum prevented the growth of bacteria, Staphylococcus spp.. Later Clutterbuck and his colleagues in 1932 studied the nature of Penicillin and found it as an organic acid that dissolves into the organic solvent at low PH. Chain et al in 1940 cultured fungus and extracted powdered form of Penicillin. Later, at the time of the Second World War, Penicillin production was done and an adequate amount was produced to treat wounded people. P. notatum gave poor results so, other species of Penicillium were tested.

As compared, P. chrysogenum NRRL 1951 gave good results which were induced by UV and other mutagenic chemicals. These selected strains produced a huge amount of Penicillin and inhibit the growth of the Oxford strain of Staphylococcus aureus. Czapek-Dox broth was used for the culture of P. notatum. Later, casein, beef extract were added for the better yield of penicillin which will be an aid in production. In 1949 chemically produced mediums like phenylacetic acid ethyl amine etc by maintaining the PH and addition of buffering agents like Calcium carbonate and also maintain the temperature.



Types of Penicillin

Two types of Penicillin are Penicillin G (Benzyl Penicillin) and Penicillin F. Penicillin F is also known as Phentenyl Penicillin. Natural Penicillin are obtained as sodium or potassium salts. These classes of antibiotics are used in treating both Gram-positive and Gram-negative infections. Penicillin G are a narrow-spectrum antibiotic. Examples of Penicillins are Ampicillin, Cloxacillin, Oxacillin, Piperacillin, etc.

Chemical structure of the Penicillin core

The structure of Penicillin includes a 4-membered β-lactam ring and thiazolidine ring. B-lactam ring contains an amide bond that is broken in an acidic and alkaline medium and that bond is hydrolyzed by beta-lactamase which is synthesized by many bacteria. Naturally occurring penicillins have different structure which is separated by R groups. The basic structure attaches to the N-acyl group in the substituted amino group. Mainly, Penicillin is categorized as natural and semisynthetic.

Biosynthesis of Penicillin

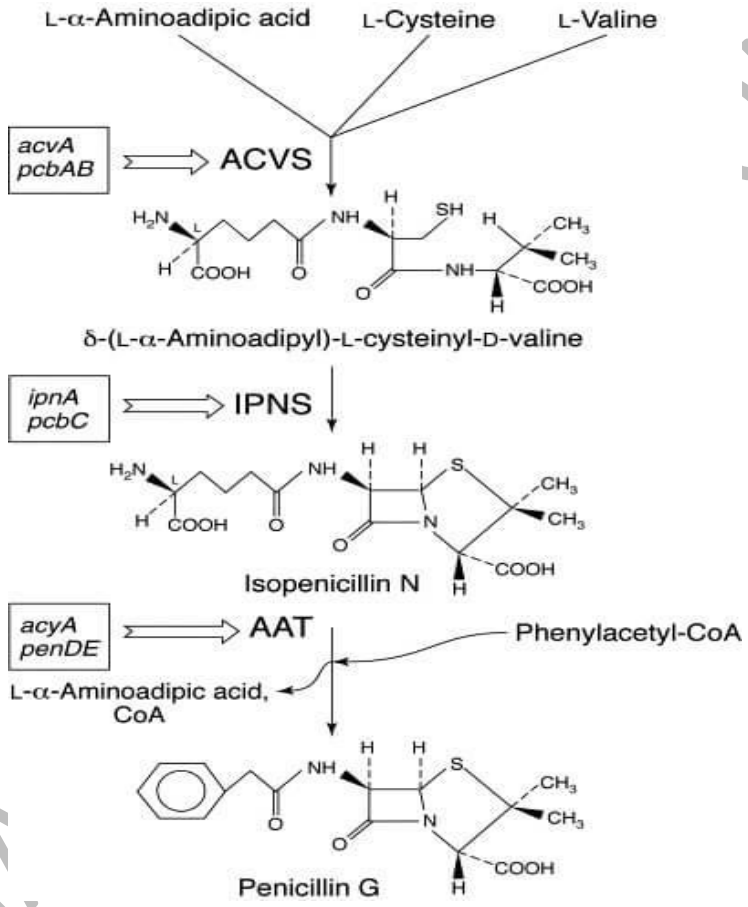


Figure: Biosynthesis of penicillin G.

Penicillins yield is done commercially by using P.chrysogenum. Although the fungus was found earlier in 1928, these biosynthesis processes were concluded later. Penicillin biosynthesis is described into three main steps; catalytic step, oxidative, and exchange of different chains.

1. The catalytic step involves an ACV synthetase enzyme that condenses the lateral chain of cysteine, valine, and alpha aminoadipate into tripeptide ACV.

2. In the second step, tripeptide ACV forms a bicyclic ring by oxidative ring closure. Isopenicillin N synthase is involved resulting in isopenicillin N which is a bioactive intermediate in the pathway.

3. The third step involves the exchange of L-aminoadipate. Acyl-CoA synthetase and Acyl-CoA racemase, a two enzyme system is involved that helps in converting isopenicillin N into Penicillin N.

Figure: Penicillin biosynthesis. Image Source: Cacycle.

Penicillin Production Process

Penicillin production is done by fermentation process in a fermenter by agitating the culture of P.chrysogenum in a suitable condition. The whole process carried out is aerobic and the method involved is fed-batch.

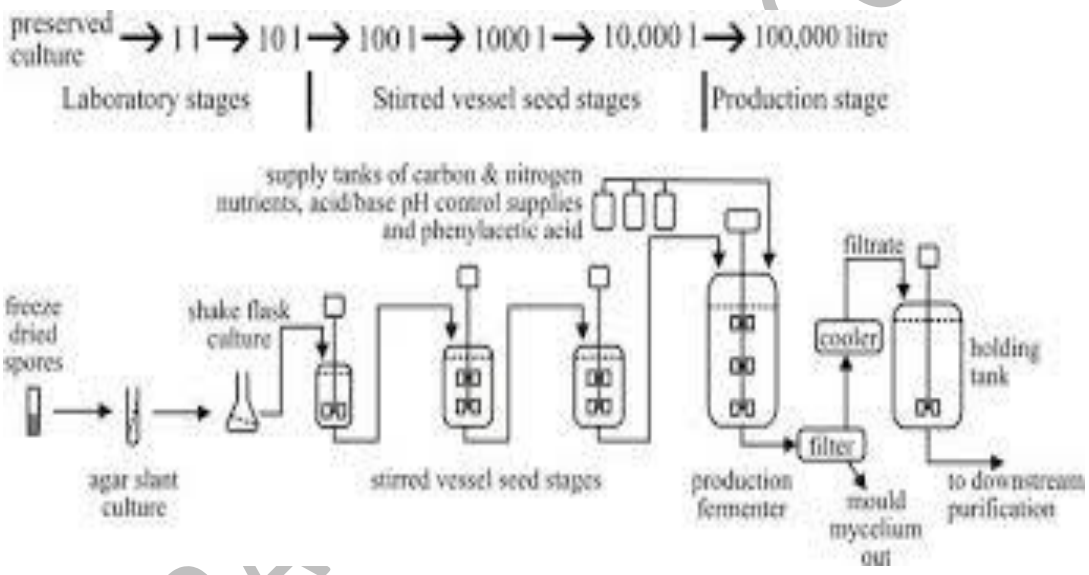


Figure: Outline diagram of current penicillin production by fed-batch submerged fermentation. The top panel shows the procedure for inoculum development. Image Source: David Moore.

This fermentation process is of Penicillin G which involves the following steps:

1. 100ml medium with spores of P.chrysogenum strains is inoculated in Erlenmeyer flask and is incubated at BOD incubator by placing them on a rotatory shaker.

2. After 4 days of incubation, the content along with two liters of medium is transferred into a flask that contains four liters and again incubates for two days.

3. Then, the content is transferred into a stainless tank containing 500 ml of the medium that provides suitable conditions for fungal growth.

4. After three days of incubation, the content is used for inoculation and kept in a fermentor that is well equipped with optimum conditions.

5. The content is filtered after six days of incubation which contains penicillin.
6. The penicillin is extracted into amyl or butyl acetate and is transferred into an aqueous solution with phosphate buffer.
7. Acidify the extract and again re-extract penicillin into butyl acetate
8. In the solvent extract potassium acetated is added to a crystallization tank to crystallize as a potassium salt.
9. Crystals were recovered and further sterilization of salt is done.

Application/Uses of Penicillin

Used in treating infections caused by both Gram-positive and Gram-negative bacteria like respiratory tract infections, throat, mouth, gum, and urine infections and also used in treating bacterial endocarditis.

TETRACYCLINE

Tetracycline is an important medicine that was found by accident in the 1940s. It's like a superhero antibiotic that fights many types of bacterial infections, like ones you can get from having sex, bladder problems, or breathing issues. Tetracycline works by stopping bacteria from making proteins, and it can fight both weak and strong bacteria. In this article, we'll learn more about tetracycline production, including its history, what it's made of, how it's used in medicine, and how it helps us fight diseases caused by bacteria. We'll also explore how it's made and how it works inside our bodies, showing why it's still important today.

Introduction of tetracycline

A crucial role in the development of medicine has been played by the broad-spectrum antibiotic tetracycline. Its discovery in the 1940s completely changed how bacteria were treated for a variety of bacterial diseases, including sexually transmitted infections, urinary tract infections, and lung infections.

General structure of tetracycline

Tetracycline is a member of a class of antibiotics that prevents the synthesis of bacterial proteins, making it effective against both Gram-positive and Gram-negative bacteria.

Tetracycline has good oral absorption and low toxicity. It needs a large dosage and is bacteriostatic. Treatment for shigellosis, salmonellosis, typhoid fever, brucellosis, etc. involves the administration of this antibiotic.

Additionally, it is utilized in feed to get rid of parasites (and aid animals in gaining weight). The antibiotic is also used to preserve fish (tetracycline is used to disinfect the ice in which the fish is kept).

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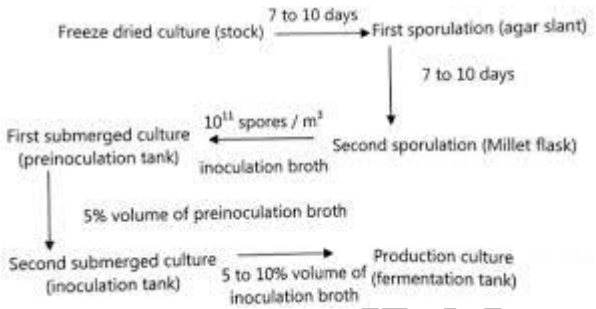
Biosynthesis of tetracycline

Biosynthesis of tetracyclines, particularly in the latter stages, has been studied with the use of mutant cultures. The involvement of pretetronid intermediates in the synthesis has been established. Tetracyclines are created from these intermediates by the bacterium Streptomyces aureofaciens.

Production of tetracycline

Both chemical and microbiologic production methods can be used to make tetracycline. All tetracyclines are produced by the same types of microbes. However, compared to other tetracyclines, chlortetracycline synthesis is much simpler. Tetracycline production is particularly sensitive to the amount of chloride in the medium because it triggers the formation of chlortetracycline rather than tetracycline.



Tetracycline antibiotics are produced by a complex fermentation process involving microbes, usually strains of the bacterium Streptomyces. Here's a simplified overview of the production process:

Microorganism Selection:

For cultivation, Streptomyces bacterial strains that can produce tetracycline compounds are isolated and selected.

Inoculation:

In a laboratory, a selected strain of Streptomyces is cultured on an agar medium that is rich in nutrients. The inoculum for the fermentation process is used once a pure culture has been obtained.

During inoculum build-up, the organism remains in the shake-flask for 24 hrs at 28°C. The most effective propagator makes use of the same kind of medium. Propagation is carried out for 19–24 hours at a pH of 5.2–6.2 with an addition of about 5% inoculum.

From the final propagator, 2-10% of the inoculum is transferred to the main fermenter. The nominal capacity of the fermenter is between 25,000 and 75,000 liters. Sterilized medium is used for fermentation (121°C for 1-2 hours).

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Fermentation:

The Streptomyces culture is grown in large-scale fermentation tanks, commonly referred to as bioreactors. The temperature, pH, and aeration are all controlled in these tanks. The bacteria are raised in a nutritional broth that also includes additional essential nutrients like carbohydrates and amino acids.

At 28°C, the primary fermentation lasts 60–65 hours. The pH is between 5.8 and 6. It is a fermentation that is submerged that needs aeration at a rate of 0.5-2 vol/vol/min.

Mechanical agitators are used for agitation. As an antifoam, lard is employed. Since glucose inhibits catabolism, it is typically not employed in the primary fermentation. Around 15000 units per milliliter of output.

Tetracycline production:

Tetracycline compounds are produced by the Streptomyces bacterium as secondary metabolites during the fermentation process. Depending on the strain and conditions, the precise chemical composition of the tetracycline generated can change.

Harvesting:

The fermentation broth is removed out of the process once enough tetracycline has been produced. Usually, the broth also contains tetracycline in addition to additional components.

Purification:

To separate the tetracycline component from contaminants and other byproducts, the recovered broth passes through a number of purification processes. These procedures frequently involve chromatography, precipitation, and filtering.

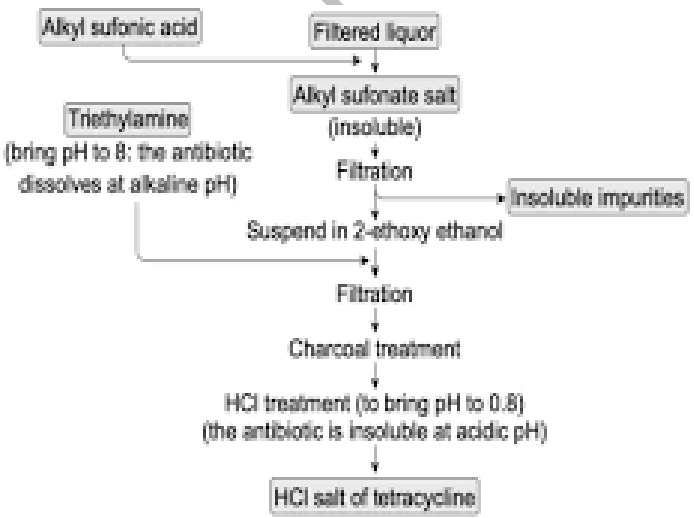

image: Purification of Chlortetracycline

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Mode of action of tetracycline

Numerous enzymatic reactions necessary for the survival of bacterial cells are inhibited by tetracyclines. The synthesis of proteins is the biological process that is hindered most delicately.

Tetracyclines prevent the aminoacyl tRNA from binding to the RNA-ribosome complex by specifically binding to the bacteria's 30S ribosome.

It simultaneously inhibits other steps of protein biosynthesis Tetracycline can also change the cytoplasmic membrane, which leads to the leakage of nucleotides and other substances from the cell. Instead of destroying the germs directly, this inhibits them.

AMPHOTERICIN

Amphotericin B is an antifungal medication used for serious fungal infections and leishmaniasis. The fungal infections it is used to treat include mucormycosis, aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, and cryptococcosis. For certain infections it is given with flucytosine. It is typically given intravenously.

Biosynthesis

The natural route to synthesis includes polyketide synthase (PKS) components.The carbon chains of amphotericin B are assembled from sixteen 'C2' acetate and three 'C3' propionate units by polyketide syntheses. Polyketide biosynthesis begins with the decarboxylative condensation of a dicarboxylic acid extender unit with a starter acyl unit to form a β-ketoacyl intermediate. A series of Claisen reactions constructs the growing chain. The extender units are loaded onto the current ACP domain by acetyl transferase (AT) within each module. The ACP-bound elongation group reacts in a Claisen condensation with the KS-bound polyketide chain. Ketoreductase (KR), dehydratase (DH), and enoyl reductase (ER) enzymes may also be present to form alcohol, double bonds, or single bonds After cyclisation, the macrolactone core undergoes further modification by hydroxylation, methylation and glycosylation.

Production

1. Microbial Fermentation Process

The commercial production of Amphotericin B (AmB) primarily follows these industrial stages:

- **Microorganism:** Strains of Streptomyces no dosus are used. High-yield mutants like ZJB2016050 are often preferred over wild-type strains to achieve higher titers.
- **Media Composition:** Fermentation media typically include glucose as a carbon source, cottonseed meal or soybean flour as nitrogen sources, and calcium carbonate to stabilize pH.
- **Fermentation Conditions:** The process is conducted in large bioreactors (up to 50 tons) at approximately 26°C with a maintained pH of 6.6–7.0.

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- **Oxygen Control:** Adequate oxygen is critical; genetic modifications such as overexpressing the Vitreoscilla hemoglobin (vhb) gene can improve yields under oxygen-limited conditions.

2. Yield Optimization & By-product Reduction

Industrialization focuses on maximizing the desired Amphotericin B while minimizing the less effective by-product Amphotericin A (AmA):

- **Precursor Feeding:** Adding metabolites like pyruvate, alanine, or isopropanol at specific times (usually 24 hours into fermentation) can significantly boost AmB production.
- **Genetic Engineering:** Modern strategies include knocking out competing metabolic pathways (such as the PKS5 gene cluster) or overexpressing transporter genes (amphG, amphH) to improve drug export from the cell.
- **Protein Synthesis Inhibitors:** Adding very low concentrations of inhibitors like chloramphenicol or streptomycin can selectively suppress the synthesis of Amphotericin A without affecting AmB yield.

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3. Extraction & Purification

After fermentation, the drug must be recovered from the bacterial mycelium:

- **Extraction:** The wet mycelium is harvested and extracted using organic solvents such as methanol or DMSO.
- **Crystallization:** The extract is purified through ceramic membranes, and the pH is adjusted (to approximately 6.0) to induce crystallization at 25°C.
- **Washing:** The resulting crystals are washed with methanol and dried to reach pharmaceutical-grade purity.

4. Advanced Pharmaceutical Formulations

Due to its high toxicity and low water solubility, raw Amphotericin B is processed into specialized delivery systems:

- **Liposomal (e.g., AmBisome):** The drug is intercalated into lipid bilayers (phospholipids and cholesterol), which significantly reduces kidney toxicity.
- **Lipid Complexes (e.g., Abelcet):** Large, ribbon-like structures formed by mixing AmB with specific lipids.
- **Colloidal Dispersions:** Microscopic "micellar" structures that help maintain the drug in an injectable

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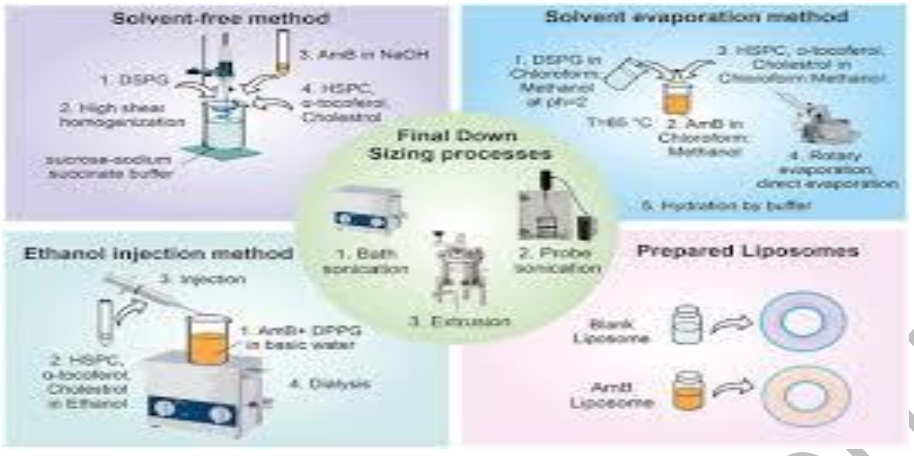

STREPTOKINASE

Streptokinase is a thrombolytic medication activating plasminogen by nonenzymatic mechanism As a medication it is used to break down clots in some cases of myocardial infarction (heart attack), pulmonary embolism, and arterial thromboembolism. The type of heart attack it is used in is an ST elevation myocardial infarction (STEMI). It is given by injection into a vein.

Side effects include nausea, bleeding, low blood pressure, and allergic reactions. A second use in a person's lifetime is not recommended.. While no harm has been found with use in pregnancy, it has not been well studied in this group. Streptokinase is in the antithrombotic family of medications and works by turning on the fibrinolytic system.

Streptokinase was discovered in 1933 from beta-hemolytic streptococci. It is on the World Health Organization's List of Essential Medicines

Mechanism of action

Streptokinase belongs to a group of medications known as fibrinolytics, and complexes of streptokinase with human plasminogen can hydrolytically activate other unbound plasminogen by activating through bond cleavage to produce plasmin. There are three domains to streptokinase, denoted α (residues 1–150), β (residues 151–287), and γ (residues 288–414). Each domain binds plasminogen, although none can activate plasminogen independently.[10]

Plasmin is produced in the blood to break down fibrin, the major constituent of blood thrombi, thereby dissolving clots once they have fulfilled their purpose of stopping bleeding. Extra production of plasmin caused by streptokinase breaks down unwanted blood clots, for example, in the lungs (pulmonary embolism). The usual activation of plasminogen is by proteolysis of the Arg561—Val562 bond.[11] The amino group of Val562 then forms a salt-bridge with Asp740, which triggers a conformational change producing the active protease plasmin. When

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streptokinase is present, it binds to plasminogen to form a complex (streptokinase·plasminogen) that converts substrate plasminogen to plasmin.

Residues 1–59 of streptokinase regulate its capacity to induce an active site in bound plasminogen by a nonproteolytic mechanism and to activate substrate plasminogen in a fibrin-independent manner. This complex subsequently rearranges to an active complex although the Arg561–Val562 bond remains intact. Therefore, another residue must substitute for the free amino group of Val562 and provide a counterion for Asp740 in this active complex.[12] Two candidates for this counterion have been suggested: Ile1 of streptokinase and Lys698 of plasminogen.

Deletion of Ile1 of streptokinase markedly inhibits its capacity to induce an active site in plasminogen, which supports the hypothesis that establishment of a salt bridge between Ile1 of streptokinase and Asp740 of plasminogen is necessary for streptokinase to induce an active site in plasminogen by a nonproteolytic mechanism.[13] In contrast with the Ile1 substitutions, the Lys698 mutations also decreased the dissociation constant of the streptokinase complex by 15 to 50 fold. These observations suggest that Lys698 is involved in formation of the initial streptokinase·plasminogen complex.

Biology

Streptokinase is naturally produced by Streptococci spp. bacteria, which use this enzyme to break up blood clots so that they can spread from the initial site of infection. It can also activate fibrin.

It is similar, both in function and in structure, to staphylokinase (Sak) found in Staphylococcus aureus. Staphylokinase is considered a virulence factor, although its presence after the establishment of infection actually decreases disease severity. Both enzymes are carried by phages.

Streptodornase

Streptodornase (SD) is an enzyme, specifically a deoxyribonuclease (DNase), produced by hemolytic streptococci that acts by breaking down DNA in necrotic tissue and pus. Its primary mechanism of action is the depolymerization of deoxyribonucleoprotein (DNP) and deoxyribonucleic acid (DNA), which are major components of viscous purulent exudates, transforming them into less viscous, smaller soluble fragments.

Mechanism of Action :

- **Depolymerization:** Streptodornase hydrolyzes high-molecular-weight DNA into smaller fragments, liquefying thick pus and fibrin.
- **Targeting Viscosity:** It works specifically on dead cells and pus within a wound, reducing the viscosity of the exudate.
- **Site of Action:** It is often used topically to treat infected wounds, lesions, or in body cavities to remove debris, usually in combination with streptokinase.

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- **Interaction with Immunity:** By breaking down the DNA in pus, it allows leukocytes to move freely and improves the effectiveness of phagocytosis, assisting the body's immune system in fighting infection.
- **Specificity:** Studies show that streptodornase exhibits DNase activity over a wide pH range (5.0–9.0) with maximum activity at pH 7.8, and does not exhibit action against RNA.

Streptodornase vs. Streptokinase

While streptodornase (SD) breaks down DNA, it is usually combined with streptokinase(SK).

- **Streptodornase** acts on dead cells and pus (DNA).
- **Streptokinase** acts on blood clots (fibrin).

Together, these enzymes facilitate debridement of infected areas and enhance the penetration of anti-infective agents.

Artemisinin

Artemisinin and its semisynthetic derivatives are a group of drugs used in the treatment of malaria due to Plasmodium falciparum.[1] It was discovered in 1972 by Tu Youyou, who shared the 2015 Nobel Prize in Physiology or Medicine for her discovery.[2] Artemisinin-based combination therapies (ACTs) have become standard treatment worldwide for P. falciparum malaria as well as malaria due to other species of Plasmodium.[3] Artemisinin can be extracted from the herb Artemisia annua (sweet wormwood), which is used in traditional Chinese medicine. Alternatively, it can be prepared by a semi-synthetic method from a precursor compound that can be produced using a genetically engineered yeast, which is much more efficient than extraction from the plant.[4]

Artemisinin and its derivatives are all sesquiterpene lactones containing an unusual peroxide bridge. This endoperoxide 1,2,4-trioxane ring is responsible for their antimalarial properties. Few other natural compounds with such a peroxide bridge are known.[5]

Artemisinin and its derivatives have been used for the treatment of malarial and parasitic worm (helminth) infections. Advantages of such treatments over other anti-parasitics include faster parasite elimination and broader efficacy across the parasite life-cycle; disadvantages include their low bioavailability, poor pharmacokinetic properties, and high cost.[6][7] Moreover, use of the drug by itself as a monotherapy is explicitly discouraged by the World Health Organization,[8] as there have been signs that malarial parasites are developing resistance to the drug.[9] Combination therapies, featuring artemisinin or its derivatives alongside some other antimalarial drug, constitute the contemporary standard-of-care treatment regimen for malaria.

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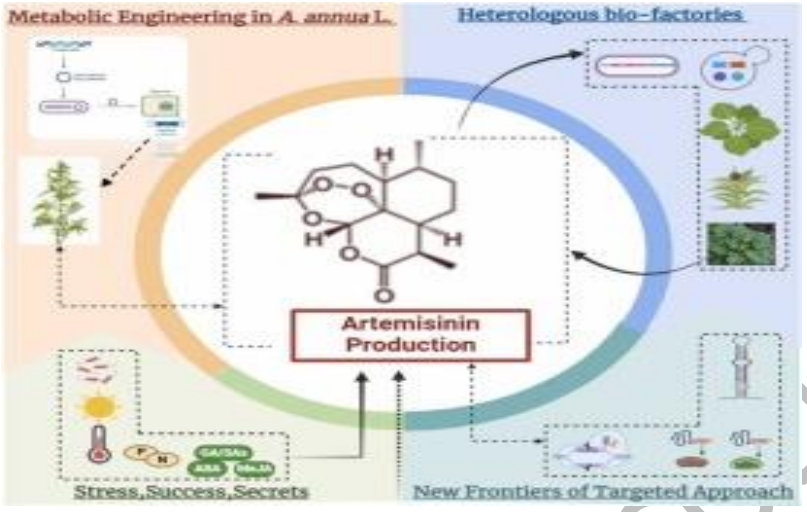
PRODUCTION OF ARTEMESININ



The process for the microbial production of artemisinin. Using synthetic biology, the metabolism of the microbe is engineered to produce artemisinic acid, a precursor to artemisinin. Starting from acetyl-CoA (an abundant product of the central metabolism of many microbes), the microbes produce, in turn, mevalonate, farnesyl pyrophosphate (FPP), amorphadiene, and finally, artemisinic acid. The artemisinic acid is released from the microbes and purified from the culture media. The artemisinic acid is chemically converted to artemisinin. Once the artemisinin is produced, it must be further chemically converted into a derivative such as artesunate or artemether, which are integrated into ACTs for the treatment of malaria

Although artemisinin can be synthesized chemically, the Analogues are unlikely to be economically competitive With that produced naturally in Artemisia due to Complexity of the process The many reaction Steps and low yields obtained by organic synthesis make It obvious that its extraction from the leafy biomass of A. Annua invariably appears to be the only viable option at The moment for producing cheap and large quantities of Artemisinin. Therefore, the increased cultivation of the Crop in plantations and the improvement of artemisinin Extraction methods are the most effective strategies for Producing artemisinin. However, one of the major Shortcomings on the production of sesquiterpenic Compounds via whole plants is the relatively lengthy Growing cycle required to obtain appreciable yields (g/100 G dry weight), which can range only from 0.2 to 0.9% in Many of the commercial varieties currently cultivated in Different parts of the world. Usually the period from time Of planting to artemisinin extraction from A. Annua is Approximately 12 –15 months. Not surprisingly, the yields Derived from dried leafy biomass after such a lengthy Period are considered low for commercial production, Where a full ton of plant materials can only produce Between 6 – 18 kg of purified artemisinin. This low yield Thus appears to be one of the most intractable problems Related to the production and use of artemisinin-derivedDrugs against malaria, especially in Africa where the plant Is not ubiquitous. A. Annua is a vigorous growing annual weed, which Stores most of its active ingredients in glandular Trichomes found in the leaves and inflorescence). The plant is Considered to have originated and occurs naturally as

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Part of the steppe vegetation in Northern China). However, it now grows effectively in other Climatic conditions. In Asia, for example, it is well Distributed and extends as a native into Southern Siberia, Vietnam, and Northern India. Outside of Asia, the plant Has adapted ubiquitously to different growth conditions as Seen in many parts of Europe, USA, Australia, and Argentina). In Africa it has been introduced into commercial-scale cultivation in Tanzania, Kenya, Uganda and Madagascar within the past six years.

And more recently in Nigeria (Brisibe, 2006), where Evaluation of optimal agronomic practices and mass Selection for late flowering and high artemisinin yielding Lines are currently in progress. For these studies, seeds Were obtained from six different countries – Brazil, China, Vietnam, India, Germany and USA. Some of these, espe- Cially the hybrid populations from Brazil, have originated Plants that had a growth span of about 192 days before Flowering and were up to 2.84 meters in height with an Average leaf biomass yield of 324 g/plant and artemisinin Concentrations as high as 0.9% (on a g/100 g dry weight Basis) under humid lowland tropical conditions .

AGRO-TECHNOLOGIES FOR ENHANCED PRODUCTION OF ARTEMISININ

The availability and cost of acts are largely functions of The artemisinin yield in A. Annua cultivars, which has sig-Nificant effect on artemisinin cost itself that is currently a Key cost driver for the production of the drugs. It is not Surprising, therefore, that there is a current surge in the Cultivation of the plant around the world, most notably in Africa, where high artemisinin-yielding lines have been Earmarked for commercial cultivation in different coun-Tries. However, African regions mostly afflicted by malaria Are within the tropics, where day lengths are short, thus Likely to induce most cultivars which are not adapted to The tropics to flower early without the accumulation of Sufficient leafy biomass (Ferreira et al., 2005). Interes-Tingly, there are currently genotypes that have been Developed by Mediplant in Switzerland (Delabays et al., 2001) and hybrid populations by Dr. Pedro M. De Magalhäes (1999) at CPQBA, University of Campinas, Campinas, Brazil, which are late flowering and produce Sufficient leafy biomass, that appear most suitable for Cultivation in the tropics. The interpretation that these Varieties, especially those from Brazil, can perform well Within the tropics has support from our own recent Studies in Nigeria which show that they can produce on Average 0.9% artemisinin and can be selected further for Adaptation to lower latitudes quite close to the Equator Though Artemisia is well suited to both small-scale and Plantation agriculture, currently the most significant Bottleneck for feasible commercial production of arte-Misinin anywhere in the world is the availability of seed.Stocks of lines suitable for the local conditions which can Produce high leafy biomass and artemisinin yields.

METRONIDAZOLE PRODUCTION

Metronidazole has another name called Metronidazole plain BP.98 99, metronidazole, metronidazole, Metronidazole etc., and chemistry Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) by name, be the nitro glyoxaline synthetic antibacterial drug.Very extensive in clinical application, there is wide spectrum anaerobe resistant and antiprotozoal effect, clinically be

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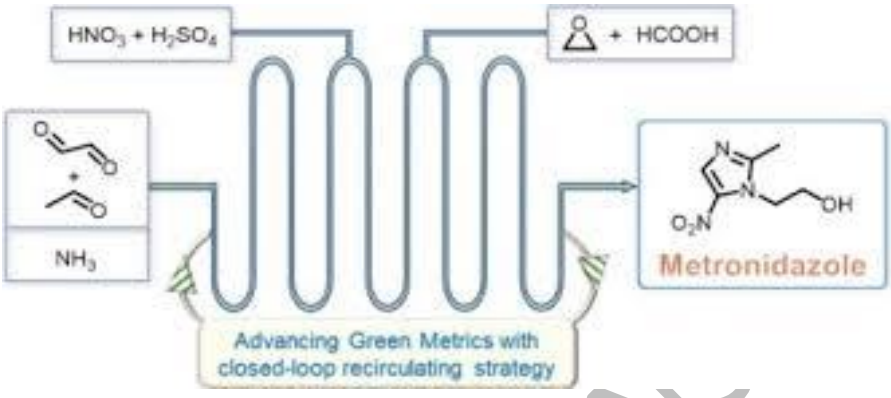
mainly used in prevention and treatment anaerobism microbial infection, as respiratory tract, digestive tube, abdominal cavity and pelvic infection, the endocarditis that the infection at the positions such as skin soft tissue, bone and osteoarthrosis and bacteroides fragilis cause, cerebral abscess, septicemia and meningitis etc., be widely used in prevention in addition and the treatment oral cavity anaerobium infects.The nitro of metronidazole is reduced into amino and has the anaerobe resistant effect in oxygen-free environment, invalid to aerophil or facultative aerobe.



The preparation method of a metronidazole is characterized in that: comprise the steps:

(1), Nitration Mixture: formic acid 100 weight parts of concentration 95%～99% are put in the reactor, added the vitriol oil 25～35 weight parts, it is for subsequent use to make nitration mixture;

(2), Building-Up Reactions: reaction raw materials 2-5-nitro imidazole 100～120 weight parts are put in the retort, the nitration mixture that adds step (1) preparation, the control temperature is at 75～80 ℃, dissolve rear insulation 10～20 minutes fully until the 2-5-nitro imidazole, add oxyethane 90～100 weight parts, vitriol oil 7-9 weight part, generate hydroxylation liquid after the reaction;

(3), Once Neutralization: the hydroxylation liquid of step (2) preparation is dropped into back in the nitre tank, and the control temperature is at 25～35 ℃, and it is 2.0～2.5 that the dropping liquid caustic soda is neutralized to pH value, then be cooled to 15～20 ℃ and carry out crystallization, after to be crystallized the finishing, centrifugal rejection filter is collected filtered liquid and is back that the nitre mother liquor is for subsequent use; This crystallisate is completely 2-5-nitro imidazole of unreacted, and it is synthetic to be used for next time;

(4), Secondary Neutralization: the nitre mother liquor that returns of step (3) preparation is joined back in the nitre tank, it is 10.0～11.0 that the dropping liquid caustic soda is neutralized to the pH value, then controls temperature and carry out crystallization under 20～25 ℃, after to be crystallized the finishing, centrifugal rejection filter, collecting filtered liquid is the crude product mother liquor; This crystallisate is completely 2-5-nitro imidazole of unreacted, and it is synthetic to be used for next time;

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(5), Metronidazole Crude Product Preparation: the crude product mother liquor of step (4) preparation is dropped into back in the nitre tank, be neutralized to pH value 6.0～7.0 with sulfuric acid after, control temperature in static crystallization below 40 ℃, after to be crystallized the finishing, rejection filter is dried, and namely obtains the metronidazole crude product;

(6), Decolouring Crystallization: add purified water in bleacher, add successively afterwards metronidazole crude product, the gac of step (5) preparation, decolour after 30 minutes, press filtration is to crystallizer, add bicarbonate of ammonia, stirred crystallization is after to be crystallized the finishing, centrifugal rejection filter, oven dry namely obtains the metronidazole highly finished product; The weight ratio of described purified water, metronidazole crude product, gac, bicarbonate of ammonia is 500:100:3:10.

ASPARGINASE

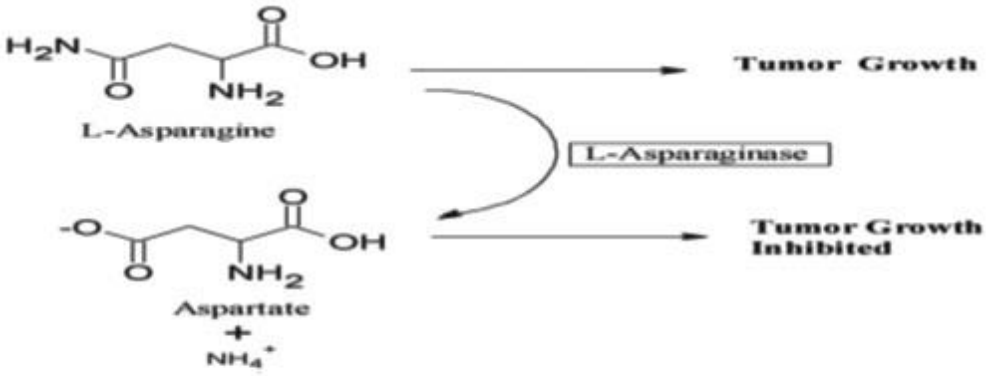

Figure 1: Schematic representation of mechanism of action of l-asparaginase

Asparaginase is an enzyme that is used as a medication and in food manufacturing.[6][7] As a medication, L-asparaginase is used to treat acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LBL).[6] It is given by injection into a vein, or muscle. [6] A PEGylated version, pegaspargase, is also available.[8] In food manufacturing it is used to decrease acrylamide.[7]

Common side effects when used by injection include allergic reactions, pancreatitis, blood clotting problems, high blood sugar, kidney problems, and liver dysfunction.[6] Use in pregnancy may harm the baby.[9] As a food it is generally recognized as safe.[7] Asparaginase works by breaking down the amino acid known as asparagine without which the cancer cells cannot make protein.[6]

Asparaginase was approved for medical use in the United States in 1978.[8] It is on the World Health Organization's List of Essential Medicines.[10] It is often made from Escherichia coli (E. coli) or Erwinia chrysanthemi.[8][11]

Development of the drug

In 1963, asparaginase (ASNase) was identified as an effective antileukemic agent, and subsequent efforts were made to isolate it from bacterial sources and scale up production for clinical

trials.[12] Clinical testing with bacterial-derived ASNase commenced in 1966, and in 1978, E. coli–derived ASNase received approval from the United States for the treatment of acute lymphoblastic leukemia.[13] Subsequently, pegylated E. coli ASNase was approved in 1994 as a second-line treatment and later in 2006 as a first-line treatment for acute lymphoblastic leukemia. Another ASNase variant, ASNase Erwinia chrysantemi, obtained authorization for use in the United Kingdom in 1985, and gained approval from the US Food and Drug Administration in 2011.

Medical

E. coli strains are the main source of medical asparaginase. Branded formulations (with different chemical and pharmacological properties) available in 1998 include Asparaginase Medac, Ciderolase, and Oncaspar.

Asparaginase produced by Dickeya dadantii (formerly called Erwinia chrysanthemi) instead is known as crisantaspase (BAN), and is available in the United Kingdom under the brand name Erwinase.

One of the E. coli asparaginases marketed under the brand name Elspar for the treatment of acute lymphoblastic leukemia is also used in some mast cell tumor protocols.

In July 2006, the US Food and Drug Administration (FDA) granted approval to pegaspargase for the first-line treatment of people with acute lymphoblastic leukemia as a component of a multiagent chemotherapy regimen. Pegaspargase was previously approved in February 1994 for the treatment of patients with acute lymphoblastic leukemia who were hypersensitive to native forms of L-asparaginase. Similar designations were later applied to calaspargase (December 2018) and asparaginase erwinia chrysanthemi (June 2021), both identified as orphan drugs.

As a food processing aid

Acrylamide is often formed in the cooking of starchy foods. During heating the amino acid asparagine, naturally present in starchy foods, undergoes a process called the Maillard reaction, which is responsible for giving baked or fried foods their brown color, crust, and toasted flavor. Suspected carcinogens such as acrylamide and some heterocyclic amines are also generated in the Maillard reaction. By adding asparaginase before baking or frying the food, asparagine is converted into another common amino acid, aspartic acid, and ammonium. As a result, asparagine cannot take part in the Maillard reaction, and therefore the formation of acrylamide is significantly reduced. Complete acrylamide removal is probably not possible due to other, minor asparagine-independent formation pathways.

As a drug

Applications of asparaginase in cancer therapy take advantage of the fact that acute lymphoblastic leukemia cells and some other suspected tumor cells are unable to synthesize the non-essential amino acid asparagine, whereas normal cells are able to make their own asparagine; thus leukemic cells require a high amount of asparagine.[23] These leukemic cells depend on circulating asparagine. Asparaginase, however, catalyzes the conversion of L-

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asparagine to aspartic acid and ammonia. This deprives the leukemic cell of circulating asparagine, which leads to cell death.

Dextrin

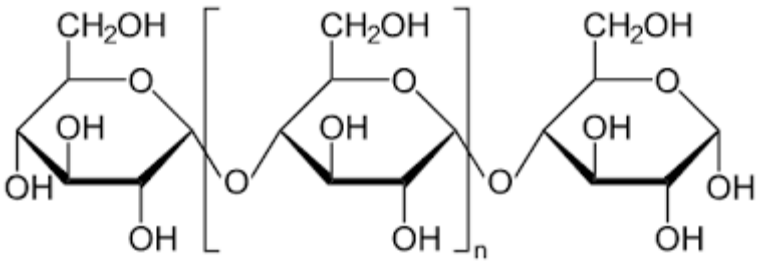

Fig:chemical structure of dextrin

Dextrin is a low molecular weight glucose polymer. It is a generic term for a variety of products obtained by heating starch in the presence of small amounts of water and acid. Dextrins can be made from any starch and are usually classified as white dextrins, yellow dextrins and English gum.

Dextrins have many industrial and food uses. The most common use of dextrin is as an adhesive base for cardboard boxes, envelopes and stamps. It is also used to polish grains and processed fruits, and to decorate ceramics. In the food sector, dextrins enter the food preparation process as thickeners and are used in brewing, baking, fruit juice and cocoa drinks, distilled spirits, confectionery products, and more.

In the pharmaceutical field, dextrin is often used as a pharmaceutical excipient, as a suspending agent, as a tablet binder, and as a diluent for tablets and capsules. It is also used as an ingredient in tablet icing and as a binder and hardener for surgical dressings

Pharmaceutical Applications of Dextrin:

- Tablet and Capsule Binder/Filler: Acts as a binding agent, Ensuring stability and integrity of tablet granules.
- Tablet Coating: Functions as a plasticizer and adhesive in sugar-coating tablets to improve finish and prevent defects.
- Drug Delivery Vehicles: Used in the form of dextrin nanoparticles to target specific cells for cancer treatment.
- Nutrient in Fermentation: Used as a nutrient in the fermentation process for producing antibiotics.
- Thickening Agent: Used in liquid preparations and suspensions.
- Medical Dressings: Applied as an adhesive and stiffening agent in surgical dressings.

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Main Types of Dextrin:

- White Dextrins: Commonly used as binders and thickeners.
- Yellow Dextrins (Canary Dextrins): Used where higher solubility and lower viscosity are required.

Synthesis of Dextrin

Dextrin is mainly prepared from starch, but can also be chemically synthesised. Commonly used starches include: corn, potato or tapioca starch.

Dextrins are made from corn starch, which is roasted and then hydrolysed by amylase, an enzyme that digests starch ingested as food. Indigestible dextrin is a water-soluble dietary fibre that is extracted and prepared from the indigestible components of the paste.

Polyethylene glycol (PEG) was further applied for fractionating dextrin prepared from cassava starch. The initial dextrin concentration and pH of the dextrin solutions were crucially considered in this study with the average molecular-weight dispersity (DMa) as the index. The results showed that the initial dextrin concentration significantly affected the mass fraction and the molecular weight distribution of each dextrin fraction obtained from gradient PEG precipitation. However, the initial dextrin concentration, which ranged from 0.9% to 3.6%, did not affect the DMa of the dextrin fractions. Furthermore, the DMa of the fractions obtained at pHs 4.00, 4.96, 6.00, 6.92, 7.99, 8.96, and 9.91, was 1.364, 1.341, 1.305, 1.286, 1.273, 1.311, and 1.404, respectively, while the dispersity of the parent dextrin was 2.052. These results suggest that the preparative approach, gradient PEG precipitation, is applicable in acidic, neutral, and alkaline environments, and that a weakly alkaline environment is optimal for dextrin fractionation.

Semi-synthetic antibiotics

Semi-synthetic antibiotics are compounds that are chemically derived from the natural antibiotics.

Oritavancin, telavancin and dalbavancin are examples of semisynthetic antibiotics. Semisynthetic antibiotics have improved pharmaceutical properties compared to their natural parent, making them more suitable for clinical use. Semisynthetic antibiotics provide a source of therapeutic candidates in the current global need to overcome antimicrobial resistance.

Antibiotics are either bacteriostatic (stop growth) or bactericidal (kill bacteria).

Humans and other eukaryotes have 80S ribosomes, while bacterial cells contain 70S ribosomes, which are smaller and structurally different.

Sources of Antibiotics:

1. **Microorganisms:** Most antibiotics currently in use are derived from Streptomyces species.
2. **Synthesis:** Chloramphenicol is typically produced through synthetic methods.

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3. **Semisynthesis:** Part of the antibiotic molecule is produced via fermentation, then chemically modified. Many penicillins and cephalosporins are manufactured this way.

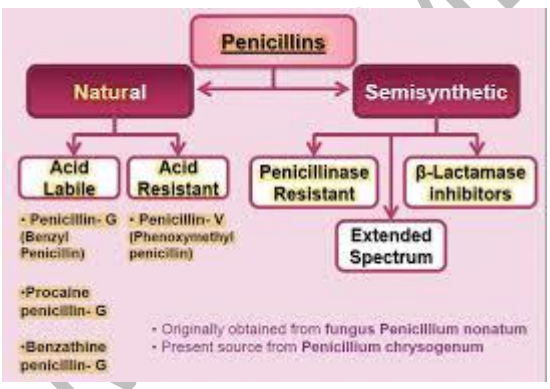
(Example: Streptomyces venezuelae)

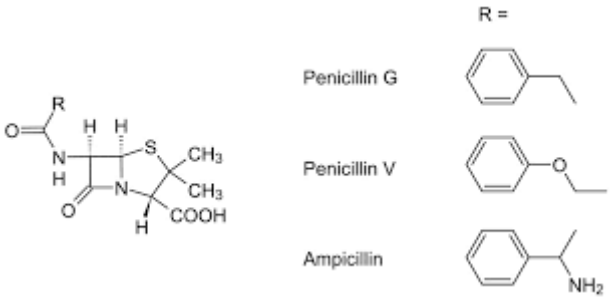
β-lactam

- Beta-lactam antibiotics target bacteria that have a cell wall.
- These bacteria build their cell walls by linking molecules together, a process that beta-lactams inhibit.
- Without a cell wall, internal pressure causes the bacterial membrane to burst, leading to cell death.
- Penicillins

Penicillins can be classified into the following types:

- Naturally occurring: Produced by fermentation of molds like Penicillium notatum and Penicillium chrysogenum. Examples include benzylpenicillin (penicillin G) and phenoxymethylpenicillin (penicillin V).
- Semisynthetic: For example, amoxicillin.





- Penicillins generally target Gram-positive bacteria.

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- Some, like ampicillin, also work against Gram-negative bacteria, but not Pseudomonas aeruginosa.
- Others, such as carbenicillin, are effective against Pseudomonas aeruginosa as well.

IMIMOBILIZATION PROCEDURES FOR PHARMACEUTICAL APPLICATIONS

Liposomes are spherical vesicles composed of phospholipid bilayers, which can encapsulate both hydrophilic and hydrophobic drugs, offering advantages like targeted delivery and reduced toxicity. Despite their benefits, liposomes face challenges such as instability, high production costs, and potential drug leakage. Various preparation methods such as solvent injection and sonication allow for the formulation of liposomes, which have therapeutic applications in drug delivery, gene therapy, and immunology.

Cephalosporin

- In the 1950s, species of Cephalosporium (now Acremonium) produced several antibiotics: a) Cephalosporin P: an acidic antibiotic later found to have a steroid-like structure. b) Cephalosporin N: another acidic antibiotic, which was later identified as a penicillin. c) Cephalosporin C: isolated during the purification of cephalosporin N; this is a true cephalosporin and the source of 7-aminocephalosporanic acid (7-ACA), the foundation for developing new cephalosporins.
- Cephalosporins interfere with the synthesis of the peptidoglycan layer, which forms the bacterial cell wall, leading to bacterial death.
- First-generation cephalosporins mainly target Gram-positive bacteria, while later generations show increased effectiveness against Gram-negative bacteria, often with reduced activity against Gram-positive ones.
- They are less vulnerable to degradation by β-lactamases.

ANTICANCER DRUGS

The available anticancer drugs have distinct mechanisms of action which may vary in their effects on different types of normal and cancer cells. A single "cure" for cancer has proved elusive since there is not a single type of cancer but as many as 100 different types of cancer. In addition, there are very few demonstrable biochemical differences between cancerous cells and normal cells. For this reason the effectiveness of many anticancer drugs is limited by their toxicity to normal rapidly growing cells in the intestinal and bone marrow areas. A final problem is that cancerous cells which are initially suppressed by a specific drug may develop a resistance to that drug. For this reason cancer chemotherapy may consist of using several drugs in combination for varying lengths of time.

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Introduction

Chemotherapy drugs, are sometimes feared because of a patient's concern about toxic effects. Their role is to slow and hopefully halt the growth and spread of a cancer. There are three goals associated with the use of the most commonly-used anticancer agents.

1. Damage the DNA of the affected cancer cells.
2. Inhibit the synthesis of new DNA strands to stop the cell from replicating, because the replication of the cell is what allows the tumor to grow.
3. Stop mitosis or the actual splitting of the original cell into two new cells. Stopping mitosis stops cell division (replication) of the cancer and may ultimately halt the progression of the cancer.

Unfortunately, the majority of drugs currently on the market are not specific, which leads to the many common side effects associated with cancer chemotherapy. Because the common approach of all chemotherapy is to decrease the growth rate (cell division) of the cancer cells, the side effects are seen in bodily systems that naturally have a rapid turnover of cells iincluding skin, hair, gastrointestinal, and bone marrow. These healthy, normal cells, also end up damaged by the chemotherapy program.

Categories of Chemotherapy Drugs

In general, chemotherapy agents can be divided into three main categories based on their mechanism of action.

- Stop the synthesis of pre DNA molecule building blocks

  These agents work in a number of different ways. DNA building blocks are folic acid, heterocyclic bases, and nucleotides, which are made naturally within cells. All of these agents work to block some step in the formation of nucleotides or deoxyribonucleotides (necessary for making DNA). When these steps are blocked, the nucleotides, which arethe building blocks of DNA and RNA, can not be synthesized. Thus the cells can not replicate because they can nnot make DNA without the nucleotides. Examples of drugs in this class include 1) methotrexate (Abitrexate®),2) fluorouracil (Adrucil®), 3) hydroxyurea (Hydrea®), and 4) mercaptopurine (Purinethol®).

- Directly damage the DNA in the nucleus of the cell

  These agents chemically damage DNA and RNA. They disrupt replication of the DNA and either totally halt replication or cause the manufacture of nonsense DNA or RNA (i.e. the new DNA or RNA does not code for anything useful). Examples of drugs in this class include cisplatin (Platinol®) and 7) antibiotics - daunorubicin (Cerubidine®), doxorubicin (Adriamycin®), and etoposide (VePesid®).

- Effect the synthesis or breakdown of the mitotic spindles

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Mitotic spindles serve as molecular railroads with "North and South Poles" in the cell when a cell starts to divide itself into two new cells. These spindles are very important because they help to split the newly copied DNA such that a copy goes to each of the two new cells during cell division. These drugs disrupt the formation of these spindles and therefore interrupt cell division. Examples of drugs in this class of 8) miotic disrupters include: Vinblastine (Velban®), Vincristine (Oncovin®) and Pacitaxel (Taxol®).

Enzyme L-asparaginase

In the 1950's a biochemical difference in metabolism related to the amino acid asparagine was found. Normal cells apparently can synthesize asparagine while leukemia cells cannot. If leukemia cells are deprived of asparagine, they will eventually die. In an almost unrecognized and parallel discovery, it was found that blood serum from guinea pigs and other South American rodents had antileukemia properties. The enzyme L-asparaginase was eventually identified as the anticancer agent. L-asparaginase was isolated and tested successfully on human leukemias. Eventually the enzyme asparaginase was also found and isolated from the bacteria, E. coli.

If the enzyme L-asparaginase is given to humans, various types of leukemias can be controlled. Tumor cells, more specifically lymphatic tumor cells, require huge amounts of asparagines to keep up with their rapid, malignant growth. This means they use both asparagine from the diet as well as what they can make themselves (which is limited) to satisfy their large asparagines demand. L-asparaginase is an enzyme that destroys asparagine external to the cell. Normal cells are able to make all the asparagine they need internally whereas tumor cells become depleted rapidly and die.The enzyme converts asparagine in the blood into aspartic acid by a deamination reaction. The leukemia cells are thus deprived of their supply of asparagine and will die.

Methotrexate

Methotrexate inhibits folic acid reductase which is responsible for the conversion of folic acid to tetrahydrofolic acid. At two stages in the biosynthesis of purines (adenine and guanine) and at one stage in the synthesis of pyrimidines (thymine, cytosine, and uracil), one-carbon transfer reactions occur which require specific coenzymes synthesized in the cell from tetrahydrofolic acid.

Tetrahydrofolic acid itself is synthesized in the cell from folic acid with the help of an enzyme, folic acid reductase. Methotrexate looks a lot like folic acid to the enzyme, so it binds to it thinking that it is folic acid. In fact, methotrexate looks so good to the enzyme that it binds to it quite strongly and inhibits the enzyme. Thus, DNA synthesis cannot proceed because the coenzymes needed for one-carbon transfer reactions are not produced from tetrahydrofolic acid because there is no tetrahydrofolic acid. Again, without DNA, no cell division.

5-Fluorouracil

5-Fluorouracil (5-FU; Adrucil®, Fluorouracil, Efudex®, Fluoroplex®) is an effective pyrimidine antimetabolite. Fluorouracil is synthesized into the nucleotide, 5-fluoro-2-deoxyuridine. This product acts as an antimetabolite by inhibiting the synthesis of 2-deoxythymidine because the

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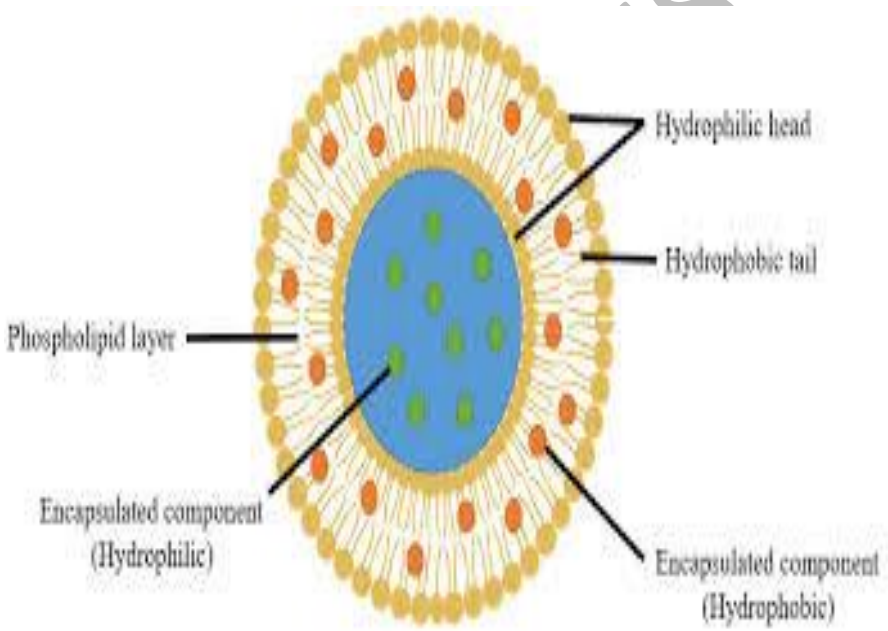
carbon - fluorine bond is extremely stable and prevents the addition of a methyl group in the 5-position. The failure to synthesize the thymidine nucleotide results in little or no production of DNA. Two other similar drugs include: gemcitabine (Gemzar®) and arabinosylcytosine (araC). They all work through similar mechanisms.

INTRODUCTION

- Liposomes are spherical vesicles with an aqueous core surrounded by one or more phospholipid bilayers.
- The first liposomes were created in England in 1961 by Alec D. Bangham during his research on phospholipids and blood clotting.
- The term "liposome" comes from Greek, where "Lipo" means fatty and "Soma" means structure.
- Liposome sizes range from about 20 nanometers to several micrometers.



Advantages

- Can load both hydrophilic and hydrophobic drugs
- Enhances efficacy and therapeutic index
- Improves stability of encapsulated drugs
- Non-toxic
- Biodegradable

- Non-immunogenic
- Reduces systemic toxicity targeted delivery
- Protection of sensitive drug molecules
- Low toxicity due to reduced exposure to sensitive tissues
- Minimal adverse drug reactions (ADR) or no side effects
- Possible formulations: suspension, emulsion, gel, cream, lotion, aerosol, reconstituted vesicles
- Targeted delivery

Disadvantages

- Long-term instability.
- Phospholipids may undergo hydrolysis and oxidation.
- Sensitive to temperature changes.
- Encapsulated drug can leak during storage.
- High production costs.

Structural components of liposomes

- Phospholipids
- Cholesterol

PHOSPHOLIPIDS

Phospholipids are the fundamental molecular components of liposomes. They are amphiphilic lipids composed of:

- Hydrophilic (polar) head
- Hydrophobic (non-polar) tails

This dual affinity allows hydrophilic drugs to be encapsulated within the aqueous phase, while hydrophobic drugs can be integrated into the lipid bilayers.

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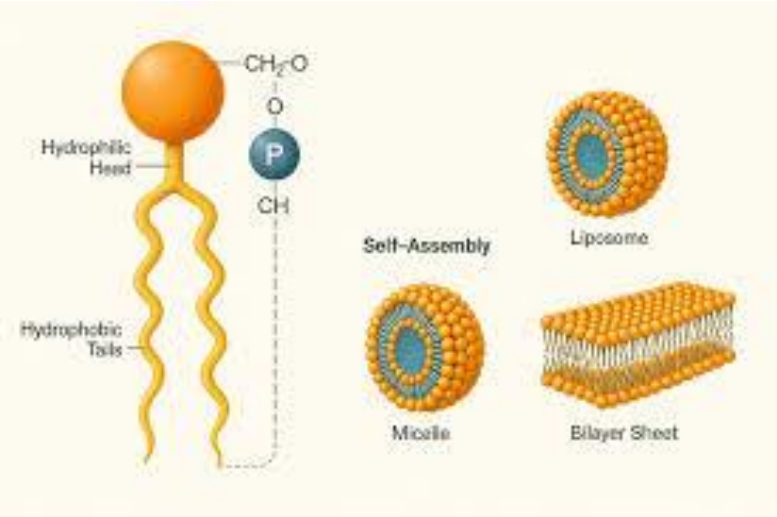

Examples:

- Dilauryl phosphotidyl choline (DLPC)
- Dimyristoyl phosphotidyl choline (DMPC)
- Dipalmitoyl phosphotidyl choline (DPPC)
- Distearoyl phosphotidyl choline (DSPC)
- Dioleolyl phosphotidyl choline (DOPC)
- Dilauryl phosphotidyl glycerol (DLPG)
- Distearoyl phosphotidyl serine (DSPS)

BIOSENSORS

Biosensors convert biological responses into electrical signals and were pioneered by Professor Leland C. Clark. They should provide accurate, precise, reproducible results using cheap, small, portable devices operable by semi-skilled users. Biosensors contain bioreceptors, transducers, signal processors and displays. Depending on the transducer, examples include electrochemical, amperometric, potentiometric, conductometric, thermometric, optical and piezoelectric biosensors. Biosensors have wide applications in medicine such as glucose monitoring, infectious disease diagnosis, and detection of cardiac markers.

BASIC CHARACTERISTICS OF BIOSENSORS

1. LINEARITY: The sensor should maintain high linearity to accurately detect high substrate concentrations.
2. SENSITIVITY: The electrode's response value relative to substrate concentration.

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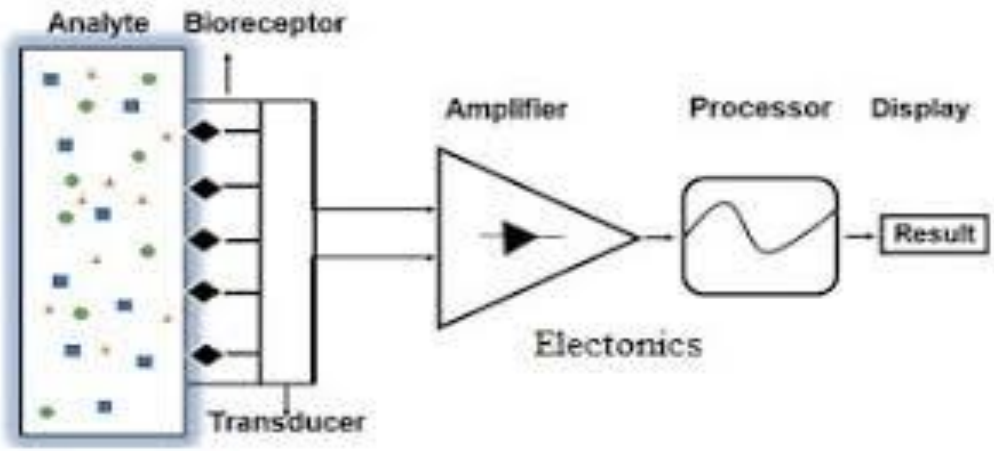
3. SELECTIVITY: Minimizing chemical interference is essential to obtain accurate results.
4. RESPONSE TIME: The time required to reach 95% of the sensor's response.

The first biosensor was invented in 1950 by American biochemist Leland C. Clark, known as the father of biosensors. He developed the glucose biosensor to measure glucose levels in samples.

Main Components of a Biosensor

1. Sensor
    - A sensitive biological element such as tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc.
2. Transducer
    - Converts biochemical activity into electrical energy by transforming one form of energy into another.
3. Amplifier
4. Processor
5. Display Unit



General Features of Biosensors:

A biosensor consists of two main components:

1. Biological component: such as enzymes or cells.
2. Physical component: including transducers and amplifiers.

The biological component detects and interacts with the analyte, generating a physical change (signal) that the transducer can detect. Typically, the biological material is immobilized onto the

transducer, allowing the biosensor to be reused multiple times (up to around 10,000 uses) over an extended period (several months).

Principle of a Biosensor

- The target biological material, typically a specific enzyme, is immobilized using standard techniques such as physical or membrane entrapment, or through non-covalent or covalent binding. This immobilized biological component is closely connected to the transducer.
- The analyte interacts with the biological material to form a bound analyte complex, which generates an electronic response that can be detected and measured.
- In certain cases, the analyte is transformed into a product, which may involve the release of heat, gas (like oxygen), electrons, or hydrogen ions.
- The transducer converts these product-related changes into electrical signals, which can then be amplified and quantified.

Types of Biosensors:

1. Electrochemical Biosensors:
    - Amperometric Biosensors
    - Potentiometric Biosensors
    - Conductometric Biosensors
2. Calorimetric Biosensors
3. Optical Biosensors
4. Piezo-electric Biosensors / Acoustic Biosensors

Electrochemical biosensors measure electric current or ionic changes using bio electrodes.

Amperometric Biosensors:

- Detect electron flow from enzyme-driven redox reactions.
- A constant voltage is applied; substrate or product transfers electrons to electrodes.
- Current changes reflect substrate concentration.
- Example: Clark oxygen electrode measures O2 reduction.
- Glucose oxidase converts glucose to gluconic acid; O2 decrease measured by electrode shows glucose level.

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Potentiometric Biosensors:

Ion-selective electrodes measure changes in ion levels. The pH electrode is most common, as many enzymes affect hydrogen ions. Other electrodes detect ammonia and CO2. The voltage difference between the sensor and reference electrode shows substrate concentration. A key limit is enzyme sensitivity to ions like H+ and NH4+. Ion-selective field effect transistors (ISFETs) are cheap, small sensors used in potentiometric biosensors. For example, ISFETs monitor heart muscle pH during surgery.

Conductometric Biosensors:

- Biological reactions change ionic species, affecting electrical conductivity.
- Conductometric biosensors measure this change.
- Example: Urea biosensor uses urease enzyme.
- Urease converts urea into NH4+ and HCO3- ions.
- NH4+ concentration, measured by an electrode, shows urea levels in the sample.

Thermal and masscalorimetric biosensors:

Thermal biosensors detect heat from biological reactions. If an enzyme reaction releases heat, two thermistors measure resistance changes to find analyte levels. The sensor has a heat-insulated box with an aluminum heat exchanger and an enzyme-packed reactor. When substrate enters, it converts to product, generating heat. Thermistors measure temperature differences, detecting even small changes.

Optical Biosensors

- Optical biosensors operate by detecting changes in light properties such as absorbance, fluorescence, and chemiluminescence. They incorporate fiber optic technology alongside optoelectronic transducers to capture these signals. The term "optrode," a blend of "optical" and "electrode," is frequently used to describe these devices.
- These biosensors typically use enzymes and antibodies as the key biological components that convert a biological response into an optical signal. One major advantage is their ability to perform safe, non-electrical, and remote sensing of substances without the need for reference sensors, since the same light source can generate both the sample and comparative signals.
- Fundamentally, an optical biosensor is a compact analytical instrument that combines a biological recognition element with an optical transducer. Its main goal is to generate a signal proportional to the concentration of a target analyte. The biological elements can vary widely, including enzymes, antibodies, antigens, receptors, nucleic acids, whole cells, or tissues.

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- Techniques such as Surface Plasmon Resonance (SPR) and optical waveguide interferometry exploit the evanescent field near the sensor surface to detect interactions between the biorecognition element and the analyte. Optical fibers require only a few microliters of sample for effective detection.
- Common applications include monitoring blood gases and measuring respiratory gases.

PIEZO-ELECTRIC BIOSENSORS/ACOUSTIC BIOSENSORS

- These biosensors use piezoelectric crystals that vibrate at set frequencies. When molecules stick to the crystal, the vibration changes and is measured electronically.
- Enzymes can be attached to detect gases or inhibitors.
- Examples:
  - Organophosphorus insecticide sensor uses acetylcholine esterase.
  - Formaldehyde sensor uses formaldehyde dehydrogenase.
  - Cocaine gas sensor uses cocaine antibodies.
- Antigen-coated electrodes detect specific antibodies by reducing noise compared to nonspecific antibodies.
- Limitation: They struggle in liquids because viscous fluids can stop crystal vibrations.

Applications of biosensor:

- Food Industry
- Medical Field
- Drug Discovery & Analysis
- Environmental Monitoring
- Paper Biosensors for Water
- Epigenetics
- Nanobiosensors

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UNIT - IV

Immunological Products

Immunological products are biological substances used to induce, enhance, or provide immunity against diseases, or for the diagnosis and treatment of immune-related conditions.

They are prepared from living organisms, their components, or immune sera and act by stimulating or supplying the immune system.

Examples of Immunological Products

Vaccines – provide active immunity

Antisera / Immunoglobulins – provide passive immunity

Toxoids – inactivated toxins (e.g., tetanus toxoid)

Antigens – used in diagnostic tests

Monoclonal antibodies – used in therapy and diagnosis

Vaccine

A vaccine is a biological preparation that stimulates the immune system to recognize and fight specific infectious agents, thereby providing active acquired immunity without causing disease. In 1796 – Edward Jenner developed the first vaccine (Smallpox).

1885 – Louis Pasteur developed Rabies vaccine

1979 – Smallpox eradicated globally

21st Century – mRNA, DNA, recombinant and vector vaccines developed

Types of vaccines

In the beginning of practice of vaccinology, only attenuated pathogens and toxoids were used for vaccination. With advances in molecular immunology and recombinant gene technology several new vaccine preparations have come into existence and are in use today. Some new exciting ideas are in the experimental stage. - Live attenuated vaccines

- Inactivated killed vaccines

- Subunit vaccines

- Recombinant vaccines

- Conjugate vaccines

- Multivalent subunit vaccines

- DNA vaccines

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- Recombinant vector vaccines

- Edible vaccines

Mechanism of Action

- Vaccine introduces antigen.
- Antigen-presenting cells activate immune response.
- B cells produce antibodies.
- Memory cells formed.
- Rapid response on future exposure.

Routes of Vaccine Administration

- Intramuscular (IM) – Hepatitis B
- Subcutaneous (SC) – MMR
- Oral – OPV
- Intradermal – BCG

Cold Chain System

- Maintains vaccine potency from manufacture to administration.
- Temperature: +2°C to +8°C
- Components:
- Ice-lined refrigerators
- Cold boxes
- Vaccine carriers

Adjuvants

- Substances added to enhance immune response.

Examples:

- Aluminium hydroxide
- Aluminium phosphate

Advantages of Vaccination

- Prevents infectious diseases.
- Reduces mortality.

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- Cost-effective public health tool.

Limitations of Vaccines

- Not 100% effective.
- Requires cold chain.
- Booster doses needed.

DNA vaccine

A DNA vaccine is a type of genetic vaccine that uses a plasmid DNA containing the gene encoding a specific antigen. When introduced into the body, host cells produce the antigen, which stimulates an immune response.

Composition

- Circular plasmid DNA
- Gene encoding the target antigen
- Promoter (to express the gene in host cells)

Mechanism of Action

- DNA vaccine is injected (usually intramuscular or intradermal).
- Plasmid DNA enters host cells.
- The cell reads the DNA instructions.
- Using its ribosomes, the cell produces the antigen protein.
- Antigen is presented via MHC I and MHC II.

Activates:

- Humoral immunity (antibodies)
- Cell-mediated immunity (T cells)
- Formation of immune memory.

Route of Administration

- Intramuscular
- Intradermal
- Needle-free injection systems

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Examples

- ZyCoV-D – first DNA vaccine approved in India (COVID-19)
- Experimental DNA vaccines for:
- HIV
- Malaria
- Cancer

Advantages

- Induces both humoral and cellular immunity.
- Stable at room temperature.
- No risk of infection.
- Easy and rapid production.
- No need for live pathogens.

Disadvantages

- Lower immunogenicity in humans.
- Requires special delivery methods (e.g., needle-free injector, electroporation).
- Long-term safety still under study.

Synthetic Peptide Vaccine

A synthetic peptide vaccine is a type of subunit vaccine made using chemically synthesized short peptide fragments (specific epitopes) of a pathogen's antigen that are capable of inducing an immune response.

Principle

- Instead of using the whole pathogen, only selected antigenic peptide sequences (epitopes) are synthesized.
- These peptides are recognized by T cells and/or B cells.
- Often combined with adjuvants or carrier proteins to enhance immunogenicity.

Types

- B-cell epitope peptide vaccines
- T-cell epitope peptide vaccines
- Multi-epitope peptide vaccines

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Working Steps or Mechanism of Action of Synthetic Peptide Vaccine

Step 1 Identification of Epitope

Scientists identify a specific antigenic region called epitope of a pathogen that can trigger an immune response.

Example A portion of a virus surface protein.

Step 2 Peptide Synthesis

The selected epitope is chemically synthesized in the laboratory using a specific amino acid sequence.

Step 3 Formulation

The synthetic peptide is combined with Adjuvants to enhance immune response.

Carriers to improve delivery and immunogenicity.

Step 4 Administration

The formulated vaccine is administered into the body usually by intramuscular injection

Step 5 Immune Response Activation

Antigen presenting cells take up the peptide.

The peptide is processed and presented on MHC molecules.

This activates T cells and stimulates a specific immune response.

This activates T cells and B cells:

- B cells produce specific antibodies.
- T cells help kill infected cells.

Step 6: Memory Cell Formation

- The immune system forms memory cells.
- On real infection, the body responds faster and stronger.

Examples

- Experimental vaccines for:
- Malaria
- HIV
- Influenza

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- Cancer immunotherapy vaccines
- COVID-19 peptide-based vaccine candidates (research stage)

Advantages

- Highly specific and safe.
- No risk of infection or reversion.
- Easy to synthesize and modify.
- Stable and well-defined composition.
- Suitable for immunocompromised individuals.

Disadvantages

- Low immunogenicity (needs adjuvant).
- Short peptides may be rapidly degraded.
- Limited immune response if epitope selection is poor.
- MHC restriction may reduce population coverage.

Adjuvants Used

- Alum
- Freund's adjuvant (experimental)
- Liposomes
- TLR agonists

Applications

- Infectious disease prevention.
- Cancer vaccines.
- Personalized immunotherapy.

Multivalent Vaccine

A multivalent vaccine is designed to protect against multiple strains or types of a pathogen, or even multiple pathogens, in a single dose.

- Different strains of the same microorganism, or

- Different microorganisms (e.g., MMR = Measles, Mumps, Rubella)

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Working Mechanism – Step by Step:

1. Vaccine Introduction

- The vaccine is injected or administered.

- It contains multiple antigens, each representing a different disease-causing organism or strain.

2. Recognition by Immune Cells

- Antigen-presenting cells (APCs) like dendritic cells or macrophages recognize all the antigens.

- APCs process each antigen and present them on their surface using MHC molecules.

3. Activation of T Helper Cells

- T helper (CD4+) cells identify these presented antigens.

- They become activated and stimulate:

- B cells to make specific antibodies for each antigen.

- Cytotoxic T cells (if the antigen is from an intracellular pathogen like a virus).

4. Antibody Production

- B cells convert into plasma cells, which produce different antibodies for each antigen.

These antibodies target and neutralize the specific pathogens when encountered.

5. Memory Formation

- The immune system also creates memory B and T cells for each antigen.

- These memory cells stay in the body for long-term immunity.

Special Notes:

- The immune system can recognize multiple antigens at once.

- There is usually no interference unless antigens are too similar or one is very dominant.

- Adjuvants may be used to boost the immune response equally for all components.

Example: MMR Vaccine Working

- Contains 3 live attenuated viruses:

- Measles virus

- Mumps virus

- Rubella virus

- Each one activates the immune system to build specific antibodies and memory cells.

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Benefits of Multivalent Vaccine Mechanism

- Fewer injections for multiple protections.

- Ensures high vaccination compliance.

- Reduces healthcare cost and logistics.

Vaccine Clinical trials

Vaccines have a long history of successfully protecting people and communities against infectious diseases.

Vaccination has improved the quality of life for many, and serious diseases like smallpox have been eliminated.

As vaccine technology advances, researchers can develop better and safer vaccines.

How new vaccines are developed

The U.S. Food and Drug Administration's (FDA's) Center for Biologics Evaluation and Research (CBER) is responsible for regulating vaccine use in the United States.

The general stages of vaccine development are:

- Research and discovery
- Proof of concept
- Testing the vaccine
- The manufacturing process
- Approving the vaccine
- Recommending the vaccine for use
- Monitoring safety after approval

Research and discovery

In this early stage of vaccine development, researchers explore their idea for a potential vaccine. Vaccine development often takes 10-15 years of laboratory research, usually at a company in private industry, but often involves collaboration with researchers at a university.

Proof of concept

Before a vaccine can be tested in people, researchers study its ability to cause an immune response with small animals, like mice. At this stage, researchers may make adjustments to the vaccine to make it more effective. Vaccine effectiveness is important because it measures how

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well vaccination protects people against outcomes such as infection, symptomatic illness, hospitalization, and death.

If the vaccine shows promising enough results, it moves forward to clinical trials for testing in people.

Testing the vaccine

Next, the vaccine enters a clinical development stage, which is also called a clinical trial. To do this, researchers submit an Investigational New Drug (IND) application to FDA, which includes data from animal studies, information on manufacturing technology, and the quality of the vaccine. Vaccine quality is important because it affects how well it will work to provide long- and short-term protection against disease.

The clinical development stage is a three-phase process, which may include a fourth phase if the vaccine is approved by FDA.

Phase 1

Small groups of people (20 to 100) receive the trial vaccine. During this phase, researchers gather information on how safe the vaccine is in people. This includes learning about and identifying side effects, and studying how well the vaccine works to cause an immune response.

Phase 2

The clinical trial expands to hundreds (100-300) of trial participants who have characteristics (such as age and physical health) similar to the intended recipients for the vaccine. They can also include groups of people from diverse backgrounds to ensure representation across different populations.

This phase provides additional safety information on side effects and risks, and more information on how well the vaccine works to cause an immune response.

Phase 3

The clinical trial expands to thousands (1,000–3,000) of people. In this phase, researchers confirm how well the vaccine works, monitor common and less common side effects, and collect information to support safe use in people.

Phase 4 (after FDA approval)

After FDA approves (also known as "licenses") a vaccine for use in the general population, it might advance to an additional clinical trial phase with thousands of participants. Phase 4 is a formal, ongoing study to evaluate the new vaccine's safety and effectiveness over a longer period of time.

Immunodiagnostics

Immunodiagnostics is a branch of laboratory medicine that uses immunological methods to diagnose diseases, monitor patients' health, and guide treatment decisions. It relies on the specific binding between antigens (substances that can induce an immune response) and

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antibodies (proteins produced by the immune system to combat antigens). This antigen-antibody interaction is the cornerstone of many immunodiagnostic tests.

Basics of antigen-antibody reactions

Antigens are substances that the body recognizes as foreign. Common examples include parts of bacteria, viruses, or other pathogens.

Antibodies (or immunoglobulins) are proteins produced by the immune system, specifically by B lymphocytes (B cells). They are released in response to an antigen. Antibodies are highly specific; each antibody binds to a particular antigen.

When antibodies bind to antigens, they do so at a region called the paratope (on the antibody) which attaches to the epitope (on the antigen). This binding can neutralize the antigen, marking it for destruction or removal from the body.

Agglutination

Agglutination of antigen-coated particles is frequently used for detection of specific antibodies. Common examples of the use of agglutination are blood-grouping techniques and identification of Rheumatoid factors.

Immunodiffusion

Ouchterlony double immunodiffusion: Also known as agar gel immunodiffusion or passive immunodiffusion, this technique is utilized to identify and quantify immunoglobulins and extractable nuclear antigens.

This method involves creating a series of wells in agar gel, where the antigen is placed in one well and the test serum in another, to assess for the presence of specific antigens. The plate is incubated for 48 hours to allow diffusion and interaction of the contents. If an antibody-antigen reaction occurs, a visible white precipitate forms in a straight line, known as a precipitin line.

Blotting techniques

Assay techniques

Immunoassays are biochemical tests that use antibodies, and occasionally antigens, to detect macromolecules in a solution. The substance being measured is referred to as the ‘analyte.’

Immunoassays typically involve the use of specific molecules, known as ‘labels,’ which can bind or conjugate to the molecule of interest. The type of label used defines the kind of immunoassay performed.

Sandwich immunoassay

In sandwich immunoassays, an antibody is first attached to a microtiter plate well. Varying amounts of antigen are then added. After washing off unbound antigen, a labeled secondary antibody specific for different epitopes of the antigen is introduced. Following another washing

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step to remove unbound secondary antibody, the amount of bound secondary antibody is measured. This amount correlates with the antigen concentration, determined via a standard curve.

Microscopy-based techniques (immunofluorescence)

These methods are primarily used on body tissues to determine specific biomolecule targets conjugated to antibodies with tagged fluorescent dyes. Immunofluorescence can be considered a subset of immunohistochemistry where fluorophores are used to determine the location of specific antigens and antibodies.

Primary (direct)

In direct immunofluorescence, a fluorophore is chemically linked to a primary antibody. The tissue sample is placed on a slide, and fluorescein-labeled antibodies specific to the antigen of interest are added. When exposed to ultraviolet light, these labeled antibodies emit visible light, indicating the presence of the antigen. The technique is applicable to both animal and human tissues.

Secondary (indirect)

In indirect immunofluorescence, two antibodies are used. The first (primary) antibody, unlabeled, binds to the target molecule. Then, a secondary antibody, labeled with a fluorophore, binds to the primary antibody. This method is commonly used for measuring ANA levels in patients with SLE, using patient serum and substrate tissue from rat liver, kidney, and stomach.

Immunoglobulin

Antibodies, also known as immunoglobulins, are proteins produced by lymphocytes as a result of interaction with antigens. Antibodies are a part of the humoral immune of the adaptive immune system where each antibody identifies a specific antigen and protects the body against it.

Antibodies are glycoproteins that bind to antigens with a high degree of specificity and affinity.

The produced antibodies circulate through the bloodstream and neutralize antigens that are identical to those that triggered the immune response.

The binding of antibodies to microorganisms or other such antigens can result in the microorganism being immobile or preventing them from penetrating the cells.

Each antibody is a Y-shaped protein where each tip of the Y contains a paratope that recognizes an epitope of a particular antigen.

Antibodies can be classified into different classes based on different structures and functions.

Immunoglobulin G (IgG)

IgG is the most abundant immunoglobin, which accounts for about 80% of the total serum antibodies. The concentration of IgG in the blood is about 10mg/ml.

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Structure of IgG

The basic structure of IgG is composed of a Y-shaped protein where the Fab arms are linked to the Fc arms by an extended region of polypeptide chain called the hinge.

The region is exposed and sensitive to attack by proteases that cleave the molecule into distinct functional units arranged in a four-chain structure.

An IgG molecule consists of two identical γ heavy chains, usually of the size 50kDa.

The light chains in IgG exist in two forms; κ and λ, where the κ form is more prevalent than λ, in the case of humans.

The Fc regions of the molecule have a highly conserved N-glycosylation site in the heavy chain.

Properties of IgG

The IgG antibodies exist in the serum in the monomeric form, and these can cross the placenta from the mother to the fetus.

Each IgG antibody has two paratopes that bind to two different epitopes on different antigens.

IgG has four subclasses classified on the basis of the subclasses of the γ heavy chains.

IgG antibodies participate predominantly in secondary immune response as these are generated as a result of class switching and maturation of the response.

Subclasses of IgG

IgG antibodies have been classified into four subclasses; IgG1, IgG2, IgG3, and IgG4.

These are named in the order of their abundance in serum, with IgG1 being the most abundant.

Functions of IgG

IgG antibodies provide long-term protection against various agents like bacteria, viruses, and bacterial toxins.

IgG is one of the most potent complement activators when compared to all other antibodies.

The binding ability of IgG to antigens is more effective as it enhances phagocytosis.

Immunoglobulin M (IgM)

IgM is the third most abundant immunoglobulin in serum, with a concentration of 1.5 mg/ml. It is the largest antibody and is the first antibody to appear in response to the initial exposure to antigen.

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Structure of IgM

IgM is secreted in a pentameric form with five distinct units, where each are composed of two µ heavy chains and two light chains.

A J chain might be present in the hexameric form of the molecule, but it isn't always present. The J chain is usually added just before the secretion of the pentamer as it helps in the polymerization of the monomers.

Each of the monomers has two antigen-binding sites, resulting in 10 binding domains in a single molecule. However, all the domains cannot be occupied at the same time due to limitations in space.

The pentameric form of IgM has a molecular weight of 900 kDa.

Properties of IgM

IgM is the largest and the only pentameric antibody in humans. It is also the first antibody to be produced in response to the initial exposure to an antigen.

IgM is the first immunoglobulin to be synthesized by the fetus, beginning at about 20 weeks of age.

IgM is a pentameric molecule with 10 antigen-binding sites and 5 Fc portions held together by disulfide linkages.

The monomeric form of IgM occurs as the major antibody receptor on the surface of B lymphocytes.

IgM is relatively short-lived and usually disappears earlier than IgG.

The large size of the molecules do not allow effective diffusion of the antibody, and thus, it is found in very low concentration in the intracellular fluids.

Functions of IgM

IgM is very effective against viruses as less IgM than IgG is enough to neutralize viral infections.

IgM is also a better agglutinin as it takes 100 to 1000 more molecules of IgG than that of IgM for the same level of agglutination.

Immunoglobulin A (IgA)

IgA or sIgA is the main immunoglobulin found in the mucous membrane in the form of secretory antibodies. The concentration of IgA is found in small quantities in blood, but it is found in high concentrations in tears, saliva, and sweat.

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Structure of IgA

The molecular size of IgA is 160 kDa with a four-chain monomeric structure, however, it can occur in dimeric and trimeric forms.

The heavy chain of IgA is divisible into three constant domains, CH1, CH2, and CH3, and a variable VH domain.

The hinge region occurs between the CH1 and CH2 domains held together by disulfide linkages.

sIgA has a secretory component as an additional component with a polypeptide chain of 75 kDa and extracellular proteolytic fragment.

The molecule also has a J-chain linked to the chains via disulfide bridges. The secretory and J chain facilitates the transport of IgA across epithelial cells and protects the molecule from proteolytic digestion by enzymes.

Properties of IgA

IgA is the second most abundant immunoglobulin in humans, with a concentration of 2-4 mg/ml. It accounts for about 10-15% of the total serum concentration but is the most abundant antibody in external secretions.

IgA is the first line of defense as it works by inhibiting bacterial and viral adhesion to epithelial cells and by neutralizing viral and bacterial toxins intracellularly.

The secretory IgA mostly occurs in dimeric form with two monomeric units linked together by a joining peptide.

Subclasses of IgA

IgA has been classified into two subclasses; IgA1 and IgA2.

IgA1 is the monomeric form, and IgA2 is the dimeric or polymeric form.

IgA1 occurs in serum IgA (about 80%), which is produced in the bone marrow and released on the mucosal surfaces.

The IgA in most locally secreted products is polymeric with a relative release of dimeric IgA2.

One of the most prominent differences between IgA1 and IgA2 is the hinge region which is quite extended in IgA1.

Functions of IgA

IgA is the first line of defense as it protects the body from the entry and colonization of mucosal surfaces by different foreign particles.

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Immunoglobulin D (IgD)

IgD is a monomeric antibody that occurs on the surface of immature B lymphocytes. It is produced in a secreted form in a small amount in the blood serum.

Structure of IgD

IgD has a structural diversity throughout evolution in the vertebrates as it is flexible to complement the function of IgM.

It is a glycoprotein with two identical δ heavy chains and two identical light chains.

IgD found on the surface of B lymphocytes has some extra amino acids at C-terminal in order to anchor to the membrane.

The light and heavy chains are linked together by disulfide links, but they have additional intrachain disulfide links that divide the chains into domains.

The IgD molecule also has an extended hinge region which increases the flexibility of the molecule but decreases its resistance against proteolytic cleavage.

Properties of IgD

IgD is found in low concentration in serum, and its exact function in the immune system is not yet clearly understood.

It represents about 0.25% of the total serum immunoglobulins with a relative molecular mass of 185 kDa and a half-life of 2.8 days.

It also accounts for about 1% of the proteins present in the plasma membranes of B lymphocytes. Here, it usually coexpressed with another cell surface antibody, IgM.

Functions of IgD

The most important function of IgD is antigen receptor on B cells. It also regulates B cell function if it encounters an antigen.

It is also secreted in some amounts in the blood, mucosal secretions, and the surface of innate immune effector cells.

Immunoglobulin E (IgE)

IgE is a type of immunoglobulin found only in mammals and synthesized by plasma cells. It occurs in a monomeric form with two ε heavy chains and two light chains.

Structure of IgE

IgE has a typical antibody structure with ε heavy chains that have a high carbohydrate content.

IgE has two identical antigen-binding sites consisting of both light and heavy chains and a valency of 2.

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Like all antibodies, heavy and light chains are further divided into variable and constant regions.

The heavy chains consist of five domains, out of which one is variable, and four are constant.

Functions of IgE

IgE is mostly associated with allergic reactions where it binds to reintroduced antigens and triggers the release of pharmacologically active agents.

It also plays an essential role in response to allergens and antigen preparation used in desensitization immunotherapy.

Quality Control (QC) in pharmaceutical

Quality Control (QC) plays a very important role in the pharmaceutical industry. QC follows systematically examining and testing pharmaceutical products at various stages of production to identify and rectify problems and variations. This makes sure that every product meets the specified quality standards before reaching the market, safeguarding patient safety.

Objectives of Quality Control

The objectives of quality control activity are to improve product quality and reduce risk in the pharmaceutical industry.

- Enhance product quality and reduce risks
- Meet specifications
- Identify & correct defects
- Minimize errors and inconsistencies
- Gain product efficiency
- Reduce waste
- Improve process control
- Cost savings
- Customer loyalty
- Customer satisfaction
- Build Brand reputation
- Competitive advantage

Steps in Pharmaceutical Quality Control

The steps in pharmaceutical quality control includes a systematic step to make sure the safety, efficacy, and consistency of pharmaceutical products.

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Raw materials testing: This step is important to ensure that the materials meet the necessary quality standard, and verify that it won't be spoiled in any way.

Testing and Analysis: The collected samples under this will go for various tests and analyses. Depending on the product type, which include chemical, physical, microbiological, and pharmacological tests.

Stability Testing: Stability testing evaluates how the product changes over time under different storage conditions.

In-process testing: It is conducted at various stages of production to ensure that the medicine meets all quality specifications.

Final testing: This is the last step in quality control to test the final product to confirm that it meets all the necessary requirements.

In-Process Quality Control

In-Process Quality Control (IPQC) refers to the systematic checks and tests performed during different stages of pharmaceutical manufacturing to ensure that the product consistently meets pre-defined quality specifications.

It is an integral part of Good Manufacturing Practices (GMP).

Objectives of IPQC

- To monitor and control the manufacturing process.
- To detect deviations at an early stage.
- To ensure uniformity and reproducibility.
- To reduce batch rejection and rework.
- To ensure patient safety and product efficacy.

Scope of IPQC

IPQC covers:

- Raw materials
- Processing stages
- Environmental conditions
- Equipment performance
- Intermediate and bulk products

IPQC Controls at Different Stages

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1 Raw Material In-Process Control

- Tests Performed
- Identification (IR, UV, chemical tests)
- Assay
- Moisture content (LOD/KF)
- Particle size distribution
- Microbial limit tests

Purpose

To ensure only approved and suitable materials enter production.

2 Environmental & Equipment Control

- Environmental Monitoring
- Temperature
- Relative humidity
- Differential pressure
- Airborne particle count
- Microbial monitoring (settle plates, air sampling)
- Equipment Checks
- Calibration status
- Cleaning validation
- Operational qualification

3 IPQC During Manufacturing Process

A. Solid Dosage Forms (Tablets)

(i) Granulation Stage

- Blend uniformity
- Bulk density & tapped density
- Angle of repose
- Moisture content

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(ii) Compression Stage

- Average weight
- Weight variation
- Hardness
- Thickness
- Friability
- Disintegration time

B. Capsules

- Fill weight variation
- Content uniformity
- Capsule locking length
- Moisture content

C. Liquid Oral Preparations

- pH
- Viscosity
- Clarity
- Specific gravity
- Fill volume
- Microbial limits (during process)

D. Semi-Solid Dosage Forms (Ointments, Creams)

- Appearance & homogeneity
- pH
- Viscosity / consistency
- Spreadability
- Fill weight

E. Parenterals (Injectables)

- pH
- Clarity

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- Particulate matter
- Fill volume
- Filter integrity test
- Environmental monitoring during aseptic processing

4 In-Process Control of Packaging

- Correct labeling
- Batch number & expiry date
- Pack integrity
- Leak test (blisters, strips)
- Overprinting accuracy

5 Documentation in IPQC

- In-Process Control Sheets
- Batch Manufacturing Records (BMR)
- Deviation reports
- Change control records

Advantages of IPQC

- Prevents costly batch failures.
- Ensures consistent product quality.
- Minimizes wastage.
- Improves manufacturing efficiency.

Limitations

- Requires skilled manpower.
- Time-consuming.
- Increased operational cost.

Final Product Quality Control (FPQC)

Final Product Quality Control (FPQC) refers to the comprehensive testing and evaluation of the finished pharmaceutical product to ensure that it complies with pharmacopoeial standards, regulatory requirements, and approved specifications before it is released for sale or distribution.

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FPQC is the last and most critical checkpoint in pharmaceutical quality assurance.

Objectives of FPQC

- To confirm safety, efficacy, and quality of the finished product.
- To ensure compliance with IP / BP / USP / EP standards.
- To verify batch-to-batch consistency.
- To decide batch release or rejection.
- To ensure patient safety and regulatory compliance.

Scope of FPQC

FPQC covers:

- Physical quality
- Chemical quality
- Microbiological quality
- Stability and shelf life
- Packaging and labeling integrity

FPQC Tests and Parameters

1. Physical Tests

- Appearance
- Color
- Odor
- Shape
- Surface texture
- Absence of visible defects
- Weight / Volume
- Weight variation (tablets/capsules)
- Net content (liquids, semisolids)
- Hardness & Friability (Tablets)
- Hardness test

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- Friability (≤ 1%)

2. Chemical Tests

Assay of Active Ingredient

- Determines actual drug content.
- Should be within 90–110% (as per monograph).

Content Uniformity

- Ensures uniform drug distribution.
- Especially critical for low-dose formulations.

Dissolution Test

- Measures drug release profile.
- Predicts in-vivo bioavailability.

pH Determination

- Important for stability and patient acceptability.
- Preservative Content
- Confirms effective antimicrobial protection.

4. Special Tests (Dosage-Form Specific)

Tablets & Capsules

- Disintegration test
- Uniformity of dosage units

Liquids

- Clarity
- Viscosity
- Specific gravity

Semisolids

- Homogeneity
- Spreadability
- Consistency

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Inhalers

- Delivered dose uniformity
- Particle size distribution

5. Stability Testing

Purpose

- To determine shelf life and storage conditions.

Types

- Accelerated stability testing
- Long-term stability testing

Parameters Checked

- Assay
- Degradation products
- Appearance
- pH
- Dissolution

6. Packaging & Labeling Control

- Container closure integrity test
- Leak test
- Correct batch number
- Manufacturing & expiry date
- Storage instructions
- Patient information leaflet

7. Documentation in FPQC

- Certificate of Analysis (CoA)
- Finished Product Specification
- Stability data reports
- Batch Release Authorization

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- Deviation & OOS reports

Advantages of FPQC

- Ensures regulatory compliance.
- Protects patient safety.
- Maintains company reputation.
- Legal safeguard for manufacturers.

Limitations

- Cannot correct manufacturing errors.
- Time-consuming.
- Batch rejection leads to financial loss.

Sterility Testing

Sterility testing is a microbiological examination method used to determine whether a product that is required to be sterile has been contaminated by microorganisms. It is commonly applied in the pharmaceutical, biotechnology, and medical device manufacturing industries.

Purpose of Sterility Testing

Sterility testing is applicable to most pharmaceutical products mandated to be sterile, including injectable drugs, eye drops, solid pharmaceuticals, and various sterile medical devices. Its scope of application is broad.

If these products are contaminated with microorganisms, they may reduce efficacy or even cause infections and other severe complications upon contact with the human body. Therefore, the primary goal of sterility testing is to ensure that products designated as sterile, after undergoing aseptic manufacturing or terminal sterilization processes, are free of viable microorganisms, ensuring safety and effectiveness in clinical use.

Definition of Sterility and International Standard

Sterility refers to the absence of viable microorganisms in a sample, which is a fundamental requirement for ensuring the safety of pharmaceutical products. The internationally recognized benchmark for sterilization efficacy is the Sterility Assurance Level (SAL). A product is considered sterile if SAL ≤ 10^{-6}, meaning the probability of microbial contamination is less than or equal to one in a million.

International Standards for Sterility Testing

Standards such as ISO 11737-2, USP <71>, EP, JP, and the Chinese Pharmacopeia define the requirements for sterility testing, including test environments, sample preparation, and operating

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procedures. These ensure compliance with global standards and provide reliability for test results.
How is Sterility Testing Performed?

Two commonly used methods for sterility testing are the Membrane Filtration Method and the Direct Inoculation Method. According to the Pharmaceutical Inspection Co-operation Scheme (PIC/S), both methods must be performed under aseptic conditions in a Grade A cleanroom within a Grade B environment. The choice of method depends on the sample characteristics.

Method 1: Membrane Filtration Method

This is one of the most widely used sterility testing methods, suitable for liquid samples, solid samples, and medical devices with pipelines that can be rinsed and filtered.

Principle: The sample is filtered through a membrane with a pore size of less than 0.45 µm to capture potential microorganisms. The membrane is then transferred to an appropriate culture medium for incubation to detect the presence of viable microorganisms.

Operating Steps for Membrane Filtration Method

The operational process of the membrane filtration method for sterility testing can generally be divided into four stages: sample pre-treatment, sample filtration, membrane incubation, and result interpretation. The following will provide an explanation of each stage in sequence.

1.Sample Pre-treatment: Depending on sample type, pre-treatment is required before filtration to improve flow rate or prepare the sample for filtration.

Sample Type: Filterable Liquids

Pre-Treatment Method: Dilute with sterile liquid if too viscous.

2.Sample Filtration: Pour the pre-treated sample into the filter funnels. Use a vacuum pump to create negative pressure, enabling vacuum filtration. For larger quantities, multi-branch filtration manifolds can be used.

3.Membrane Incubation: Transfer the membrane to a culture medium using sterile tweezers, seal, and incubate at an appropriate temperature for 14+ days.

4.Result Interpretation: Observe the culture medium for microbial growth. If no changes occur, the sample is sterile and complies with sterility testing requirements. If the medium becomes turbid, it indicates the presence of viable microorganisms, meaning the sample does not meet sterility requirements.

Method 2: Direct Inoculation Method

Direct Inoculation Method is designed for samples that cannot be processed using membrane filtration, such as insoluble solids or medical devices that are unsuitable for filtration.

The principle involves directly placing the sample into a culture medium and monitoring for microbial growth to assess sterility.

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Operating Steps for Direct Inoculation Method

The operational process of the direct inoculation method can generally be divided into three stages: Sample preparation, culture medium incubation, and result interpretation. The following provides an explanation of each stage in sequence.

1.Sample Preparation: After preparing the sample, place it into the culture medium.

2.Culture medium incubation: Seal the culture medium and incubate for 14+ days.

3.Result Interpretation: Observe the culture medium for turbidity to determine sterility. If no changes occur, the sample is sterile and complies with sterility testing requirements. If the medium becomes turbid, it indicates the presence of viable microorganisms, meaning the sample does not meet sterility requirements.

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UNIT - V

Good Manufacturing practices (GMP)

GMP stands for Good Manufacturing Practices, a system that ensures that manufactured products—such as food, cosmetics, and pharmaceutical goods—are consistently produced and controlled according to set quality standards. Implementing GMP can help cut losses and waste, and avoid recalls, fines, and jail time. Overall, it protects both the company and the consumer from negative food safety events.

GMPs examine and cover every aspect of the manufacturing process to guard against any risks that can be catastrophic for products, such as cross-contamination, adulteration, and mislabeling. Some areas that can influence the safety and quality of products that GMP guidelines and regulations address are the following:

- Quality management
- Sanitation and hygiene
- Building and facilities
- Equipment
- Raw materials
- Personnel
- Validation and qualification
- Complaints
- Documentation and recordkeeping
- Inspections & quality audits

PRINCIPLES / ELEMENTS OF GOOD MANUFACTURING PRACTICES (GMP)

GMP is based on the principle that quality must be built into the product, not tested only at the end.

1. Quality Assurance (QA)

* Establishes the overall quality system.

* Ensures products are designed, manufactured, and controlled correctly.

* Prevents errors through SOPs, training, audits.

* Approves batch records, validation, and product release.

* Handles deviations, CAPA, and change control.

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*QA is responsible for GMP compliance.

2. Quality Control (QC)

* Concerned with testing and inspection.

* Sampling and testing of:

* Raw materials

* In-process samples

* Finished products

* Uses validated analytical methods.

* Ensures only approved materials and products are used or released.

*QC detects defects; QA prevents them.

3. Personnel

* Adequate number of qualified and trained staff.

* Clearly defined roles and responsibilities.

* Regular GMP training.

* High level of personal hygiene.

* Use of protective clothing (gowns, gloves, masks).

4. Premises & Equipment

Premises:

* Designed to prevent cross-contamination.

* Adequate space, lighting, ventilation.

* Smooth, easy-to-clean surfaces.

Equipment:

* Properly designed, installed, and maintained.

* Qualified and calibrated.

* Cleaned and validated before use.

5. Documentation

* SOPs, manuals, batch records, logs.

* Clear, accurate, and approved documents.

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* Changes controlled through document control.

Golden rule: If it is not documented, it did not happen.

6. Validation

* Documented evidence that processes and systems perform consistently
* Types:
    * Process validation
    * Equipment validation
    * Cleaning validation
    * Analytical method validation
* Includes IQ, OQ, PQ

7. Materials Management

* Approved and qualified suppliers
* Proper receipt, labeling, storage
* FIFO / FEFO followed
* Segregation of:
* Approved
* Quarantined
* Rejected materials

8. Production & In-Process Control

* Manufacturing strictly as per approved SOPs.
* In-process checks to ensure uniformity.
* Recording of every step in Batch Manufacturing Records.
* Prevention of mix-ups and contamination.

9. Sanitation & Hygiene

* Clean premises and equipment.
* Validated cleaning procedures.
* Pest control programs.
* Personnel hygiene practices.

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10. Packaging & Labeling

* Correct labels to avoid mix-ups.
* Line clearance before packaging.
* Proper storage of printed packaging materials.
* Traceability of batch numbers.

11. Complaints & Product Recall

* System to receive and investigate complaints.
* Identification of root cause.
* Effective recall system to withdraw defective products.
* Recall records maintained.

12. Self-Inspection / Internal Audit

* Periodic GMP audits.
* Identification of non-compliance.
* Implementation of corrective actions.
* Continuous improvement.

13. Contract Manufacturing & Analysis

* Written agreements defining responsibilities.
* Compliance with GMP by both parties.
* Quality oversight maintained.

IMPORTANCE OF GMP PRINCIPLES

* Ensures consistent product quality.
* Protects patient safety.
* Reduces recalls and rejections.
* Mandatory for regulatory approval.

GOOD LABORATORY PRACTICES (GLP)

Introduction

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Good Laboratory Practices (GLP) is a quality system concerned with the organizational process and conditions under which laboratory studies are planned, performed, monitored, recorded, archived, and reported.

GLP ensures that laboratory data are:

* Accurate
*Reliable
*Traceable
*Reproducible

Objectives of GLP

1. To ensure authenticity and integrity of data.
2. To minimize experimental errors.
3. To ensure proper documentation.
4. To improve confidence in laboratory results.
5. To facilitate regulatory acceptance of data.
6. To ensure safety of personnel and environment.

Scope of GLP

GLP applies to non-clinical laboratory studies, including:

* Toxicological studies
* Pharmacological studies
* Environmental safety studies
* Pesticide and chemical testing
* Food and cosmetic testing
* Biotechnology and microbiology labs

Organization & Personnel

i) Organizational Structure

* Well-defined hierarchy
* Clear authority and responsibility
* Adequate number of personnel

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ii) Personnel Responsibilities

* Staff must be qualified and trained.
* Continuous training programs.
* Training records maintained.
* Personnel must follow SOPs strictly.

Quality Assurance Unit (QAU)

Role of QAU

* Independent from study conduct
* Conducts:
* Study-based inspections
* Facility inspections
* Process audits
* Reports findings to management

Importance

* Ensures compliance with GLP
* Prevents data falsification
* Maintains study credibility

Facilities

I) Facility Requirements

* Adequate space and layout
* Proper ventilation and lighting
* Clean and contamination-free environment

Separate Areas For

* Sample receipt
* Sample preparation
* Instrumentation
* Storage of chemicals and reagents
* Waste disposal

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Equipment, Apparatus & Materials

Equipment Management

* Suitable design and capacity
* Proper installation
* Regular calibration
* Preventive maintenance

Documentation

* Equipment log books
* Calibration records
* Maintenance records

Standard Operating Procedures (SOPs)

SOPs are written, authorized instructions describing how specific laboratory activities should be performed.

Types of SOPs

* Sample collection SOP
* Instrument operation SOP
* Cleaning and sanitation SOP
* Safety SOP
* Waste disposal SOP
* Data recording SOP

Test Systems

Types

*Biological(cells, tissues, animals)

*Chemical (reagents, standards)

*Physical (instruments, devices)

Requirements

* Proper identification
* Controlled environmental conditions

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* Health and stability monitoring

Test & Reference Items

Test Item

* Substance under investigation

Reference Item

* Standard or control substance

GLP Requirements

* Proper labeling
* Storage conditions documented
* Stability testing
* Expiry monitoring

Study Plan / Protocol

Contents of Study Plan

* Study title and objective
* Test and reference items
* Experimental design
* Methods and procedures
* Statistical methods
* Study schedule
* Authorized signatures

Conduct of Study

* Study conducted as per protocol
* SOPs strictly followed
* Any deviations documented and justified
* Raw data recorded immediately

Data Recording & Raw Data

Raw Data Includes

* Lab notebooks

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* Instrument printouts
* Electronic data
* Photographs

Data Integrity Rules

* No overwriting
* Corrections with date & signature
* Traceable and legible records

Final Study Report

Contents

* Study objectives
* Materials and methods
* Results and discussion
* Statistical analysis
* Conclusion
* QA statement
* Signatures

Storage & Archiving

Items Archived

* Raw data
* Samples
* SOPs
* Study reports
* QA inspection records

Archiving Period

* As per regulatory requirements
* Secure, controlled access

Safety & Waste Management

* Proper PPE usage

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* Chemical labeling
* Biohazard handling
* Safe disposal of waste
* Emergency procedures

Importance of GLP

* Ensures data credibility
* Reduces experimental errors
* Facilitates regulatory approval
* Improves laboratory discipline
* Enhances global acceptance

Advantages of GLP

- High-quality data
- Reduced repetition of studies
- Legal and regulatory compliance
- Improved lab efficiency

Regulatory Aspects of Quality Control in Pharmaceutical Industry

Quality Control (QC) in pharmaceuticals is governed by national and international regulatory authorities to ensure that every medicine is safe, effective, and of standard quality.

1. Purpose of Regulatory Control in QC

Regulations ensure:

*Identity of the drug
*Strength & purity
*Safety and efficacy
*Consistency between batches
*Patient protection

2. Major Regulatory Authorities

India

- CDSCO – Central Drugs Standard Control Organization

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- DCGI – Drug Controller General of India
- Drugs & Cosmetics Act, 1940
- Drugs & Cosmetics Rules, 1945

International

- US FDA – United States Food and Drug Administration
- WHO – World Health Organization
- EMA – European Medicines Agency
- ICH – International Council for Harmonisation
- MHRA – UK

3. Regulatory Guidelines Affecting QC

a) Good Manufacturing Practices (GMP)

QC must follow Schedule M (India)and cGMP (US FDA)

QC responsibilities under GMP:

* Approval or rejection of raw materials
* In-process testing
* Finished product testing
* Stability studies
* Environmental monitoring
* Documentation control

b) Pharmacopoeial Standards

- QC testing must comply with:
- IP – Indian Pharmacopoeia
- USP – United States Pharmacopoeia
- BP – British Pharmacopoeia
- EP – European Pharmacopoeia

These define:

* Test methods

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* Acceptance limits
* Reference standards

4. Regulatory Requirements for QC Activities

1. Raw Material Control

* Approved suppliers only
* Testing for identity, purity, and potency
* Certificate of Analysis (CoA) verification

2. In-Process Quality Control (IPQC)

* Weight variation
* pH, hardness, friability
* Uniformity of content
* Monitoring critical process parameters

3. Finished Product Testing

Mandatory tests:

* Assay
* Dissolution / disintegration
* Sterility (for injectables)
* Microbial limits
* Stability testing

4. Stability Studies

As per ICH Q1A(R2):

* Accelerated
* Long-term
* Intermediate studies

Used to fix shelf life & storage conditions

5. Documentation & Records (Regulatory Mandatory)

QC must maintain:

* SOPs (Standard Operating Procedures)

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* Batch testing records

* Analytical method validation reports

* Stability reports

* Out-of-specification (OOS) records

* Change control records

6. Validation & Calibration

Regulations require:

*Analytical method validation (ICH Q2)

*Instrument calibration

*Cleaning validation

*Computer system validation

7. Quality Audits & Inspections

* Internal QC audits

* Regulatory inspections by DCGI / FDA / WHO

* Data integrity checks (ALCOA principles)

8. Handling Deviations & OOS

Regulatory expectation:

* Root cause analysis

* Corrective and Preventive Actions (CAPA)

* Proper documentation and closure

9. Regulatory Submissions Related to QC

QC data included in:

*Drug Master File (DMF)

*CTD / eCTD

*ANDA / NDA

10. Importance of Regulatory Compliance in QC

* Product approval & market authorization

* Avoidance of product recall

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* Company reputation

* Patient safety

* Legal compliance

BIS (IS) in Pharmaceutical Industry

BIS = Bureau of Indian Standards

IS = Indian Standards

BIS is the national standards body of India, responsible for developing Indian Standards (IS) to ensure quality, safety, and reliability of products, including pharmaceuticals.

* Established under BIS Act, 2016

* Headquarters: New Delhi

* Earlier known as ISI

* BIS formulates and publishes Indian Standards (IS)

Role of BIS in Pharmaceutical Industry

BIS ensures:

*Quality of pharmaceutical products

*Standardization of drugs & excipients

*Safety of medical devices

*Uniform testing methods

Indian Standards (IS) in Pharmaceuticals

BIS standards apply mainly to:

*Pharmaceutical raw materials

*Excipients

*Packaging materials

*Medical devices

*Water quality

IS standards are used when Pharmacopoeial standards are not available.

Areas Covered by BIS (IS)

1. Drugs & Pharmaceutical Chemicals

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* Specifications for bulk drugs
* Limits for impurities
* Physical & chemical test methods

2. Pharmaceutical Excipients

Examples:

* Starch
* Talc
* Lactose
* Gelatin
* Glycerin

IS provides:

* Identity tests
* Purity limits
* Microbial limits

3. Packaging Materials

IS standards for:

* Glass containers
* Plastic containers
* Rubber closures
* Aluminum foils

Ensures:

* No interaction with drug
* Protection from contamination

4. Medical Devices & Surgical Items

* Syringes
* Needles
* Catheters
* IV sets

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5. Water Standards

IS specifications for:

* Purified water
* Drinking water
* Water for pharmaceutical use

6. BIS Certification Mark (ISI Mark)

* Indicates product conforms to **Indian Standards**
* Mandatory for some products
* Voluntary for many pharma-related items

BIS in Quality Control (QC)

QC labs use IS standards for:

* Testing non-pharmacopoeial materials
* Raw material approval
* Packaging material testing
* Vendor qualification

Legal Importance

* BIS standards are recognized by Drugs & Cosmetics Act
* Used by regulators when no IP standard exists
* Helps in regulatory compliance

Advantages of BIS in Pharma

* Uniform quality across manufacturers
* Consumer safety
* Supports exports
* Improves credibility of products

ISI (Indian Standards Institution)

ISI (Indian Standards Institution) was the national organization of India responsible for the formulation, publication, and promotion of Indian Standards (IS) for various products, including those used in the pharmaceutical industry.

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* Established in 1947

* Functioned as the standard-setting authority in India

* In 1987, ISI was replaced by the Bureau of Indian Standards (BIS)

* However, the ISI certification mark is still used and recognized

In pharmaceuticals, ISI standards helped ensure quality, safety, uniformity, and reliability of drugs and pharma-related materials.

Objectives of ISI in Pharmaceutical Industry

The main objectives were:

1. To ensure standard quality of pharmaceutical products
2. To protect public health and safety
3. To establish uniform testing methods
4. To support regulatory control of drugs
5. To promote good manufacturing practices
6. To facilitate trade and exports

Scope of ISI in Pharmaceutical Industry

ISI standards were applied mainly to non-pharmacopoeial products and materials related to pharmaceutical manufacturing.

Major areas covered:

* Pharmaceutical chemicals (bulk drugs)

* Pharmaceutical excipients

* Packaging materials

* Medical devices and surgical items

* Water and utilities used in pharma industry

ISI Standards for Pharmaceutical Chemicals (Bulk Drugs)

ISI laid down Indian Standards (IS) for various pharmaceutical chemicals where Indian Pharmacopoeia (IP) standards were not available.

These standards included:

* Chemical name and description

* Identification tests

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* Purity requirements
* Limits for impurities
* Assay methods
* Storage conditions

Purpose:

To ensure identity, purity, strength, and safety of bulk drugs.

ISI Standards for Pharmaceutical Excipients

ISI developed standards for commonly used excipients such as:

* Lactose
* Starch
* Talc
* Glycerin
* Liquid paraffin
* Gelatin

ISI specifications included:

* Physical characteristics
* Chemical purity limits
* Microbial limits
* Test methods

These standards ensured excipients were safe for human consumption and suitable for pharmaceutical use.

ISI Standards for Packaging Materials

Packaging plays a critical role in maintaining drug quality. ISI standards were developed for:

a) Glass Containers

* Ampoules
* Vials
* Bottles

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b) Plastic Containers

* HDPE bottles
* Plastic strips

c) Rubber Closures

* Stoppers for injectables

d) Metal Containers

* Aluminum foils

ISI standards ensured:

* No chemical interaction with drugs
* Protection from moisture, light, and contamination
* Mechanical strength and durability

ISI Standards for Medical Devices & Surgical Products

ISI provided standards for:

* Syringes
* Hypodermic needles
* IV sets
* Catheters
* Surgical gloves

These standards ensured:

* Sterility
* Safety
* Performance reliability

Many of these items required **mandatory ISI certification.

ISI Mark (Certification Mark)

ISI Mark:

* Indicates conformity to Indian Standards
* Granted after inspection and testing
* Ensures product meets required quality specifications

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In pharmaceuticals:

* Mainly used for packaging materials and medical devices
* Not commonly applied to finished drug formulations

Legal Importance of ISI in Pharmaceuticals

* ISI standards were **recognized under the Drugs & Cosmetics Act**
* Regulatory authorities used ISI standards for quality assessment
* QC laboratories referred to ISI standards for testing
* Supported enforcement of quality regulations

Replacement of ISI by BIS

* In 1987, ISI was replaced by Bureau of Indian Standards (BIS)
* BIS took over all functions of ISI
* IS standards continued under BIS
* ISI mark continues to be used under BIS certification

Importance of ISI Standards in Quality Control

In pharmaceutical QC, ISI standards were used for:

* Raw material testing
* Packaging material approval
* Vendor qualification
* Compliance during inspections

ISI	BIS
Indian Standards Institution	Bureau of Indian Standards
Established in 1947	Established in 1987
Old standard body	Present standard body
Developed IS standards	Maintains & updates IS standards
ISI mark	ISI mark continued under BIS

Advantages of ISI in Pharmaceutical Industry

* Uniform quality standards
* Improved patient safety

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* Reduction in substandard products

* Support for exports

* Enhanced credibility of manufacturers

Conclusion

ISI (Indian Standards Institution) played a significant role in establishing quality standards in the pharmaceutical industry of India. By setting Indian Standards for drugs, excipients, packaging materials, and medical devices, ISI ensured safety, uniformity, and regulatory compliance. Although ISI was replaced by BIS, its standards and certification mark continue to influence pharmaceutical quality control in India.

ISO (International Organization for Standardization)

ISO (International Organization for Standardization) is an independent, non-governmental international body that develops international standards to ensure quality, safety, efficiency, and consistency of products and services worldwide.

* Established in 1947

* Headquarters: Geneva, Switzerland

* Members from 160+ countries

* ISO standards are voluntary, but widely adopted in the pharmaceutical industry

In pharmaceuticals, ISO standards support quality systems, manufacturing control, testing, documentation, and continual improvement.

Objectives of ISO in Pharmaceutical Industry

ISO aims to:

1. Ensure consistent quality of pharmaceutical products
2. Improve patient safety
3. Enhance global acceptance of pharma products
4. Standardize quality management systems
5. Support regulatory compliance
6. Promote continuous improvement

Scope of ISO in Pharmaceuticals

ISO standards apply to:

* Pharmaceutical manufacturing units

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* Quality Control (QC) laboratories
* Quality Assurance (QA) systems
* Medical device manufacturers
* Packaging and storage
* Environmental and safety management

Important ISO Standards Relevant to Pharmaceutical Industry

4.1 ISO 9001 – Quality Management System (QMS)

Most important ISO standard for pharma

Purpose:

* Establishes a systematic approach to quality
* Focuses on customer satisfaction and continual improvement

Key requirements:

* Documented procedures (SOPs)
* Management responsibility
* Process control
* Corrective & Preventive Actions (CAPA)
* Internal audits
* Risk-based thinking

4.1 ISO 9001 complements GMP, but does not replace it.

4.2 ISO 13485 – Medical Devices Quality Management

Applied to:

* Syringes
* Needles
* IV sets
* Catheters
* Surgical instruments

Ensures:

* Product safety

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* Regulatory compliance
* Risk management
* Traceability

Mandatory in many countries for medical device manufacturers.

4.3 ISO/IEC 17025 – Testing & Calibration Laboratories

Applicable to:

* Pharmaceutical QC laboratories
* Analytical testing labs

Ensures:

* Technical competence
* Accuracy and reliability of test results
* Method validation
* Instrument calibration
* Analyst competency

Very important for pharma QC labs.

4.4 ISO 14001 – Environmental Management System (EMS)

Focus:

* Environmental protection
* Waste management
* Pollution control

Applied in pharma for:

* Effluent treatment
* Hazardous waste disposal
* Environmental compliance

4.5 ISO 45001 – Occupational Health & Safety

Ensures:

* Worker safety
* Accident prevention

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* Safe working environment
* Risk assessment

Important for:

* Chemical handling
* Manufacturing plants
* QC laboratories

Role of ISO in Pharmaceutical Quality Control

ISO standards help QC by:

* Standardizing test procedures
* Ensuring documentation accuracy
* Improving laboratory competence
* Enhancing data integrity
* Supporting regulatory inspections

ISO Certification in Pharmaceutical Industry

Certification process:

1. Gap analysis
2. Documentation preparation
3. Implementation
4. Internal audit
5. External audit
6. Certification

Certification is given by accredited certification bodies, not ISO itself.

ISO vs GMP

ISO	GMP
Voluntary	Mandatory
System-based	Product-based
Focus on quality management	Focus on manufacturing

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| International standard | Regulatory requirement |

| Certification available | No certification |

Pharma companies follow both ISO + GMP together.

Advantages of ISO in Pharmaceutical Industry

* Improves product quality
* Reduces errors and deviations
* Enhances global market acceptance
* Strengthens documentation system
* Builds customer and regulator confidence
* Promotes continuous improvement

Limitations of ISO

* Does not specify product quality standards
* Cannot replace pharmacopoeial or GMP requirements
* Requires continuous maintenance

Legal Status of ISO in Pharmaceuticals

* ISO standards are **not legally mandatory**
* Often required by:
* Export markets
* Tenders
* Multinational companies
* Strongly recommended by regulatory bodies

WHO (World Health Organization)

WHO (World Health Organization) is a specialized agency of the United Nations responsible for international public health.

In the pharmaceutical industry, WHO plays a major role in setting global quality standards, guidelines, and norms to ensure safe, effective, and quality medicines worldwide.

* Established: 1948
* Headquarters: Geneva, Switzerland

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* Member countries: 194

* WHO guidelines are not laws, but they are globally accepted and widely followed.

Objectives of WHO in Pharmaceuticals

WHO aims to:

1. Ensure availability of safe, effective, and quality medicines.
2. Promote Good Manufacturing Practices (GMP).
3. Prevent circulation of substandard and counterfeit drugs.
4. Support regulatory authorities of member countries.
5. Harmonize international drug standards.
6. Protect public health.

Scope of WHO

WHO guidelines apply to:

* Pharmaceutical manufacturing
* Quality Control (QC) laboratories
* Quality Assurance (QA) systems
* Storage and distribution
* Clinical trials
* Vaccines and biologicals
* Traditional medicines

WHO–GMP

WHO Good Manufacturing Practices (GMP)

WHO-GMP provides minimum global requirements for:

* Manufacturing
* Processing
* Packaging
* Testing
* Storage

Key elements of WHO-GMP:

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* Qualified personnel
* Premises and equipment
* Sanitation and hygiene
* Documentation and SOPs
* Quality control system
* Validation and calibration
* Self-inspection and audits
* Complaints and recalls

WHO-GMP is the foundation for:

* Schedule M (India)
* US cGMP
* EU GMP

WHO Technical Reports & Guidelines

WHO publishes guidelines through:

WHO Technical Report Series (TRS)

Includes guidance on:

* GMP
* Quality Control
* Validation
* Stability studies
* Water systems
* Sterile manufacturing
* Biological products

WHO Prequalification Programme (WHO-PQ)

Purpose:

* To ensure quality of medicines supplied to UN agencies.
* Especially for:
* HIV/AIDS

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* Tuberculosis
* Malaria
* Vaccines

Covers:

* Product evaluation
* Manufacturing site inspection
* QC testing
* Ongoing monitoring

WHO and Quality Control Laboratories

WHO guidelines specify:

* Sampling procedures
* Analytical method validation
* Reference standards
* Stability testing
* Microbiological testing

WHO also supports:

* Establishment of National Control Laboratories
* Training of analysts

WHO and Pharmacopoeias

WHO supports:

* International Pharmacopoeia
* Harmonization between:
* IP
* USP
* BP
* EP

Used when national pharmacopoeia standards are not available.

WHO and Drug Regulation

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WHO assists countries by:

* Developing drug regulatory frameworks.
* Strengthening National Regulatory Authorities (NRAs).
* Providing training and technical assistance.
* Promoting rational use of medicines.

WHO and Counterfeit Medicines

WHO:

* Monitors substandard and falsified medicines.
* Provides alert systems.
* Helps countries improve surveillance.
* Promotes awareness.

Legal Status of WHO Guidelines

* WHO guidelines are not legally binding
* Adopted by:
* National drug authorities
* Regulatory bodies
* Pharmaceutical industries
* Often treated as mandatory in practice

Importance of WHO in Pharmaceutical Industry

* Global quality benchmark
* Basis for GMP worldwide
* Supports medicine access in developing countries
* Ensures patient safety
* Enhances global trade acceptance

Advantages of WHO Guidelines

* Uniform international standards
* Improves product quality
* Reduces substandard medicines

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* Helps regulatory harmonization
* Supports exports

Conclusion

The World Health Organization (WHO) plays a vital role in the pharmaceutical industry by establishing globally accepted guidelines for manufacturing, quality control, and regulation of medicines. WHO-GMP forms the backbone of pharmaceutical quality systems worldwide, ensuring safe, effective, and quality medicines for public health protection.

US Certification in Pharmaceutical Industry (US FDA)

In the pharmaceutical industry, US certification commonly refers to regulatory approval and compliance by the US FDA (United States Food and Drug Administration).

The US FDA is the national regulatory authority of the United States responsible for ensuring that drugs, biologics, medical devices, and cosmetics marketed in the US are safe, effective, and of good quality.

Important note for exams:

US FDA does NOT issue GMP certificates" like ISO.

It approves products and inspects facilities for cGMP compliance.

* Full form: United States Food and Drug Administration
* Parent body: Department of Health and Human Services (HHS)
* Headquarters: Silver Spring, Maryland, USA
* Governing law: Federal Food, Drug, and Cosmetic Act (FD&C Act)

US Certification

In pharmaceuticals, US certification includes:

1. US FDA product approval
2. cGMP compliance
3. FDA facility inspection
4. Registration and listing
5. Regulatory filings (DMF, ANDA, NDA, etc.)

US FDA cGMP

cGMP = Current Good Manufacturing Practices

cGMP regulations are given under:

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21 CFR Parts 210 & 211

Purpose:

* Ensure drugs are consistently produced and controlled
* Prevent contamination, mix-ups, and errors

Key cGMP Requirements:

* Qualified personnel
* Suitable premises & equipment
* Validated processes
* Quality Control & Quality Assurance
* Proper documentation
* Change control
* CAPA system
* Stability studies
* Complaint handling & recalls

FDA inspects manufacturing sites for cGMP compliance.

US FDA Facility Inspection

Types of inspections:

* Pre-approval inspection (PAI)
* Routine surveillance inspection
* For-cause inspection

Outcomes:

* NAI – No Action Indicated
* VAI – Voluntary Action Indicated
* OAI – Official Action Indicated (Warning letter)

FDA Product Approvals (Core Certification Concept)

NDA – New Drug Application

* For new drugs

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* Requires:
* Safety data
* Efficacy data
* Quality & stability data
* Clinical trial results

ANDA – Abbreviated New Drug Application

* For generic drugs
* Demonstrates:
* Bioequivalence
* Same strength, dosage form, route
* cGMP compliance

No clinical trials required (except bioequivalence).

BLA – Biologics License Application

* For:
* Vaccines
* Blood products
* Monoclonal antibodies

Drug Master File (DMF)

* Submitted to FDA
* Contains confidential manufacturing information
* Types:
* Type II – Drug substance
* Type III – Packaging materials
* Type IV – Excipients

Supports NDA / ANDA submissions.

Establishment Registration & Drug Listing

Mandatory for companies exporting to the US:

* FDA Establishment Registration

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* Drug Listing

* NDC (National Drug Code) assignment

Must be renewed annually.

US FDA Certification for Medical Devices

For devices, FDA approvals include:

a) 510(k) Clearance

* Demonstrates substantial equivalence

b) PMA (Premarket Approval)

* For high-risk devices

US FDA Warning Letters & Import Alerts

If non-compliance is found:

* Warning letters issued

* Products may be banned

* Company placed under **Import Alert**

* Severe impact on exports

US Certification vs ISO Certification

US FDA	ISO
Regulatory authority	Standard organization
Mandatory for US market	Voluntary
Product approval + inspection	System certification
No GMP certificate issued	Certificate issued
Legal enforcement power	No legal enforcement

Importance of US FDA Certification

* Access to US pharmaceutical market

* Global reputation

* Higher credibility

* Essential for exports

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* Ensures patient safety

Advantages of US FDA Approval

* International recognition
* Higher product acceptance
* Quality benchmark worldwide
* Competitive advantage

Conclusion

US certification in the pharmaceutical industry refers to compliance with US FDA regulations, including cGMP adherence, product approvals (NDA/ANDA/BLA), facility inspections, and registration requirements. Although the FDA does not issue formal certificates, its approval and inspection outcomes are considered the highest regulatory standard globally.

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