PREFACE

In a bid to standardize higher education in the country, the University Grants Commission (UGC) has introduced Choice Based Credit System (CBCS) based on five types of courses viz. *core, general elective, ability and skill enhancement* for graduate students of all programmes at Honours level. This brings in the semester pattern, which finds efficacy in sync with credit system, credit transfer, comprehensive continuous assessments and a graded pattern of evaluation. The objective is to offer learners ample flexibility to choose from a wide gamut of courses, as also to provide them lateral mobility between various educational institutions in the country where they can carry their acquired credits.

UGC Open and Distance Learning (ODL) Regulations, 2017 have mandated compliance with CBCS for U.G. programmes for all the HEIs in this mode. Welcoming this paradigm shift in higher education, Netaji Subhas Open University (NSOU) has resolved to adopt CBCS from the academic session 2021-22 at the Bachelors Degree Programme (BDP) level. The present syllabus, framed in the spirit of syllabi recommended by UGC, lays due stress on all aspects envisaged in the curricular framework of the apex body on higher education. It will be imparted to learners over the six semesters of the Programme.

Self Learning Materials (SLMs) are the mainstay of Student Support Services (SSS) of an Open University. From a logistic point of view, NSOU has embarked upon CBCS presently with SLMs in English/Bengali. Eventually, the English version SLMs will be translated into Bengali too, for the benefit of learners. As always, all of our teaching faculties contributed in this process. In addition to this we have also requisitioned the services of the best academics in each domain in preparation of the new SLMs. I am sure they will be of commendable academic support. We look forward to proactive feedback from all stakeholders who will participate in the teaching-learning based on these study materials. It has been a very challenging task well executed, and I congratulate all concerned in the preparation of these SLMs.

I wish the venture a grand success.

Professor (Dr.) Subha Sankar Sarkar
Vice-Chancellor

Netaji Subhas Open University

Under Graduate Degree Programme
Choice Based Credit System (CBCS)
Subject: Honours in Chemistry (HCH)
Skill Enhancement Courses-2
Course Code: SE-CH-21
Course Title: PHARMACEUTICAL CHEMISTRY

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NETAJI SUBHAS OPEN UNIVERSITY

SEC-2
UG CHEMISTRY
(HCH)

Course Code: SE-CH-21
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Unit 1 ❐ Drug Discovery

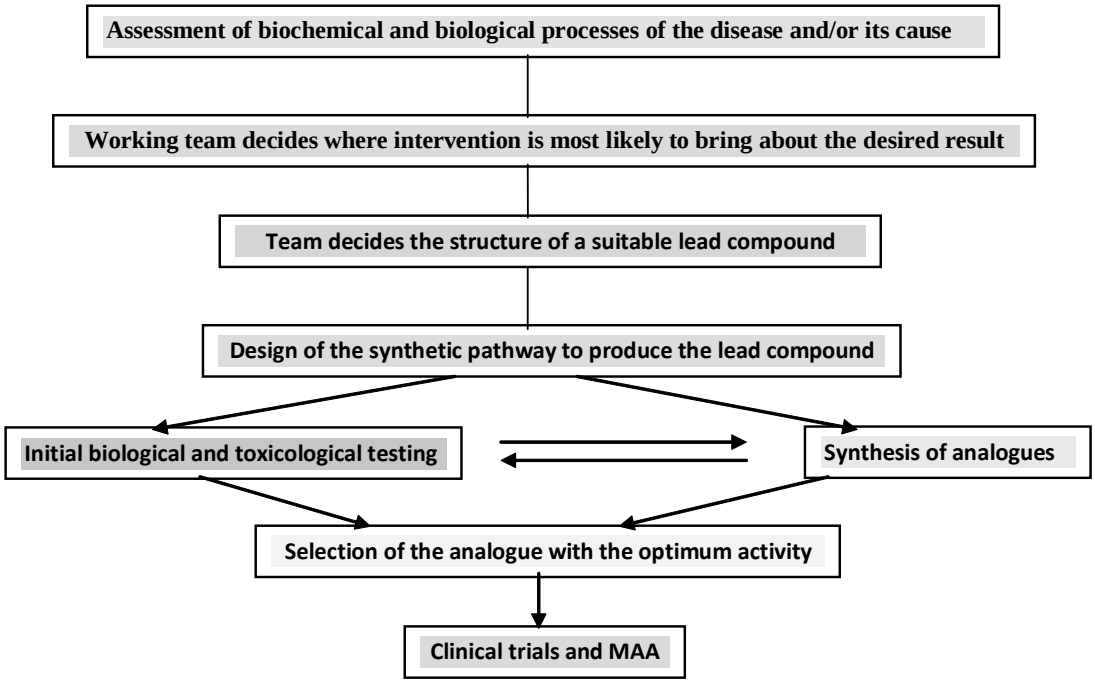
1.0 Objectives

In this self-learning material very briefly a venture of pharmaceutical chemistry was described so that learner will have a brief idea about drug discovery, development, medication, synthesis and mechanism of action of different therapeutics which normally used in our domestic life. This material also focused on application oriented and most relevant topic i.e. medicinal chemistry, now a day of human life. This unit deals with the Drug discovery, design and development along with the Classification of drugs, Synthesis of the representative drugs in different categories which were discussed lucidly. The synthetic route and mechanism of different drugs molecules wherever applicable were also explained. End of this chapter model questions will also involve actively all learners. Through this assignment learners can examine themselves how much knowledge they gained on this course.

1.1 Introduction

Pharmaceutical chemistry (medicinal chemistry) is the study of drugs, and its development. This also includes drug discovery, delivery, absorption, metabolism, and more. There are many of biomedical analysis, pharmacology, pharmacokinetics, and pharmaco dynamics. Medicinal chemistry work is usually done in a laboratory framework. This chemistry involves cures and therapies for disease, analytical techniques, pharmacology, metabolism, quality assurance, and drug chemistry. Now the question is what are drugs? And why do we need new ones? In this regards we can say Drugs are strictly defined as chemical substances that are used to prevent or cure diseases in humans, animals and plants. The activity of a drug is its pharmaceutical effect on the subject, for example, analgesic or β-blocker, whereas its *potency* is the measurable nature of that effect. Regrettably, the term drug is also used by the media and the general public to describe the substances taken for their psychotic rather than medicinal effects. However, this does not mean that these substances cannot be used as drugs. *Heroin*, for example, is a very effective painkiller and is used as such in the form of diamorphine in late-stage cancer cases. In drug discovery process the new class of medications are discovered. The discovery of a new drug includes not only a discovery or design process but also the synthesis of drug, a method of administration, the improvement of tests and procedures to establish how it operates in the body and its safety assessment. Discovery of a new drug may also require fundamental research

into the chemical and biological nature of the diseased state. Recent drug discovery involves the identification of target hit, medicinal chemistry and optimization of those hits to increase the affinity, selectivity i.e. to reduce the potential of side effects, oral bioavailability and metabolic stability i.e. to increase the half-life. The process of drug development can continue once a compound fulfills all of these requirements. A flowchart includes the general steps followed in the discovery of a new drug for a specific disease state is shown below.



1.2 Historical outline

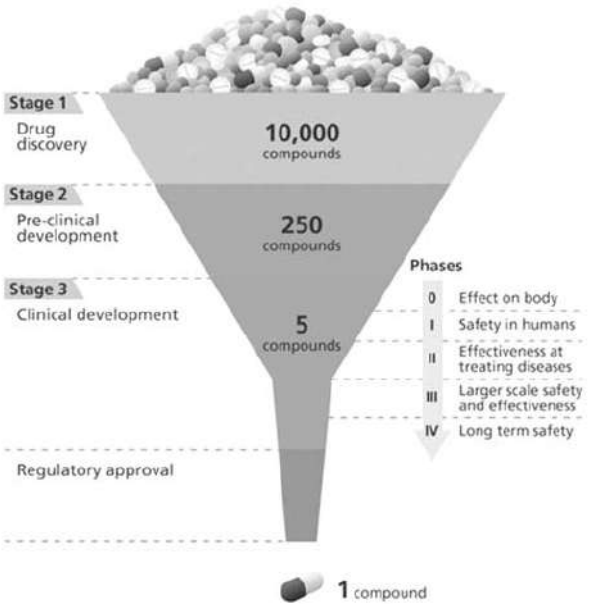
Long ago the peoples of the world have had a wide range of natural products that they use for medicinal purposes. These products obtained from mineral sources, vegetable and from animals. However, these products were sometimes very effective but many of the products were toxic also. Information about these ancient remedies was not readily to users until the invention of the printing press in the fifteenth century. The early nineteenth century saw the extraction of pure substances from the plant materials. Though these substances were of consistent quality but only a few of the isolated compound proved to be satisfactory as therapeutic agents. Although, morphine and cocaine for example, were extensively prescribed by the physicians but the majority were found to be too toxic. In the late 19th the search to find fewer toxic

medicines than those based on natural sources resulted in the introduction of synthetic substances as drugs. More recently, discovery of a drug includes classical pharmacology (i.e. chemical libraries of synthetic small molecules, natural products were screened in intact cells or whole organism to identify substances that had a desirable therapeutic effect), after that reverse pharmacology and efficacy. Modern drug discovery is thus usually a capital-intensive process that involves large investments by pharmaceutical industry and governments corporations.

The scientists led to the conclusion that individual chemicals are required for the biological activity of the drug from the effect of a drug in the human body is mediated by specific interactions of the drug molecule with biological macromolecules. This made for the beginning of the modern era in pharmacology, as pure chemicals, instead of crude extracts of medicinal plants, became the standard drugs. Later, small molecules were synthesized to specifically target a known physiological pathway, avoiding the mass screening of banks of stored compounds. This led to great success, such as the work of Gertrude Elion and George H. Hitchings on purine metabolism. Now-a-days, reverse pharmacology is the most frequent approach used, is the cloning of human proteins made possible the screening of large libraries of compounds against specific targets thought to be linked to specific diseases.

1.3 Different Stages of Drug Discovery

Introducing a new drug to market is a complex and time-consuming procedure that can cost pharmaceutical companies an average $2.6 billion and ten years of research and development. There are multiple defined stages for this process, each with their own associated challenges, timelines, and costs. In general, to find out one effective drug molecule more than 10000 molecules need to take into consideration during this procedure. Before preclinical development target discovery and its validation followed by Lead Compound Identification and optimization are



the primary criteria of drug discovery. In preclinical development i.e in stage-2 the molecules are examined on different states of animals. In stage-3 i.e clinical trial scientists need to consider many phases to find out right choice. During this time different parameter like effect on human bodies, safety, efficacy, effectiveness on diseases, large scale safety and effectiveness and of course long-term safety carefully checked. Finally, regulatory approval from FDA or CDC could lead the drugs in the market. During this time Post-Approval Research & Monitoring also been carried out because what most of the population does not realize is the amount of post-approval monitoring that pharmaceutical companies need to conduct while their drug is on the market. Some data obtained from this phase are unpredicted serious side effects, interactions with other drugs, potential alternate uses, and modifications to dosage.

1.4 Design & Development

1.4.1. Drug Designing:

The approach of finding drugs by design, based on their biological targets is known as drug design, sometimes referred to as rational drug design. Typically, a drug target is a key molecule involved in a particular metabolic pathway that is specific to a disease condition. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as protein, which in turn results in a therapeutic benefit to the patient.

What is really meant by drug design is ligand design i.e. design of a small molecule that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it. In medicine, biotechnology and pharmacology, drug discovery is the process by which drugs are designed.

There are three different methodologies commonly used in the drug designing:

1. Ligand-Based Drug Design: Relies on knowledge of other molecules that bind to the biological target of interest. It is used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.

2. Structure-Based Drug Design: Relies on knowledge of three-dimensional (3D) structure of the biological target obtained through X-ray crystallography and Nuclear Magnetic Resonance (NMR) spectroscopy.

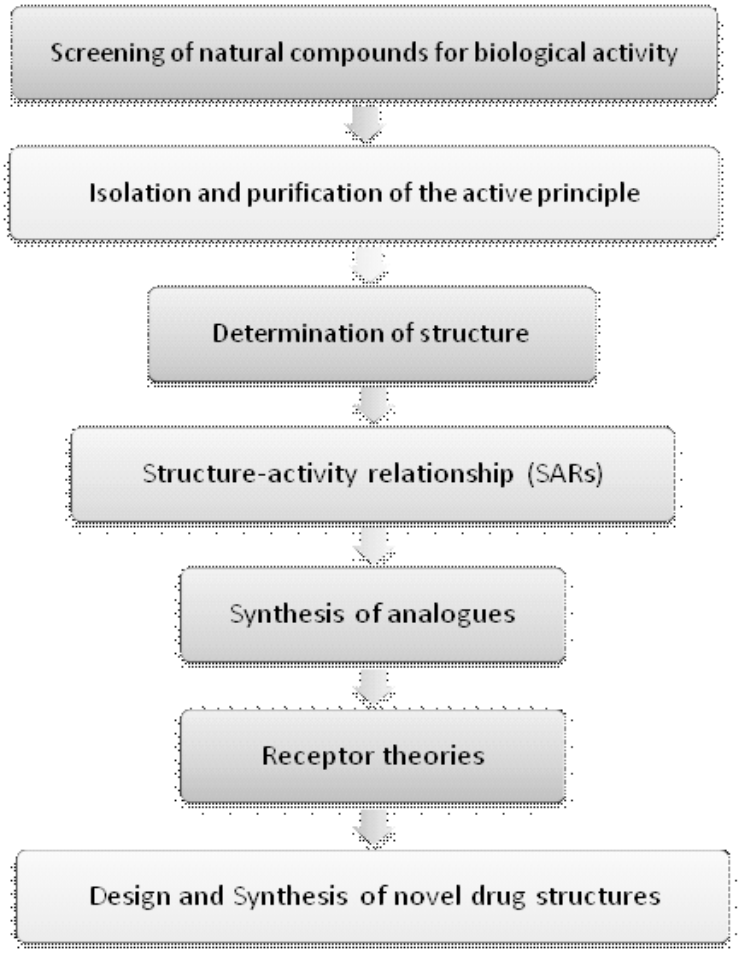
3. Mechanism-Based Drug Design: When the disease process is understood at the molecular level and the target molecule(s) are defined, drugs can be designed specifically to interact with the target molecule.

4. Computer-Assisted Drug Design (CADD): Drug design frequently but not necessarily relies on computer modeling techniques. CADD represents computational methods and resources that are used to facilitate the design and discovery of new therapeutic solutions. Drug design with the help of computers may be used at any of the following stage of drug discovery.

- Hit identification using virtual screening.
- Hit-to-lead optimization of affinity and selectivity.
- Optimization of other pharmaceutical properties while maintaining affinity.

1.4.2. Drug Development:

It is the process of taking a new chemical lead (drug) through the stages necessary to allow it to be tested in human clinical trials, in its broadest definition this encompasses the entire process of finding a suitable molecular target and clinical testing of novel



drug candidates. Since ancient time peoples have used herbs and potions as medicines and since then serious efforts were made to isolate and purify the active principles of these remedies and a large variety of biologically active compounds have been obtained and their structures determined (e.g. quinine from the bark of the cinchona tree, morphine of opium, cocaine from coca leaves). These natural products became the lead compounds for a major synthetic effort where chemists literally made thousands of analogues in an attempt to improve on with Nature had provided. Now-a-days, the development of a novel drug from natural sources might follow the following pattern.

1.5 Classification of Drugs:

Drugs can be grouped together in different ways – by the way they affect the body or by how or where they are used. Though different text books grouped drugs in different ways thus their classifications are quite confusing. Normally drugs are classified in following four ways-

1. Classification on the basis of pharmacological effect or therapeutic action:

How a drug or medicine affects or influences the cells of an organism is referred to as the pharmacological effect. Drugs are grouped depending on the biological effect they have. Different types of drugs have various pharmacological effects on an organism. For example, an analgesic reduces pain while an anti-inflammatory drug reduces inflammation in the body.

2. Classification on the basis of drug action:

Drugs are grouped according to the enzyme or receptor with which they interact. Different drugs act differently i.e. each drug has its own way of generating a response. For example, there are lots of medicines to treat hypertension but they have different drug action such as all medicines of hypertension reduces the blood pressure but in a different way.

3. Classification on the basis of chemical structure:

Drugs which have a common skeleton are grouped together. Generally, drugs that have the same pharmacological effect and drug action have a basic skeleton structure and a minute variation in the branching. For example, all sulphonamides have the same skeleton structure.

4. Classification on the basis of molecular targets:

These are compounds which are grouped according to whether they affect a certain target system in the body. Usually, drugs target the macromolecules inside the body to generate a biological response. Drugs that have the same mechanism of action will have the same target.

1.6 Basic Retro-synthetic Approach

When you plan the synthesis of a molecule all you know for certain in the structure of the molecule you are trying to make. We need to follow the following steps to synthesis a target molecule from easily available starting molecules.

a) Analysis:

(i) Recognize the functional groups in the target molecule.

(ii) Disconnect with known reliable reactions in mind.

(iii) Repeat as necessary to find out the starting material.

b) Synthesis:

(i) Write out the plan adding reagents and conditions.

(ii) Modify the plan according to unexpected failure and success in the laboratories.

1.7 Synthesis of the representative drugs

1.7.1. Analgesics Agents

The term analgesic encompasses a class of drugs that are designed to relieve pain without causing the loss of consciousness. This selectivity is an important distinction between an analgesic and an anesthetic.

- **ANALGESICS**: A drug that selectively relieves pain by acting in CNS or on peripheral pain mechanism, without significantly altering consciousness.
- **ANESTHESIA**: Anesthesia means loss of sensation. Anesthetic agent is one which bring about loss of all modalities of sensation, particularly pain, along with a reversible loss of consciousness.
- **PAIN (ALGESIA)**: An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage.

There are several types of analgesics are available like

- Opioids
- Nonopioids and NSAIDs
- Corticosteroids
- Neurological analgesia
- Anesthetic nerve blockade

Analgesics may be classified into two groups:

A. Opioids / narcotic / morphine like analgesics.

B. Nonopioids / non-narcotic / antipyretic / aspirin like analgesics or nonsteroidal anti-inflammatory drugs. (NSAIDs).

The anti-inflammatory drugs, which alleviate pain by reducing local inflammatory responses; and the opioids, which act on the brain. The opioid analgesics were once called narcotic drugs because they can induce sleep. The opioid analgesics can be used for either short-term or long-term relief of severe pain. In contrast, the anti-inflammatory compounds are used for short-term pain relief and for modest pain, such as that of headache, muscle strain, bruising, or arthritis.

1.7.2. Anti-inflammatory agent

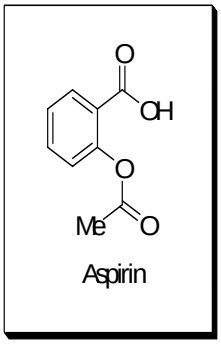
Most anti-inflammatory analgesics are derived from three compounds discovered in the 19th century—Salicylic Acid, Pyrazolone, and Phenacetin (or Acetophenetidin). Although chemically unrelated, the drugs in these families have the ability to relieve mild to moderate pain through actions that reduce inflammation at its source. Acetylsalicylic acid or aspirin, which is derived from salicylic acid, is the most widely used mild analgesic. It is considered the prototype for anti-inflammatory analgesics. The two other major types of which include acetaminophen (a derivative of phenacetin) and the aspirin like drugs, or nonsteroidal anti-inflammatory drugs (NSAIDs), which include compounds such as Ibuprofen, Naproxen, and Fenoprofen. Pyrazolone derivatives, with some exceptions, are no longer widely used in many countries, because of their tendency to cause an acute infection known as Agranulocytosis.

Aspirin and NSAIDs appear to share a similar molecular mechanism of action—namely, inhibition of the synthesis of prostaglandins (natural products of inflamed white blood cells) that induce the responses in local tissue. It has two forms, COX-1 (Cyclooxygenase-1), which is found in most normal tissues and COX-2, which is induced in the presence of inflammation. Because COX-2 is not normally expressed in the stomach, the use of COX-2 inhibitors (e.g., Rofecoxib, Celecoxib) seems to result in less gastric ulceration than occurs with other anti-inflammatory analgesics, particularly aspirin. However, COX-2 inhibitors do not reduce the ability of platelets to form clots, a benefit associated with aspirin and other nonselective COX inhibitors that include pain and inflammation. In fact, aspirin and all aspirin like analgesics, including Indomethacin and Sulindac, which are derived from a heterocyclic organic compound known as indole, inhibit prostaglandin synthesis and release. All these agents can be further divided into nonselective COX inhibitors and selective COX inhibitors. COX, or cyclooxygenase, is an enzyme responsible for the synthesis of prostaglandins and related compounds.

1.7.3. Aspirin (Acetylsalicylic acid)

Aspirin is an effective analgesic (pain reliever), antipyretic (fever reducer) and anti-inflammatory agent and is one of the most widely used non-prescription drugs. The use of aspirin had its origin in the 18th century, when it was found that an extract from the bark of willow trees was useful in reducing pain and fever. The active ingredient in willow bark was later found to be salicylic acid, was effective at reducing pain and fever, it also had some unpleasant side effects- it is irritating to the lining of the mouth, esophagus, stomach and can cause hemorrhaging of the stomach lining. Aspirin is also used long-term to help prevent further heart attacks, is chaemic strokes and blood clots in people at high risk.

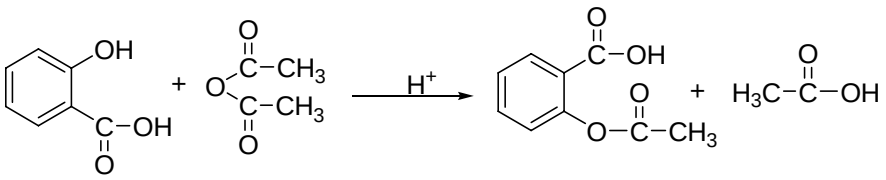
Structure:



Aspirin

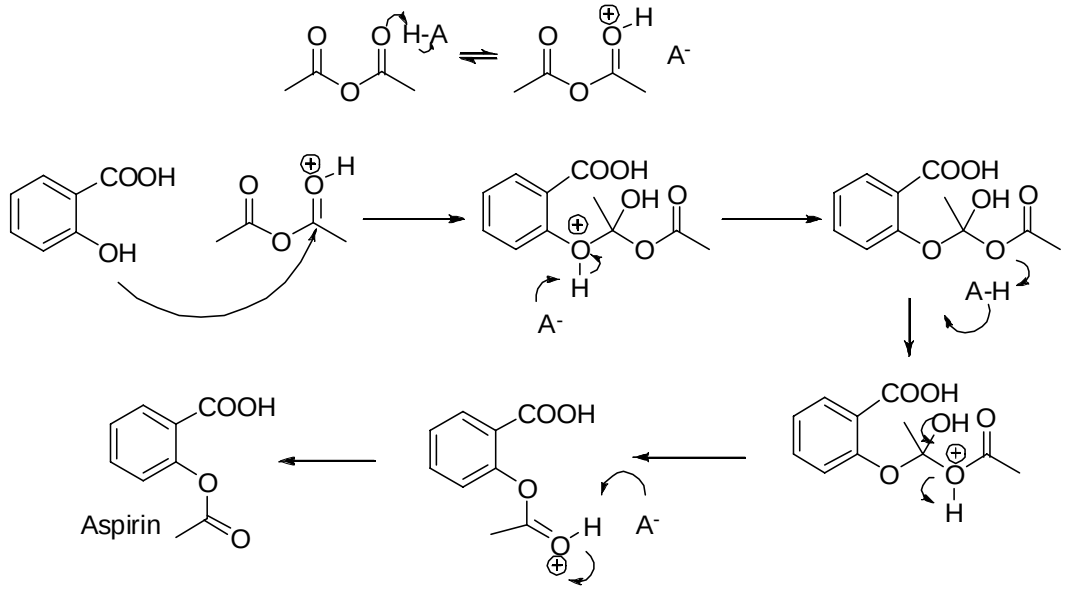
Synthesis:

The synthesis of aspirin is classified as an "Esterification Reaction". Salicylic acid is treated with acetic anhydride, an acid derivative, undergoes a chemical reaction that turns hydroxyl group of salicylic acid into an ester group (R-OH $\rightarrow$ R-OCOCH$_3$), yields aspirin and acetic acid (by product of this reaction) and a small amount of sulfuric acid (and occasionally phosphoric acid) are almost frequently used as a catalyst.



Salicyclic acid Acetic anhydride Acetylsalicylic acid (aspirin) Acetic acid

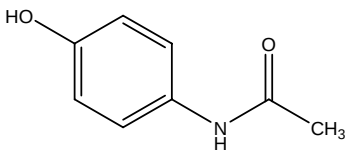
Reaction mechanism:



1.7.4. Paracetamol (Acetaminophenol)

It is an effective antipyretic and analgesic. It is also active against arthritic and rheumatic disorders involving musculoskeletal pain as well as the pain occurred due to headache, myalgia, dysmenorrhea and neuralgia. Paracetamol also known as Acetaminophenol. In combination with Opioid pain medication, Paracetamol is now used for more severe pain such as cancer pain and after surgery, typically used either by mouth or rectally but is also available intravenously. Paracetamol is generally safe at recommended doses.

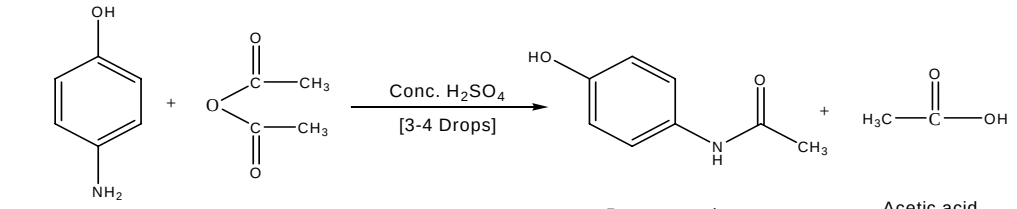
Structure:



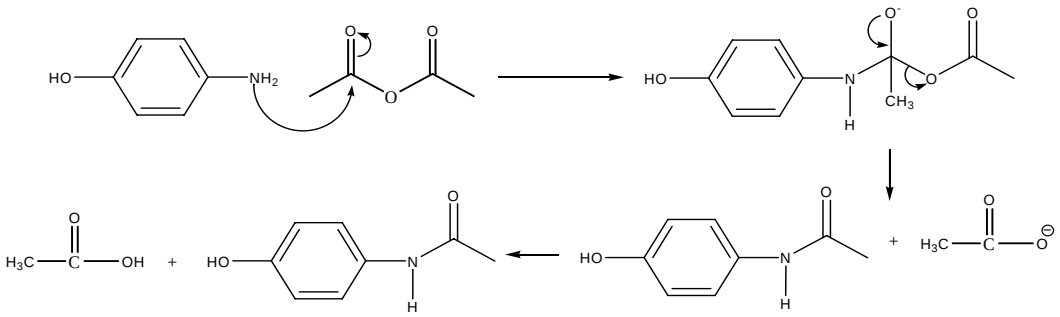
Paracetamol

Synthesis:

Paracetamol is prepared from p-aminophenol by acetylating it with acetic anhydride in the presence of 3-4 drops of concentrated sulfuric acid as catalyst.



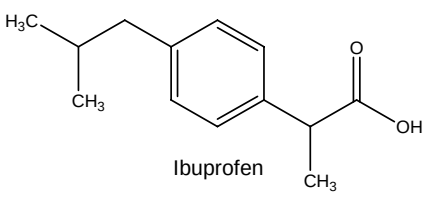
Reaction mechanism:



1.7.5. Ibuprofen:

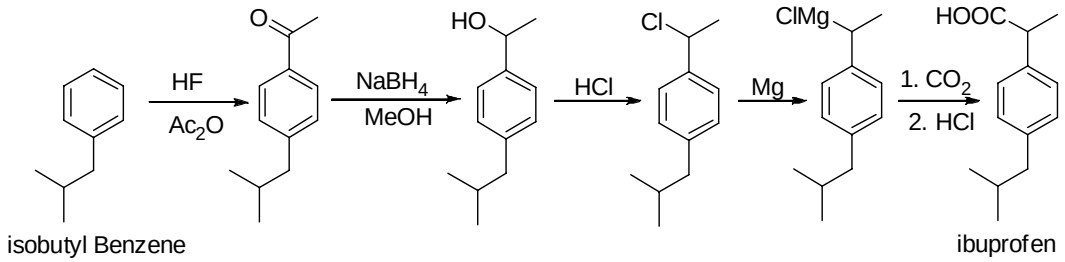
Ibuprofen is a non-steroidal anti-inflammatory drug used to relieve pain and reduce swelling, among other common treatments. It is somewhat short-lived and relatively mild. However, it is known to have an anti-platelet effect. In addition, ibuprofen acts as a vasoconstrictor because it inhibits the vasodilating prostacyclin that is produced by cyclooxygenase-2-enzymes. Ibuprofen is not only existing in tablet or capsule forms, but they can also be in topical gel form that can simply absorb through the skin, which are commonly use during sport injuries because it does not cause a high risk of digestive problems.

Structure:

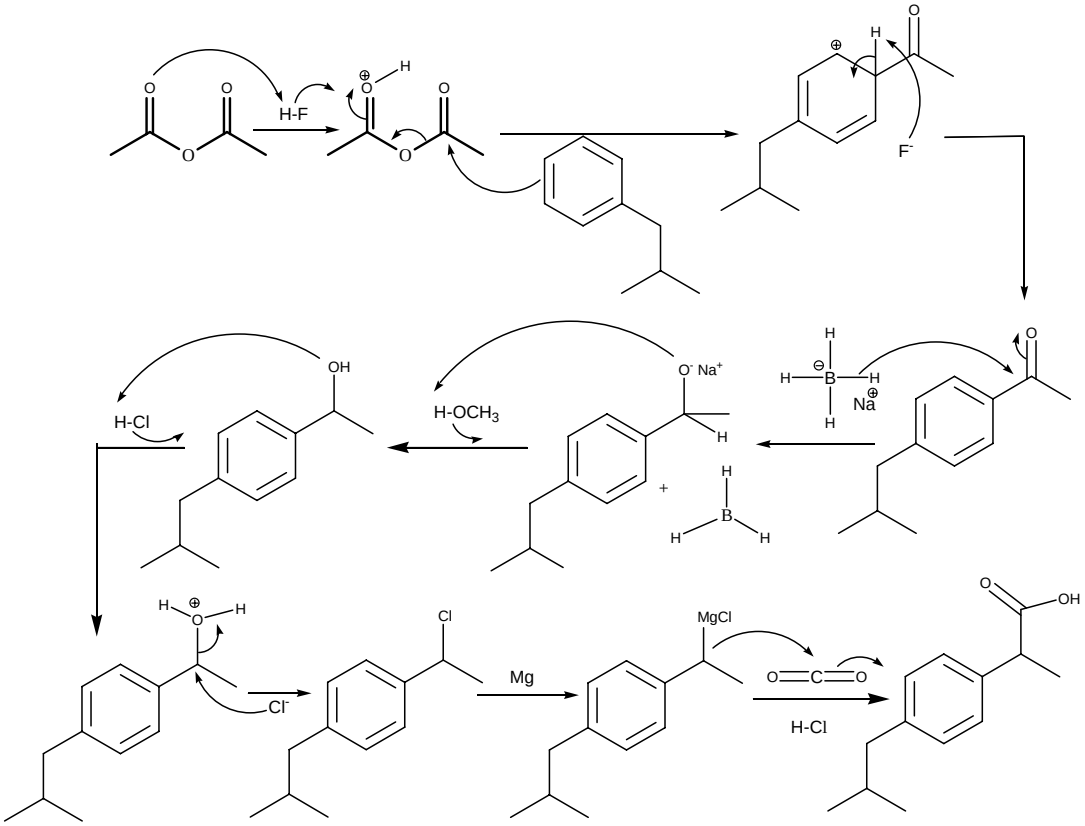


Synthesis:

The synthesis of ibuprofen was accomplished from isobutyl benzene. The synthetic process included a Friedel-Crafts acylation of isobutylbenzene afforded para-isobutyl Acetophenone, which under reduction condition followed by chloride substation form chlorinated product. After preparation of Grignard reagent, the precursor was trapped with CO_2 and after hydrolysis Ibuprofen was prepared.



Reaction mechanism:



1.7.6 Preparation of magnesium silicate (Antacid)

Antacids are generally weak bases or basic salts which neutralizes stomach acidity. They are used to relieve acid indigestion, upset stomach, sour stomach and heartburn. Most antacids are weak inorganic bases. More specifically, magnesium silicate acts as a neutralizing and astringent agent. It is the magnesium salt of silicic acid containing an unspecified amount of water. The molecular formula can be expressed more clearly as $MgSiO_3.XH_2O$. It is known as 'Talc' and its presents many uses in the cosmetic industry, food industry and pharmaceutical industry.

Magnesium silicate is prepared by the precipitation reaction between sodium silicate (Na_2SiO_3) dissolved in water and a magnesium salt [$Mg(NO_3)_2.6H_2O$] was dissolved in the mixture of propylene glycol-400 and ethanol. After those two solutions were mixed, a white precipitate was formed. The aqueous suspension of the precipitate is filtered and the product was collected.

1.8 Antibiotics

Many micro-organisms produce among themselves chemical substances which when extracted interfere with the growth or metabolism of other micro-organism. Such compounds are known as, **"antibiotics".** An antibiotic is therefore defining as follow:

It is a chemical substance produced by or derived from living cells which is capable in small concentration to inhibit the life processes or even destroying some other micro-organisms. Antibiotics are required in very low concentration to bring about their therapeutic action and so they are also sometimes termed as, Chemotherapeutics agents. All chemical substances produced by or derived from living cells are however cannot be antibiotics, they have to satisfy certain conditions in order to be designated as antibiotics.

In order for a particular antibiotic to act as therapeutic agent, it should have to satisfies the following conditions-

a) It must be effective against a pathogen.

b) It must not cause significant toxic side effect.

c) Its stability must be appreciably high, so that, it can be isolated and processed into suitable forms of doses, which are readily absorbed.

d) It could be stored for a long-time period without appreciable loss of its activity.

e) The rate of detoxification and elimination from the body must be such that there exists sufficient time interval between two successive doses and during

that period a proper concentration level has to be maintained.

f) The antibiotic should be completely eliminated from the body system soon after its administration has been stopped.

[**Chemotherapeutic drugs:** are harmful to invading bacteria, virus but harmless to the host.]

1.8.1 Classification of antibiotics:

The antibiotics covered a wide range of compounds of different chemical structures and hence a rational classification of them is very difficult and many schemes has been suggested as follows-

A. First types of classification:

Here, antibiotics are divided into two types,

(i) Broad spectrum: They include such antibiotics which may be used as curative agents against several ailments. There is considerable overlapping amongst them but each is usually superior to others, against some specific diseases. E.g. of broad spectrum antibiotics are- *Penicillin, Chloramphenicol, Tetra cyclones* etc.

(ii) Narrow spectrum: These antibiotics are quite specific or highly selective in their mode of action to kill bacteria. E.g. are *Braciticin, Nystatin etc*.

B. Second types of classification:

These types of classification described antibiotics are: gram-positive and gram-negative according to their ability to kill the bacterial strains.

In the gram-positive/negative method, the fixed bacterial smear is made to treat with a solution of crystal violet, followed by treatment with iodine solution. This smear is then washed with alcohol. The bacteria with retained the color of crystal violet and appeared deep violet in color are known as gram-positive bacteria. On the other hand, bacteria which lose violet color and get counter strained by safranin and appeared in color are called, gram-negative bacteria. E.g., are-

(i) Gram positive antibiotics: That kills the Gram-positive bacterial strains e.g., *diphtheria bacillus, leprosy bacillus, pneumococcus, staphylococcus, streptococcus, tubercle coccus*.

(ii) Gram negative antibiotics: That kills the Gram negative bacterial strains e.g. *coli* and *typhoid bacillus, gonococcus, meningococcus, plague bacillus, H. pyllorii*.

C. Third types of classification:

This classification of antibiotics is based on their chemical structures or therapeutic actions, although here exists great variations in the structural moieties of antibiotics yet there are certain similarities in their structure as well and it has been observed that such structurally similar antibiotics exert their therapeutic action also in a similar manner. These different classes are-

a) **Penicillin:** these are derived from amino acids, e.g.- Cephalosporin.

b) **Chloramphenicol** and its synthetic analogue.

c) **Aminoglycoside:** they usually contain a sugar molecule glycoside alkali linked to amino compound. e.g.- *Streptomycin, Vancomycin etc*.

d) **Tetracyclines:** the content four 6-membered fused ring system.

e) **Macrolides:** discounted a large lactone ring. e.g.- *Erythromycin.*

f) **Lincomycin:** these are S containing antibiotics in which S atom is not present in a ring.

g) **Polypeptide:** they contain 6-12 amino acid residues and they include some very potent bactericident antibiotics. e.g.-*Bracitracin.*

h) **Antineoplastic antibiotics:** these groups of antibiotics are used to control cancer. e.g.- *Mitomycin.*

i) **Antitubercular antibiotic:** these are a group of drugs used to treat tuberculosis e.g., *Rifampin, Isoniazid, Pyrazinamide, and Ethambutol.*

j) **Polyacetylene antibiotics:** this compound in addition of acyclic bonds also contain ethylene bond with another functional group. They are usually very toxic and have limited application.

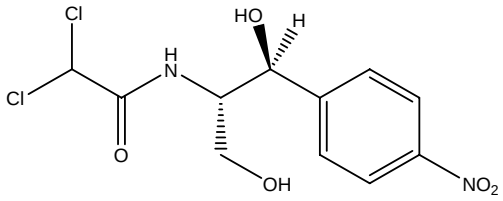
k) **Unclassified antibiotics:** this includes a number of antibiotics which not related to any one the classes described above.

1.8.2 Chloramphenicol:

Chloramphenicol is a broad-spectrum antibiotic isolated from Streptomyces Venezuelae. It is a levorotatory compound. It has a nitrobenzene moiety that is responsible for antibacterial activity and the bitter taste. It is the first natural product found to contain a $-NO_2$ group. Chloramphenicol has a wide range activity that induces gram-positive, gram-negative, aerobic and anaerobic bacteria. It is very effective in the treatment typhoid fever and in some other bacterial infections which are insensitive to other antibacterial agents.

Structure:

Chloramphenicol has two possible pair of enantiomers only the levorotatory isomer is the biologically active isomer.

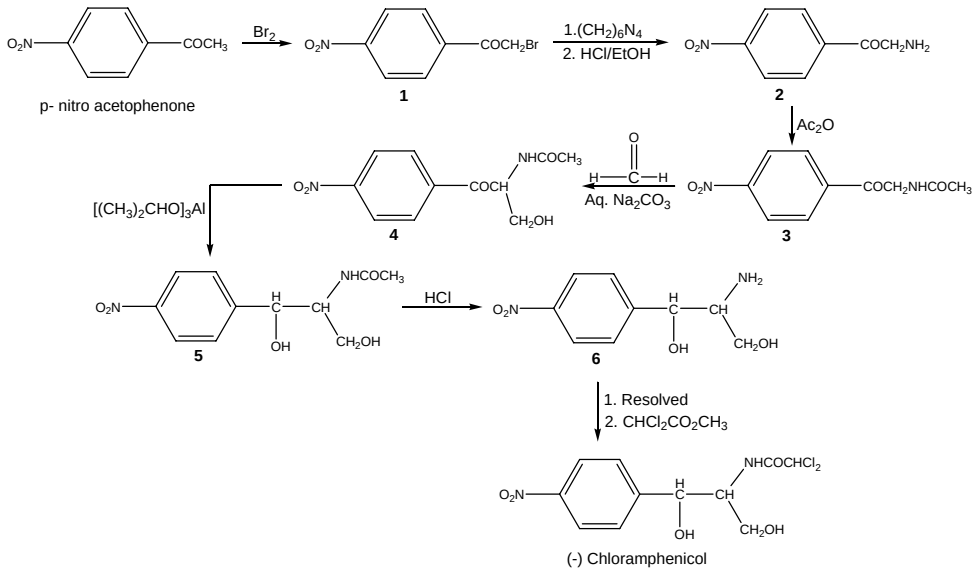


2,2-dichloro-N-[(1R, 2R)-1,3-dihydroxy-1-(4-nitrophenyl)propane-2-yl]acetamide

Mechanism of action:

Chloramphenicol is a bacteriostatic by inhibiting protein synthesis. It prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome. It inhibits protein synthesis by binding to 50s ribosomal subunit of the microbe.

Synthesis:



Reduction of **4** with $[(CH_3)_2CHO]_3Al$ (Aluminium isopropoxide) get predominantly the threo isomer of **5** together with a small amount of its erythro isomer, this are separated by fractional crystallization. Compound **6** is obtained as a mixture of (±) form is resolved by (+)Champhor sulfonic acid, the D(-)*erythro* isomer of **6** was then converted to (-)Chloramphenicol on treatment with methyl dichloro acetate.

1.9 Antibacterial agents

Antibacterial agents are a group of materials that fight against pathogenic bacteria-by killing or reducing the metabolic activity of bacteria, their pathogenic effect in the biological environments will be minimized. To cure infectious diseases, researchers discovered antibacterial agents, which are considered to be the most promising chemotherapeutic agents. The antibacterial agents can be classified into four major groups-

I. Type of action
II. Source
III. Spectrum of activity
IV. Chemical structure

1.9.1. Classification based on the type of action

Generally, antibacterial agents can be classified on the basis of type of action i.e.-"bacteriostatic and bactericidal". Antibacterials, those that slow or inhibit the growth of bacteria are called, bacteriostatic and the antibacterials, which destroy bacteria by targeting the cell wall or cell membrane of the bacteria, are referred to as bactericidal. The inhibition of phenomenon of bacteriostatic agents involves inhibition of protein synthesis or some bacterial metabolic pathways. Since, bacteriostatic agents just prevent the growth of the pathogenic bacteria, sometimes it is very difficult to a clear distinction between bacteriostatic and bactericidal, and especially when high concentrations of some bacteriostatic agents are used then they may work as bactericidal. Examples of bacteriostatic and bactericidal antibacterial along with their mode of action are presented in following table:

A. Bacteriostatic antibacterial	**Function**
Sulphonamides	They act to inhibit folate synthesis at initial stages
Amphenicols, e.g.- Chloramphenicol	They inhibit protein synthesis
Spectinomycin	It binds to the 30S ribosomal subunit, thereby interrupting protein synthesis
Trimethoprim	It disturbs the tetrahydrofolate synthesis pathway
B. Bactericidal antibacterial	**Function**
Penicillins, Oxacillin, Cloxacillin and Flucloxacillin. They belong to â-lactams antibiotic class	Penicillin antibiotics stop bacteria from multiplying by preventing bacteria from forming the walls that surround them. The walls are necessary to protect the bacteria from their environment, and to keep the contents of the bacterial cell together. Bacteria cannot survive without a cell wall. This class of antibiotics also used to treat skin infections, external ear infections, infections of leg ulcers, diabetic foot infections, and infection of bone.
Carbapenems like Imipenem, Meropenem, Aztreonam, Tricaracillinclvulnate, and Piperaciintazobactam- these are â-lactam inhibitors. Some others â- lactam inhibitors are cephalosporin, e.g.- Cefotaxime, Ceftriaxone and Cefepime.	They interfering the synthesis of the bacterial cell wall
Gentamicin, Tobramycin and Amikacin are aminoglycosides	They inhibit protein synthesis
Quinolones and Flouroquinolones such as Levofloxacin, Ciprofloxacin and Oxifloxacin	They block bacterial DNA replication

1.9.2. Classification based on source of antibacterial agents:

Antibacterial agents can be naturally obtained from fungal sources, semi- synthetic members which are chemically altered natural product or synthetic. Antibacterials are the subclass of antibiotics. Cephalosporins, Benzylpenicillin and Gentamicin are well-known examples of natural antibiotics/antibacterials. Natural antibacterial often exhibits higher toxicity than synthetic antibacterials. Ampicillin and Amikacin are semi-synthetic antibiotics, which were developed to show lower toxicity and increase effectiveness. Synthetic antibiotics are also designed to have even greater effectiveness and less toxicity and have an advantage over the natural antibiotics that the bacteria are not exposed to the compounds until they are released.

1.9.3. Classification based on spectrum of activity:

This classification based on their (antibacterial agents) target specification. In this category, the antibacterials may be either narrow or broad spectrum. The narrow spectrum antibacterials are considered to be those which can work on a narrow range of microorganism, i.e. they act against gram-positive and gram-negative bacteria only. Usually, the narrow spectrum antibacterials are considered ideal antibacterial and preferred over the broad spectrum antibacterials, because the narrow spectrum antibiotics do not kill as many of the normal microorganism in the body as the broad-spectrum antibiotics and thus has less ability to cause superinfection. Also, the narrow spectrum antibiotic will cause less resistance of the bacteria as it will ideal with only specific bacteria.

1.9.4. Classification based on chemical structure:

Different skeleton containing antibiotics show different therapeutic behavior; therefore, it is an ultimate need to classify antibacterials on the basis of their chemical structure. This classification is also very important as similar structural units have similar structural units have similar patterns of toxicity, effectiveness and other related properties. On the structural basis, antibacterials have been classified into two groups: group A (â- lactams) and group B (aminoglycoside). However, in a more elaborated way, the antibacterials can be classified into â-lactams, â-lactam/â-lactamase inhibitor combinations, aminoglycoside, macrolides, quinolones and flouroquinolones.

1.10 Antifungal agents

Most fungal infections (mycoses) involve superficial invasion of the skin or mucous membrane of the body orifices. These diseases, which can usually be controlled by

local application of antifungal agents, are divided into two etiologic groups:

1. The Dermatophytes are contagious superficial epidermal infections caused by various Epidermophyton, Microsporum and Trichophyton species.
2. Mycoses caused by pathogenic saprophytic yeasts (Aspergillus, Blastomyces, Candida, Cryptococcus and Histoplasma), which are contagious and usually superficial infections involving the skin and mucous membranes. Under certain conditions, these are capable of invading deeper body cavities and causing systematic mycoses. Such infections may become serious and occasionally life-threatening. Moreover, they are difficult to treat.

1.10.1. Classification

1. Antibiotics: Amphotericin B, Nystatin, Griseofulvin.
2. Azole (imidazole, triazole) derivatives:

a. Systematic: Ketoconazole, Fluconazole and Itraconazole.

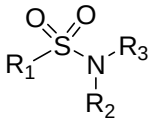
b. Locally acting: Clotrimazole, Econazole, Miconazole, Terconazole and Butoconazole.

3. Pyrimidine derivatives: 5-Flucytosine.
4. Miscellaneous: Ciclopirox, Tolnaftate, Niftifine and Terbinafine.

1.10.2. Sulphonamides

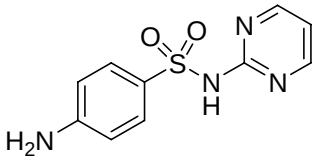
Sulphonamides are synthetic, broad spectrum bacteriostatic antibiotics. The sulphonamide drugs were the first effective chemotherapeutic agents to be employed systematically for the prevention and are of bacterial infections in human beings. These are totally synthetic substances that are produced by relatively simple chemical synthesis. Because of associated toxicity and high rates of resistance, their use is now very limited. The sulphonamide-antibacterials are primarily used for the treatment of uncomplicated urinary tract infections caused by *E.coli*, Enterobacter and proteus and only seldom for middle ear infections caused by *Haemophilus influenzae*. Sulphonamides are also used for the prophylaxis of recurrent rheumatic fever associated with streptococcal infection and are also useful in burn therapy.

Structure:



Basic structure of sulphonamides

(R_1, R_2, and R_3 can be alkyl, aryl and heteroaryl)



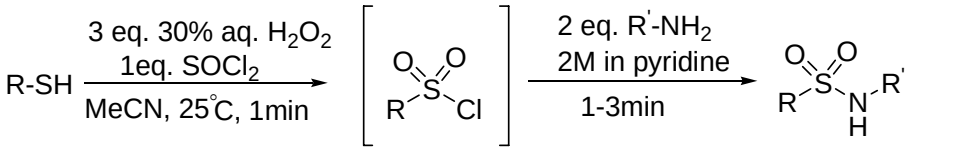
Sulphadiazine

Synthesis:

The classic approach of synthesis of sulphonamides, is the reaction of sulphonyl chlorides with an amine. The reaction of primary and secondary amines with benzenesulphonyl chloride is the basis of Hinsberg reaction, a method for detecting primary and secondary amines.

$RSO_2Cl + R_2NH \rightarrow RSO_2NR_2 + HCl$

The combination of H_2O_2 and $SOCl_2$ is a highly reactive agent for the direct oxidative conversion of thiol derivatives to the corresponding sulphonyl chlorides through oxidative chlorination. Upon reaction with amines, the corresponding sulphonamides were obtained in excellent yields in very short reaction times.

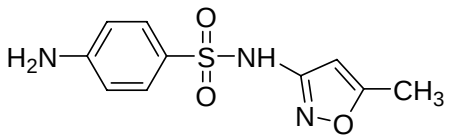


R= Ar, alkyl, Bn
R'= Ar, alkyl

1.10.3. Sulphamethoxazole

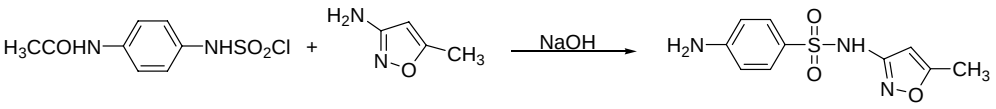
Sulphamethoxazole is a bacteriostatic antibiotic- interferes with folic acid synthesis insusceptible bacteria. It is generally given in combination with trimethoprim, which inhibits a sequential step in bacterial folic acid synthesis- these agent works synergistically to block two consecutive steps in the biosynthesis of nucleic acids and proteins which are necessary for bacterial growth and division, and using them in conjunction helps to slow the development of bacterial resistant. In this combination, sulphamethoxazole is useful for the treatment of a variety of bacterial infections, including those of the urinary, respiratory and gastrointestinal tracts.

Structure:



4-amino-N-(5-methyl-1,2-oxazol-3-yl)benzene-1-sulphonamide

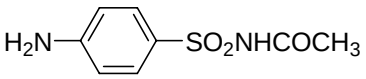
Synthesis:



1.10.4. Sulphacetamide

An anti-infective agent that is used to treat skin infections and orally urinary tract infections. Sulphacetamide is asulphonamide antibiotic with bacteriostatic actions and broad spectrum activity against most gram-positive and many gram-negative organisms. Most of the sulphonamides are readily absorbed orally. However, parenteral administration is difficult, since the soluble sulphonamides salts are highly alkaline and irritating to the tissues. The sulphonamides are widely distributed throughout all tissues. High levels are achieved in pleural, peritoneal, synovial and ocular fluids. Although these drugs are no longer used to treat meningitis, CFS levels are high in meningeal infections. Their antibacterial action is inhibited by pus.

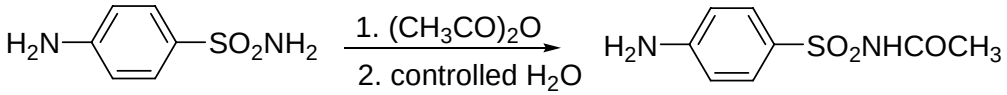
Structure:



N-(4-aminobenzenesulphonyl)acetamide

Synthesis:

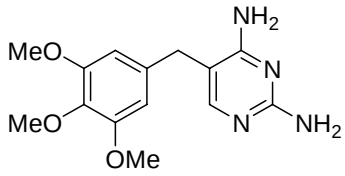
Sulphacetamide is synthesized by reacting 4-aminobenzenesulphonamide with acetic anhydride under controlled hydrolysis.



1.10.5. Trimethoprim

Trimethoprim is an antifolate antibacterial agent – inhibits bacterial dihydrofolate reductase (DHFR), a critical enzyme that catalyzes the formation of tetrahydrofolic acid (THF) -in doing so, it prevents the synthesis of bacterial DNA and ultimately continued bacterial survival. Trimethoprim is often used in combination with sulphamethoxazole due to their complementary and synergistic mechanisms but may be used as a monotherapy in the treatment and/or prophylaxis of urinary tract infections. It is structurally and chemically related to pyrimethamine, another antifolate antimicrobial used in the treatment of plasmodial infections.

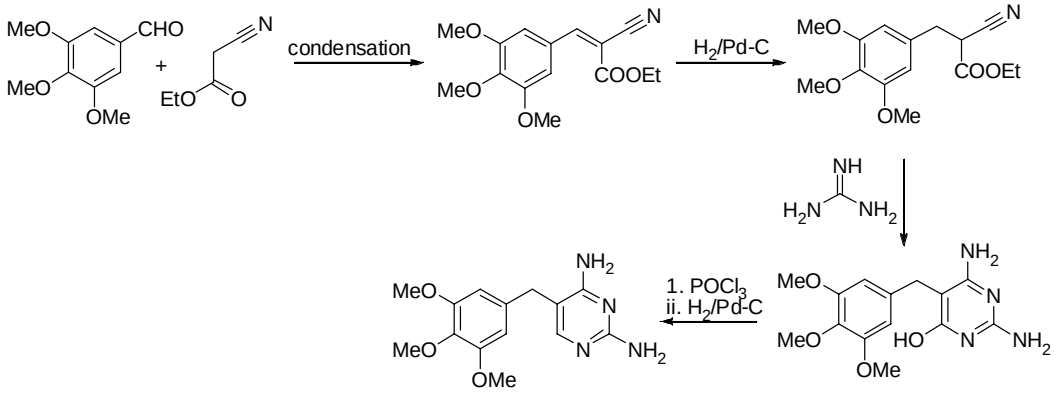
Structure:



5-[(3,4,5-trimethoxyphenyl)methyl]pyrimidine-2,4-diamine

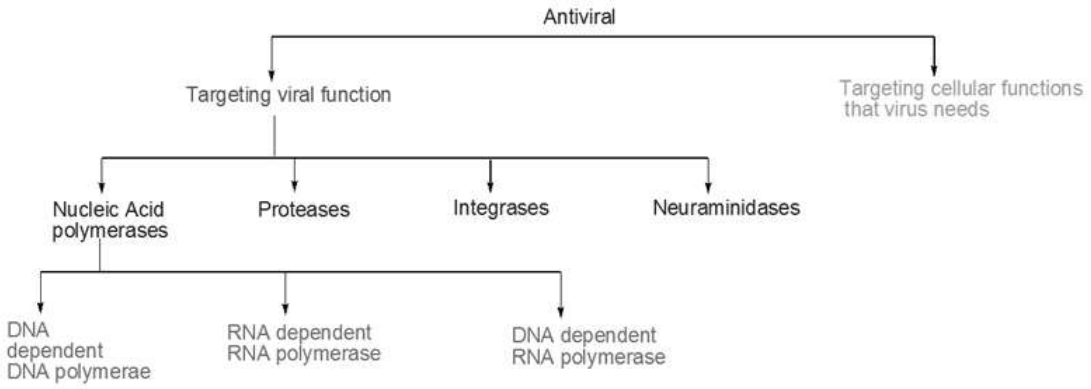
Synthesis:

Trimethoprim is synthesized form 3,4,5-trimethoxybenzaldehyde. Condensation of 3,4,5-trimethoxybenzaldehyde with 3-ethoxy or 3-anilinopropionitrile gives the corresponding benzylidene derivative, which upon direct reaction with guanidine gives trimethoprim.



1.11 Antiviral agents

Antiviral agents are a class of medication used specifically for treating or controlling viral infections. Like antibiotics for bacteria, specific antivirals are used for specific viruses. Unlike, most antibiotics, antivirals do not destroy their target pathogen; instead, they inhibit their development.

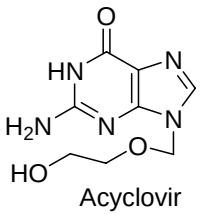


Classification of the Antiviral drugs based on their targets

Acyclovir (an Antiviral agents):

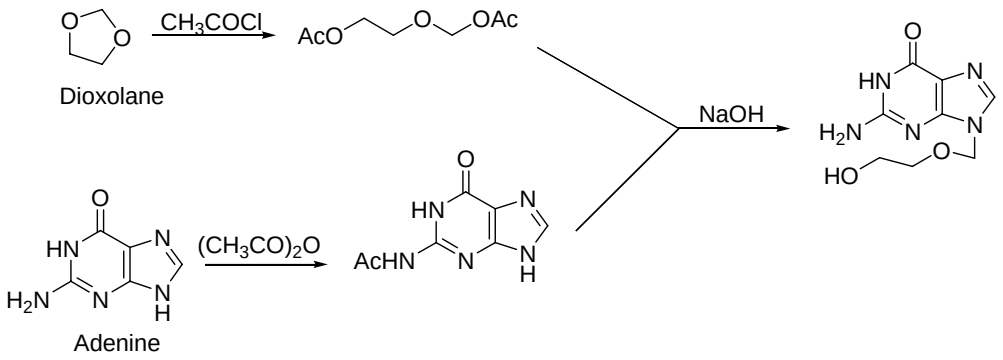
Acyclovir is an antiviral medication. Acyclovir is used to treat infections caused by certain types of viruses. It treats cold sores around the mouth (caused by herpes simplex), shingles (caused by herpes zoster) and chickenpox. This medication is also used to treat outbreaks of genital herpes. The viruses that cause these infections continue to live in the body even between outbreaks. Acyclovir decreases the severity and length of these outbreaks. It helps the sores heal faster, keeps new sores from forming and decreases pain/ itching. This medication may also help reduce how long pain remains after the sores heal. In addition, in people with a weakened immune system, acyclovir can decrease the risk of the virus spreading to other parts of the body and causing serious infections.

Structure:



Acyclovir

Synthesis:



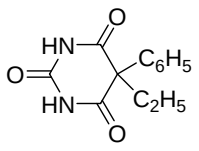
1.12 Central Nervous System (CNS) agents

The central nervous system consists of the brain and spinal cord. The brain plays a central role in the control of most bodily functions, including awareness, movements, sensations, thoughts, speech and memory. Some reflex movements can occur via spinal cord pathways without the participation of brain structures. Drugs works on CNS, including anesthetics, anticonvulsants, antimetics, antiparkinson agents, CNS stimulants, muscle relaxants, pain relievers, nonnarcotic analgesics (such as actamiophen and NSAIDs) and sedatives.

1.12.1. Phenobarbital

Phenobarbital is a prescription medicine used to treat and prevent the symptoms of seizures, sedation, hypnotics, Insomina and Status Epilepticus. Phenobarbital may be used alone or with other medications. Phenobarbital belongs to a class of drugs called Anticonvulsants, Barbiturates.

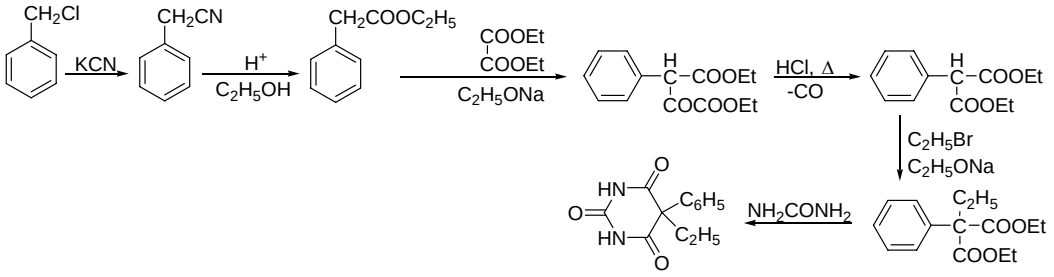
Structure:



Phenobarbital

Synthesis:

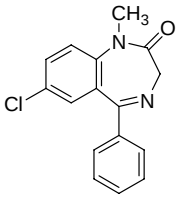
Barbiturate drugs are obtained via condensation reactions between a derivative of diethylmalonate and urea in the presence of a strong base. The methods consist of a Pinner reaction of benzyl cyanide, giving phenylacetic acid ethyl ester. Subsequently, this ester undergoes cross claisen condensation using diethyl oxalate, giving diethyl ester of phenyloxobutandioic acid. Upon heating this intermediate easily loses carbon monoxide, yielding diethyl phenylmalonate. Malonic ester synthesis using ethyl bromide leads to the formation of á-phenyl-á-ethylmalonic ester. Finally a condensation reaction with urea produced phenobarbital.



1.12.2. Diazepam

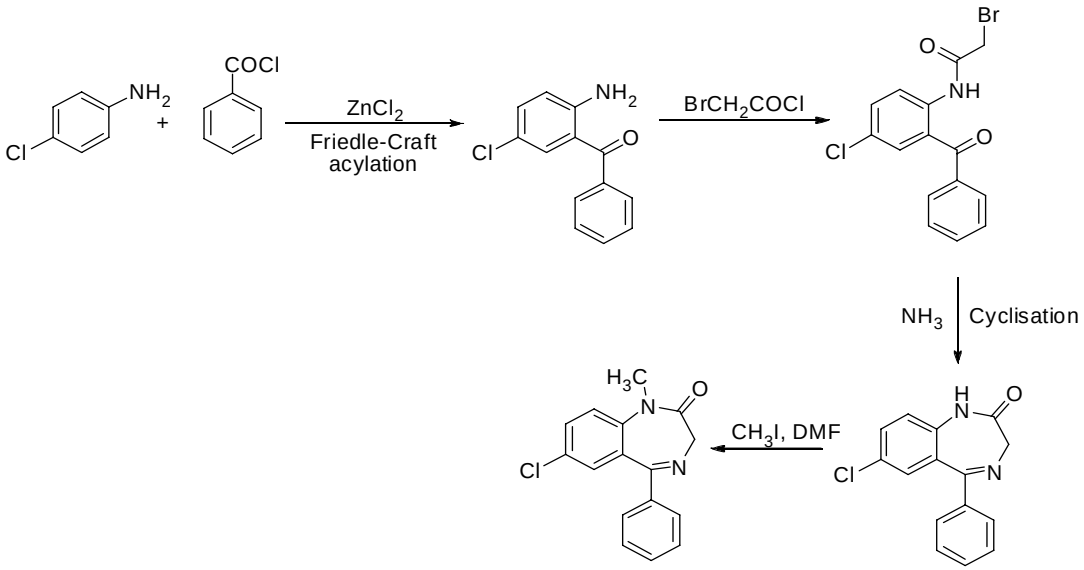
Diazepam is used to treat anxiety, alcohol withdrawal and seizures. It is also used to relieve muscle spasms and to provide sedation before medical procedures. This medication works by claiming the brain and nerves. Diazepam belongs to a class of drugs known as benzodiazepines.

Structure:



Diazepam
(7-Chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2-one)

Synthesis:



1.13 Drugs for Cardiovascular disease

Relating to the circulatory system, which comprises the heart and blood vessels and carries nutrients and oxygen to the tissues of the body and removes carbon dioxide and other wastes from them. Cardiovascular diseases are conditions that affect the heart and blood vessels and include arteriosclerosis, coronary artery disease, heart value disease, arrhythmia, heart failure, hypertension, shock, endocarditis and congenital heart disease. E.g.

Glyceryl Trinitrate:

Nitroglycerin, also known as glyceryl trinitrate, is a medication used for heart failure, high blood pressure, anal fissures, and painful periods and to treat and prevent chest pain caused by decreased blood flow to the heart or due to the recreational use of cocaine.

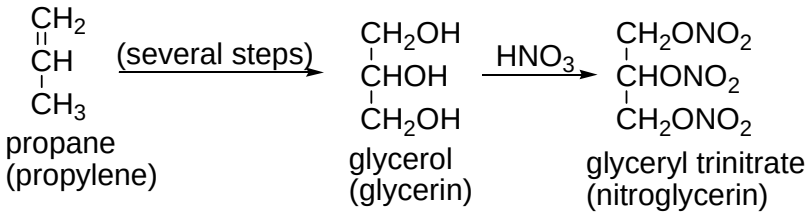
Structure:

CH_2ONO_2
|
$CHONO_2$
|
CH_2ONO_2

Glyceryl trinitrate
(nitroglycerine)

Synthesis:

Glyceryl trinitrate, is synthesized by nitrating glycerol with nitric acid.



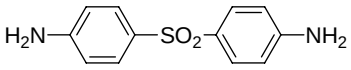
1.14 Drugs for Anti-Leprosy

Substances that suppress Mycobacterium leprae, ameliorate the clinical manifestations of leprosy and/or reduce the incidence and severity of leprous reactions. E.g.

Dapsone:

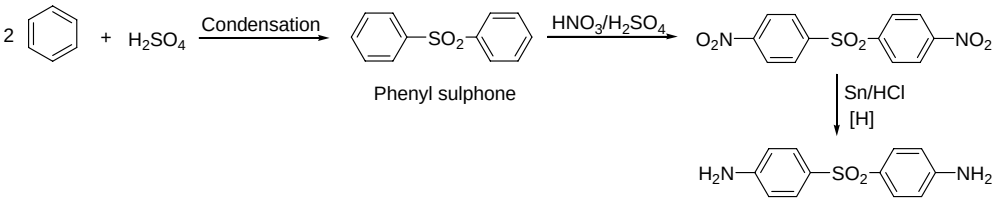
Dapsone, also known as diaminodiphenyl sulfone (DDS), is used for the treatment of infection caused by Mycobacterium leprae. It is used in the treatment of both lepromatous and tuberculoid types of leprosy. Dapsone is used in combination with Rifampicin and Clofazimine. Dapsone is also the drug of choice for dermatitis herpetiformis, with pyrimethamine for the treatment of malaria, with trimethoprim for pneumocystis carnii and has been used for rheumatoid arthritis.

Structure:



Dapsone
(4,4'-sulphonyl bis benzenamine)

Synthesis:



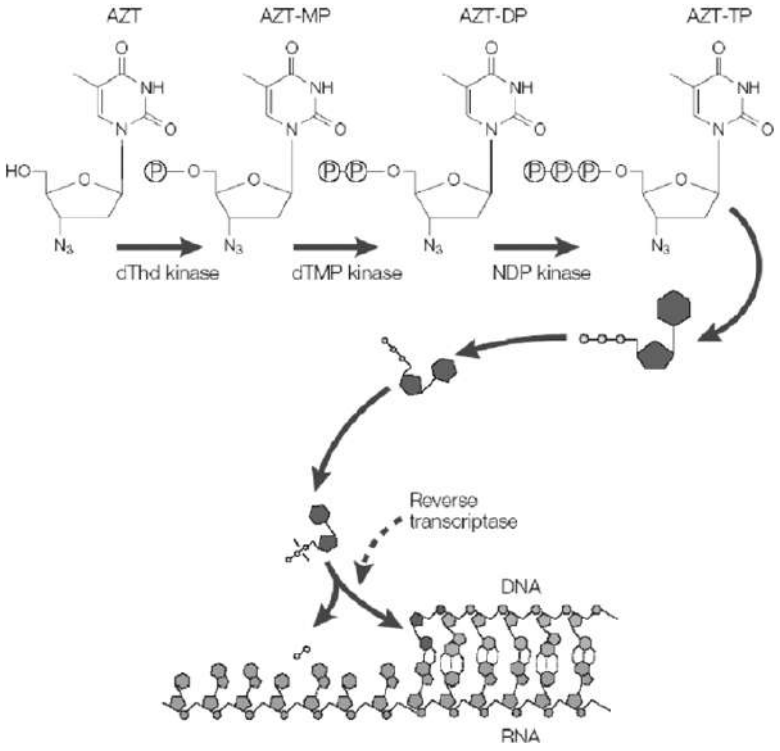
Phenyl sulphone

1.15 Anti-HIV agents

Acquired immune deficiency syndrome (AIDS) is caused by the retrovirus, HIV. The HIV infection that targets the lymphocytes, the monocytes and macrophages expressing the surface CD-4 receptors, eventually produces profound defects in cell-mediated immunity. Overtime infection leads to severe depletion of CD-4 T-lymphocytes resulting in opportunistic infection like tuberculosis, fungal, viral, protozoal and neoplastic diseases and ultimately death. E.g.

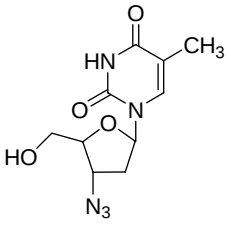
Zidovudine:

Zidovudine, also known as Azidothymidine (AZT), is a nucleoside derivative of reverse transcriptase inhibitors and an antiretroviral medication used to prevent and treat HIV/AIDS. AZT is only active against HIV. When the virus is replicating into proviral DNA (viral DNA synthesized prior to integration into host DNA). This is because the active compound AZT, has a high affinity for an enzyme called, reverse transcriptase, which is used by HIV to replicate viral single-stranded RNA into proviral double-stranded DNA. AZT contains a N_3 group in place of the usual nucleoside -OH group. As a result, reverse transcriptase incorporates AZT into growing strands of HIV proviral DNA and DNA synthesis and replication are terminated (see following figure), since subsequent nucleoside cannot bind to the nitrogen group of AZT.



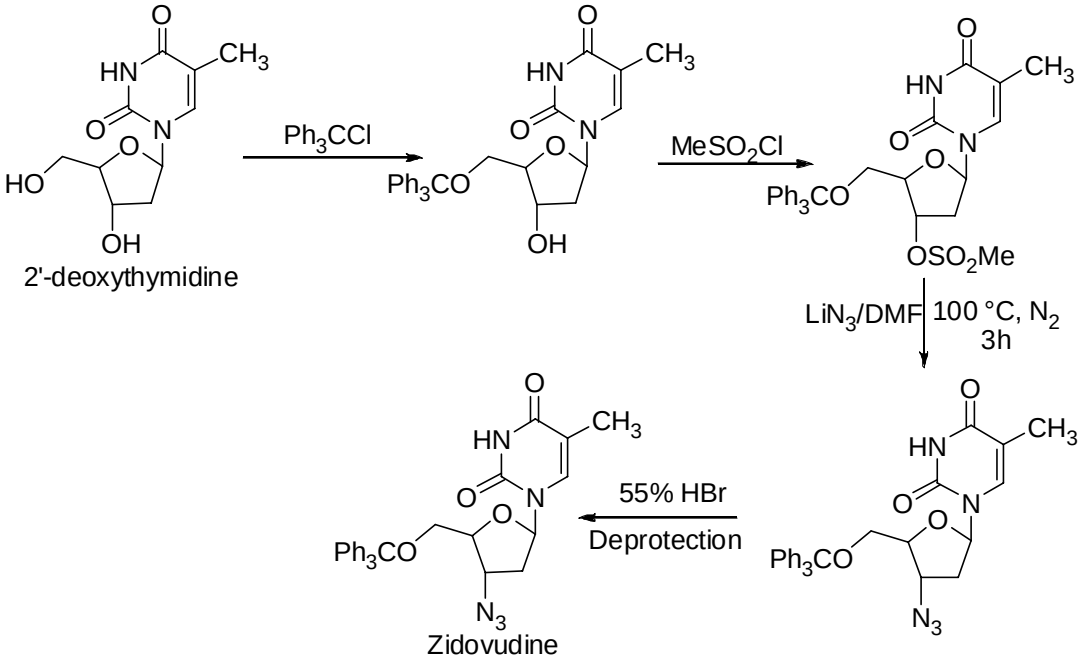
It is generally recommended for use with other anti-retrovirals. It may be used to prevent mother-to-child spread during birth or after a needle stick injury or other potential exposure. It is sold both by itself and together as Lamivudine/Zidovudine and Abacavir/Lamivudine/Zidovudine. It can be used by mouth or by slow injection into vein. Common side effects include headaches, fever and nausea. Serious side effects include liver problems, muscle damage and high blood lactate levels. It is commonly used in pregnancy and appears to be safe for the baby.

Structure:



Zidovudine

Synthesis:



2'-deoxythymidine

Zidovudine

1.16 Summary

In this unit mainly we discussed about topic "Drug Discovery". After carefully reading learners could inform about following things,

- Historical outline of drug discovery and different stages of drug discovery process.
- You also learned how the drug design have been done in laboratory framework based on different methodologies like ligand based, structure based, mechanism based and Computer-assisted drug design process.
- You have also learned how Drugs can be grouped together in different ways – by the way they affect the body or by how or where they are used. Mainly overall drugs are classified on the basis of its therapeutic action, chemical structure and molecular targets.
- Synthesis of different types of drugs of analgesic categorized molecules have been demonstrated with mechanism e.g., Aspirin, Paracetamol, Ibuprofen etc.
- A brief idea about antibiotics, about its characteristic features, classification and synthesis (Chloramphenicol) was discussed.
- Different type of antibacterial agents and its function was mentioned. Classification of different antibacterial agents on source, spectrum of activity, type of action and chemical structure was also demonstrated in this section.
- Students also learned about antifungal agents, anti-leprosy agent, CNS-agents, cardiovascular agent and their synthetic approach.
- A brief classification about antiviral agents (anti-herpes, anti-HIV) and their synthesis (Acyclovir, Zidovudine) were discussed. The mode of action of AZT on T-Lymphocyte cell against HIV was also discussed.

1.17 Model Questions

1. Answer the following questions (MCQ): (*1 Mark for Each*)

I. People should not take acyclovir, those having problems in ——

A) Kidney.
B) Heart.
C) Brain.
D) Lungs.

II. Which of the following medicinal disorder is commonly treated by Diazepam?

A) Glaucom.

B) Indigestion.
C) Anxiety.
D) Myasthenia Gravis.

III. Which of the following is a plausible side effect of Diazepam?

A) Drowsiness.
B) Confusion.
C) Muscle Weakness.
D) All of these.

IV. Which of the following is primarily used for the treatment of uncomplicated urinary tract infections caused by *E. coli*,

A) Sulphacetamide
B) Sulphamethoxazole
C) Sulphonamide
D) Trimethoprim

V. Knowledge of three-dimensional structure of the biological target obtained through X-Ray crystallography in ————————

A) Ligand based drug design.
B) Structure based drug design.
C) Mechanism based drug design.
D) Computer-Assisted drug design.

2. Answer the following questions: (*1 Mark for Each*)

i. What are the three main parts of Central Nervous System?
ii. For which symptoms Phenobarbital is prescribed?
iii. Cardiovascular disease mainly affects which part of human body?
iv. Mention a medication that is used for heart failure or high blood pressure.
v. Give one example of Gram-negative bacteria

3. Answer the following questions (MCQ): (*2 Marks for Each*)

i. Briefly explain how does HIV works on T-lymphocyte?
ii. Explain the use of acyclovir medication.
iii. What are anti-leprosy drugs? Give an example.
iv. Give the basic differences between Analgesic and Anti-inflammatory agent.
v. What is Chloramphenicol? Give its proper structure.
vi. Write down the functions of Spectinomycin and Trimethoprim?
vii. What is Lincomycin? How does it differ from penicillin?
viii. Write down the conditions which is needed for a particular antibiotic to act as therapeutic agent.

4. Answer the following questions (MCQ): (*3 Marks for Each*)

i. What are antiviral agents? Give examples.

ii. What is the main difference between antiviral and antibiotic agent?

iii. Classified antiviral drugs based on their targets.

iv. Write down the synthetic route of Phenobarbital from benzyl chloride?

v. Write down the synthesis of AZT.

vi. What are Antibiotics? Give its classifications with proper Example.

vii. Write down the mechanistic study of IBUPROFEN.

viii. Give an example of Antifungal Agent and note down its plausible mechanism of synthesis.

ix. Give the basic classifications of Drug. Explain with proper example.

x. Write down the synthetic route of Chloramphenicol.

5. Answer the following questions (MCQ): (*4 Marks for Each*)

i. What is AZT? How does it work against HIV?

ii. What is HIV? Mention its target in human body.

Unit 2 ❐ Fermentation

2.0 Objectives

In this unit fermentation process was elaborately discussed and, in this section, also different features of fermentation process for preparing antibiotics, amino acids, vitamins were demonstrated. End of this chapter model questions will also involve actively all learners. Through this assignment learners can examine themselves how much knowledge they gained on this course.

2.1 Introduction

Fermentation is the chemical transformation of organic substances into simple compounds by the action of enzymes, complex organic catalysts, which are produced by the microorganism such as molds, yeasts or bacteria. This process converts sugar

to acids, gases or alcohol. In fermentation molecules such as glucose are broken down anaerobically. More broadly, in food production it is the foaming that occurs during the manufacture of wine and beer. In biochemistry, it is narrowly defined as the extraction of energy from carbohydrates in the absence of oxygen. Humans have used fermentation to produce foodstuffs and beverages since the Neolithic age. There are two types of fermentation based on respiration: Aerobic fermentation and An-aerobic fermentation.

2.2 Aerobic Fermentation

Aerobic fermentation is also known as aerobic glycolysis, it occurs in the presence of oxygen. It is a metabolic process by which cells metabolize sugars via fermentation and occurs through the repression of normal respiratory metabolism (also referred to as the Crabtree effect in yeast). Oxygen limitation is the major problem in aerobic fermentation because oxygen has a low solubility in water. Dissolved oxygen (DO) concentration is generally kept as high as possible by increasing the oxygen transfer rate (OTR). Aerobic fermentation is usually a shorter and more intense process than anaerobic fermentation.

2.3 Anaerobic Fermentation

Anaerobic fermentation is a process in which cells use to extract energy from carbohydrates when oxygen or other electron acceptors are not available in the surrounding environment. This differentiates it from anaerobic respiration, which doesn't use oxygen but does use electron-accepting molecules that come from outside of the cell. Anaerobic fermentation has a broad range of applications. It could be used for the production of various industrial chemicals, such as ethanol, butyl alcohol, acetic acid, lactic acid, hydrogen gas and various nutraceutical molecules with medical benefit.

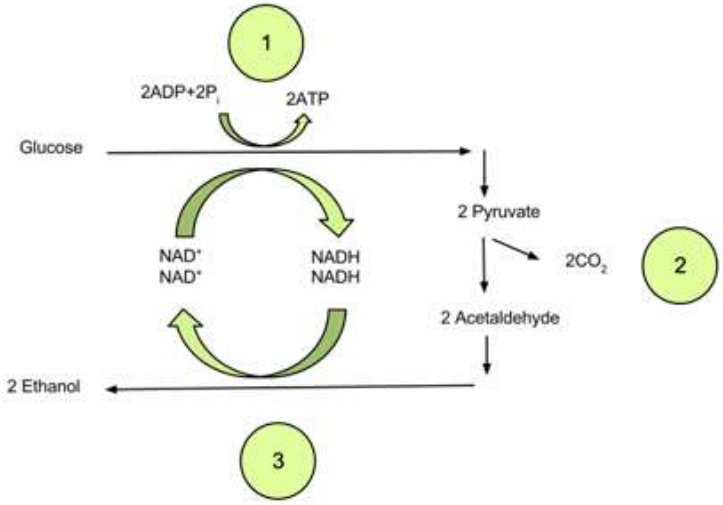
This process can follow gyclolysis as the next step in the breakdown of glucose and other sugars to produce molecules of adenosine triphosphate (ATP) which create an energy source for the cell. Through this method, a cell is able to regenerate nicotinamide adenine dinuleotide (NAD+) from the reduced form of nicotinamide adenine dinucleotide (NADH). It also relies on enzymes to add a phosphate group to an individual diphosphate (ADP) to produce ATP.

2.4 Production of Ethyl alcohol

Production of ethyl alcohol by fermentation is also chemically known as Ethanol fermentation. It is a biotechnological process accomplished by yeast in the absence of oxygen, thus alcoholic fermentation is considered as an anaerobic process. In this process sugars such as glucose, sucrose and fructose are converted into cellular energy and thereby produced ethanol and carbon dioxide as metabolic waste product. This conversion can also be performed by some kinds of bacteria or a few other microorganisms. Among the yeasts, *Saccharomyces cerevisiae* is the most commonly used while among the bacteria, *Zymomonasmobilis* is the most frequently employed for ethanol production. Alcoholic fermentation produces beer and wine.

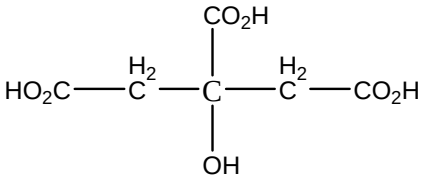
Alcoholic fermentation includes three steps discussed below:

1. Alcoholic fermentation begins with the breakdown of sugars (glucose) by yeasts to form two pyruvate molecules, which is also known as glycolysis. The energy released by this exothermic reaction is used to phosphorylate two ADP molecules, yielding two ATP molecules. Two molecules of NAD^+ are also reduced to NADH.
2. The two pyruvate molecules are broken down, yielding two acetaldehyde molecules and giving off two molecules of carbon dioxide.
3. The two molecules of NADH reduce the two acetaldehyde molecules to two molecules of ethanol and this converts NAD^+ back into NADH. The flowchart is given below.



2.5 Production of citric acid

Citric acid (2-hydroxy-1,2,3-propane tri-carboxylic acid), a natural constituent and common metabolite, is the most important organic acid produced in tonnage and is extensively used in food and pharmaceutical industries.



Chemical structure of citric acid

Citric acid found primarily in several varieties of varieties of fruits and vegetables with citrus fruits such as lemons and limes containing the highest amounts of citric acid. This acid has many uses, including as a food additive / preservative, ingredient in cosmetic products and as a powerful cleaving agent. A large number of micro-organisms including bacteria, fungi and yeasts have been employed to produce citric acid.

For the production of citric acid, fermentation is the most economical and widely used way. The industrial citric acid production can be carried out in three different ways:

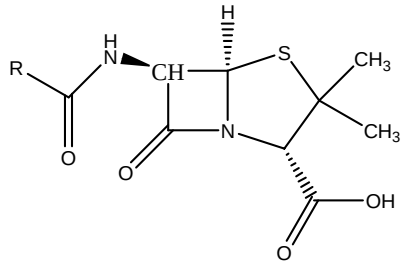
- **Surface Fermentation:** Surface fermentation using *Aspergillus niger* may be done on rice bran as is the case in Japan, or in liquid solution in flat aluminium pans. Special strains of *Aspergillus niger* which can produce citric acid despite the high content of trace metals in rice bran are used. The citric acid is extracted from the bran by leaching and is then precipitated from the resulting solution as calcium citrate.
- **Solid state Fermentation:** It is also known as Koji process and it is the simplest method for the production of citric acid.
- **Submerged Fermentation:** Citric acid produced mainly by submerged fermentation using *Aspergillus niger* from different sources of carbohydrates, such as molasses and starch-based media. In this case, the strains are inoculated of about 15cm depth in fermentation tank. The culture is enhanced by giving aeration using air bubbles and it is allowed to grow for about 5-14 days at 27-30 degree Celsius. The citric acid is produced in the fermentation tank and it is purified.

2.6 Antibiotics

An antibiotic is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of such infections. They may either kill or inhibit the growth of bacteria. Here we will discuss about the production process of few antibiotics using fermentation process.

2.6.1. Production of Penicillin

Penicillin is the oldest and one of the most commonly used groups of antibiotics at present. They are derived from the mold/fungi *Penicillium* and it is also known as *Penicillium notatum.* It can be found on salted food products, but it is mostly found in indoor environments, especially in damp or water-damaged buildings. It is common in temperature and subtropical. Penicillin is the source of several â-lactum antibiotics, which inhibits biosynthesis of bacterial cell wall.



Penicillin

Penicillin can be divided into two groups, namely natural and semisynthetic penicillins. Semisynthetic penicillins are prepared from (+)-6-aminopenicillanic acid, on the other hand, natural Penicillins are produced from the fermentation of the fungus *Penicillium chrysogenum.* Penicillin production is previously achieved by surface process i.e. Solid state fermentation and surface liquid fermentation. Nowadays, a commercial production is carried out by fed batch process mentioned below.

Inoculum (Organism): *Penicillium chrysogenum* is high yielding strain and therefore most widely used as production strain. In Inoculum preparation the purpose is to develop a pure Inoculum in an adequate amount. To do so various sequential steps are necessary like:

1. For inoculation a starter culture is needed.
2. After getting growth on solid media, one or two growth stages should allowed in shaken flask cultures to create a suspension, which can be transferred to

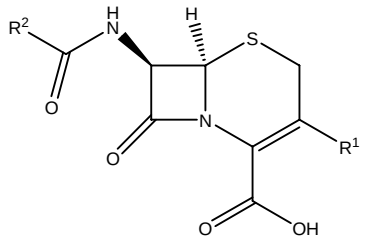
seed tanks for further growth.

3. The content of the seed tanks is transferred to the primary fermentation tanks after about 24-28 hours.
4. All the bio parameters like temperature, pH, aeration, agitation etc. should be properly maintained.

Adding corn syrup, selection of strain, mutation and selection and sexual reproduction should be maintained properly for the increasing yield of penicillin.

2.6.2. Production of Cephalosporin

Cephalosporins are the second major group of â-lactum, derived from the micro-organism *Acrimonium chrysogenum.* These are closely related to the Penicillins in both the structure and mode of action and are used to treat bacterial infections.



Core structure of cephalosporins

Cephalosporin is a popular antibiotic because of its excellent characteristics such as broad spectrum of activity, low toxicity, high safety profile, oral and parenteral preparations, and resistance to â-lactamase than Penicillin.

Classification of Cephalosporins: According to generations Cephalosporins are following types-

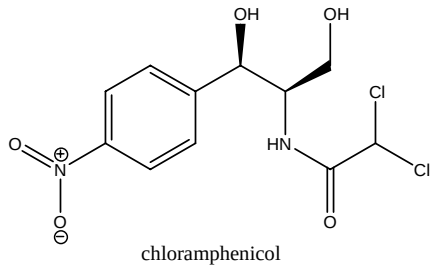
- **First Generation:** It's a narrow spectrum antibiotic e.g. *Streptococcus*.
- **Second Generation:** This class of cephalosporin is known as intermediate spectrum antibiotics e.g. *E. coli*.
- **Third Generation:** This class is known as broad spectrum antibiotics e.g. *Pseudomonas*.
- **Fourth Generation:** This class of Cephalosporins is an extended version of third generation antibiotics with the increased activity against Gram-positive and Gram-negative bacteria with high resistance to beta lactamase.
- **Fifth Generation:** This class of Cephalosporins is also extended with activities against MRSA.

Fermentation Process:

Cephalosporin C is easily produced from the fungus *Acremonium chrysogenum* by the process of fermentation in a bioreactor under optimum reaction conditions to obtain maximum yield of the antibiotic. The fermentation process concerned with the production of cephalosporin is similar to that of penicillin. The culture media consists of corn steep liquor and soy flour-based media in a continuous feeding system. The other ingredients of the medium include sucrose, glucose and ammonium salts. Methionine is added as a source of sulfur. The fermentation is carried out at temperature 25-28°C and at pH 6-7. The growth of micro-organisms substantially increases with good oxygen supply, although during production phase, oxygen consumption declines. Cephalosporin C from the culture broth can be recovered by ion-exchange resins and by using column chromatography. Cephalosporin C can be precipitated as zinc, sodium or potassium salt and isolated.

2.6.3. Production of Chloromycetin (Chloramphenicol)

Chloramphenicol is an antibiotic produced by *Streptomycetes venezuelae* in 1947. It is useful for the treatment of a number of bacterial infections.



chloramphenicol

Chloramphenicol is a broad spectrum antibiotic that typically stops bacterial growth by stopping the production of proteins. It can act on both Gram-positive and Gram-negative, actinomycetes, rickettsiae and chlamydias. Because of its toxic effect on bone marrow, Chloramphenicol has not been widely used but its toxicity can be reduced if therapy is conducted carefully. Chloramphenicol can be produced by fermentation:

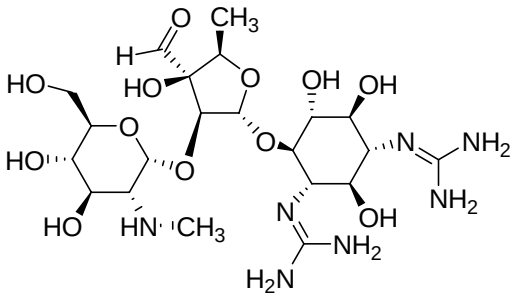
Fermentation Process:

The fermentation is carried out in 30 liters Fermenter containing 18-liter medium consisting of glycerol (1%), yeast extract, sodium chloride (0.5%) and pH is adjusted to 7.5. The fermentation is carried out at 25°C for 3-4 days. Chloramphenicol is extracted from the clarified broth. The filtrate is extracted either with ethyl or diluted with kerosene and then washed with dilute acetic acid, sodium carbonate and water.

The lipids are removed by petroleum ether and the crude product is decolorized by passing the organic solution through a column of charcoal or alumina. The purified product is recrystallized from ethylene or ether and petroleum ether mixture.

2.6.4. Production of Streptomycin

Streptomycin is an antibiotic, produced by *Streptomyces griseus*. It is used to treat a number of bacterial infections, this includes tuberculosis, endocarditis, plague, tularemia, *Mycobacterium avium* complex, brucellosis, *Burkholderia* infection and rat bite fever.



Streptomycin

It is particularly active against Gram-negative bacteria and against tuberculosis organism, *Mycobacterium tuberculosis*. However, it proved to be useful in the treatment of infections caused by Gram-positive especially resistant to penicillin. It is also useful in the control of plant diseases caused by bacteria as it acts systemically in plants.

Fermentation Process:

Spores of *S. griseus* are inoculated into medium to establish a culture with high mycelia biomass for introduction into inoculums tank, using inoculums to initiate the fermentation process. Yield in this production vessel responds to high aeration and agitation condition. The optimum fermentation temperature is in the range of 25-30°C and the optimum pH range in between 7-8. High rate to streptomycin production, however, occurs in the pH range of 7.6-8. The process of fermentation is highly aerobic and lasts approximately for 5 to 7 days and passes through 3 phases:

a) The first phase (it takes about 24-28 hours)

b) The second phase (it lasts for 2 days)

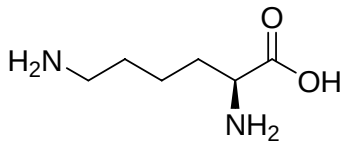
c) The third phase (harvested before cell lysis)

After completion of fermentation the mycelium is separated from the broth by filtration and streptomycin is recovered then the fermentation broth is acidified, filtered

and neutralized. It is then passed through a column containing a cation exchange resin so that streptomycin is adsorbed from the broth. The column is then washed. The streptomycin is dissolved in methanol and filtered. Then acetone is added to the filtrate to precipitate the antibiotic. The precipitate is again washed with acetone and vacuum dried and purified further by dissolving in methanol. The pure form of streptomycin is extracted as calcium chloride complex.

2.7 Production of Lysine

Lysine is an essential, economically important amino acid. Since it is need for bone formation so children and growing animals have a high requirement of lysine. It is used medically as a nutrient in form of supplements and medicines. Lysine also has some pharmaceutical applications in the formulation of diets with balanced amino acid composition and in amino acid infusions. Lysine is present in two forms, L-form and D-form. L-lysine is biologically active.



L-lysine

Two major biotechnological processes are used to manufacture lysine, one is the enzymatic conversion of dl-á-amino-å-caprolactam and the other one is fermentation. L-lysine is mainly produced by fermentation using strains of coryne bacteria, especially *Corynebacterium glutamicum*. The process can be divided into three main parts: fermentation; product recovery; product concentration, drying and packaging. The optimum fermentation temperature is 35-37°C and the optimum pH is 7.2.

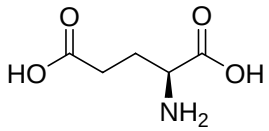
Fermentation Process:

- The culture media used in the batch and fed-batch phases of fermentation are prepared by mixing process water, glucose and nutrients.
- The fermentation step is performed in fed-batch mode and under aerobic conditions.
- In the batch phase, the micro-organism seed is fed into the fermenters, which have been filled previously with the fermentation batch medium. After glucose exhaustion, the batch phase is finished and the fed-batch phase is started.
- Glucose and nutrients are continuously supplied, during the fed-batch phase until the desired L-lysine concentration is achieved.

- The broth is sent to a buffer tank to provide a continuous flow to the downstream process steps, at the end of the fermentation.
- For product recovery, the fermentation broth is sent to an ultra-filtration system for the removal of cell debris and other suspended solids.
- Subsequently, the liquor from ultra-filtration is fed to ion-exchange columns where L-lysine is selectively adsorbed.
- Then the adsorbed L-lysine is eluted from the ion-exchange resins by washing with an aqueous ammonia solution.
- The eluted L-lysine is mixed with mother liquor from the product filtration step and concentrated by evaporation.
- Free L-lysine is converted to L-lysine HCL by adding hydrochloric acid to the concentrated lysine.
- The L-lysine HCL solution is then sent to the crystallizer and the salt is filtered and thus the product is obtained.

2.8 Production of Glutamic acid

L-glutamic acid is one of the major amino acid used in the biosynthesis of proteins. It is present in a wide variety of foods. L-glutamic acid is mainly used as a food additive and flavor enhancer in the form of sodium salt. Glutamic acid is important in brain metabolism hence various analogues of glutamic acid are used in treating various neuropathic diseases.



L-glutamic acid

Chemical mode of synthesis of L-glutamic acid is not widely preferred due to the formation of racemic mixture. In biotechnological processes, *Corynebacterium glutamicium*is used for the production of glutamic acid by submerged fermentation. The process of fermentation comprises: fermentation; crude isolation and purification of product.

Fermentation Process:

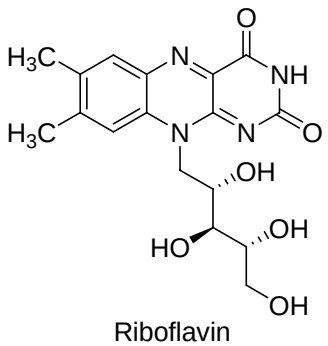
The usual culture medium for glutamic acid fermentation contains a carbon source such as glucose and nitrogen source such as urea. The prepared culture medium is sterilized in a fermenter by steam. When the temperature of the medium cools down

to 30°C, the micro-organism is added to the fermenter in a proper inoculums size. The micro-organism is incubated for 36-48 hours during which time the pH should be in between 7-7.8, temperature should be in between 30-35°C and the aeration rate are carefully controlled.

2.9 Production of Vitamin B_2 (Riboflavin)

Riboflavin is a water-soluble vitamin, which is essential for growth and reproduction of humans and animals and it is one of the most important applications. Riboflavin performs its biochemical function as a precursor for the enzymes, Flavin Adenine Dinucleotide (FAD) and Flavin Mononucleotide (FMN), which are mostly involved in redox reactions of all organisms. These flavor-coenzymes participate in the metabolism of carbohydrate, lipids, ketone bodies and proteins from which living organisms derive most of their energy.

Riboflavin is produced by all plants and most micro-organisms. Industrial production of riboflavin can be performed by both chemical synthesis and fermentation.



Riboflavin

The fermentation route is cost-effective as the production of vitamin B2 occurs in a single-step. In contrast, chemical processes are multistage and expensive. Thus, nowadays, the fermentation production of Riboflavin is economically and ecologically more feasible. Most of the producers like BASF, Roche, ADM/Aventis, Hubei Gaungii prefer fermentive production.

Fermentation Process:

Although bacteria (Clostridium species) and yeasts (Candida species) are good producers, two closely related *Ascomycete fungi, Eremothecium ashbyii* and *Ashbya gossypii*, are considered the best Riboflavin producers.

The necessary seed cultures are prepared in different seed fermenters in several

steps. The last seed culture is the start inoculum for the main fermentation. The duration of a seed-fermentation is around 50 hours, while the main fermentation lasts about 500 hours. During this time the strain produces 27 g/L riboflavin. Fermentation requires aeration accomplished by a gas compressor and a sterile filter and exhaust gases are filtered by a second filter. Then a small fraction of the harvested broth is put into another tank and is used as inoculums for the next batch. After fermentation the broth is harvested into the harvest tank. Crystallization of the obtained product is completed by evaporation of some of the water and the powder product is obtained by drying in the last step.

2.10 Production of Vitamin B_{12}

Vitamin B12 is also known as Cyanocobalamin, belongs to the Cobalamin family of compounds. It is a water-soluble vitamin that has a key role in the normal functioning of the brain and nervous system, and the formation of red blood cells. It is involved in the metabolism of every cell of the human body especially affecting DNA synthesis, fatty acid and amino acid metabolism. Vitamin B12 is an essential vitamin that is widely used in medical and food industries. People with vitamin B12 deficiency may eventually develop pernicious anemia.

It is synthesized only by micro-organisms and not by animals and plants. It is the largest and most structurally complicated vitamin and can be produced industrially only through bacterial fermentation synthesis. It is usually manufactured by submerged culture process which completed in 3-4 days.

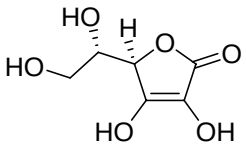
Fermentation Process:

B_{12} fermentation process use glucose as a carbon source and the micro-organisms that may be employed in the industrial production process are *Streptomyces griseus, Streptomyces olivaceus, Pseudomonas denitrificans, Propionibacterium shermanii, Propionibacterium freudenreichii, Bacillus magaterium* and *Bacillus coagulans*. Steps involved in the production process are-

- Formulation of the medium.
- Sterilization of the medium.
- Making starter culture.
- Anaerobic fermentation.
- Aerobic fermentation.
- Recovery.

2.11 Production of Vitamin C

Vitamin C is also known as Ascorbic Acid and Ascorbate, is an essential nutrient for humans and a few other mammals. Vitamin C is widely used in the food, beverage, animal feed and pharmaceutical industries. It is required for the functioning of several enzymes, important for immune system function and also functions as an antioxidant.



Vitamin C

Vitamin C has been produced commercially by extraction from plants, by chemical synthesis, by fermentation and by mixed fermentation method. The manufacture of vitamin C is now carried out in two ways, traditional Reichstein process and newer two-stage fermentation process. But Reichstein process is not ecofriendly as-

1. explosive gas H_2 is used
2. Requires high pressure and temperature
3. Also, acetone and $KMnO_4$ are toxic to environment.

Today the two-stage fermentation process is widely used.

Two-stage Fermentation Process:

A second Fermentation step replaces the chemical reactions used to produce keto-gulonic-acid in the Reichstein method. When compared with classic process, a mixed fermentation consisting of *Ketogulonicigenium vulgare* and *Bacillus spp*. is conducted to convert L-sorbose to intermediate 2-Keto-L-Gulonic acid (2-KLG) in the two-step fermentation process. It is developed in china and is used by all Chinese producers. Though two-stage fermentation process gives high quality product but yield is less compared to Reichstein process.

2.12 Summary

In this unit mainly we discussed about topic “Fermentation”. After carefully reading you could inform about following things,

- Fermentation is the chemical transformation of organic substances into simple compounds by the action of enzymes, complex organic catalysts, which are produced by the microorganism such as molds, yeasts or bacteria.
- There are two types of fermentation based on respiration: Aerobic fermentation and An-aerobic fermentation.

- The production of ethanol by fermentation process is considered as an anaerobic process as it accomplished by yeast and in absence of oxygen. The details steps involved in this process was discussed in this unit.
- The industrial citric acid production by different fermentation process was briefly described in this section.
- Through this section students also learned about different techniques of fermentation process which were used for the synthesis of different antibiotics (Penicillin, Cephalosporin, Chloromycetin and Streptomycin), amino acids (Lysine, Glutamic acid) and vitamins (Vitamin B2, Vitamin B12 and Vitamin C).

2.13 Model Questions

1. Answer the following questions (MCQ) (*1 Mark for Each*)

I. In fermentation which of the following is the correct pairing between initial reactant and major product?
   A) Glucose; Carbon dioxide.
   B) Acetate; ethylene glycol.
   C) Glucose; Lactate.
   D) Ethanol; Lactate.

II. Which of the following is the primary function of fermentation?
   A) Production of ethanol to be used as a fuel source.
   B) Regeneration of NAD^+.
   C) Production of oxygen to be used in aerobic pathways in the future.
   D) Production of Carbon dioxide gas.

III. Streptomycin is produced by ——
   A) S. griseoflavus.
   B) S. griseus.
   C) S. ramosus.
   D) S. aerofaciens.

IV. Upon which of the following the yield of the antibiotic depends?
   A) Age of the inoculums.
   B) pH of the medium.
   C) Composition of the medium.
   D) All of the above.

V. Lysine is produced by ——
   A) Aerobic fermentation.
   B) Anaerobic fermentation.
   C) Aerobic fermentation followed by anaerobic fermentation.
   D) Anaerobic fermentation followed by aerobic fermentation.

VI. Riboflavin is soluble in ——
   A) Ether.
   B) Water.
   C) Organic solvents.
   D) All of these.

VII. Vitamin B_{12} deficiency causes ——
   A) Anemia.
   B) Kwashiorkor.
   C) Both A and B.
   D) Hypocalcemia.

2. Answer the following questions: (*1 Mark for Each*)
   i. How many types of fermentation present based on respiration?
   ii. What are the optimum fermentation conditions for the production of Lysine?

3. Answer the following questions: (*2 Marks for Each*)
   i. What is fermentation?
   ii. Write down the use of fermentation?
   iii. What is the main difference between aerobic and anaerobic fermentation?
   iv. Write down the name of yeast and bacteria, which are commonly used in ethanol fermentation?
   v. Explain submerged fermentation for the production of Citric acid.
   vi. Which type of fermentation is most effective, aerobic or anaerobic? Explain your answer.
   vii. What are the media used for the production of Chloramphenicol in fermentation?
   viii. What is the advantage of Vitamin B_{12} – fermentation process over chemical process?
   ix. Write down some riboflavin producers in fermentation.
   x. What is the key role of Vitamin B_{12} in human body?

4. Answer the following questions: (*4 Marks for Each*)
   i. Classified Cephalosporin according to generation.
   ii. How Streptomycin is recovered after fermentation?
   iii. What are the disadvantages of Reichstein Process for the production of Vitamin C? How it is overcome in two-stage fermentation process?

References and Further Readings

1. G.L. Patrick: Introduction to Medicinal Chemistry, Oxford University Press, UK.
2. Hakishan, V.K. Kapoor: Medicinal and Pharmaceutical Chemistry, Vallabh Prakashan, Pitampura, New Delhi.
3. William O. Foye, Thomas L., Lemke, David A. William: Principles of Medicinal Chemistry, B.I. Waverly Pvt. Ltd. New Delhi.
4. Arthur Raphael Miller, MichealH.Davis; Intellectual Property: Patents, Trademarks and Copyright in a Nutshell, West Group Publishers (**2000**).
5. Jayashree Watal, Intellectual property rights in the WTO and developing countries, Oxford University Press, Oxford
6. G. Thomas: Medicinal Chemistry (Second Edition) John Wiley & Sons Ltd, (**2007**)
7. Mukund S. Chorghade Drug Discovery and Development (*Vol-1*) John Wiley & Sons Ltd, (**2006**)