KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

LECTURE PLAN
DEPARTMENT OF BIOTECHNOLOGY

STAFF NAME: Dr. C. Rajkuberan
SUBJECT NAME: Pharmaceutical Biotechnology SUB.CODE:19BTP305A
SEMESTER: II CLASS: I M.Sc (BT)

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	Classification of pharmaceutical – solutions, suspension, tablets and caapsules	T1: 87
2	1	Drug and its sources	T1: 117
3	1	Routes of drug administration	T1: 135
4	1	Drug – absorbtion, bioavailability and distribution	T1: 153
5	1	Drug metabolism and theories	T1: 20
6	1	Drug receptor interactions – prodrug concept	T2: 25
	Total No of Hours Planned For Unit 1=6		
		UNIT-II	
1.	1	Drug design and development	T3: 31
2.	1	Drug screenup; target identification and validation	T3: 33
3.	1	Drug discovery and drug delivery	T3: 35
4.	1	Drug abuse and self poisoning	T3: 45
5.	1	Pharmacogenomics and biochip	T1: 117
	Total No of Hours Planned For Unit II=5		
		UNIT-III	
1.	1	Genetically engineered proteins and peptide agents	T4: 37

2.	1	Novel drug delivery systems – routes of drug administration	T4: 311
3.	1	Anti AIDS drug development	T4: 402
4.	1	Drug development - Oncogenes	T4: 401
5.	1	Multidrug resistance	T4: 457
6.	1	Vaccine development – infectious diseases	T4: 531
7.	1	Drug development by genetic engineering – infectious diseases	T4: 627
8.	1	Gene theraphy	T4: 444
9.	1	Stem cell theraphy	T4: 585
	Total No of Hours Planned For Unit III=9		
		UNIT-IV	
1	1	Enzyme technology	T5: 3
2	1	Enzyme sources, extraction, purification and application	T5: 25
3	1	Enzymes – applications (pharmaceuticals, therapeutic and clinical)	T5: 93
4	1	Production of aminoglucosidase	T5: 173
5	1	Production of glucose isomerase	T5: 183
6	1	Production of amylase and trypsin	T5: 199
7	1	Immobilization of enzymes - techniques	T5: 333
8	1	Immobilization of enzymes - applications	T5: 339
9	1	Immobilization of enzymes – Reactors	T5: 361
10	1	Enzyme engineering	T5: 393
	Total No of Hours Planned For Unit IV=10		
		UNIT-V	
1	1	Drug carrier	J1
2	1	Drug carrier - liposomes	J1
3	1	Drug carrier – biodegradable polymer	J1
4	1	Drug release	J2
5	1	Drug release – kinetics	J2
6	1	Drug release- sustained release	J2
7	1	Drug release – first order release approximation; multiple dosing	J2

8	1	Unit test	
9	1	ESE Question paper revision	
10	1	ESE Question paper revision	
	Total No of Hours Planned for unit V=10		
Total Planned Hours			**40**

References (R) /Text Books (T) / Journal (J) / Website (W):

1. **T1:** JAY RHO, P., STAN LOUIE, G (2009). HANDBOOK OF PHARMACEUTICAL BIOTECHNOLOGY (11TH ED). NEW YORK. PHARMACEUTICAL PRODUCT PRESS.
2. **T2**: DRUG METABOLISM- CURRENT CONCEPT EDITED BY – CORINA IONESCO AND MINO.R.CAIRA; 2009- SPRINGER PUBLICATIONS
3. **T3**: COMMONLY ISED DRUGS- RAFIK KARAMAN. 2015 – PUBLISHED BY NOVA PUBLISHERS Inc.,
4. **T4:** PHARMACEUTICAL BIOTECHNOLOGY – CONCEPTS AND APPLICANTS – GARLY WALSH- 2012 – JOHN WILEY & SONS
5. **T5**: ENZYMES: A PRACTICAL INTRODUCTION TO STRUCTURE AND MECHANISM BY ROBERT COPELAND – 2012- JOHN WILEY & SONS
6. **J1**: POLYMER AND DRUG DELIVERY SYSTEMS; CURRENT DRUG DELIVERY – 2016
7. **J2**: KINETICS AND MECHANISM OF DRUG RELEASE FROM SWELLABLE AND NON SWELLABLE MATRICES. RESEARCH JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCS. 2017

UNIT-1

SYLLABUS

Introduction to pharmacy – Classification of pharmaceutical – solutions, suspension, tablets and capsules Drug and its sources Routes of drug administration Drug – absorption, bioavailability and distribution Drug metabolism and theories Drug receptor interactions – prodrug concept

Drug

According to WHO, a drug is defined as any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient.

Drug dosage form ?

A dosage form is the way of identifying the drug in its physical form.

Factors to be considered while determining drug dosage form?

1) Physical appearance of the drug product
2) Physical form of the drug product prior to dispensing to the patient.
3) The way the product is administered
4) Frequency of dosing,
5) How pharmacists and other health professionals might recognize and handle the product.

Qualities of an ideal drug dosage form

1) Resist action by gastric fluids
2) Prevent vomiting and nausea.
3) Reduce or alleviate the undesirable taste and smells.
4) Achieve a high concentration of drug at target site.
5) Produce a delayed or long-acting drug effect.

Types of dosage forms

1) Solid dosage forms
2) Liquid dosage forms
3) Gaseous dosage forms

Examples of solid dosage forms

1. Capsules
2. Tablets
3. Powder
4. Lozenge
5. Pill
6. Suppository

Capsule

- A solid oral dosage form consisting of a shell and a filling. The shell is composed of a single sealed enclosure, or two halves that fit together and which are sometimes sealed with a band.
- Capsule shells may be made from gelatin, starch, or cellulose, or other suitable materials, may be soft or hard, and are filled with solid or liquid ingredients that can be poured or squeezed.

Types of capsule ?

A. Coated capsules

B. Liquid filled capsules

Coated capsule?

- A solid dosage form in which the drug is enclosed within either a hard or soft soluble container or "shell" made from a suitable form of gelatin; additionally, the capsule is covered in a designated coating.

Extended release capsule

- The capsule is covered in a designated coating, and which releases a drug (or drugs) in such a manner to allow at least a reduction in dosing frequency as compared to that drug (or drugs) presented as a conventional dosage form.
- Eg: Wellbutrin(Bupropion)

Delayed released capsule

- A solid dosage form in which the drug is enclosed within either a hard or soft soluble container made from a suitable form of gelatin, and which releases a drug (or drugs) at a

time other than promptly after administration. Enteric-coated articles are delayed release dosage forms.

- Manoprolol (Propranolol)

Film coated capsule

- The capsule is covered in a designated film coating, and which releases a drug (or drugs) in such a manner to allow at least a reduction in dosing frequency as compared to that drug (or drugs) presented as a conventional dosage form.

Gelatine

- A solid dosage form in which the drug is enclosed within either a hard or soft soluble container made from a suitable form of gelatin; through a banding process, the capsule is coated with additional layers of gelatin so as to form a complete seal.Alcochep (Metadoxine,L-Ornithine aspartate& thiamine)

Liquid filled capsule

- A solid dosage form in which the drug is enclosed within a soluble, gelatin shell which is plasticized by the addition of a polyol, such as sorbitol or glycerin, and is therefore of a somewhat thicker consistency than that of a hard shell capsule; typically, the active ingredients are dissolved or suspended in a liquid vehicle.

Tablet

- A tablet is a mixture of active substances and excipients, usually in powder form, pressed or compacted into a solid.
- The excipients include binders, glidants (flow aids) and lubricants to ensure efficient tabletting; disintegrants to ensure that the tablet breaks up in the digestive tract; sweeteners or flavours to mask the taste of bad- tasting active ingredients; and pigments to make uncoated tablets visually attractive.
- A coating may be applied to hide the taste of the tablet's components, to make the tablet smoother and easier to swallow, and to make it more resistant to the environment, extending its shelf life.
- Eg:paracip-500 (Acetaminophen)

Types of tablet

a. Chewable

b. Coated – (i) Enteric coated

- (ii)Sugar coated

- (iii)Film coated c. Extended release

d. Delayed release e. Dispersible

f. Multilayer

g. Orally disintegrating h. Soluble

i. Effervescent

Chewable tablet

- A solid dosage form containing medicinal substances with or without suitable diluents that is intended to be chewed, producing a pleasant tasting residue in the oral cavity that is easily swallowed and does not leave a bitter or unpleasant after-taste.
- Eg: Cisapride MPS (Chloradiazepoxide&Clidinium Br)

Coated tablet

- A solid dosage form that contains medicinal substances with or without suitable diluents and is covered with a designated coating.

Enteric coated tablet

- Enteric coated tablets are coated with a material which will dissolve in the intestine but remain intact in the stomach.

  Pantocid (Pantoprazole)

Sugar coated tablet

- A solid dosage form that contains medicinal substances with or without suitable diluents and is coated with a colored or an uncolored water-soluble sugar.

Film coated tablet

- A solid dosage form that contains medicinal substances with or without suitable diluents and is coated with a thin layer of a water-insoluble or water-soluble polymer. Eg: Gaspaz (Domperidone & rantidine)

Extended release tablet

- A solid dosage form containing a drug which allows at least a reduction in dosing frequency as compared to that drug presented in conventional dosage form

Delayed release tablet

- A solid dosage form which releases a drug (or drugs) at a time other than promptly after administration. Enteric-coated articles are delayed release dosage forms.

Powder

- An intimate mixture of dry, finely divided drugs and/or chemicals that may be intended for internal or external use.

  Eg : Cremaffin –FS(Ispaghula)

Types of powder

1. **Powder for solution**: An intimate mixture of dry, finely divided drugs and/or chemicals, which, upon the addition of suitable vehicles, yields a solution.

2. **Powder for suspension** - An intimate mixture of dry, finely divided drugs and/or chemicals, which, upon the addition of suitable vehicles, yields a suspension (a liquid preparation containing the solid particles dispersed in the liquid vehicle).

3. **Powder dentifrice** A powder formulation intended to clean and/or polish the teeth, and which may contain certain additional agents.

4. **Powder metered**- An powder dosage form that is situated inside a container that has a mechanism to deliver a specified quantity.

Lozenge

- A solid preparation containing one or more medicaments, usually in a flavored, sweetened base which is intended to dissolve or disintegrate slowly in the mouth.

  Eg : Alex (Dextromethorphan)

Pill

- A small, round solid dosage form containing a medicinal agent intended for oral administration.

  Eg : Ovaral(Levonorgestrel&Ethinyl estradiol)

Suppository

- A solid body of various weights and shapes, adapted for introduction into the rectal orifice of the human body; they usually melt, soften, or dissolve at body temperature.

  Eg: Anusol-HC Suppository(Hydrocortisone)

Liquid dosage form

A dosage form consisting of a pure chemical in its liquid state.

Examples of liquid dosage forms

1. Solution
2. Suspension
3. Liniment
4. Lotion
5. Oil
6. Spray
7. Syrup

8 Tincture

9. Concentrate
10. Douche
11. Elixir

Solution

- `A clear, homogeneous liquid dosage form that contains one or more chemical substances dissolved in a solvent or mixture of mutually miscible solvents.

  Eg : Balvidine M solution (Metronidazole,Povidone & Iodine)

Types of solutions

1. Concentrated
2. For flush
3. Drops Gel forming

Suspension

- A liquid dosage form that contains solid particles dispersed in a liquid vehicle. Eg: Aviband (Albendazole)

Types of suspension

1. Extended released
2. Drops

Liniment

- A solution or mixture of various substances in oil, alcoholic solutions of soap, or emulsions intended for external application.

Lotion

- An emulsion, liquid dosage form.This dosage form is generally for external application to the skin.Eg: Caladryl lotion (Calamine& Diphenhydramine hydrochloride)

Oil

- An unctuous, combustible substance which is liquid, or easily liquefiable, on warming, and is soluble in ether but insoluble in water. Such substances, depending on their origin, are classified as animal, mineral, or vegetable oils.

  Eg : Oliade bath oil (Vit A,Vit D,Vit E,Olive oil)

Spray

- A liquid minutely divided as by a jet of air or steam

  Eg : Lignox spray (Lidocaine)

Types of spray

1.**Spray metered**-A non-pressurized dosage form consisting of valves which allow the dispensing of a specified quantity of spray upon each activation.

2.**Spray suspension**-A liquid preparation containing solid particles dispersed in a liquid vehicle and in the form of coarse droplets or as finely divided solids to be applied locally, most usually to the nasal-pharyngeal tract, or topically to the skin.

Syrup

- An oral solution containing high concentrations of sucrose or other sugars; the term has also been used to include any other liquid dosage form prepared in a sweet and viscid vehicle, including oral suspensions.

  Eg : Avicot O (Sucralfate& oxythazine)

Tincture

- An alcoholic or hydroalcoholic solution prepared from vegetable materials or from chemical substances.

  Eg : Iodine tincture

Concentrate

- A liquid preparation of increased strength and reduced volume which is usually diluted prior to administration

Examples for gaseous dosage form

1. Aerosol
2. Inhalants
3. Gas

Aerosol

- A product that is packaged under pressure and contains therapeutically active ingredients that are released upon activation of an appropriate valve system; it is intended for topical application to the skin as well as local application into the nose (nasal aerosols), mouth (lingual aerosols), or lungs (inhalation aerosols)

Types of aerosols

Aerosol foam

- A dosage form containing one or more active ingredients, surfactants, aqueous or nonaqueous liquids, and the propellants; if the propellant is in the internal (discontinuous) phase (i.e., of the oil-in-water type), a stable foam is discharged, and if the propellant is in the external (continuous) phase (i.e., of the water-in-oil type), a spray or a quick-breaking foam is discharged.

  Eg : Entofoam (Hydrocortisone acetate)

Aerosole metered :

- A pressurized dosage form consisting of metered dose valves which allow for the delivery of a uniform quantity of spray upon each activation.

Aerosole powder:

- A product that is packaged under pressure and contains therapeutically active ingredients, in the form of a powder, that are released upon activation of an appropriate valve system.

Aerosol spray:

- An aerosol product which utilizes a compressed gas as the propellant to provide the force necessary to expel the product as a wet spray; it is applicable to solutions of medicinal agents in aqueous solvents.

Inhalant

- A special class of inhalations consisting of a drug or combination of drugs, that by virtue of their high vapor pressure can be carried by an air current into the nasal passage where they exert their effect.

Gas

- Any elastic aeriform fluid in which the molecules are separated from one another and so have free paths.

Semisolid dosage form

A semisolid dosage form is not pourable; it does not flow or conform to its container at room temperature. It does not flow at low shear stress and generally exhibits plastic flow behavior.

Examples of semisolid drug dosage forms

1 Cream
2 Gel
3 Ointment
4 Paste
5 Jelly

Cream

- An emulsion, semisolid dosage form, usually containing > 20% water and volatiles5 and/or < 50% hydrocarbons, waxes, or polyols as the vehicle. This dosage form is generally for external application to the skin or mucous membranes.

  Eg –,Prilox(Lidocaine&prilocaine)

Gel

- A semisolid dosage form that contains a gelling agent to provide stiffness to a solution or a colloidal dispersion. A gel may contain suspended particles.

  Eg – Acidine(Dried Al(OH)3 gel,Mg, activated dimethicone)

Types of gel

1.Gel dendrifice A combination of a dentifrice (formulation intended to clean and/or polish the teeth, and which may contain certain additional agents), and a gel. It is used with a toothbrush for the purpose of cleaning and polishing the teeth.

2.Gel metered- A gel preparation, with metered dose valves, which allow for the delivery of a uniform quantity of gel upon each activation.

Ointment

- A semisolid dosage form, usually containing <20% water and volatiles and >50% hydrocarbons, waxes, or polyols as the vehicle. This dosage form is generally for external application to the skin or mucous membranes

  Eg : Nitrogesic(Glyceryl trinitrate)

Paste

- A semisolid dosage form, containing a large proportion (20 – 50%) of solids finely dispersed in a fatty vehicle. This dosage form is generally for external application to the skin or mucous membranes.

  Eg : TESS (Triamcinolone)

Jelly

- A class of gels, which are semisolid systems that consist of suspensions made up of either small inorganic particles or large organic molecules interpenetrated by a liquid--in which the structural coherent matrix contains a high portion of liquid, usually water.

  Eg:, Gesicain (Lidocaine)

Emulsion

- A dosage form consisting of a two-phase system comprised of at least two immiscible liquids, one of which is dispersed as droplets (internal or dispersed phase) within the other liquid (external or continuous phase), generally stabilized with one or more emulsifying agents

  Eg: Cremaffin (Milk of magnesia& liquid paraffin)

ROUTES OF DRUG ADMINISTRATION

Routes of Drug Administration (Enteral, Parenteral, Topical)

Enteral Routes:

- Placement of drug directly into any part of the GIT is called an 'enteral' mode of administration

Oral: Swallowing a drug through mouth

Advantages:

- Most commonly used method as it is safe, convenient & painless procedure
- Economical as sterilization of drug products is not essential
- No need of any assistant

Disadvantages:

- Onset of action is slower
- Polar drugs can't be given as they are not absorbed (eg: Streptomycin)
- Drugs are destroyed by the digestive juices (Eg: Penicillin-G, Insulin, Oxytocin)
- 1st pass effect (those destroyed in liver before reaching systemic circulation) (eg: Morphine, Isoprenaline)
- Bad test & Bad smell & irritant drugs can't be given
- Drugs can't be given to unconscious & uncooperative patients
- Drugs can't be given during emesis

Sublingual / Buccal

The drug is place beneath the tongue (sublingual) or crushed in mouth and spread over the buccal mucosa (Buccal).

Advantages:

- Quick onset of action because of rapid absorption due to more blood supply in that region
- Bypasses the portal circulation no 1st pass metabolism
- Drug action can be terminated at any time when side effects are observed

Disadvantages:

- Distasteful, irritant drugs can't be given
- Higher molecular weight drugs can't be absorbed (eg; insulin)

Examples

Isosorbide dinitrate tablets & Nitroglycerin tablets (for Angina), Isopranline sulfate tablets (for Bronchial Asthma), Nifedipine in powder form (in Hypertension)

Rectal: Through Rectum (Suppositories, Enema)

Advantages:

- Useful in patients with nausea and vomiting
- 1st pass metabolism is greatly bypassed as a major portion of the drg is absorbed from external haemorrhoidal veins
- Useful for gastric irritant drugs

Disadvantages:

- Chances of rectal inflammation
- Absorption is irregular
- Inconvenient and embarrassing to the patient

Examples

Dulcolax & Glycerine suppositories, enemas, ointments for Local action

Aminophylline (Bronchodillator) & Indomethacin (Anti-inflammatory agent) Suppositories for Systemic action

Parental Routes:

- Routes other than "Enteral" are called 'Parenteral' routes of administration. Administration of drugs by injection, by topical application to skin or by inhalation through the lungs are all parenteral.

Intravenous: Through lumen of the veins

Advantages:

- Directly enters into the systemic circulation & no 1st pass effect & quicker onset of action
- Less dose is needed to achieve greater therapeutic effects
- Valuable in emergency
- Can be given evening unconscious, uncooperative patients those are having nausea, vomiting & diarrhea
- Hypertonic solutions & GIT irritant drugs can be infused
- Large volume of fluids can be infused at a uniform rate
- Amount of the drug can be controlled with an accuracy

Disadvantages:

- Strict aseptic conditions are needed
- Patient has to depend upon other person for administration of drug
- Painful
- Risky because once the is injected it can't be recalled
- Introduction of any air or particulate matter produce embolism which is fatal
- Drugs in suspensions & Oily drugs can't be given
- Depot injections can's be given
- Venous thrombosis & Thrombophlebitis of the vein injected
- Necrosis around the site of action

Examples: Glucose, Glucose normal saline, Dopamine & Norepinephrine drips

Intramuscular

Deltoid muscle or gluteal mass of left or right buttock

Vastus muscle underlying the lateral surface of the thigh

Advantages:

Absorption is more predictable, less variable & rapid compared to Oral route

Depot injections can be given

Disadvantages:

- Perfect aseptic conditions are needed
- Chances of abscess at the site of injection
- Chances of nerve damage leading to paresis of muscle supplied by it
- Large volumes can't be given (maximum 5 – 10 ml)

Examples: Depot injection of Testosterone, Antibiotics, Antiemetics

Intraperitoneal

1. Into the peritoneal space
2. Rapid absorption due to large surface area
3. Painful, risky
4. Antirabies injection can be given
5. Intrathecal (Intraspinal)
6. Into the subarachnoid space
7. They crosses BBB & Blood CSF barrier
8. Strict aseptic conditions & grater expertise is needed
9. Its painful & risky procedure
10. Many radiopaque contrast media for myelography (to visualize spinal cord) are given through this route
11. Xylocaine injection for providing Spinal Anesthesia

Intramedullary:

Injection into the tibial or sternal bone marrow

Intra-arterial: into the lumen of the desired artery

Intra-articular: injection directly into the joint space

Subcutaneous: Injection into the subcutaneous tissue under the skin

Inhalation: Inspiration through nose or mouth

Topical Routes

Transdermal

Transdermal Patches

- In these adhesive patches, the drug is incorporated into a polymer (usually Polyisobutylene) which in turn is bonded to an adhesive plaster
- The drug is delivered at the skin surface by diffusion, for percutaneous absorption into circulation
- These preparations are designed to provide steady & smooth plasma concentration of the drug for a period ranging from 1-3 days from the site of their application
- Site of application: Chest, Abdomen, Upper arm or Mastoid region
  Examples: Transdermal Patches of Nitroglycerine, Scopalamine, Clonidine, Estradiol

Conjuctival: into the conjuctiva for local effects eg: Sulfacetamide

Vaginal and Urethral: Pessaries are used for local actions

Inunction (Rubbing): rubbing onto the skin

Newer Drug Delivery Systems:

- To improve drug delivery and to prolong its duration of action, special drug delivery systems have recently been developed.
- These include: Ocuserts, Progestaserts, Transdermal Adhesive Patches, Prodrugs, Computerised Miniature Pumps, Use of Monoclonal Antibodies and Liposomes as drug carriers.

Sources of Drugs

Drugs are obtained from six major sources:

- Plant sources
- Animal sources
- Mineral/ Earth sources
- Microbiological sources
- Semi synthetic sources/ Synthetic sources
- Recombinant DNA technology

Plant Sources:

Plant source is the oldest source of drugs. Most of the drugs in ancient times were derived from plants. Almost all parts of the plants are used i.e. leaves, stem, bark, fruits and roots.

Leaves:

a. The leaves of Digitalis Purpurea are the source of Digitoxin and Digoxin, which are cardiac glycosides.

b. Leaves of Eucalyptus give oil of Eucalyptus, which is important component of cough syrup.

c. Tobacco leaves give nicotine.

d. Atropa belladonna gives atropine.

Flowers:

Poppy papaver somniferum gives morphine (opoid)

Vinca rosea gives vincristine and vinblastine

Rose gives rose water used as tonic.

Photo of Papaver somniferum by Evelyn Simak

Fruits:

Senna pod gives anthracine, which is a purgative (used in constipation)

Calabar beans give physostigmine, which is cholinomimetic agent.

Seeds:

Seeds of Nux Vomica give strychnine, which is a CNS stimulant.

Castor oil seeds give castor oil.

Calabar beans give Physostigmine, which is a cholinomimetic drug.

Roots:

Ipecacuanha root gives Emetine, used to induce vomiting as in accidental poisoning. It also has amoebicidal properties.

Rauwolfia serpentina gives reserpine, a hypotensive agent.

Reserpine was used for hypertension treatment.

Bark:

Cinchona bark gives quinine and quinidine, which are antimalarial drugs. Quinidine also has antiarrythmic properties.

Atropa belladonna gives atropine, which is anticholinergic.

Hyoscyamus Niger gives Hyosine, which is also anticholinergic.

Stem:

Chondrodendron tomentosum gives tuboqurarine, which is skeletal muscle relaxant used in general anesthesia.

2. Animal Sources:

Pancreas is a source of Insulin, used in treatment of Diabetes.

Urine of pregnant women gives human chorionic gonadotropin (hCG) used for the treatment of infertility.

Sheep thyroid is a source of thyroxin, used in hypertension.

Cod liver is used as a source of vitamin A and D.

Anterior pituitary is a source of pituitary gonadotropins, used in treatment of infertility.

Blood of animals is used in preparation of vaccines.

Stomach tissue contains pepsin and trypsin, which are digestive juices used in treatment of peptic diseases in the past. Nowadays better drugs have replaced them.

3. Mineral Sources:

i. Metallic and Non metallic sources:

Iron is used in treatment of iron deficiency anemia.

Mercurial salts are used in Syphilis.

Zinc is used as zinc supplement. Zinc oxide paste is used in wounds and in eczema.

Iodine is antiseptic. Iodine supplements are also used.

Gold salts are used in the treatment of rheumatoid arthritis.

ii. Miscellaneous Sources:

Fluorine has antiseptic properties.

Borax has antiseptic properties as well.

Selenium as selenium sulphide is used in anti dandruff shampoos.

Petroleum is used in preparation of liquid paraffin.

4. Synthetic/ Semi synthetic Sources:

i. Synthetic Sources:

When the nucleus of the drug from natural source as well as its chemical structure is altered, we call it synthetic.

Examples include Emetine Bismuth Iodide

ii. Semi Synthetic Source:

When the nucleus of drug obtained from natural source is retained but the chemical structure is altered, we call it semi-synthetic.

Examples include Apomorphine, Diacetyl morphine, Ethinyl Estradiol, Homatropine, Ampicillin and Methyl testosterone.

Most of the drugs used nowadays (such as antianxiety drugs, anti convulsants) are synthetic forms.

5. Microbiological Sources:

Penicillium notatum is a fungus which gives penicillin.

Actinobacteria give Streptomycin.

Aminoglycosides such as gentamicin and tobramycin are obtained from streptomycis and micromonosporas.

6. Recombinant DNA technology:

Recombinant DNA technology involves cleavage of DNA by enzyme restriction endonucleases. The desired gene is coupled to rapidly replicating DNA (viral, bacterial or plasmid). The new genetic combination is inserted into the bacterial cultures which allow production of vast amount of genetic material.

Advantages:

Huge amounts of drugs can be produced.

Drug can be obtained in pure form.

It is less antigenic.

Disadvantages:

Well equipped lab is required.

Highly trained staff is required.

It is a complex and complicated technique.

Drug – Absorption, Distribution, Metabolism and Excretion

The four processes involved when a drug is taken are absorption, distribution, metabolism and elimination or excretion (ADME).

Pharmacokinetics is the way the body acts on the drug once it is administered. It is the measure of the rate (kinetics) of absorption, distribution, metabolism and excretion (ADME). All the four processes involve drug movement across the membranes. To be able to cross the membranes it is necessary that the drugs should be able dissolve directly into the lipid bilayer of the membrane; hence lipid soluble drugs cross directly whereas drugs that are polar do not.

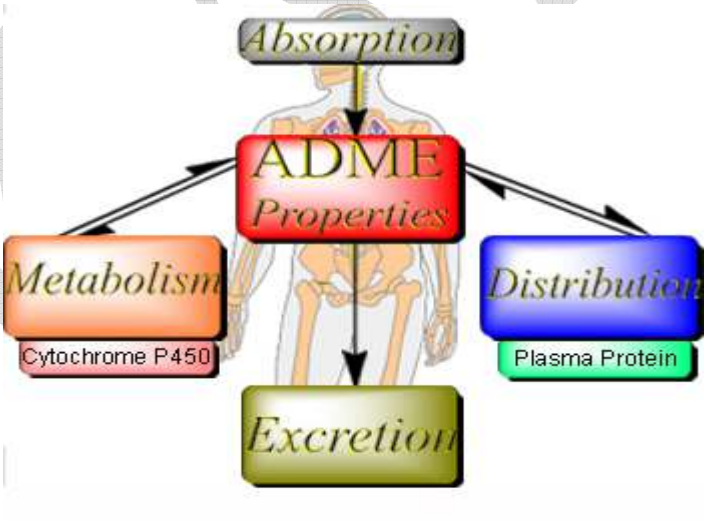


Figure showing the interplay between absorption, distribution, metabolism and excretion (ADME).

Absorption

- Absorption is the movement of a drug from its site of administration into the blood. Most drugs are absorbed by passive absorption but some drugs need carrier mediated transport. Small molecules diffuse more rapidly than large molecules.
- Lipid soluble non – ionized drugs are absorbed faster. Absorption is affected by blood flow, pain stress etc.Acidic drugs such as asprin will be better absorbed in the stomach whereas basic drug ike morphine will be absorbed better in the intestine.
- Most of the absorption of the drug takes place in the small intestine. Since the surface area of the stomach is much smaller than that of the intestine. Most of the drugs are absorbed in the small intestine since the amount of time that the drugs spend in the stomach is less and also the surface area of the stomach is small.
- If a basic drug is taken after a meal then the activity of the drug can be reduced whereas if an acidic drug is taken after a meal then the action of the can be noticed much more quickly, owing to the gastric absorption.
- For a drug even though lipophilic to be absorbed in the intestine some portion of it needs to be dissolved in the intestinal juices which are aqueous. There are some substances that are partly soluble in water and it is these that will be absorbed and then an equivalent amount will be absorbed from the undissolved portion. Thus complete absorption will take place.
- There are bile salts present in the intestine which will aid in salvation of the drug and their resultant absorption. Drugs that are amphipathic have no problem in getting absorbed. There are some drugs that are completely insoluble in water such drugs float as globules in the intestine but the bile salts will emulsify these into small enough particles such that absorption can take place. E.g. vitamins.
- Some of the drugs are similar to compounds found in the body for e.g. thyroxine and such drugs can be absorbed into the system by active transport.
- When drugs are injected into the muscle, subcutaneous layer absorption still has to take place but it is less dependent on the chemical nature of the drugs since the drugs are absorbed into the circulatory system through the small pores in the capillary walls.

Distribution

- Distribution is the movement of drugs throughout the body. Determined by the blood flow to the tissues, it is ability of the drug to enter the vasculature system and the ability of the drug to enter the cell if required.

Plasma Protein Binding

- The blood stream has the ability to transport relatively insoluble substances. These substances are transferred by binding to the proteins which have a very amphipathic structure.
- The hydrophilic group renders the protein soluble in water and the lipophilic compounds are attracted to the lipophilic group and are loosely bound to the protein molecule hence protein bound.
- Most of the drugs travel in the plasma are partly in solution and partly bound to the plasma protein. The bound drug is inactive and the unbound drug is active. The ratio of bound to the unbound drug varies. Binding is reversible.
- Generally acidic drugs bind to albumin and basic drugs to α1 – acid glycoprotein. Diseased state can cause a problem on the effectiveness of the drug. As globin levels increase with age this factor should be taken into account when treating an elderly person with a basic drug.
- The protein bound drug is in equilibrium with the free drug. That means that once the free drug enters the target tissue then the protein bound drug will be released to maintain equilibrium.
- If two drugs bind at the same site of the protein and are administered together then there can be problems. e.g. Warfarin and asprin.. asprin displaces warfarin from its bound protein as a result of which there are elevated levels of warfarin in the unbound state and this can lead to warfarin toxicity.

Tissue Distribution

- After absorption most drugs are distributed in the blood to the body tissue where they have their effect. The degree to which the drug is likely to accumulate in the tissue is dependent on the lipophilicity and local blood flow to the tissue. Highly perfused organs receive most of the drugs.

The role of the liver in drug distribution

- After the drug is absorbed by the GI tract, it is taken up by the part of the bloodstream called the hepatic portal system.
- Most of the drugs are absorbed into this system except for the lipids which are absorbed into the lymphatic system and then delivered into the blood by the thoracic duct into the superior vena cava.
- The hepatic portal system is designed to take digested foodstuff into the liver where it can be processed, in some cases it is stored before being distributed and it is possible that this may happen to the drug and the drug would be metabolized before reaching the rest of the body. Such drugs that metabolized by the liver are said to have a high hepatic first pass. Hence drugs with a very high hepatic first pass cannot be given orally.

The Blood–Brain Barrier (BBB)

- The capillaries in the CNS are different they have pores which are sealed by the connective tissue and hence only small molecules can cross the blood brain barrier and the substances that can cross over have to be very lipophilic in nature.
- The blood-brain barrier (BBB) is the protective mechanism of the CNS and is not present everywhere in the brain. This is sometimes useful as it avoids some drugs from crossing the CNS and causing deleterious effects. E.g. neuromuscular blocking agents. Sometimes the blood brain barrier allows the transport of drugs resulting in unwanted effect for e.g. antihistamines cross the bbb and result in drowsiness, now there are antihistamines that are made that are not so lipophilic in nature.

Metabolism or Biotransformation

- It is the process of transformation of a drug within the body to make it more hydrophilic so that it can be excreted out from the body by the kidneys. This needs to be done since drugs and chemicals are foreign substances in our body.
- If the drug continues to be in the lipohilic state and is going to be filtered by the glomerulus then it will be reabsorbed and remain in the body for prolonged periods. Hence metabolism deals with making the drug more hydrophilic such that it can be excreted out from the body. In some cases the metabolites can be more active than the drug itself e.g. anxiolytic benzodiazepines.

- Some enzymes are highly specific and will breakdown only compounds that they recognize for e.g. glucose dehydrogenase.
- But there are some enzymes such as pepsin which are not specific and will breakdown most soluble proteins into smaller polypeptides or amino acids. This enzyme and many other proteolytic enzymes attack the peptide bond that joins the amino acids to make proteins, and in this way break the protein down.

Two types of enzymes are involved in metabolism:

Phase I Metabolism

These enzymes modify the drug chemically by processes such as oxidation, reduction and hydrolysis or by the removal and addition of an active group.

Phase II Metabolism

These include the conjugation of a drug or a phase I metabolite with a polar group to render it possible for excretion. e.g. sulphates and glucuronide

The deconjugation of the drug by bacterial enzymes is called the enterohepatic cycle. Sometimes this deconjugation can lead to increased levels of drugs in the body. But some times due to treatment with antibiotics there may be less or no deconjugation as a result of which there will be less drug in the body.

Principal sites of metabolism are Liver and Kidney and once the drug is rendered hydrophilic they can be easily excreted out by the bile and urine without significant reabsorption.

Enzyme Induction

There are some drugs that can lead to an increase in the production of the enzyme and as a result speed up the metabolism of the drug and hence a higher dose of the drug is required to achieve the therapeutic effect.

Enzyme Inhibition

Some drugs result in the inhibition of certain enzymes and as a result there is an accumulation of the drug in the body and can lead to drug toxicity. This is also a form of drug – drug interaction.

Excretion

Excretion is the removal of the substance from the body. Some drugs are either excreted out unchanged or some are excreted out as metabolites in urine or bile. Drugs may also leave the body by natural routes such as tears, sweat, breath and saliva. Patients with kidney or liver problem can have elevated levels of drug in the system and it may be necessary to monitor the dose of the drug appropriately since a high dose in the blood can lead to drug toxicity.

.**Drug Receptor Interactions**

- Receptors are typically glycoproteins located in cell membranes that specifically recognize and bind to ligands. These are smaller molecules (including drugs) that are capable of 'ligating' themselves to the receptor protein. This binding initiates a conformational change in the receptor protein leading to a series of biochemical reactions inside the cell (‘signal transduction’), often involving the generation of ‘secondary messengers’ that is eventually translated into a biological response (e.g. muscle contraction, hormone secretion).
- Although the ligands of interest to prescribers are exogenous compounds (i.e. drugs), receptors in human tissues have evolved to bind endogenous ligands such as neurotransmitters, hormones, and growth factors. Formation of the drug-receptor complex is usually reversible and the proportion of receptors occupied (and thus the response) is directly related to the concentration of the drug.
- Reversibility enables biological responses to be modulated and means that similar ligands may compete for access to the receptor. The term 'receptor' is usually restricted to describing proteins whose only function is to bind a ligand, but it is sometimes used more widely in pharmacology to include other kinds of drug target such as voltage-sensitive ion channels, enzymes and transporter proteins.

Main types of drug targets and their mechanisms of action		
Drug Target	**Description**	**Example(s)**
Receptors		
Channel-linked receptors	Coupled directly to an ion channel. Activation opens the channel, making a cell membrane permeable to specific ions. These channels are known as 'ligand-gated' because it is receptor binding that operates them (in contrast to 'voltage-gated' channels that respond to changes in membrane potential) (see B in figure).	Nicotinic acetylcholine receptors; gamma-Aminobutyric acid (GABA) receptors
G-Protein coupled receptors	Coupled to intracellular effector mechanisms via a family of closely related 'G-proteins' that participate in signal transduction by coupling receptor binding to intracellular enzyme activation or the opening of an ion channel. Secondary messenger systems include the enzymes, adenylyl cyclase and guanylyl cyclase, which generate cyclic AMP and cyclic GMP, respectively (see A in figure).	Muscarinic acetylcholine receptors; beta-Adrenoceptors; Dopamine receptors; 5-hydroxytryptamine (Serotonin) receptors; Opioid receptors
Kinase-linked receptors	Linked directly to an intracellular protein kinase that triggers a cascade of phosphorylation reactions.	Insulin receptors
Nuclear hormone receptors	Intracellular and also known as 'nuclear receptors'. Binding of a ligand promotes or inhibits synthesis of new proteins, which may take hours or days to promote a biological effect.	Steroid hormone receptors; Thyroid hormone receptors; Vitamin D receptors
Other targets		
Voltage-sensitive ion channels	Found in excitable tissues and a potential target for drugs that can block the channel or interfere with conductance in other ways.	Na+ channels that are blocked by local anesthetics such as lidocaine
Enzymes	Catalyze biochemical reactions, some of which involve the production of key mediators of physiological processes in body systems. Drugs interfere with the active site of the enzyme or affect co-factors required by the enzyme for activity. In most cases inhibition of the active site is competitive although in some cases it may be long-lasting and effectively irreversible (e.g. aspirin) (see C in figure)	Inhibitors of cyclooxygenase such as aspirin; Inhibitors of angiotensin converting enzyme such as enalapril; Inhibitors of xanthine oxidase such as allopurinol
Transporter	Specialized proteins that carry ions or molecules	Inhibitors of serotonin

proteins	across cell membranes. Movement may be in either direction, and may involve exchange of one substance for another, co-transport of two or more substances in the same direction, or 'pumping' of a single substance into or out of a cell or organelle. Drugs may act on transporters to inhibit their activity or may also act as 'false substrates', preventing the transport of the normal biological substrate (see D in figure).	reuptake transporter such as fluoxetine
Cell adhesion proteins	Type-1 membrane glycoproteins that mediate cell-cell and cell-matrix adhesion by acting as transmembrane linkers to connect ligands on the outside of the cell (other cell membrane molecules, ECM components) to the actin cytoskeleton. Includes the adherins and integrins.	β_2 **integrins** on leukocytes which are essential for effective immune responses. **Adhesion class GPCRs**. Cadherins such as E-cadherin required for endothelial cell-cell contact, and tissue morphogenesis during embryonic development.

Prodrug concept

- A prodrug is a medication or compound that, after administration, is metabolized (i.e., converted within the body) into a pharmacologically active drug.
- Inactive prodrugs are pharmacologically inactive medications that are metabolized into an active form within the body. Instead of administering a drug directly, a corresponding prodrug might be used instead to improve how a medicine is absorbed, distributed, metabolized, and excreted (ADME). Prodrugs are often designed to improve bioavailability when a drug itself is poorly absorbed from the gastrointestinal tract.
- A prodrug may be used to improve how selectively the drug interacts with cells or processes that are not its intended target. This reduces adverse or unintended effects of a drug, especially important in treatments like chemotherapy, which can have severe unintended and undesirable side effects.

- Prodrugs can be classified into two major types, based on how the body converts the prodrug into the final active drug form:
- Type I prodrugs are bioactivated inside the cells (intracellularly). Examples of these are anti-viral nucleoside analogs that must be phosphorylated and the lipid-lowering statins.
- Type II prodrugs are bioactivated outside cells (extracellularly), especially in digestive fluids or in the body's circulatory system, particularly in the blood. Examples of Type II prodrugs are salicin (described above) and certain antibody-, gene- or virus-directed enzyme prodrugs used in chemotherapy or immunotherapy.
- Both major types can be further categorized into subtypes, based on factors such as (Type 1) whether the intracellular bioactivation location is also the site of therapeutic action, or (Type 2) whether or not bioactivation occurs in the gastrointestinal fluids or in the circulation system.

UNIT-1

SYLLABUS

Drug design and development screen up; target identification validation. Drug discovery and drug deliver Drug abuse and self poisoning Pharmacogenomics and biochip

Target Identification & Validation for Early Drug Discovery

Early stages of drug discovery start with initial steps of target identification and moves to the later stages of lead optimization. Multiple sources including academic research, clinical works and commercial sector help in the identification of a suitable disease target. The chosen target is then used by the pharmaceutical industry and more recently by some academic centers to identify molecules for making acceptable drugs. The process involves various early steps.

1. Target Identification & Validation

Target Identification & Characterization

Target identification and characterization begins with identifying the function of a possible therapeutic target (gene/protein) and its role in the disease. Identification of the target is followed by characterization of the molecular mechanisms addressed by the target. A good target should be efficacious, safe, meet clinical and commercial requirements and be 'druggable'.

Approaches:

Data mining using bioinformatics

— identifying, selecting and prioritizing potential disease targets

Genetic association

— genetic polymorphism and connection with the disease

Expression profile

— changes in mRNA/protein levels

Pathway and phenotypic analysis

— In vitro cell-based mechanistic studies

Functional screening

— knockdown, knockout or using target specific tools

Tools for Target Identification & Validation

- Disease association (genetics and expression changes)
- Bioactive molecules
- Cell based models
- Protein interactions (pull-down assays, yeast 2 hybrid)
- Analysis of signaling pathways
- Functional analysis (overexpression, transgenics, antisense RNA, gene variants)

Target Validation

Target Validation shows that a molecular target is directly involved in a disease process, and that modulation of the target is likely to have a therapeutic effect. The most important criteria for target validation is to take multi-validation approach.

Approaches:

Genetic manipulation of target genes (in vitro)

— knocking down the gene (shRNA, siRNA, miRNA), knocking out the gene (CRISPR, ZFNs), knocking in the gene (viral transfection of mutant genes)

Antibodies

— interacting to the target with high affinity and blocking further interactions

Chemical genomics

— chemical approaches against genome encoding protein

Development and evaluation of new drugs:

The ultimate aim of pharmacological studies in animals is to find out a therapeutic agent suitable for clinical evaluation in man. No doubt, animal studies provide analogies and serve as useful models. The administration of biologically active agent to human beings is associated with an element of risk, which cannot be predicted by even the most careful and exhaustive animal experiments. Scientists all over the world are in a continuous effort to develop new drugs although drug development is an extremely technical and enormously expensive operation. Among the contributors to new drug development, pharmacologists are more concerned in evaluating “new chemical entities” (NCE). Synthesis and evaluation of thousands of NCEs are usually necessary for new drugs to be introduced in the market. Research and development of

new drugs have been done under strict government regulations which have greatly increased over the past couple of decades.

Drug development comprises of two steps.

a) Preclinical development and

b) Clinical development

A) Preclinical development: Synthesis of new chemical entities is done as per research policy decision which is based on:

(i) Random synthesis

(ii) Structure activity relationship (SAR)

(iii) Biochemical and pharmacological insight and

(iv) Chance finding.

The aim of the preclinical development phase for a potential new medicine is to explore the drug's efficacy and safety before it is administrated to patients. In this preclinical phase, varying drug doses are tested on animals and/or in vitro systems.

If active compounds are found, then studies on animals are done which include pharmacodynamics, pharmacokinetics, toxicology and special toxicological studies (mutagenicity and carcinogenicity) have to be done. In this study single dose is used for acute toxicity and repeated doses for sub chronic and chronic toxicity studies. Most of the preclinical tests have to be conducted in accordance with the standards prescribed.

B) Clinical development: About one in 1000 NCEs reach this stage. The steps to be studied in this stage include:

a) Pharmaceutical study

b) Pharmacological study

c) Clinical trial.

a) Pharmaceutical study covers stability of formulation and compatibility of the NCEs with other tablet or infusion ingredients.

b) Pharmacological study includes further chronic toxicological study in animal, initially animal metabolic and pharmacokinetic study. When studies in animals predict that a NCE may be useful medicine i.e. effective and safe in relation to its benefits, then the time has come to put it to the test in man i.e. clinical trial.

c) Studies on human or Clinical Trial:

Clinical trial is a means by which the efficacy of drug is tested on human being. It may also give some idea about the risk involved. It is divided into 4 phases. With each phase, the safety and efficacy of the compound are tested progressively.

Phase - I: This is the first exposure of the new drug on man which is usually conducted in healthy volunteers and which is designed to test the tolerable dose, duration of action. This phase is usually carried out in only one centre on 20 to 50 subjects.

Phase - II: This phase comprises small scale trials on patients used to determine dose level and establish that the treatment offers some benefit. It usually involves 100-500 patients and is usually conducted in several centres.

Phase - III: Full scale evaluation of treatment comparing it with standard treatment is done in this phase. It involves randomised control trials on 250 to 2000 patients and is done in multiple centres. Information from all studies are received by the "Committee of safety of medicines" (CSM). If the drug is satisfied by the CSM, the product license is issued then the drug is marketed.

Phase - IV: It is also called as phase of post marketing surveillance. Reports about efficacy and toxicity are received from the medical practitioners and reviewed by the committee of review of medicines. Renewal or cancellation of the product license depends on the comment of the review committee.

Drug Abuse

Drug addiction is a chronic, relapsing brain disease that is characterized by compulsive drug seeking and use, despite harmful consequences (National Institute on Drug Abuse, 2014). Drug addiction is associated with impairment in various aspects of physical, psychological and socio-occupational functioning. Drug addiction is a growing problem in India and the world. The global problem of addiction and drug abuse is responsible for millions of deaths and HIV cases.

The use of the term "Addiction" has now been dropped from the scientific literature because of its derogatory connotation and instead the use of "Substance use disorder" is preferred. Drugs are any chemical (psychoactive) substances that affect physical, mental, emotional or behavioral states of an individual. Drug abuse, a form of substance use disorder is a patterned use of a drug in which the user consumes the substance (drugs) in amount or with method which are harmful.

The pattern of substance use and substance use related syndrome can be described as following -

- Substance intoxication is a reversible, substance-specific syndrome due to the recent ingestion of a substance of abuse. Signs of intoxication often include confusion, impaired judgment, inattention, and impaired motor and spatial skills.
- Tolerance is a state of physical habituation to a drug, resulting from frequent use, such that higher doses are needed to achieve the same effect. The person can increase the amount of drug use to the point that can be lethal for non drug users.
- Dependence is a set of physiological, behavioural, and cognitive symptoms. For an individual when drug use takes much priority over other behaviours that previously had greater value, the person can be called dependent on the drug. Dependence syndrome is characterized by a strong desire or sense of compulsion to take drug, difficulty in controlling drug use behaviour, withdrawal, tolerance, neglect of alternative pleasures and persistent use of drug despite clear evidence of harmful consequences of drug.
- Withdrawal syndrome (also called an abstinence syndrome) is a cluster of symptoms that occur when a dependent person abruptly stops using a particular substance following heavy, prolonged use. Some common withdrawal symptoms include anxiety, restlessness and body aches while some withdrawal symptoms are drug specific. Thus, withdrawal symptom varies from one drug to another

Types of drugs

Drugs can broadly be classified into Depressants, Narcotics, Stimulants and Hallucinogens Depressants (Downers)

Depressants, also known as sedatives and tranquilizers, are substances that can slow brain activity. These include alcohol, hypnotics to induce sleep, anxiolytic to reduce anxiety, sedatives for relaxation and anticonvulsants such as barbiturates. Alcohol is the most commonly used depressant. Officially, Indians are still among the world's lowest consumers of alcohol—only 21% of men and around 2% of women drink. But up to a fifth of this group amounting to about 14 million people—are dependent drinkers requiring "help". The percentage of the drinking population aged under 21 years has increased from 2% to more than 14% in the past 15 years, according to studies in Kerala by Alcohol and Drugs Information Centre India (NGO).

Alarmingly, the study found that the "average age of initiation" had dropped from 19 years to 13 years in the past two decades.

Barbiturates

Barbiturates such as amobarbital, pentobarbital, phenobarbital, and secobarbital are depressants, or sedatives. These drugs have several medical uses, including easing anxiety and tension, dulling pain, and treating epilepsy and high blood pressure. At the highest risk for prescription drug abuse are anesthesiologists, emergency medicine physicians, family practitioners, psychiatrists and nurses. The ease of access and frequency of exposure to prescription drugs is one factor that increases the probability of these professionals to abuse these drugs. Other factors that contribute to the abuse of prescription drugs include stress, anxiety and depression, often associated with the long working hours and high stress levels of healthcare jobs.

Narcotics

Narcotics or opioids are drugs that are used medically for pain relief but that have strong addictive potential. Opioids produce a rush, or intense feelings of pleasure, which is the primary reason for their popularity as street drugs. They also dull awareness of one's personal problems, which is attractive to people seeking a mental escape from stress. Their pleasurable effects derive from their ability to directly stimulate the brain's pleasure circuits— the same brain networks responsible for feelings of sexual pleasure or pleasure from eating a satisfying meal.

Stimulants (Uppers)

Stimulants act on the central nervous system to increase energy and alertness while suppressing appetite and fatigue. They include cocaine (such as freebase and 'crack'), amphetamines (for example Dexedrine, Benzedrine), methamphetamine (methedrine: 'speed', 'crystal', 'ice', 'crank'), MDMA (ecstasy), nicotine, caffeine and amphetamine like products (preludin or Ritalin.) Some of these are discussed below. Continued use of some stimulants can result in changes in how the brain operates and an inability to experience pleasure naturally. For example, chronic use of amphetamines (and cocaine) may result in the temporary loss of approximately 20% of dopamine receptors in the nucleus accumbens, at least for 4 months since the last exposure.

Amphetamines

Amphetamine (contracted from alpha-methylphenethylamine) is a central nervous system (CNS) stimulant. Amphetamines are used in high doses for their euphoric rush. They are often taken in pill form or smoked in a relatively pure form called "ice" or "crystal meth"Amphetamines are also used for therapeutic purposes e.g., for the treatment of attention deficit and hyperactivity disorder (ADHD), narcolepsy, and obesity. At therapeutic doses, it induces physical effects such as decreased reaction time, fatigue resistance, and increased muscle strength. Larger doses of amphetamine may impair cognitive function and induce rapid muscle breakdown.

Ecstasy

The drug ecstasy, or MDMA (3,4-methylenedioxymethamphetamine) is a designer drug, similar in chemical structure to amphetamine. It produces mild euphoria and hallucinations and has become especially popular on college campuses and in clubs and "raves" in many cities.

Cocaine

Cocaine is a natural stimulant extracted from the leaves of the coca plant. Cocaine is usually snorted in powder form or smoked in the form of crack. In 2008, 5.3 million Indians age 12 and older had abused cocaine in any form and 1.1 million had abused crack at least once in the year prior to being surveyed. (NIDA, 2008).

Nicotine

Nicotine is found in tobacco products including cigarettes, cigars, and smokeless tobacco. Tobacco is used by smoking, chewing, sucking and applying to the teeth and gums etc. In India, there is a wide availability of smoking (e.g., bidi, cigarette, hookah etc) and smokeless tobacco (e.g., gutkha, khaini, zarda). The WHO estimates that 1 billion people worldwide smoke and more than 3 million die each year from smoking-related causes. Jha et al (2008) have estimated that around 1 million deaths a year in India will be attributable to smoking by the early 2010s. India's tobacco problem is very complex, with a large use of a variety of smoking forms and an array of smokeless tobacco products. Many of these products are manufactured as cottage and small-scale industries using varying mixtures and widely differing processes of manufacturing. Highest prevalence of stimulant injection was observed in the state of Goa, considered as the 'party capital' of India. Though there are no reports in the scientific literature, there have been

reports in the popular press suggesting that Goa has become a principal hub of drug trade and consumption in India, potentially due to its relatively unprotected coastline.

Hallucinogens

Hallucinogens, also called psychedelics, are a class of drugs that produce sensory distortionsor hallucinations, including major alterations in color perception and hearing. Hallucinogens may also have additional effects, such as relaxation and euphoria or, in some cases, panic. Hallucinogens include lysergic acid diethylamide (LSD), psilocybin, and mescaline. PCP, Marijuana, PCP, LSD are the most commonly used hallucinogens.

Marijuana/Cannabis

Marijuana is derived from the Cannabis sativa plant. It is generally classified as a hallucinogen because it can produce perceptual distortions or mild hallucinations. It is also known by various street names such as bhaang, gaanja, charas, hashish, pot, weed. Cannabis can produce anxiety, paranoia, and a sense of derealisation. It is the most prevalent illegal drug used around the world. About 40% of the U.S. population age 12 or older has tried cannabis at least once, and about 10% has tried it within the last year.

PCP (Phencyclidine)

Phencyclidine was developed as an anesthetic in the 1950s but was discontinued as such when its hallucinatory side effects were discovered. Use of this substance causes hallucinations, accelerates the heart rate and blood pressure and causes sweating, flushing, and numbness. PCP is classified as a deliriant—a drug capable of producing states of delirium. It also has dissociating effects, causing users to feel as if there is some sort of invisible barrier between themselves and their environments. It is also called “angel dust”. Its popularity has since waned, largely because of its unpredictable effects.

Effect of Drugs

As already mentioned, drugs act on the central nervous system (i.e., they affect the individual's neurological functioning). The physiological correlates and effects vary according to each substance; there are specific mechanisms that involve precise receptors for each substance type. In this section we present some common aspects to the physiological effects of drugs.

When a substance enters the body it first affects the neuronal receptors, which are structures located within a neuron or in its membrane and are characterized by selective binding to a substance and the physiological effect that accompanies the union.

The presence of a drug in the body affects the presynapse, altering the production/ release of neurotransmitters. During the next step, the drug affects the synapses, by increasing the presence of neurotransmitters in the synaptic space. There can be a reuptake inhibition, blockade of reuptake channels, or inhibition of degradation.

The activity of the drug in the body over a period of time comprises the processes of absorption, distribution, localization in tissues, biotransformation and excretion.

Physical dependence emerges as the need to maintain certain levels of a substance in the body. Therefore, it involves the development of a drugorganism link and neuroadaptation process.

Central Nervous System Depressants

Alcohol

The two main types of alcohol based on their chemical composition are: methyl alcohol (methanol), which is the simplest of the alcohols and is used as a solvent, antifreeze and in industrial applications; and ethyl alcohol (ethanol), which is what alcoholic beverages contain. Ethyl alcohol is obtained through the fermentation of sugars from different plants. After the distillation process, the amount of alcohol can be concentrated and significantly increased. From a physical standpoint, the short term effects of alcoholic beverage intake are impaired balance, movement, speech and vision, hurried and unintelligible expression, decreased ability to concentrate, drowsiness, and despondency, irritability, nausea, vomiting and/or headaches.

Chronic alcohol consumption leads to serious physical and psychological problems.

a) Physical problems:

The mortality attributed to diseases brought about by alcohol abuse is estimated at between 20,000 and 25,000 cases per year. Some of the physical problems related to alcohol abuse occur in the short term. Others, however, such as coronary heart disease appear after ongoing use. Alcohol, consumed abusively, is harmful to all the body´s organs. Among the consequences at the physical level the following stand out:

− Alcoholic dependency syndrome or alcoholism.

− Digestive tract disorders (esophagus and stomach): gastritis,

gastrointestinal bleeding, varices in the esophagus, etc.

− Pancreas: pancreatitis, diabetes, etc.

− Liver disease: cirrhosis, hepatitis, etc.

− Cancers: considered the second leading cause of death among alcoholics.

The most common types are of the stomach, esophagus, liver and pancreas.

− Cardiovascular disorders: cardiac abnormalities such as arrhythmias or mitral valve insufficiency are associated with the prolonged use of alcohol.

− Malformations in the fetus: the use of any toxic substance, including alcohol, provokes alterations in fetal development, and may even cause an abortion. In the case of mothers who consume alcohol, a condition known as fetal alcohol syndrome appears. Children with this syndrome show morphological changes in the head, skeleton, heart and genitals, as well as mental retardation in approximately 50% of cases.

− Neurological disorders: various types of brain damage, such as Korsakoff's Syndrome, have been found to result from a history of alcoholism.

b) Psychological problems:

− Acute alcohol intoxication (drunkenness): although alcohol is a neurological depressant, at low doses it produces a generalized

Drug Poisoning

What is an overdose?

An overdose occurs when too much of a drug, medication or poison is taken, which may result in a toxic effect on the body. Many substances can cause harm when taken in excess including alcohol, illegal (and 'party') drugs, prescription and over-the-counter medication, and some herbal remedies.

Why do people overdose?

The are two main reasons people overdose.

• Accidental – a person takes the wrong substance (a drug or medication) or the wrong combination, in the wrong amount or at the wrong time, not knowing that it could cause them harm.

• Intentional misuse – a person takes an overdose to get 'high' or to cause deliberate harm. Any deliberate harm may be a cry for help or a suicide attempt. Intentional misuse of drugs may

indicate a serious mental health problem and help should be sought even if the overdose has not caused you harm.

What are the symptoms?

A wide variety of symptoms is possible. Symptoms will depend on the substance, the amount taken and your health. Some poisons only cause minor distress or harm while others can cause serious problems and possibly death.

Symptoms can include:

• nausea and vomiting

• burning in the throat or stomach (oesophagitis or gastritis) after drinking a corrosive substance

• dizziness

• high or low blood pressure

• fitting (seizures)

• drowsiness, confusion or coma (the person is unconscious)

• organ damage or failure (especially the liver or kidneys)

• breathing problems

• respiratory or cardiac arrest – when the person stops breathing or their heart stops beating/pumping blood around the body

• There may be no symptoms, or only minor symptoms, even when severe damage is occurring in some overdoses (for example: paracetamol), so always seek medical help.

Tests and treatment

The tests and treatment given depend on what the person took and their medical needs. There are a number of possible tests and treatments. Some treatments need to be given soon after an overdose to prevent serious harm. It is important to seek medical advice as soon as possible, even if there are no symptoms.

• Blood tests, ECG (heart rhythm trace).

• Intravenous fluids (into the vein) or medication – usually to improve low blood pressure.

• Observation in hospital and monitoring of the person's vital signs and heart rhythms (if necessary).

• Removing the substance from the body (such as using activated charcoal, which binds to the drug so the body cannot absorb it – this must be given within an hour of substance ingestion). Charcoal is rarely given and works only on certain types of poisons.

• An antidote may be given to reverse the effect of the toxic substance (for some drugs).

• Admission to hospital for further treatment.

If you are discharged home, you may need to follow up with your local doctor for more blood tests within a couple of days to make sure there is no delayed damage to the liver or kidneys. Your doctor will advise you if this is necessary.

What to expect

• Many overdoses do not cause serious harm or long–term damage and most people make a full recovery.

• Some overdoses can cause damage to organs such as the liver and kidneys, and may result in death.

• If the overdose was an attempt at self-harm or suicide, ongoing treatment and follow up will be arranged before the person is sent home.

Prevention

To avoid a drug overdose in future:

• avoid illegal drugs of any kind

• take prescription medications as directed

• tell a doctor or health care professional of any previous medication problems

• ask your GP for available support systems in your area

• keep all medications and poisons locked away in a safe secure place out of reach of children

• keep all medications in original packaging

• if you take a number of medications each day, a pharmacy webster pack may be helpful to avoid overdose.

Biochips

Biochip technology is highly effective method that allows monitoring of thousands of genes/alleles at a time in computerized automatic operations with minimal volumes of necessary reagents. Biochips promise an important shift in molecular biology, DNA diagnostics, and

pharmacology, research in carcinogenesis and other diseases and also the possibility of a better understanding of the world of biology in its globality.

A new revolution technology that may become promising for research, diagnostics and therapy enters into biology and medicine. Biochips containing microarrays of genetic information promise to be important research tools in the post genomic era (Jain, 2001).

The basic idea of the biochip technology is to convert the chemistry of life into a static form programmed to monitor genes, A new revolution technology that may become promising for research, diagnostics and therapy enters into biology and medicine.

Biochips containing microarrays of genetic information promise to be important research tools in the post genomic era (Jain, 2001). The basic idea of the biochip technology is to convert the chemistry of life into a static form programmed to monitor genes, proteins and relations between them.

Biochip programmed by known sequences of DNA/RNA or proteins can recognized the real genes, mutations and levels of expression. Biochips are collections of miniaturized test sites (microarrays) arranged on a solid substrate onto which a large number of biomolecules are attached with high density. The word "biochip" derives from the computer term "chip". Although silicon surfaces bearing printed circuits can be used for DNA binding, the term biochip is now broadly used to describe all surfaces bearing microscopic spots, each one being formed by specific capture probes. The capture probes are chosen to complement the target sequence to be detected. Each capture probe will bind to its corresponding target sequence. Like a computer chip performing millions of mathematical operations in a few split seconds, a biochip allows for simultaneous analyses of thousands of biological reactions, such as decoding genes, in a few seconds. Biochip technologies can be applied to numerous fields including genomic, proteomic, and glycomic research, as well as pharmacology and toxicology. However, one of the most common applications is in the determination of gene expression in human cells and tissues. Global gene expression analysis has helped to identify important genes and signalling pathways in human malignant tumours.

Biochips are formed by in situ (on chip) synthesis of oligonucleotides (Braun et al., 2005) or peptide nucleic acids (PNAs) or spotting of DNA fragments. Hybridisation of RNA- or DNA-derived samples on chips allows the monitoring of expression of mRNAs or the occurrence of

polymorphisms in genomic DNA. Basic types of DNA chips are the sequencing chip, the expression chip and chips for comparative genomic hybridisation. Like a computer chip performing millions of mathematical operations in a few split seconds, a biochip allows for simultaneous analyses of thousands of biological reactions, such as decoding genes, in a few seconds. Advanced technologies used in automated microarray production are photolithography, mechanical microspotting and ink jets.

Bioelectronic microchips contain numerous electronically active microelectrodes with specific DNA capture probes linked to the electrodes through molecular wires. Several biosensors have been used in combination with biochips.

The purpose of the chips is to detect many genes present in a sample in one assay rather than performing individual gene assays as is the practice e.g. in so-called multiwells, plates with 96 wells, where the reactions take place. The huge amount of information coming from the genome sequence and other research genome programs cannot be utilised to the full without the availability of methods such as biochips which enable these genes or specific DNA sequences to be detected in biological samples. DNA chip technology is an example of the enormous efforts undertaken in the genomic field in the last few years

Applications of Biochips

The complete sequence of the human genome and subsequent intensive searches for polymorphic variations are providing the prerequisite markers necessary to facilitate elucidation of the genetic variability in drug responses. Improvements in the sensitivity and precision of DNA microarrays permit a detailed and accurate scrutiny of the human genome.

These advances have the potential to significantly improve health care management by improving disease diagnosis and targeting molecular therapy. Pharmacogenetic approaches, in limited use today, will become an integral

part of therapeutic monitoring and health management, permitting patient stratification in advance of treatments, with the potential to eliminate adverse drug reactions Biochip technologies can be applied to numerous fields including genomic, proteomic, and glycomic research, as well as pharmacology and toxicology.

However, one of the most common applications is in the determination of gene expression in human cells and tissues. Global gene expression analysis has helped to identify

important genes and signaling pathways in human malignant tumors. And there is hope that microarrays will make the step from "the (laboratory) bench to the bedside (of the patient)".

Biochips (eg, GeneChip, CYP450, electrochemical biochips, protein biochips, microfluidic biochips and nanotechnology-based biochips) are assuming an important role in molecular diagnostics, and their application in point-of-care diagnosis is expected to facilitate the development of personalized medicine.

Gene expression profiling by microarrays should advance the progress of personalized cancer treatment based on the molecular classification of subtypes. Refinements in biochip miniaturization with the advent of nanotechnology will further contribute to molecular diagnostics and the development of personalized medicine.

UNIT-III

SYLLABUS

Genetically engineered proteins and peptide agents. Novel drug delivery systems – routes of drug administration. Anti AIDS drug development Drug development – Oncogenes Multidrug resistance Vaccine development – infectious diseases Drug development by genetic engineering – infectious diseases. Gene therapy- Stem cell therapy

Genetically engineered proteins and peptide agents

Drugs developed using living organisms with the help of biotechnology or genetic engineering are known as biologics, biopharmaceuticals, recombinant DNA expressed products, bioengineered, or genetically engineered drugs. The recombinant technique was developed by Cohen in 1973. The general procedure starts from the identification of the gene responsible for the production of the desired product. The gene is isolated from human cells and inserted into other carrier or vector cells like bacteria (Escherichia coli) or yeast (Saccharomyces cerevisiae, Hansenulla polymorpha, Pichia pastoris) that proliferate and produce large amounts of the desired product.The central theme of recombinant DNA technology is the process of gene cloning which consists of the production of a defi nedfragment of DNA and its propagation and amplification in a suitable host cell. Recombinant technology was only possible after the discovery of restriction endonucleases, the enzymes used as cutters for a desired segment of genes known as recognition sequences. There are various other enzymes that have great value in recombinant technology such as DNA polymerases, ligases, kinases, alkaline phosphatases, and nucleases. For thousands of years, living microorganisms have been used to produce gases, leaven bread, ferment alcoholic liquids, and for other desired by-products, but their use in recombinant DNA technology is often described as a relatively new science.

Recombinant technology, thus, is a new method for the development of drugs and other life-saving products that involves the blending of discoveries in molecular biology, DNA alteration, gene splicing, immunology, and immunopharmacology. Now days, various human diseases are treated with the help of drugs developed by recombinant technology, such as erythropoietin, coagulation modulators, enzymes, hormones, interferons, interleukins, granulocyte colony-

stimulating factors, anti-rheumatoid drugs, and various other agents like Tumor necrosis factor (TNF), becaplermin, hepatitis-B vaccine, antibodies etc. This article provides general as well as recent pharmacological information on different aspects like a brief description of recombinant products, pharmacological actions, and mechanism of action, adverse effects, doses, contraindications, interactions and therapeutic applications. The pharmacological information of bio-engineered products may be useful for better understanding by users and professionals in the medical and health-related sciences.

MANUFACTURING PROCESS FOR RECOMBINANT PRODUCTS

The following steps are involved in the production of recombinant products

Extraction of the DNA from a donor organism and the plasmid from bacteria

↓

Cleaved with restriction endonucleases

↓

Joined to form a recombinant DNA molecule or vector

↓

Insert this vector into host cell or expression system (E. coli or Yeast)

↓

Multiplication of recombinant DNA molecules

↓

Formation of clone

↓

Scale-up (harvesting or fermentation) of cell to produce recombinant drugs

↓

Purification (centrifugation or filtration)

↓

Preformulation

↓

Preclinical studies (animal testing)

↓

Clinical studies (human testing)

↓

Final product

CLASSIFICATION OF RECOMBINANT PRODUCTS

The following products or biologics are obtained by recombinant or genetic engineering technology

(I) Recombinant erythropoietin
Epoetin-α
Epoetin-β
Epoetin-ω
Darbepoetin-α

(II) Recombinant blood coagulation modulators
(a) Coagulants
Factor-VII A
Factor-VIII
Factor-IX
(b) Anticoagulants
Lepirudin
Desirudin
Drotecogin alfa

(III) Recombinant enzymes
(a) Thrombolytic enzymes
Alteplase
Anistreplase
Duteplase
Reteplase
Saruplase
Streptase or streptokinase
Tenecteplase
Tissue plasminogen activator
Urokinase
(b) Therapeutic enzymes
Agalsidase beta
Aeromonas aminopeptidase
Alpha-glactosidase-A
Dornase alpha
Imiglucerase or glucocerebrosidase or alglucerase
Laronidase or alpha-L-iduronidase
Galsulfase or N-acetylgalactosamine-4-sulfatase

(IV) Recombinant hormones
(a) Insulin and its analogs
Human insulin
Insulin aspart
Insulin glargine
Insulin lispro
Insulin glulisine
Insulin-like growth factor-I
Insulin-like growth factor-II
(b) Growth hormone and its analogs
Growth hormone
Growth hormone (ovine)
Growth hormone binding protein
Growth hormone-20K
Somatrem
Somatropin
Sermorelin
(c) Gonadotropins
Recombinant follitropin-α
Recombinant follitropin-β
Recombinant choriogonadotropin
Follicle-stimulating hormone
Luteinizing hormone
(d) Other hormones
Calcitonin
Glucagon

(V) Recombinant interferons
Interferon alfa-2a
Interferon alfa-2b
Interferon beta-1a
Interferon beta-1b
Interferon-gamma-1a
Interferon-gamma-1b
Interferons-ω
Consensus interferon

(VI) Recombinant interleukins
Interleukin-1 alpha
Interleukin-1 beta
Interleukin-2 (aldesleukin)
Interleukin-3
Interleukin-4
Interleukin-10
Interleukin-11 (oprelvekin)
Interleukin-13
Interleukin-15
Interleukin-17
Interleukin-18

(VII) Recombinant granulocyte colony stimulating factors
Filgrastim
Lenograstim
Molgramostim
Sargramostim
Regrasmostim
Pegfi lgrastim

(VIII) Recombinant anti-rheumatoid agents
Infl iximab
Adalimumab
Basiliximab
Daclizumab
Anakinra
Etanercept

(IX) Miscellaneous agents
Recombinant antibodies
Recombinant albumin
Fc purifi ed recombinant protein
Becaplermin
Beta-nerve growth factor
Bone morphogenetic protein-2
Brain-derived neurotrophic factor
Ciliary neurotrophic factor
Chymosin
Endostatin human

	Hepatitis-B vaccine	Relaxin
Enteropeptidase/Enterokinase	Hepatocyte growth factor	TNF
Epidermal growth factors	Influenza A, nucleoprotein, recombinant protein	Recombinant vaccines
Fibroblast growth factor	Lung surfactant protein	α-antitrypsin
		Thrombopoietin

Recombinant erythropoietin

Erythropoietin is a 165 amino acid glycoprotein hormone that is synthesized naturally in the kidney and the liver but is now prepared by recombinant technology. It stimulates erythrocyte formation and is available as epoetin-α, epoetin-β, and darbepoetin-α.

Mechanism of action: It stimulates proliferation and differentiation of erythrocytes. Dose: 5-100 units/ kg/ s.c. or i.v./three times a week.

Adverse effects: Allergic reaction, deficiency of iron and folic acid, disorientation, encephalopathy, fl u-like symptoms, headache, mild hypertension, seizures, and thrombosis.

Uses: It is preferred in cases of anemia due to AIDS, cancer chemotherapy, chronic renal failure, and anemia in premature babies. It is also useful in hematopoietic disorders and is used to increase the benefits of autologous blood transfusion.

Anticoagulants

Lepirudin and Disirudin are thrombin-inhibiting agents, developed by recombinant technology and are also known as derivatives of hirudin. Precautions: used cautiously in patients with renal failure. Mechanism of action: They directly bind to clot-bound and fl uid-phase thrombin.

Dose: 30 mg/ kg/i.v./ day. Uses: They are preferred in cases of heparin-induced thrombocytopenia and deep vein thrombosis.

Thrombolytic enzymes

Alteplase is an enzyme that catalyzes tissue plasminogen to plasmin and is commonly used to prevent clot-related myocardial disorders. Mechanism of action: It dissolves blood clots by converting plasminogen into plasmin that digests fibrin, fibrinogen, and other proteins. **Interactions**: Its action accelerates in combination with heparin. Contraindicated in bleeding, defective homeostasis, trauma, surgical procedures, stroke, acute pericarditis, hypoglycemia and hyperglycemia. Dose: 100 mg/i.v. infusion.

Adverse effects:

Alteplase may cause adverse effects like nausea, vomiting, fever, arrhythmias, allergy, hypotension, intracranial hemorrhage, and GIT bleeding. Uses: It is preferred in cases of angina pectoris, as an anticoagulant, intravascular thrombosis, ischemic diseases, myocardial infarction, pulmonary embolism, and to dissolve thrombins

Growth hormones and their analogs

Growth hormones and their analogs are synthesized by recombinant technology, and are commonly used as growth-promoting agents. These agents are not effective in bone disorders, cardiac diseases, cartilage disorders, Down syndrome, families of short stature, GIT problems, and renal complications. They are available as somatrem, somatropin, and sermorelin. These

agents are recommended for the treatment of complications due to the deficiency of growth hormone. Growth hormone deficiency may occur due to skull fracture, excessive exposure to radiation, cancer disease, and due to intracranial infections like tuberculosis or meningitis. Pharmacological actions: Stimulate the growth of connective
tissues and skeletal muscles and enhance protein synthesis and fatty acid metabolism. They also decrease the use of glucose in the body. Contraindicated in severe obesity, severe respiratory syndrome, and after renal transplantation.

Adverse effects: Growth hormones may cause acromegaly, antibody formation, arthralgia, atherosclerosis, diabetes mellitus, fluid retention, gigantism, glucose intolerance, headache, hypertension, hypoglycaemia, hypothyroidism, insulin resistance, myalgia, nausea, vomiting, pain and pruritis at injecting point, paraesthesia, and visual disturbances.

Uses: They are preferred in anti-aging and performance enhancers, chronic renal deficiency, dwarfism, gonadal dysgenesis, growth hormone deficiency, idiopathic short stature, and Sheehanís syndrome.

Novel drug delivery systems – Routes of drug administration

- Drug delivery is the method or process of administering pharmaceutical compound to achieve a therapeutic effect in humans or animals.
- Most common methods of delivery include the preferred non-invasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal, sublingual, vaginal, ocular and rectal) and inhalation routes.
- Many medications such as peptide and protein, antibody, vaccine and gene based drugs, in general may not be administered using these routes because they might be susceptible to enzymatic degradation or can not be absorbed into the systemic circulation efficiently due to molecular size and charge issues to be therapeutically effective.
- Protein and peptide drugs have to be delivered by injection

Why do we need NDDS?

- The conventional dosage forms provide drug release immediately and it causes fluctuation of drug level in blood depending upon dosage form.
- Therefore to maintain the drug concentration within therapeutically effective range need novel drug delivery system.

Novel Drug delivery System (NDDS)

- It refers to the approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effects. It may involve scientific site-targeting within the body, or it might involve facilitating systemic pharmacokinetics; in any case, it is typically concerned with both quantity and duration of drug presence".
- Novel Drug delivery is often approached via a drug's chemical formulation, but it may also involve medical devices or drug-device combination products.

- Drug delivery is a concept heavily integrated with dosage form and route of administration. NDDS is advanced drug delivery system which improves drug potency, control drug release to give a sustained therapeutic effect, provide greater safety; finally it is to target a drug specifically to a desired tissue.
- **Dissolution & Diffusion Controlled Release System-** in which drug is encapsulated in partially soluble membrane, pores are created due to soluble parts of coating film which permits entry of aqueous medium into core and drug dissolution starts by diffusion of dissolved drug out of system. Mixture of water soluble PVP and water insoluble ethyl cellulose is used for this purpose).
- Water penetration/Osmotic Pressure Controlled NDDS (in which drug may be osmotically active or drug may be combined with osmotically active salts like NaCl).
- Chemically controlled NDDS (in which systems change their chemical nature/structure when exposed to biological fluids
- Hydrogels (in which three dimensional structures of hydrophilic polymers having chemical and physical cross links provide a network structure to hydrogels.
- These are insoluble due to network structure and provide desirable protection of liable drugs, proteins and peptides).
- **Ion Exchange Resins Controlled Release Systems** - in these systems, ionisable drug is absorbed on ion-exchange resins granules then granules are coated with water permeable polymers using spray dryer technique).

1. Nanosomes
2. Liposomes
3. Niosomes
4. Nanoparticle
5. Nanosphere
6. Microsphere
7. Microparticle
8. Microemulsion
9. Nanosuspension
10. Micelles

Advantages of NDDS

- Decreased dosing frequency.
- Reduced rate of rise of drug concentration in blood.
- Sustained and consistent blood level within the therapeutic window.
- Enhanced bioavailability.
- To achieve a targeted drug release.
- Reduced side effects.
- Improved patient compliance

Anti AIDS drug development

- AIDS was initially described in the U.S. in 1981, although sporadic cases probably occurred for at least two decades prior to this. By 1983, the causative agent, now termed HIV, was identified.
- HIV is a member of the lentivirus subfamily of retroviruses. It is a spherical, enveloped particle, 100–150 nm in diameter, and contains RNA as its genetic material (Figure 13.13).
- The viral surface protein, gp 120, is capable of binding to a specifi c site on the CD4 molecule,found on the surface of susceptible cells (Table 13.10). Some CD4 negative cells may (rarely) also become infected, indicating the existence of an entry mechanism independent of CD4.
- Infection of CD4 cells commences via interaction between gp 120 and the CD4 glycoprotein, which effectively acts as the viral receptor. Entry of the virus into the cell, which appears to require some additional cellular components, occurs via endocytosis and/or fusion of the viral and cellular membranes. The gp 41 transmembrane protein plays an essential role in this process.
- Once released into the cell, the viral RNA is transcribed (by the associated viral reverse transcriptase) into double-stranded DNA. The retroviral DNA can then integrate into the
- host cell genome (or, in some instances, remain unintegrated). In resting cells, transcription of viral genes usually does not occur to any significant extent.
- However, commencement of active cellular growth/differentiation usually also triggers expression of proviral genes and, hence, synthesis of new viral particles. Aggressive expression of viral genes usually leads to cell death.
- Some cells, however (particularly macrophages), often permit chronic low-level viral synthesis and release without cell death. Entry of the virus into the human subject is generally accompanied by initial viral replication, lasting a few weeks.
- High-level viraemia (presence of viral particles in the blood) is noted and p24antigen can be detected in the blood. Clinical symptoms associated with the initial infection include an influenza-like illness, joint pains and general enlargement of the lymph nodes. This primary viraemia is brought under control within 3–4 weeks.
- This appears to be mediated largely by HIV specific cytotoxic T-lymphocytes, indicating the likely importance of cell-mediated immunity in bringing the initial infection under control.
- Although HIV-specific antibodies are also produced at this stage, effective neutralizing antibodies are detected mainly after this initial stage of infection.
- After this initial phase of infection subsides, the free viral load in the blood declines, often to almost undetectable levels.

- This latent phase may last for anything up to 10 years or more. During this phase, however, there does seem to be continuous synthesis and destruction of viral particles. This is accompanied By a high turnover rate of (CD4) T-helper lymphocytes.
- The levels of these T-lymphocytes decline with time, as does antibody levels specifi c for viral proteins. The circulating viral load often increases as a result, and the depletion of T-helper cells compromises general immune function.
- As the immune system fails, classical symptoms of AIDS-related complex (ARC) and, finally, full-blown AIDS begin to develop. In excess of 40 million individuals are now thought to be infected by HIV. In 2001 alone, it was estimated that 3 million people died from AIDS and a further 5 million became infected with the virus.
- Over 20 million people in total are now thought to have died from AIDS. The worst affected geographical region is the southern half of Africa (Table 13.11). Some 90 percent of sufferers live in poorer world regions. So far, no effective therapy has been discovered, and the main hope of eradicating this disease lieswith the development of safe, effective vaccines.
- The first such putative vaccine entered clinical trials in 1987; but, thus far, no effective vaccine has been developed.

Difficulties associated with vaccine development

- A number of attributes of HIV and its mode of infection conspire to render development of an effective vaccine less than straightforward. These factors include:
- HIV displays extensive genetic variation even within a single individual. Such genetic variation is particularly prominent in the viral env gene whose product, gp 160, is subsequently proteolytically processed to yield gp 20 and gp 41.
- HIV infects and destroys T-helper lymphocytes, i.e. it directly attacks an essential component of the immune system itself.
- Although infected individuals display a wide range of antiviral immunological responses, these ultimately fail to destroy the virus. A greater understanding of what elements of immunity are most effective in combating HIV infection is required.
- After initial virulence subsides, large numbers of cells harbour unexpressed proviral DNA. The immune system has no way of identifying such cells. An effective vaccine must thus induce the immune system to (a) bring the viral infection under control before cellular infection occurs or (b)destroy cells once they begin to produce viral particles and destroy the viral particles released.
- The infection may often be spread not via transmission of free viral particles, but via direct transmission of infected cells harbouring the proviral DNA.

AIDS vaccines in clinical trials

- A number of approaches are being assessed with regard to developing an effective AIDS vaccine.

- No safe attenuated form of the virus has been recognized to date, nor is one likely to be developed in the foreseeable future. The high level of mutation associated with HIV would, in any case, heighten fears that spontaneous reversion of any such product to virulence would be possible.
- The potential of inactivated viral particles as effective vaccines has gained some attention, but again fears of accidental transmission of disease if inactivation methods are not consistently 100 percent effective have dampened enthusiasm for such an approach. In addition, the stringent containment conditions required to produce large quantities of the virus render such production
- Processes expensive. Not withstanding the possible value of such inactivated viral vaccines, the bulk of products assessed to date are subunit vaccines. Live vector vaccines expressing HIV
- Genes have also been developed and are now coming to the fore (Table 13.12). Much of the preclinical data generated with regard to these vaccines entailed the use of one of two animal model systems: simian immunodeficiency virus infection of macaque monkeys and HIV
- Infection of chimpanzees. Most of the positive results observed in such systems have been inassociation with the chimp–HIV model. However, no such system can replace actual testing in humans.
- Most of the recombinant subunit vaccines tested in the first half of this decade employed gp 120 or gp160 expressed in yeast, insect or mammalian (mainly CHO) cell lines. Eukaryotic systems facilitate glycosylation of the protein products.
- Like all subunit vaccines, these stimulate a humoral based immune response but fail to elicit a strong T-cell response.
- The failure to elicit a cell-based response, in particular a cytotoxic T-cell response (now seen as critical to mounting an effective immune response), explains at least in part why subunit vaccines were a clinical disappointment.
- Several HIV vaccine systems based upon live vectors have also been developed in an attempt to stimulate a signifi cant T-cell, as well as B-cell, immune response. Both envelope and core antigens have been expressed in a number of recombinant viral systems. The clinical efficacy of these remains to be established.
- Expression in engineered vaccinia has been undertaken, but its use as an HIV vaccine is likely precluded by the fact that the virus can apparently disseminate and cause fatal encephalitis in immunosuppressed infected individuals.
- Modified vaccinia Ankara, canarypox and fowlpox viruses have come to the fore as vectors. These likely can produce sufficient protein to initiate both a humoral and cellular immune response during an abortive replication cycle in humans.

- A vaccination schedule variation of potential interest entails the use of a vector-based primary dose (to induce a cellular response in particular) followed by a subunit-based booster (to induce mainly a humoral response).
- Whatever the schedule however, the induction of effective (i.e. broadly neutralizing) antibodies remains a challenge, as the regions of HIV envelope proteins that are most highly conserved seem to be shielded from antibody access by loop structures and sugar side-chains on these surface proteins.
- Large-scale clinical trials are likely to be the only way by which any HIV vaccine may be properly assessed. In addition, a greater understanding of the molecular interplay between the virus and immune system may provide clues as to the development of novel vaccine and/or therapeutic products.
- For example, a small proportion of infected individuals remain clinically asymptomatic for periods considerably greater than the average 10–15 years.
- An understanding of the immunological or other factors that delay onset of ARC/full-blown AIDS in these individuals may aid in the design of more effective vaccines.

UNIT-1V

SYLLABUS

Enzyme technology - Enzyme sources, extraction, purification and application; Enzymes – applications (pharmaceuticals, therapeutic and clinical) Production of aminoglucosidase - Production of glucose isomerase - Production of amylase and trypsin. Immobilization of enzymes – techniques; Immobilization of enzymes – applications Immobilization of enzymes – Reactors. Enzyme engineering

ENZYME TECHNOLOGY

Enzymes are the biocatalysts synthesized by living cells. They are complex protein molecules that bring about chemical reactions concerned with life. It is fortunate that enzymes continue to function (bring out catalysis) when they are separated from the cells i.e. in vitro. Basically, enzymes are non-toxic and biodegradable. They can be produced in large amounts by microorganisms for industrial applications.

Enzyme technology broadly involves production, isolation, purification and use of enzymes (in soluble or immobilized form) for the ultimate benefit of humankind. In addition, recombinant DNA technology and protein engineering involved in the production of more efficient and useful enzymes are also a part of enzyme technology.The commercial production and use of enzymes is a major part of biotechnology industry. The specialties like microbiology; chemistry and process engineering, besides biochemistry have largely contributed for the growth of enzyme technology.

ENZYME SOURCES, EXTRACTION ,PURIFICATION AND APPLICATION

Commercial Production of Enzymes:

Microbial enzymes have been utilized for many centuries without knowing them fully. The first enzyme produced industrially was taka-diastase (a fungal amylase) in 1896, in United States. It was used as a pharmaceutical agent to cure digestive disorders.

In Europe, there existed a centuries old practice of softening the hides by using feces of dogs and pigeons before tanning. A German scientist (Otto Rohm) demonstrated in 1905 that extracts from

animal organs (pancreases from pig and cow) could be used as the source of enzymes-proteases, for leather softening.

The utilization of enzymes (chiefly proteases) for laundry purposes started in 1915. However, it was not continued due to allergic reactions of impurities in enzymes. Now special techniques are available for manufacture, and use of enzymes in washing powders (without allergic reactions). Commercial enzymes can be produced from a wide range of biological sources. At present, a great majority (80%) of them are from microbial sources.

The different organisms and their relative contribution for the production of commercial enzymes are given below:

Fungi – 60%

Bacteria – 24%

Yeast – 4%

Streptomyces – 2%

Higher animals – 6%

Higher plants – 4%

A real breakthrough for large scale industrial production of enzymes from microorganisms occurred after 1950s.

Enzymes from animal and plant sources:

In the early days, animal and plant sources largely contributed to enzymes. Even now, for certain enzymes they are the major sources

Animal organs and tissues are very good sources for enzymes such as lipases, esterases and proteases. The enzyme lysozyme is mostly obtained from hen eggs. Some plants are excellent sources for certain enzymes-papain (papaya), bromelain (pineapple).

Enzymes from mammalian cell cultures:

There exists a possibility of producing commercial enzymes directly by mammalian cell cultures. But the main constraint will the cost factor which will be extremely high. However, certain therapeutic enzymes such as tissue plasminogen activator are produced by cell cultures.

Enzymes from microbial sources:

Microorganisms are the most significant and convenient sources of commercial enzymes. They can be made to produce abundant quantities of enzymes under suitable growth conditions.

Microorganisms can be cultivated by using inexpensive media and production can take place in a short period.

In addition, it is easy to manipulate microorganisms in genetic engineering techniques to increase the production of desired enzymes. Recovery, isolation and purification processes are easy with microbial enzymes than that with animal or plant sources.

In fact, most enzymes of industrial applications have been successfully produced by micro-organisms. Various fungi, bacteria and yeasts are employed for this purpose

***Aspergillus niger*— A unique organism for production of bulk enzymes:**

Among the microorganisms, A. niger (a fungus) occupies a special position for the manufacture of a large number of enzymes in good quantities. There are well over 40 commercial enzymes that are conveniently produced by A. niger. These include a-amylase, cellulase, protease, lipase, pectinase, phytase, catalase and insulinase.

The Technology of Enzyme Production—General Considerations:

In general, the techniques employed for microbial production of enzymes are comparable to the methods used for manufacture of other industrial products .The salient features are briefly described.

1. Selection of organisms
2. Formulation of medium
3. Production process
4. Recovery and purification of enzymes.

An outline of the flow chart for enzyme production by microorganisms is depicted in Fig. 1

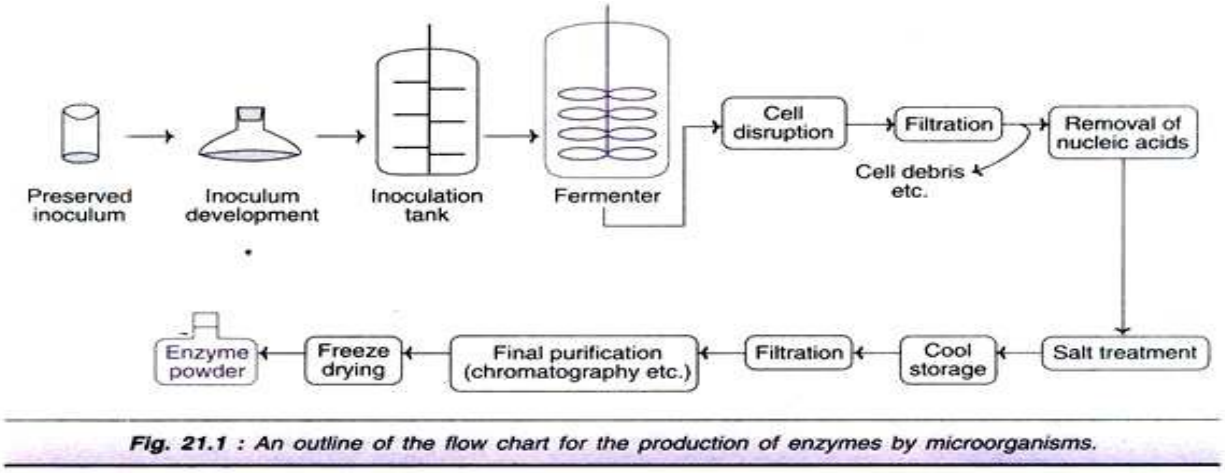


Fig. 21.1 : An outline of the flow chart for the production of enzymes by microorganisms.

Selection of organism:

The most important criteria for selecting the microorganism are that the organism should produce the maximum quantities of desired enzyme in a short time while the amounts of other metabolite produced are minimal. Once the organism is selected, strain improvement for optimising the enzyme production can be done by appropriate methods (mutagens, UV rays). From the organism chosen, inoculum can be prepared in a liquid medium.

Formulation of medium:

The culture medium chosen should contain all the nutrients to support adequate growth of microorganisms that will ultimately result in good quantities of enzyme production. The ingredients of the medium should be readily available at low cost and are nutritionally safe. Some of the commonly used substrates for the medium are starch hydrolysate, molasses, corn steep liquor, yeast extract, whey, and soy bean meal. Some cereals (wheat) and pulses (peanut) have also been used. The pH of the medium should be kept optimal for good microbial growth and enzyme production.

Production process:

Industrial production of enzymes is mostly carried out by submerged liquid conditions and to a lesser extent by solid-substrate fermentation. In submerged culture technique, the yields are more and the chances of infection are less. Hence, this is a preferred method. However, solid substrate fermentation is historically important and still in use for the production of fungal enzymes e.g. amylases, cellulases, proteases and pectinases.

The medium can be sterilized by employing batch or continuous sterilization techniques. The fermentation is started by inoculating the medium. The growth conditions (pH, temperature, O_2 supply, nutrient addition) are maintained at optimal levels. The froth formation can be minimised by adding antifoam agents.

The production of enzymes is mostly carried out by batch fermentation and to a lesser extent by continuous process. The bioreactor system must be maintained sterile throughout the fermentation process. The duration of fermentation is variable around 2-7 days, in most production processes. Besides the desired enzyme(s), several other metabolites are also produced. The enzyme(s) have to be recovered and purified.

Recovery and purification of enzymes:

The desired enzyme produced may be excreted into the culture medium (extracellular enzymes) or may be present within the cells (intracellular enzymes). Depending on the requirement, the commercial enzyme may be crude or highly purified. Further, it may be in the solid or liquid form. The steps involved in downstream processing i.e. recovery and purification steps employed will depend on the nature of the enzyme and the degree of purity desired.

In general, recovery of an extracellular enzyme which is present in the broth is relatively simpler compared to an intracellular enzyme. For the release of intracellular enzymes, special techniques are needed for cell disruption. The reader must invariably refer them now and learn all the details, as they form part of enzyme technology. Microbial cells can be broken down by physical means (sonication, high pressure, glass beads). The cell walls of bacteria can be lysed by the enzyme lysozyme. For yeasts, the enzyme β-glucanase is used. However, enzymatic methods are expensive.

The recovery and purification (briefly described below) steps will be the same for both intracellular and extracellular enzymes, once the cells are disrupted and intracellular enzymes are released. The most important consideration is to minimise the loss of desired enzyme activity.

Removal of cell debris:

Filtration or centrifugation can be used to remove cell debris.

Removal of nucleic acids:

Nucleic acids interfere with the recovery and purification of enzymes. They can be precipitated and removed by adding poly-cations such as polyamines, streptomycin and polyethyleneimine.

Enzyme precipitation:

Enzymes can be precipitated by using salts (ammonium sulfate) organic solvents (isopropanol, ethanol, and acetone). Precipitation is advantageous since the precipitated enzyme can be dissolved in a minimal volume to concentrate the enzyme.

Liquid-liquid partition:

Further concentration of desired enzymes can be achieved by liquid-liquid extraction using polyethylene glycol or polyamines.

Separation by chromatography:

There are several chromatographic techniques for separation and purification of enzymes. These include ion-exchange, size exclusion, affinity, hydrophobic interaction and dye ligand chromatography .Among these, ion- exchange chromatography is the most commonly used for enzyme purification.

Drying and packing:

The concentrated form of the enzyme can be obtained by drying. This can be done by film evaporators or freeze dryers (lyophilizers). The dried enzyme can be packed and marketed. For certain enzymes, stability can be achieved by keeping them in ammonium sulfate suspensions.

All the enzymes used in foods or medical treatments must be of high grade purity, and must meet the required specifications by the regulatory bodies. These enzymes should be totally free from toxic materials, harmful microorganisms and should not cause allergic reactions.

Regulation of Microbial Enzyme Production —General Considerations:

A maximal production of microbial enzymes can be achieved by optimising the fermentation conditions (nutrients, pH, O_2, temperature etc.). For this purpose, a clear understanding of the genetic regulation of enzyme synthesis is required. Some of the general aspects of microbial enzyme regulation are briefly described.

Induction:

Several enzymes are inducible i.e. they are synthesized only in the presence of inducers. The inducer may be the substrate (sucrose, starch, galactosides) or product or intermediate (fatty acid, phenyl acetate, xylobiose). A selected list of inducible enzymes and the respective inducers is given in Table 21.4.

Table 21.4 Selected examples of inducible enzymes along with the inducers

Enzyme	*Inducer*
Invertase	Sucrose
Amylase	Starch
Lipase	Fatty acids
β-Galactosidase	Galactosides
Penicillin G amidase	Phenylacetate
Xylanase	Xylobiose

The inducer compounds are expensive and their handling (sterilization, addition at specific time) also is quite difficult. In recent years, attempts are being made to develop mutants of microorganisms in which inducer dependence is eliminated.

Feedback repression:

Feedback regulation by the end product (usually a small molecule) significantly influences the enzyme synthesis. This occurs when the end product accumulates in large-quantities. Large scale production of feedback regulated enzymes is rather difficult. However, mutants that lack feedback repression have been developed to overcome this problem.

Nutrient repression:

The native metabolism of microorganism is so devised that there occurs no production of unnecessary enzymes. In other words, the microorganisms do not synthesize enzymes that are not required by them, since this is a wasteful exercise. The inhibition of unwanted enzyme production is done by nutrient repression. The nutrients may be carbon, nitrogen, phosphate or sulfate suppliers in the growth medium. For large scale production of enzymes, nutrient repression must be overcome.

Glucose repression is a classical example of nutrient (more appropriately catabolite) repression. That is in the presence of glucose, the enzymes needed for the metabolism of rest of the compounds are not synthesized. Glucose repression can be overcome by feeding of carbohydrate to the fermentation medium in such a way that the concentration of glucose is

almost zero at any given time. In recent years, attempts are being made to select mutants that are resistant to catabolite repression by glucose. For certain microorganisms, other carbon sources such as pyruvate, lactate, citrate and succinate also act as catabolite repressors.

Nitrogen source repression is also observed in microorganisms. This may be due to ammonium ions or amino acids. Most commonly inexpensive ammonium salts are used as nitrogen sources. The repression by ammonium salts can be overcome by developing mutants resistant to this nitrogen source.

Genetic Engineering for Microbial Enzyme Production:

Enzymes are the functional products of genes. Therefore, theoretically, enzymes are good candidates for improved production through genetic engineering. During the past 15 years, the advances in the recombinant DNA technology have certainly helped for increasing the microbial production of commercial enzymes. It is now possible to transfer the desired enzyme genes from one organism to the other. Once an enzyme with a potential use in industry is identified, the relevant gene can be cloned and inserted into a suitable production host.

Cloning strategies:

A diagrammatic representation of a cloning strategy for industrial production of enzymes This involves the development of cDNA library for the mRNA, and creation of oligonucleotide probes for the desired enzyme. On hybridization with oligonucleotide probes, the specific cDNA clones

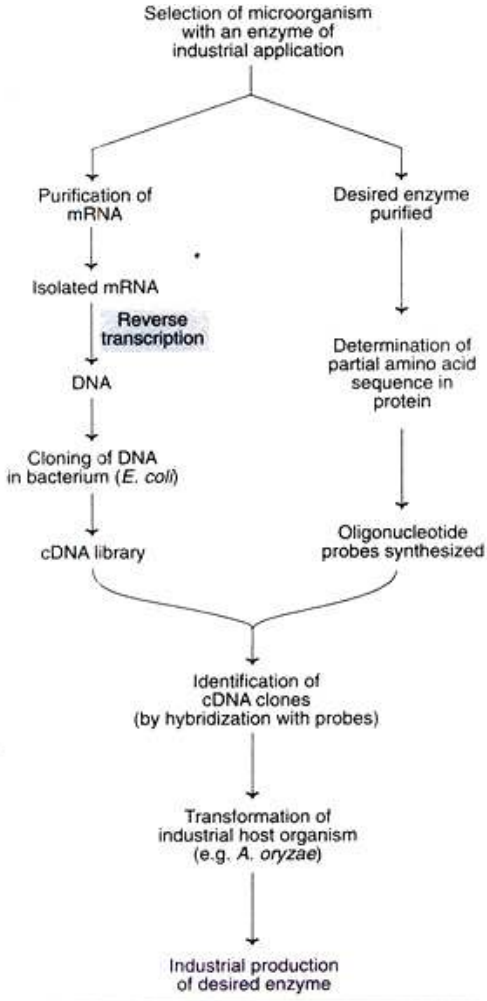


Fig. 21.2 : Schematic representation of a cloning strategy for industrial production of enzymes.

The next step is the transformation of industrially important host organism (e.g. Aspergillus oryzae) for the production of the desired enzyme. By this approach, it is possible to manufacture high quality industrial enzymes. A couple of enzymes produced by employing cloning strategies are described below.

1. The enzyme lipolase, found in the fungus Humicola languinosa is very effective to remove fat stains in fabrics. However, industrial production of lipolase by this organism is not possible due to a very low level of synthesis. The gene responsible for lipolase was isolated, cloned and inserted into Aspergillus oryzae.

Thus, large scale production of this enzyme was successfully achieved. Lipolase is very stable and resistant to degradation by proteases that are commonly used in detergents. All these properties make lipolase a strong candidate for its use fabric washing.

2. Rennet (chymosin) is an enzyme widely used in making cheese. It is mainly obtained from the stomachs of young calves. Consequently, there is a shortage in its supply. The gene for the synthesis of chymosin has been cloned for its large scale production.

Protein engineering for modification of industrial enzymes:

It is now possible to alter the structure of a protein/enzyme by protein engineering and site- directed mutagenesis .The changes in the enzymes are carried out with the objectives of increased enzyme stability and its catalytic function, resistance to oxidation, changed substrate preference and increased tolerance to alkali and organic solvents.

By site- directed mutagenesis, selected amino acids at specific positions (in enzyme) can be changed to produce an enzyme with desired properties. For instance, protein engineering has been used to structurally modify phospholipase A_2 that can resist high concentration of acid. The modified enzyme is more efficiently used as a food emulsifier. Genetic engineering has tremendous impact on the industrial production of enzymes with desired properties in a cost-effective manner.

ENZYMES-APPLICATIONS

Enzymes have wide range of applications. These include their use in food production, food processing and preservation, washing powders, textile manufacture, leather industry, paper industry, medical applications, and improvement of environment and in scientific research.

As per recent estimates, a great majority of industrially produced enzymes are useful in processes related to foods (45%), detergents (35%), textiles (10%) and leather (3%)

Enzyme	Source(s)	Application(s)
α-Amylase	*Aspergillus oryzae* *Aspergillus niger* *Bacillus subtilus* *Bacillus licheniforms*	Production of beer and alcohol, Preparation of glucose syrups, As a digestive aid Removal of starch sizes
Amyloglucosidase	*Aspergillus niger* *Rhizopus niveus*	Starch hydrolysis
Cellulase	*Aspergillus niger* *Tricoderma koningi*	Alcohol and glucose production
Glucoamylase	*Aspergillus niger* *Bacillus amyloliquefaciens*	Production of beer and alcohol Starch hydrolysis
Glucose isomerase	*Arthrobacter* sp *Bacillus* sp	Manufacture of high fructose syrups
Glucose oxidase	*Aspergillus niger*	Antioxidant in prepared foods
Invertase	*Saccharomyces cerevisiae*	Surcose inversion Preparation of aritficial honey, confectionaries
Keratinase	*Streptomyces fradiae*	Removal of hair from hides
Lactase	*Kluyveromyus* sp *Saccharomyces fragilis*	Lactose hydrolysis Removal of lactose from whey
Lipase	*Candida lipolytica* *Asperigillus niger*	Preparation of cheese Flavour production
Pectinase	*Aspergillus* sp *Sclerotina libertina*	Clarification of fruit juices and wines Alcohol production, coffee concentration
Penicillin acylase	*Escherichia coli*	Production of 6-aminopenicillanic acid
Penicillanase	*Bacillus subtilis*	Removal of penicillin
Protease, acid	*Aspergillus niger*	Digestive aid Substitute for calf rennet

PRODUCTION OF AMINOGLUCOSIDASE

The extracellular enzyme, amyloglucosidase (AMG) (EC3.2.1.3), which converts starch to dextrins and glucose is used in the starch-processing industry. Even though the enzyme is found in a wide variety of microorganisms, the commercial AMG is mainly obtained from fungi such as Aspergillus and Rhizopus spp. Methods of cultivation greatly influence the production and properties of the enzyme

.The most common methods of production involve either solid-state or submerged cultivation .Recently, aqueous, two-phase systems have been used and these have the advantage that a highly concentrated stream of pure product is easily obtained. The semi-continuous production of a-amylase and cellulase has been studied in Bacillus subtilis and Trichoderma reesei respectively. Improvements in yield were observed in both cases.

The fermentation medium consisted of (g/l): soluble starch, 50; yeast extract 10; KCI, 0.5; KH,PO,, 5; (NH,),SO,, 5; MgS0,.7H,O, 0.5; FeS0,.7H,O, 0.01; ZnS0,.7H,O, 0.001; and CuS0,.5H,O, 0.001. Starch and salt solutions were sterilized separately. Batch-fermenter cultivations were carried out in a laboratory fermenter at 30°C with a working volume of 2 1.

The inoculum used was 100 ml of a 12-h culture grown in shake-flasks as above. The pH was maintained at 6.0 by automatic titration with 2 M NaOH. The stirrer speed was increased from 300 to 600 rev/min after 12 h of cultivation, in steps of 40 rev/min at 6-h time intervals and the air flow was 2.6 Urnin. The fermented bran was mixed with distilled water in the ratio of 1:9 (w/v) and agitated at 30°C for 30 min.

The solids were separated from the extract by centrifuging at 3000 x g for 10 min. The yield was 7.5 to 8.0 ml extract from 1 g wet wt. Enzyme activities were calculated per ml extract or per g fermented moist solids. AMG activity in 1 ml extract was compared with that in 1 ml submerged-culture filtrate.

PRODUCTION OF GLUCOSE ISOMERASE

A microorganism that produces glucose isomerase was isolated from soil and identified as a strain of Streptomyces flavogriseus. The organism produced a large quantity of glucose isomerase when grown on straw hemicellulose, xylan, xylose, and H2SO4 hydrolysate of ryegrass straw. The organism produced glucose isomerase both intra- and extra-cellularly. The

highest level of intracellular glucose isomerase (3.5 U/ml) was obtained in about 36 h by a culture grown on straw hemicellulose; the extracellular enzyme (1.5 U/ml) appeared in cultures grown for about 72 h. About equal levels of enzyme were produced in cultures grown on straw hemicellulose, xylan, xylose, and H2SO4 hydrolysate of straw, but production of the enzyme was drastically reduced when the organism was grown on other carbon sources.

As a nitrogen source, corn steep liquor produced the best results. Soy flour extract, yeast extract, and various peptones also were adequate substrates for glucose isomerase production. Addition of Mg2+, Mn2+, or Fe2" to the growth medium significantly enhanced enzyme production. Theorganism, however, did not require Co2+, which is commonly required by microorganisms used in the production of glucose isomerase.

For production of glucose isomerase, the Streptomyces flavogriseus were grown on a medium containing 1% straw hemicellulose, 2.5% corn steep liquor, and 0.1% MgSO4. 7H20. The pH of the medium was 7.0. Corn steep liquor was also used .Yeast extract-malt extract agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar were used for morphological studies and determinations of mycelium color.

PRODUCTION OF AMYLASE AND TRYPSIN

In recent years, the potential of using microorganisms as sources of industrially relevant enzymes has stimulated our interest, where these enzymes can be used in a large number of industries like food, feed, leather, textile, and paper industries. Starch degrading enzymes like amylase have received a great deal of attention because of their technological significance and economic benefits.

They are among the most important enzymes and are of great significance in many areas. There are various types of amylases, namely α, β, and glucoamylases. α-amylases (endo-1,4-α-D-glucan glucohydrolase, EC 3.2.1.1) are extra cellular enzymes that randomly cleave the 1,4-α-D-glycosidic bonds between adjacent glucose units in the linear amylase chain and are classified according to their action and properties. β-amylases (β-1,4-glucan maltohydrolase, EC 3.2.1.2) are usually of plant origin, but a few microbial strains are also known to produce them. It is an exoacting enzyme that cleaves nonreducing ends of amylose, amylopectin, and glycogen molecule. Glucoamylase (amyloglucosidase, glucanogenic enzymes, starch glucogenase, and

exo-1,4-α-D-glucan glucanohydrolase (EC 3.1.2.3)) hydrolyses single glucose units from the nonreducing ends of amylose and amylopectin in stepwise manner.

Amylases can be derived from various sources, such as plants, animals, and microorganisms, but microbial sources generally meet industrial demands as there is a possibility of increasing the levels of microbial enzyme synthesized by classical genetic techniques, continuous culture selection, induction, or optimization of growth conditions for the enzyme of interest.

The fungal amylases are preferred over other microbial sources because of their more acceptable GRAS (generally regarded as safe) status, the hyphal mode of growth, and good tolerance to low water activity (a w), and high osmotic pressure conditions make fungi most efficient for bioconversion of solid substrates and thus attracting increasing attention as source of amylolytic enzymes suitable for industrial applications

Aspergillus fumigatus , exhibiting the best amylase activity among the isolate, produced 341.7 U/mL of amylases under optimized conditions of incubation period (6 days), incubation temperature (35°C), and incubation pH (6.0), using nutrient salt solution (NSS) of (potassium dihydrogen orthophosphate 5, ammonium nitrate 5, sodium chloride 1, and magnesium sulphate) as the moistening agent, beef extract as the nitrogen source, and wheat bran as the solid substrate.

The crude enzyme preparation, so obtained from the test strain under optimized conditions of SSF, was found to be thermoalkali stable, as it retained about 90% of its maximum activity at pH 8.0 and more than 50% of its maximum activity at a temperature of 75°C. UV-mutated and chemical-mutated strains were, respectively, found to exhibit an increase of 44.52 and 58.03% in amylase activity as compared to wild strain of *Aspergillus fumigatus* .

The crude amylase preparation is also found to contain important industrial accessory enzymes, which add to the value of the said enzyme preparation for industrial applications. Use of a single enzyme preparation in multiple steps of industrial processing has the potential to improve the economics of the overall process. Thus, the future work deals with application of the crude enzyme of Aspergillus fumigatus NTCC1222 in multiple sections of textile wet processing so as to explore cost-effective usage of enzymes.

Trypsin (EC 3.4.31.4) is a crucial enzyme in the serine protease family, which can specifically hydrolyze the carboxy-terminal peptide bonds of arginine or lysine residues . Nowadays, it has been widely used in several industrial applications, especially in the pharmaceuticals, food and leather industry.

In the pharmacy field, it can be used as the main material of some digestives, which can digest the denatured proteins for detumescence . Also, trypsin has been used for extraction of collagen, which is a natural material harboring excellent biocompatibility and biodegradability . In the food industry, it can hydrolyze the raw materials of proteins into low molecular peptides and amino acids Furthermore, trypsin is a typical enzyme that plays a critical role in the bating process of the leather industry .

The optimized culture temperature and initial pH for B. licheniformis were proven to be 37 °C and pH 6.0, respectively Low temperature, acidic and alkali pH conditions strongly reduced the trypsin activity. The inoculum size had slight influence on enzyme activity and 1% of inoculum size was selected for further study.

In these optimization experiments, the results of a previous optimized step would be used in the subsequent one. Corn flour and soy peptone were proven to be the suitable carbon and nitrogen sources, respectively .Their concentrations were further optimized and results showed that both of them were 15 g/L. High carbon or nitrogen source concentration stimulated cell growth but reduced enzyme activity, while the opposite result was observed with low concentrations. Moreover, addition of 5 mM Fe3+ or 5 mM Mg2+ can improve the enzyme activity, while other ions showed no positive effects on B. licheniformis trypsin .

Further increase of Mg2+ concentration supported the cell growth but slightly inhibited the enzyme activity. However, interestingly, the trypsin suffered from complete inhibition by Fe3+ with concentrations of higher than 10 mM. To sum up, the total trypsin activity was significantly improved to 140 U/mL by optimization, while the initial value was about 20 U/mL. *N*α-Benzoyl-l-arginine ethylester (BAEE), a specific substrate for trypsin, was used to mine potential trypsin-producing strains.

The trypsin-producing strain was a rod shaped Gram-positive bacterium. Colonies on Luria-Bertani (LB) medium were flat, dry, and opaque. Gills and irregular edge of the colonies were observed . Furthermore, it developed light yellow colonies on the skim milk medium.

Transmission electron microscope image showed flagella as one of its morphological features, which proved the motility of this bacteria.

IMMOBILIZATION OF ENZYMES-TECHNIQUES

Traditionally, enzymes in free solutions (i.e. in soluble or free form) react with substrates to result in products. Such use of enzymes is wasteful, particularly for industrial purposes, since enzymes are not stable, and they cannot be recovered for reuse.

Immobilization of enzymes (or cells) refers to the technique of confining/anchoring the enzymes (or cells) in or on an inert support for their stability and functional reuse. By employing this technique, enzymes are made more efficient and cost-effective for their industrial use. Some workers regard immobilization as a goose with a golden egg in enzyme technology. Immobilized enzymes retain their structural conformation necessary for catalysis.

There are several advantages of immobilized enzymes:

a. Stable and more efficient in function.

b. Can be reused again and again.

c. Products are enzyme-free.

d. Ideal for multi-enzyme reaction systems.

e. Control of enzyme function is easy.

f. Suitable for industrial and medical use.

g. Minimize effluent disposal problems.

There are however, certain disadvantages also associated with immobilization.

a. The possibility of loss of biological activity of an enzyme during immobilization or while it is in use.

b. Immobilization is an expensive affair often requiring sophisticated equipment.

Immobilized enzymes are generally preferred over immobilized cells due to specificity to yield the products in pure form. However, there are several advantages of using immobilized multi-enzyme systems such as organelles and whole cells over immobilized enzymes. The immobilized cells possess the natural environment with cofactor availability (and also its regeneration capability) and are particularly suitable for multiple enzymatic reactions.

Methods of Immobilization:

The commonly employed techniques for immobilization of enzymes are—adsorption, entrapment, covalent binding and cross-linking.

Adsorption:

Adsorption involves the physical binding of enzymes (or cells) on the surface of an inert support. The support materials may be inorganic (e.g. alumina, silica gel, calcium phosphate gel, glass) or organic (starch, carboxymethyl cellulose, DEAE-cellulose, DEAE-sephadex).

Adsorption of enzyme molecules (on the inert support) involves weak forces such as van der Waals forces and hydrogen bonds (Fig. 21.3). Therefore, the adsorbed enzymes can be easily removed by minor changes in pH, ionic strength or temperature. This is a disadvantage for industrial use of enzymes.

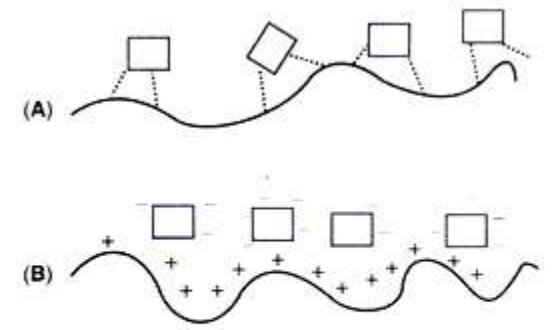


*Fig. 21.3 : Immobilization of enzymes by adsorption (A) By van der Waals forces (B) By hydrogen bonding (**Note** : Cloured blocks represent enzymes)*

Entrapment:

Enzymes can be immobilized by physical entrapment inside a polymer or a gel matrix. The size of the matrix pores is such that the enzyme is retained while the substrate and product molecules pass through. In this technique, commonly referred to as lattice entrapment, the enzyme (or cell) is not subjected to strong binding forces and structural distortions.

Some deactivation may however, occur during immobilization process due to changes in pH or temperature or addition of solvents. The matrices used for entrapping of enzymes include polyacrylamide gel, collagen, gelatin, starch, cellulose, silicone and rubber. Enzymes can be entrapped by several ways.

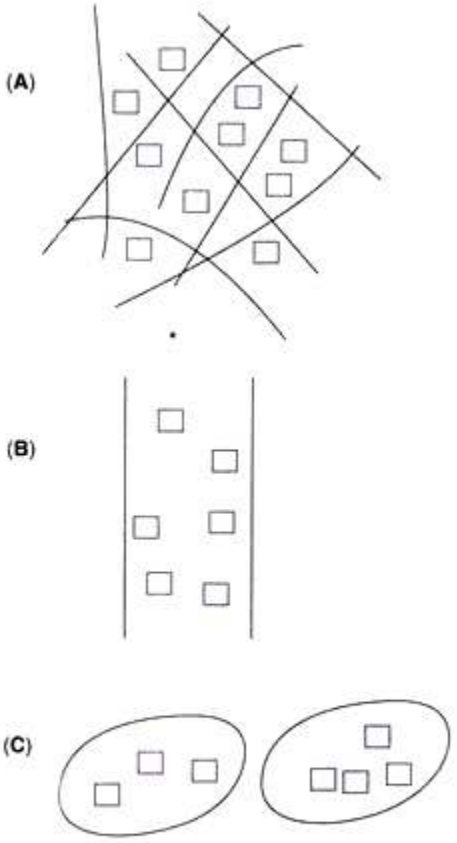


*Fig. 21.4 : Immobilization of enzymes by entrapment (A) Inclusion in gels (B) Inclusion in fibres (C) Inclusion in microcapsules (**Note** : Coloured blocks represent enzymes)*

1. Enzyme inclusion in gels:

This is an entrapment of enzymes inside the gels (Fig. 21.4A).

2. Enzyme inclusion in fibres:

The enzymes are trapped in a fibre format of the matrix (Fig. 21.4B).

3. Enzyme inclusion in microcapsules:

In this case, the enzymes are trapped inside a microcapsule matrix (Fig. 21.4C). The hydrophobic and hydrophilic forms of the matrix polymerise to form a microcapsule containing enzyme molecules inside. The major limitation for entrapment of enzymes is their leakage from the matrix. Most workers prefer to use the technique of entrapment for immobilization of whole cells. Entrapped cells are in use for industrial production of amino acids (L-isoleucine, L-aspartic acid), L-malic acid and hydroquinone.

Microencapsulation:

Microencapsulation is a type of entrapment. It refers to the process of spherical particle formation wherein a liquid or suspension is enclosed in a semipermeable membrane. The membrane may be polymeric, lipoidal, lipoprotein-based or non-ionic in nature. There are three distinct ways of microencapsulation.

1. Building of special membrane reactors.
2. Formation of emulsions.
3. Stabilization of emulsions to form microcapsules.

Microencapsulation is recently being used for immobilization of enzymes and mammalian cells. For instance, pancreatic cells grown in cultures can be immobilized by microencapsulation. Hybridoma cells have also been immobilized successfully by this technique.

Covalent Binding:

Immobilization of the enzymes can be achieved by creation of covalent bonds between the chemical groups of enzymes and the chemical groups of the support (Fig. 21.5). This technique is widely used. However, covalent binding is often associated with loss of some enzyme activity. The inert support usually requires pretreatment (to form pre-activated support) before it binds to enzyme. The following are the common methods of covalent binding.

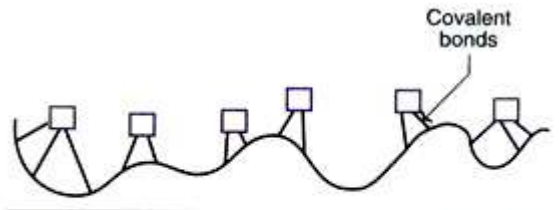


*Fig. 21.5 : A general representation of immobilization of enzymes by covalent binding (**Note** : coloured blocks represent enzymes).*

1. Cyanogen bromide activation:

The inert support materials (cellulose, sepharose, sephadex) containing glycol groups are activated by CNBr, which then bind to enzymes and immobilize them (Fig. 21.6A).

2. Diazotation:

Some of the support materials (amino benzyl cellulose, amino derivatives of polystyrene, aminosilanized porous glass) are subjected to diazotation on treatment with $NaNO_2$ and HCI. They, in turn, bind covalently to tyrosyl or histidyl groups of enzymes (Fig. 21.6B).

3. Peptide bond formation:

Enzyme immobilization can also be achieved by the formation of peptide bonds between the amino (or carboxyl) groups of the support and the carboxyl (or amino) groups of enzymes (Fig. 21.6C). The support material is first chemically treated to form active functional groups.

4. Activation by bi- or poly-functional reagents:

Some of the reagents such as glutaraldehyde can be used to create bonds between amino groups of enzymes and amino groups of support (e.g. aminoethylcellulose, albumin, amino alkylated porous glass). This is depicted in Fig. 21.6D.

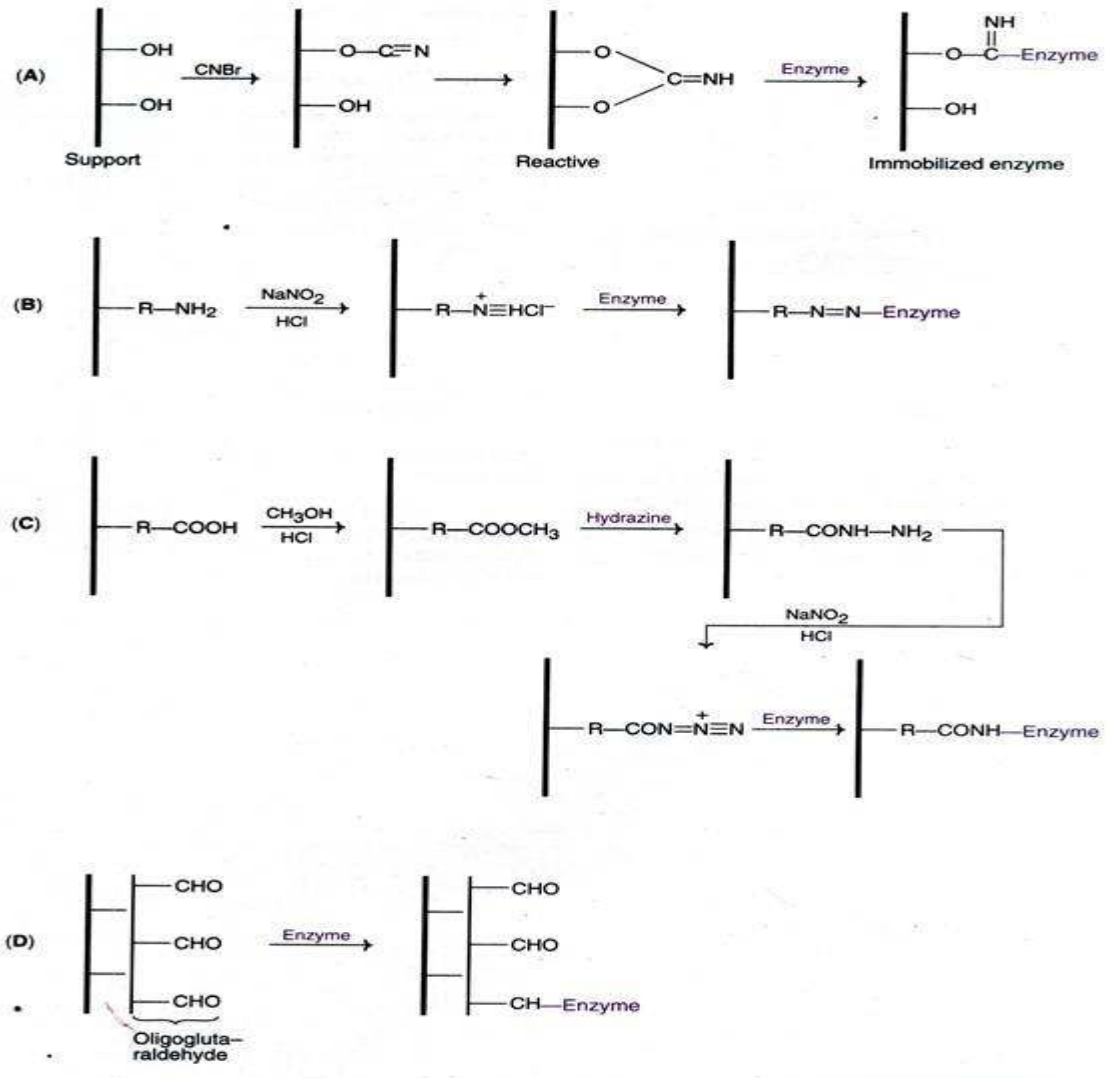


Fig. 21.6 : Immobilization of enzymes by covalent binding (A) Cyanogen bromide activation, (B) Diazotation, (C) Peptide bond formation, (D) Activation by bifunctional agent.

Cross-Linking:

The absence of a solid support is a characteristic feature of immobilization of enzymes by cross-linking. The enzyme molecules are immobilized by creating cross-links between them, through the involvement of poly-functional reagents. These reagents in fact react with the enzyme molecules and create bridges which form the backbone to hold enzyme molecules (Fig. 21.7). There are several reagents in use for cross-linking. These include glutaraldehyde, diazobenzidine, hexamethylene diisocyanate and toluene di- isothiocyanate.

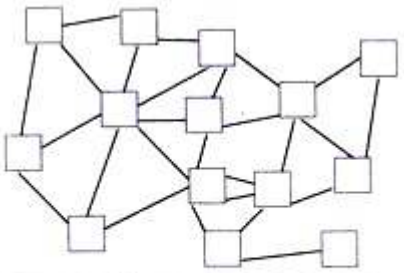


Fig. 21.7 : Immobilization of enzyme molecules by cross linking.

Glutaraldehyde is the most extensively used cross-linking reagent. It reacts with lysyl residues of the enzymes and forms a Schiff's base. The cross links formed between the enzyme and glutaraldehyde are irreversible and can withstand extreme pH and temperature. Glutaraldehyde cross- linking has been successfully used to immobilize several industrial enzymes e.g. glucose isomerase, penicillin amidase. The technique of cross-linking is quite simple and cost-effective. But the disadvantage is that it involves the risk of denaturation of the enzyme by the poly-functional reagent.

Choice of Immobilization Technique:

The selection of a particular method for immobilization of enzymes is based on a trial and error approach to choose the ideal one. Among the factors that decide a technique, the enzyme catalytic activity, stability, regenerability and cost factor are important.

Immobilization of L-amino acid acylase:

L-Amino acid acylase was the first enzyme to be immobilized by a group of Japanese workers (Chibata and Tosa, 1969). More than 40 different immobilization methods were attempted by

this group. Only three of them were found be useful. They were covalent binding to iodoacetyl cellulose, ionic binding to DEAE-Sephadex and entrapment within polyacrylamide.

Stabilization of Soluble Enzymes:

Some of the enzymes cannot be immobilized and they have to be used in soluble form e.g. enzymes used in liquid detergents, some diagnostic reagents and food additives. Such enzymes can be stabilized by using certain additives or by chemical modifications. The stabilized enzymes have longer half-lives, although they cannot be recycled. Some important methods of enzyme stabilization are briefly described.

Solvent Stabilization:

Certain solvents at low concentrations stabilize the enzymes, while at high concentrations the enzymes get denatured e.g. acetone (5%) and ethanol (5%) can stabilize benzyl alcohol dehydro-genase.

Substrate Stabilization:

The active site of an enzyme can be stabilized by adding substrates e.g. starch stabilizes a-amylase; glucose stabilizes glucose isomerase.

Stabilization by Polymers:

Enzymes can be stabilized, particularly against increased temperature, by addition of polymers such as gelatin, albumin and polyethylene glycol.

Stabilization by Salts:

Stability of metalloenzymes can be achieved by adding salts such as Ca, Fe, Mn, Cu and Zn e.g. proteases can be stabilized by adding calcium.

Stabilization by Chemical Modifications:

Enzymes can be stabilized by suitable chemical modifications without loss of biological activity. There are several types of chemical modifications.

a. Addition of poly-amino side chains e.g. polytyrosine, polyglycine.

b. Acylation of enzymes by adding groups such as acetyl, propionyl and succinyl.

Stabilization by Rebuilding:

Theoretically, the stability of the enzymes is due to hydrophobic interactions in the core of the enzyme. It is therefore, proposed that enzymes can be stabilized by enhancing hydrophobic

interactions. For this purpose, the enzyme is first unfold and then rebuilt in one of the following ways (Fig. 21.8).

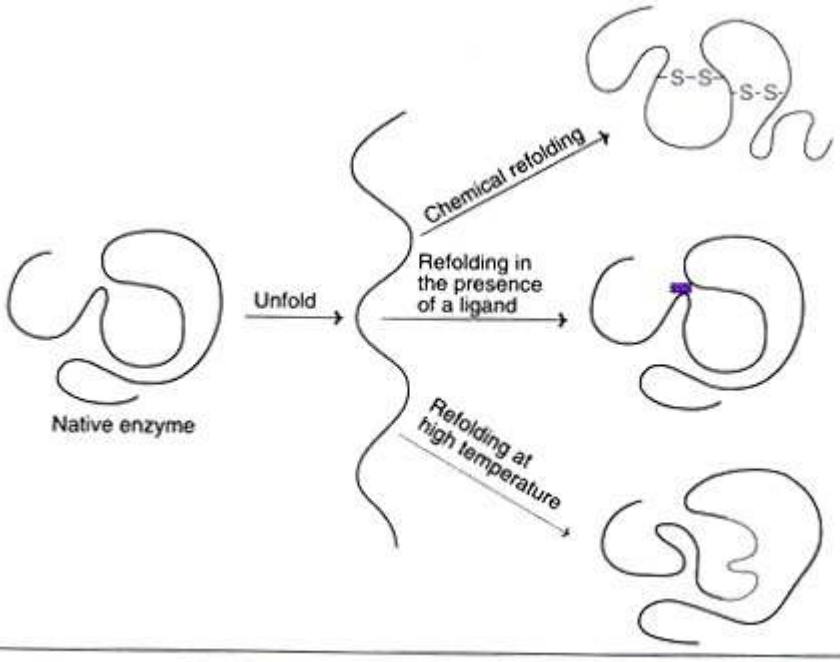


Fig. 21.8 : Stabilization of an enzyme by refolding.

1. The enzyme can be chemically treated (e.g. urea and a disulfide) and then refolded.
2. The refolding can be done in the presence of low molecular weight ligands.
3. For certain enzymes, refolding at higher temperatures (around 50°C) stabilize them.

Stabilization by Site-Directed Mutagenesis:

Site-directed mutagenesis has been successfully used to produce more stable and functionally more efficient enzymes e.g. subtilisin E.

Immobilization of Cells:

Immobilized individual enzymes can be successfully used for single-step reactions. They are, however, not suitable for multi-enzyme reactions and for the reactions requiring cofactors. The whole cells or cellular organelles can be immobilized to serve as multi-enzyme systems. In addition, immobilized cells rather than enzymes are sometimes preferred even for single reactions, due to cost factor in isolating enzymes. For the enzymes which depend on the special arrangement of the membrane, cell immobilization is preferred.

Immobilized cells have been traditionally used for the treatment of sewage. The techniques employed for immobilization of cells are almost the same as that used for immobilization of enzymes with appropriate modifications. Entrapment and surface attachment techniques are commonly used. Gels, and to some extent membranes, are also employed.

Immobilized Viable Cells:

The viability of the cells can be preserved by mild immobilization. Such immobilized cells are particularly useful for fermentations. Sometimes mammalian cell cultures are made to function as immobilized viable cells.

Immobilized Non-viable Cells:

In many instances, immobilized non-viable cells are preferred over the enzymes or even the viable cells. This is mainly because of the costly isolation and purification processes. The best example is the immobilization of cells containing glucose isomerase for the industrial production of high fructose syrup. Other important examples of microbial biocatalysts and their applications are given in Table 21.5.

TABLE 21.5 Selected examples of immobilized cells (to bring out one or two enzyme reactions) in industrial applications

Immobilized microorganism (microbial biocatalyst)	*Application(s)*
Escherichia coli	For the synthesis of L-aspartic acid from fumaric acid and NH_3
Escherichia coli	For the production of L-tryptophan from indole and serine
Pseudomonas sp	Production of L-serine from glycine and methanol
Saccharomyces cerevisiae	Hydrolysis of sucrose
Saccharomyces sp	Large scale production of alcohol
Zymomonas mobilis	Synthesis of sorbitol and gluconic acid from glucose and fructose
Anthrobacter simplex	Synthesis of prednisolone from hydrocortisone
Pseudomonas chlororaphis	Production of acrylamide from acrylonitrile
Humicola sp	For the conversion of rifamycin B to rifamycin S
Bacteria and yeasts (several sp)	In biosensors

Limitations of Immobilizing Eukaryotic Cells:

Prokaryotic cells (particularly bacterial) are mainly used for immobilization. It is also possible to immobilize eukaryotic plant and animal cells. Due to the presence of cellular organelles, the metabolism of eukaryotic cells is slow. Thus, for the industrial production of biochemical, prokaryotic cells are preferred. However, for the production of complex proteins

(e.g. immunoglobulin's) and for the proteins that undergo post- translational modifications, eukaryotic cells may be used.

Effect of Immobilization on Enzyme Properties:

Enzyme immobilization is frequently associated with alterations in enzyme properties, particularly the kinetic properties of enzymes.

Some of them are listed below:

1. There is a substantial decrease in the enzyme specificity. This may be due to conformational changes that occur when the enzyme gets immobilized.

2. The kinetic constants K_m and V_{max} of an immobilized enzyme differ from that of the native enzyme. This is because the conformational change of the enzyme will affect the affinity between enzyme and substrate.

IMMOBILIZATION OF ENZYMES-APPLICATIONS

Applications of Immobilized Enzymes and Cells:

Immobilized enzymes and cells are very widely used for industrial, analytical and therapeutic purpose, besides their involvement in food production and exploring the knowledge of biochemistry, microbiology and other allied specialties. A brief account of the industrial applications of immobilized cells is given in Table 21.5.

Manufacture of Commercial Products:

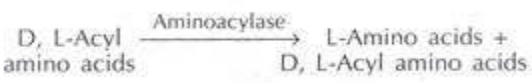
A selected list of important immobilized enzymes and their industrial applications is given in Table 21.6. Some details on the manufacture of L-amino acids and high fructose syrup are given hereunder.

TABLE 21.6 A selected list of important immobilized enzymes and their industrial applications

Immobilized enzyme	*Application(s)*
Aminoacylase	Production of L-amino acids from D, L-acyl amino acids
Glucose isomerase	Production of high fructose syrup from glucose (or starch)
Amylase	Production of glucose from starch
Invertase	Splitting of sucrose to glucose and fructose
β-Galactosidasp	Splitting of lactose to glucose and galactose
Penicillin acylase	Commercial production of semi-synthetic penicillins
Aspartase	Production of aspartic acid from fumaric acid
Fumarase	Synthesis of malic acid from fumaric acid
Histidine ammonia lyase	Production of urocanic acid from histidine
Ribonuclease	Synthesis of nucleotides from RNA
Nitrilase	Production of acrylamide from acrylonitrile

Production of L-Amino Acids:

L-Amino acids (and not D-amino acids) are very important for use in food and feed supplements and medical purposes. The chemical methods employed for their production result in a racemic mixture of D- and L-amino acids. They can be acylated to form D, L-acyl amino acids. The immobilized enzyme aminoacylase (frequently immobilized on DEAE sephadex) can selectively hydrolyse D, L-acyl amino acids to produce L-amino acids.



The free L-amino acids can separated from the un-hydrolysed D-acyl amino acids. The latter can be recemized to D, L-acyl amino acids and recycled through the enzyme reactor containing immobilized aminoacylase. Huge quantities of L-methionine, L-phenylalanine L-tryptophan and L-valine are produced worldwide by this approach.

Production of High Fructose Syrup:

Fructose is the sweetest among the monosaccharide's, and has twice the sweetening strength of sucrose. Glucose is about 75% as sweet as sucrose. Therefore, glucose (the most abundant monosaccharide) cannot be a good substitute for sucrose for sweetening. Thus, there is a great demand for fructose which is very sweet, but has the same calorific value as that of glucose or sucrose.

High fructose syrup (HFS) contains approximately equivalent amounts of glucose and fructose. HFS is almost similar to sucrose from nutritional point of view. HFS is a good substitute for sugar in the preparation of soft drinks, processed foods and baking.

High fructose syrup can be produced from glucose by employing an immobilized enzyme glucose isomerase. The starch containing raw materials (wheat, potato, corn) are subjected to hydrolysis to produce glucose. Glucose isomerase then isomerizes glucose to fructose (Fig. 21.11). The product formed is HFS containing about 50% fructose. (Note: Some authors use the term high fructose corn syrup i.e. HFCS in place of HFS).

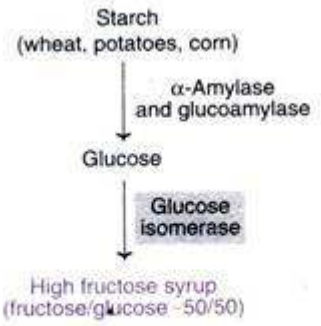


Fig. 21.11 : Production of high fructose syrup from starch (glucose isomerase is the immobilized enzyme).

Glucose isomerase:

This is an intracellular enzyme produced by a number of microorganisms. The species of Arthrobacter, Bacillus and Streptomyces are the preferred sources. Being an intracellular enzyme, the isolation of glucose isomerase without loss of biological activity requires special and costly techniques. Many a times, whole cells or partly broken cells are immobilized and used.

Immobilized Enzymes and Cells- Analytical Applications:

In Biochemical Analysis:

Immobilized enzymes (or cells) can be used for the development of precise and specific analytical techniques for the estimation of several biochemical compounds. The principle of analytical assay primarily involves the action of the immobilized enzyme on the substrate.

A decrease in the substrate concentration or an increase in the product level or an alteration in the cofactor concentration can be used for the assay. A selected list of examples of immobilized enzymes used in the assay of some substances is given in Table 21.7. Two types of detector systems are commonly employed.

TABLE 21.7 Selected examples of immobilized enzymes used in analytical biochemistry

Immobilized enzyme	*Substance assayed*
Glucose oxidase	Glucose
Urease	Urea
Cholesterol oxidase	Cholesterol
Lactate dehydrogenase	Lactate
Alcohol oxidase	Alcohol
Hexokinase	ATP
Galactose oxidase	Galactose
Penicillinase	Penicillin
Ascorbic acid oxidase	Ascorbic acid
L-Amino acid oxidase	L-Amino acids
Cephalosporinase	Cephalosporin
Monoamine oxidase	Monoamine

Thermistors are heat measuring devices which can record the heat generated in an enzyme catalysed reaction. Electrode devices are used for measuring potential differences in the reaction system. In the Fig. 21.12, an enzyme thermistor and an enzyme electrode, along with a specific urease electrode are depicted.

In Affinity Chromatography and Purification:

Immobilized enzymes can be used in affinity chromatography. Based on the property of affinity, it is possible to purify several compounds e.g. antigens, antibodies, cofactors.

IMMOBILIZATION OF ENZYMES-REACTORS

Immobilized Enzyme Reactors:

The immobilized enzymes cells are utilized in the industrial processes in the form of enzyme reactors. They are broadly of two types — batch reactors and continuous reactors. The frequently used enzyme reactors are shown in Fig. 21.9.

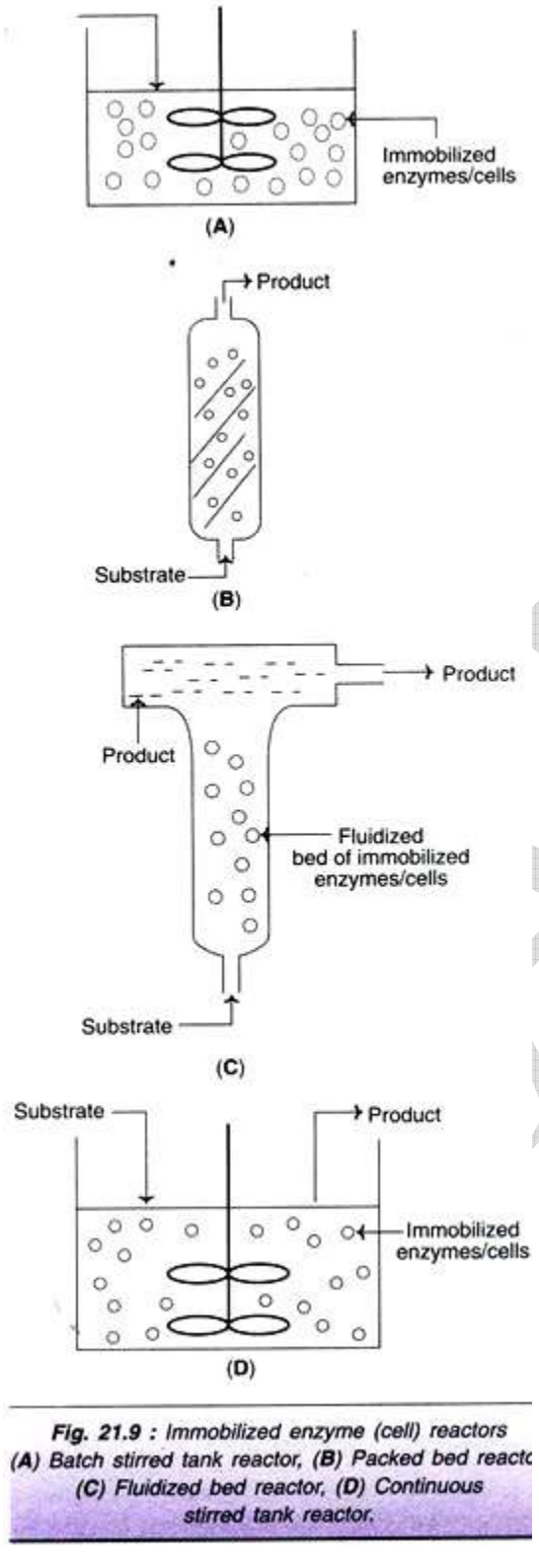


Fig. 21.9 : Immobilized enzyme (cell) reactors (A) Batch stirred tank reactor, (B) Packed bed reactc (C) Fluidized bed reactor, (D) Continuous stirred tank reactor.

Batch Reactors:

In batch reactors, the immobilized enzymes and substrates are placed, and the reaction is allowed to take place under constant stirring. As the reaction is completed, the product is separated from the enzyme (usually by denaturation).

Soluble enzymes are commonly used in batch reactors. It is rather difficult to separate the soluble enzymes from the products; hence there is a limitation of their reuse. However, special techniques have been developed for recovery of soluble enzymes, although this may result in loss of enzyme activity.

Stirred tank reactors:

The simplest form of batch reactor is the stirred tank reactor (Fig. 21.9A). It is composed of a reactor fitted with a stirrer that allows good mixing, and appropriate temperature and pH control. However, there may occur loss of some enzyme activity. A modification of stirred tank reactor is basket reactor. In this system, the enzyme is retained over the impeller blades. Both stirred tank reactor and basket reactor have a well-mixed flow pattern.

Plug flow type reactors:

These reactors are alternatives to flow pattern type of reactors. The flow rate of fluids controlled by a plug system. The plug flow type reactors may be in the form of packed bed or fluidized bed (Fig. 21.9B and 21.9C). These reactors are particularly useful when there occurs inadequate product formation in flow type reactors. Further, plug flow reactors are also useful for obtaining kinetic data on the reaction systems.

Continuous Reactors:

In continuous enzyme reactors, the substrate is added continuously while the product is removed simultaneously. Immobilized enzymes can also be used for continuous operation. Continuous reactors have certain advantages over batch reactors. These include control over the product formation, convenient operation of the system and easy automation of the entire process. There are mainly two types of continuous reactors-continuous stirred tank reactor (CSTR) and plug reactor (PR). A diagrammatic representation of CSTR is depicted in Fig. 21.9D. CSTR is ideal for good product formation.

Membrane Reactors:

Several membranes with a variety of chemical compositions can be used. The commonly used membrane materials include polysulfone, polyamide and cellulose acetate. The biocatalysts

(enzymes or cells) are normally retained on the membranes of the reactor. The substrate is introduced into reactor while the product passes out. Good mixing in the reactor can be achieved by using stirrer (Fig. 21.10A). In a continuous membrane reactor, the biocatalysts are held over membrane layers on to which substrate molecules are passed (Fig. 21.10B).

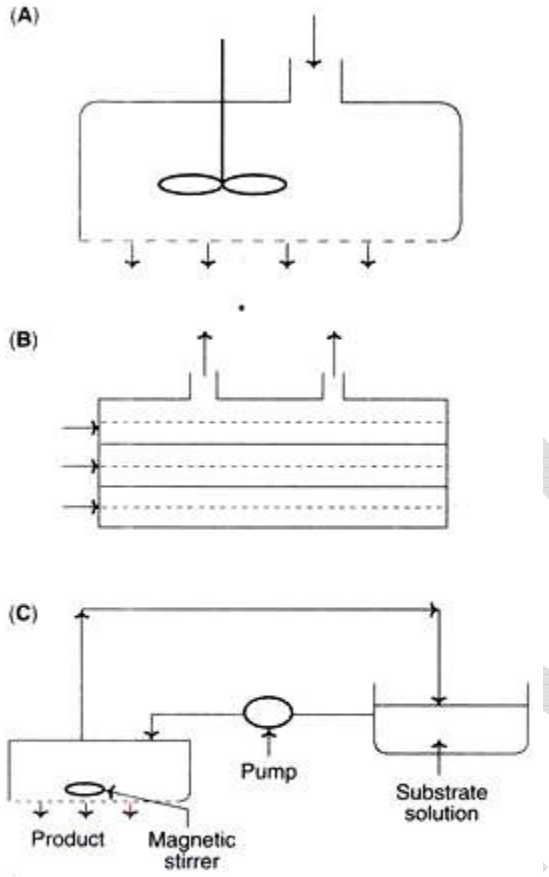


Fig. 21.10 : Membrane reactors (A) Batch membrane reactor, (B) Continuous membrane reactor, (C) Recycle membrane reactor (Coloured lines indicate membranes).

In a recycle model membrane reactor, the contents (i.e. the solution containing enzymes, cofactors, and substrates along with freshly released product are recycled by using a pump (Fig. 21.10C). The product passes out which can be recovered.

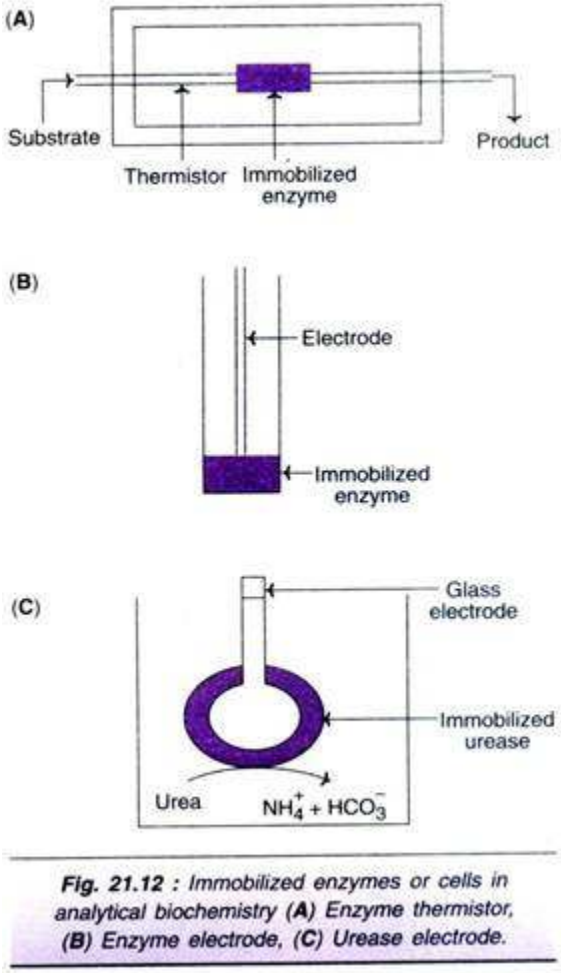


Fig. 21.12 : Immobilized enzymes or cells in analytical biochemistry (A) Enzyme thermistor, (B) Enzyme electrode, (C) Urease electrode.

ENZYME ENGINEERING

Improvement in the activity and usefulness of an existing enzyme or creation of a new enzyme activity by making suitable changes in its amino acid sequence is called Enzyme Engineering. When this approach is used to modify the properties of any protein, whether enzyme or nonenzyme, it is termed as protein engineering. Since enzymes are proteins, enzyme engineering is a part of the larger activity of protein engineering. Enzyme engineering utilizes r-DNA technology to introduce the desired changes in amino acid sequences of enzymes. In addition, the level of production of an enzyme may be increased by introducing more copies of the gene into the concerned organism.

OBJECTIVES OF ENZYME ENGINEERING

The chief objective of enzyme engineering is to produce an enzyme that is more useful for industrial and other applications. The various properties of an enzyme that may be modified to achieve this objective are as follows :-

1. Improved kinetic properties.
2. Elimination of allosteric regulation.
3. Enhanced substrate and reaction specificity.
4. Increased thermostability.
5. Alteration in optimal pH.
6. Suitability for use in organic solvents.
7. Increased/decreased optimal temperature.

PRINCIPLES OF ENZYME ENGINEERING

The structure and function of an enzyme molecule, for that matter of any protein molecule, are chiefly determined by its amino acid sequence, i.e, its primary structure. Therefore, any change in the properties of an enzyme is always reflected in its primary structure. Conversely, a change in the amino acid sequence should alter the properties of the enzyme. But, this is not always the case because the enzymatic properties, etc. are changed only when amino acid changes are introduced in certain critical regions of the protein. Therefore, it is of great importance to know the critical regions for the various functions of an enzyme, and to be able to predict the effect of specific amino acid changes in these areas on the various functions. However, the present knowledge of the relationships between amino acid sequences, 3D structure of protein and properties of enzymes, obtained from a large database is only partially operative. It allows an explanation of the changes in structure and function on the basis of the changes in amino acid sequence, but it does not allow a dependable prediction of the influences of specific amino acid changes on the structure and function of the enzymes.

It may, however, be reasonable to anticipate that as more elaborate databases and improved softwares become available, it should become possible to predict with a far greater confidence the structural and functional changes in enzymes produced by the specified changes

in their amino acid sequences. The effectiveness of enzyme engineering will be greatly enhanced then, and this activity may have a tremendous influence on enzyme technology.

STEPS IN ENZYME ENGINEERING

The strategies for enzyme engineering and their theoretical considerations are quite involved. The steps involved in enzyme engineering are briefly described in simple terms :-

1. The first step consists of isolation of the concerned enzyme and determination of its structure and properties. Both amino acid sequence and the 3D structure are usually obtained from X-ray diffraction, nuclear magnetic resonance (NMR), etc.
2. The data so obtained are analyzed together with the database of known and putative structural effects of amino acid substitutions on enzyme structure and function. Molecular modelling is performed to determine a possible change in amino acid sequence for the desired improvement in the structure/function of an enzyme.
3. The next step consists of constructing a gene that will encode the amino acid sequence specified at the end of step 2. This is best achieved by isolation and cloning of the endogenous gene encoding the concerned enzyme, and using this gene for site directed mutagenesis.
4. Once the appropriated gene is constructed, it is introduced and expressed in a suitable host, e.g E.coli.
5. The recombinant or mutant enzyme so produced is isolated, purified and used for determination of its structure and properties. The information so obtained is added to the database. If the enzyme structure and function are not altered as desired, the next cycle of experimentation (step 2-5) is undertaken

UNIT-V

SYLLABUS

Drug carrier – liposomes; Drug carrier – biodegradable polymer Drug release – kinetics. Drug release- sustained release Drug release – first order release approximation; multiple dosing

DRUG CARRIER

A drug carrier is any substrate used in the process of drug delivery which serves to improve the selectivity, effectiveness, and/or safety of drug administration. Drug carriers are primarily used to control the release of a drug into systemic circulation. This can be accomplished either by slow release of the drug over a long period of time (typically diffusion) or by triggered release at the drug's target by some stimulus, such as changes in pH, application of heat, and activation by light. Drug carriers are also used to improve the pharmacokinetic properties, specifically the bioavailability, of many drugs with poor water solubility and/or membrane permeability. A wide variety of drug carrier systems have been developed and studied, each of which has unique advantages and disadvantages. Some of the more popular types of drug carriers include liposomes, polymeric micelles, microspheres, and nanoparticles. Different methods of attaching the drug to the carrier have been implemented, including adsorption, integration into the bulk structure, encapsulation, and covalent bonding.

Different types of drug carrier utilize different methods of attachment, and some carriers can even implement a variety of attachment methods

Surface modification of pharmaceutical carriers, such as liposomes, micelles, nanocapsules, polymeric nanoparticles, solid lipid particles, and others can be used to control their biological properties in a desirable fashion and make them perform various therapeutically or diagnostically important functions in chorus. Various methods for immobilizing proteins on the surface of such carriers were previously described and to date, targeting of drugs using an antibody or other vector bound to a drug carrier has continued to be developed in the fields of cancer and tumor diagnostics and therapy as well as in cardiovascular research and other clinical manifestations. Along with the development of multifunctional pharmaceutical carrier concepts and designs mostly for cancer research, the occurrence of cardiovascular disease and especially

atherosclerosis, the challenges of restenosis of arteries after angioplasty, the growth of plaques on stent implants, and additional aspects of inflammatory processes in cardiovascular disease have led to the need for a vascular targeted therapeutic approach. Along with the ability of the vascular endothelium to act both as a barrier and as a potential target for drug delivery, this approach is based on vasculature physical–chemical characteristics (e.g., surface charge), its physiology characteristics, and on the unique endothelial determinants exerted from, or present on, the endothelium lumen. To facilitate targeting, drug carriers comprised of stimuli-sensitive and/or conjugated with affinity moieties that bind to endothelial cells (ECs) can be effective for targeting either normal and/or pathologically altered EC. The use of cells such as neutrophils as drug carriers, red blood cells as carriers of antineoplastic drugs (i.e., in the treatment of red blood cell-consuming tumors), and lymphocytes as carriers of drugs to treat lymphoid tumors are also potentially promising drug carriers

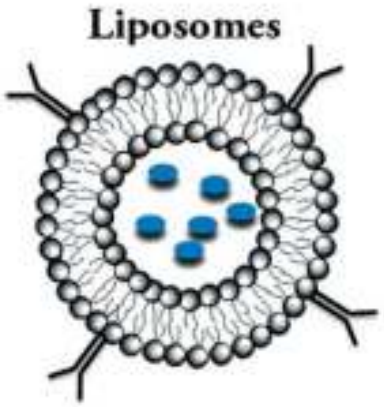
DRUG CARRIER- LIPOSOMES

Liposomes are well-recognized drug delivery carriers (3). They are artificial monolamellar or multilamellar phospholipid vesicles of different size and composition that have been recognized as pharmaceutical carriers with great practical potential. To date, liposomes have been approved by regulatory agencies to carry a range of chemotherapeutics. These carriers can entrap practically hydrophilic agents within their internal water compartment or small hydrophobic agents in their membrane. Liposomes have also shown the ability to deliver pharmaceuticals into cells or inside individual cellular compartments. In general, liposomes are able to passively accumulate into areas with increased vascular permeability and, therefore, are experimentally suitable to passively target the vascular system under pathological conditions such as atherosclerotic lesions, vascular inflammation, thrombosis, and cancer. The use of liposomes for targeted delivery of a variety of agents to the vasculature was previously reviewed by selected authors . One example is the early work by Nguyen et al., which investigated the thrombolytic efficacy of liposome encapsulated streptokinase in a canine model of myocardial infarction. The time required to restore vessel patency was reduced by more than 50% with a liposomal enzyme compared with free enzyme. In addition to a lower dose needed for the encapsulated agent, a smaller remnant of thrombi was observed with this formulation. Another example demonstrates the great potential to reduce both intravascular platelet aggregation and thrombosis sequelae by

entrapping antiplatelet peptides into liposomes. In this work, CD-39-containing liposomes effectively inhibited platelet aggregation, when platelets were activated by various agents. Another approach of liposomal delivery to the vasculature is the delivery of the antioxidant enzymes, SOD and catalase. The delivery of these agents in an animal model of lung oxidative stress was shown to modulate the tissue damage. A common approach for liposomal targeting, however, involves the use of liposomes modified with specific targeting moieties that have a specific affinity for the affected organ or tissue. These modifications include the addition of antibodies or specific targeting peptide moieties on the liposomal surface. The use of cationic liposomes to enhance their target to the tumor vasculature will also be briefly discussed. The use of modified liposomes with specific monoclonal antibodies against some components characteristic of the cardiovascular and tumor vascular system was, and is still, a widely popular targeting concept and previously reviewed . Although there is a vast literature regarding the characteristics and potential applications for the use of monoclonal antibodies to specifically target a variety of carriers, an antibody-modified carrier has not been approved for human use yet. When designing immune liposomes, antibodies are conjugated either to the liposomal surface or to the distal end of the liposomal PEG .

Khaw et al. described 30 years ago the specificity of localization of myosin-specific antibody fragments in experimental myocardial infarction and showed the preservation of this antibody activity after covalent coupling to liposomes . Later on, this group also demonstrated suppressed hypoxic cardiocyte death by sealing membrane lesions with antimyosinliposomes . Another interesting platform for immune liposomes is their use as an acoustically reflective carrier that can be targeted for site-specific acoustic enhancement. Ultrasound parameters to enhance the delivery of therapeutic-loaded echogenic immune liposomes into the arterial wall for the treatment of atherosclerosis were investigated in an ex vivo mouse aorta model. By using anti-ICAM-targeted echogenic liposomes and following the 1-MHz wave ultrasound, greater adherence of the targeted liposomes to vascular endothelium and greater passage across the vessel wall was shown . It was also previously observed that targeting liposomes to ICAM-1, VCAM-1, fibrin, fibrinogen, and TF, in addition to application of ultrasound waves, was able to produce the targeted enhancement in the vessel walls 5 min after intravenous administration of targeted liposomes. Several in vivo studies have investigated the tumor vascular targetability of

liposomes decorated with a variety of peptides. These peptides includes RGD (Arg-Gly-Asp) motifs binding to avb3 and avb5 integrins , NGR motifs that bind to aminopeptidase-N, CREKA that binds to fibrinogen or fibrin, GPLPLR that binds to membrane type 1 matrix metalloproteinase, peptides binding to unknown receptors such as APRPG, and the synthetic angiostatic peptide anginex that binds to galectin-1, which is a carbohydrate-binding protein with affinity to b-galactosidase. Much research is being carried out with peptide modified liposomes. Unlike antibodies, peptides have been shown to have slower clearance from the circulation, and



various researchers have shown the advantage of using these peptides as targeting moieties on the liposomes surface. RGD peptide, for example, showed a significant improvement in targeting liposomes to vascular lesions and to activated platelets . In addition to gene therapy, cationic liposomes were shown to be a promising carrier system for the delivery of anticancer agents to tumor ECs. These positively charged carriers take advantage of the natural affinity of cationic molecules at the carrier's surface toward anionic molecules on the targeted cells surface (e.g., glycoproteins, anionic phospholipids, and proteoglycans) in the tumor microvasculature

DRUG CARRIER-BIODEGRADABLE POLYMERS

Biodegradable polymers are the polymers that are degradable in vivo, either enzymatically or nonenzymatically, to produce biocompatible or non toxic by-products. Now they are becoming very demanding in pharmaceutical applications especially in the field of drug delivery. The word biodegradation is a natural process in the environment by which complex organic chemicals are converted into simpler compounds, mineralized and redistributed in the environment through material cycles such as the Carbon, nitrogen and Sulphur cycles.

Biodegradation takes place by two processes, either by the action of enzymes and/or chemical deterioration associated with living organisms. For the past few decades, biodegradable polymers (either synthetic or natural) are capable of being cleaved into biocompatible by-products through chemical or enzyme-catalyzed hydrolysis. This property makes it possible to implant them into the body without any subsequent removal by the surgical operation. Polymers are also range from their use as binders in tablets to viscosity and flow controlling agents in liquids, emulsions and suspensions; also can be used as film coatings for the following reasonsA. To enhance drug stability. B. To camouflage the unpleasant taste of a drug and C. To modify the release characteristics.

Need for Biodegradable Polymers

1) Leaving non-biodegradable foreign materials in the body for an indefinite time period caused toxicity problem that's why surgical removal of a drug depleted delivery system was difficult.

2) Avoid reduction in bone density.

3) No need for a second surgery for removal of polymers.

4) Give excellent potential as the basis for controlled drug delivery.

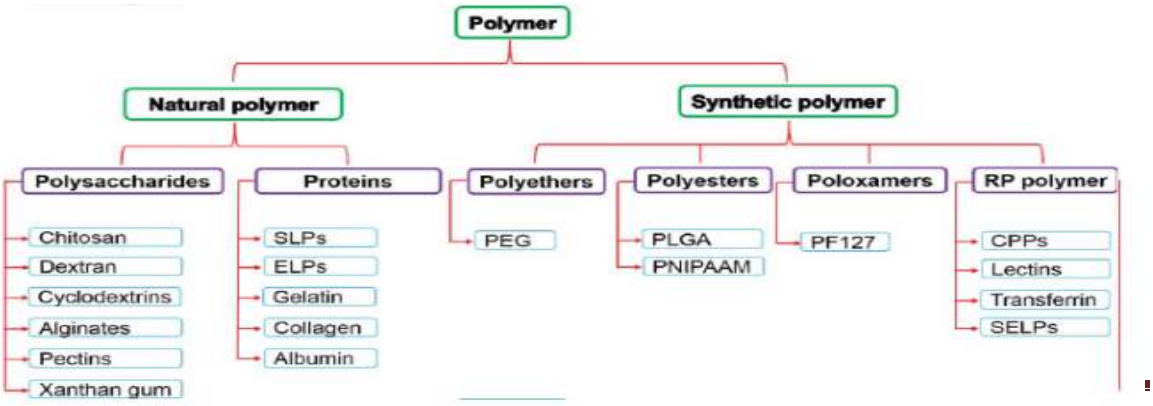
Advantage of Biodegradable polymers

1) Biodegradable polymers play the role of a drug depot providing a more or less long-term supply of drug to the blood at a constant rate.

2) As biocompatible materials as a rule

3) The polymer carrier would degrade into non toxic, absorbable subunits which would be subsequently metabolized.

4) Degradable system eliminates the necessity for surgical removal of implanted device following depletion of a drug.



The majority of research in this field was done using poly (D,Llactide), PLA, poly(D,L-glycolide), poly(lactide-co-glycolide), and poly(cyanoacrylate) polymers. Muzykantov and coworkers tested the in vitro and in vivo parameters of targeting to ECs of anti-intercellular adhesion molecule-1 (ICAM-1)/polymeric nanocarriers consisting of either prototype polystyrene or biodegradable poly(lactic-co-glycolic) acid polymers. Anti-ICAM-1-modified particles bound specifically to tumor necrosis factor-activated ECs in a dosedependent manner and accumulated in the pulmonary vasculature after i.v. injection in mice.

Dextran.

Dextran is a non-toxic and highly water-soluble polysaccharide. It predominantly contains linear a-1,6-linked glucopyranose units with some degree of 1,3-branching (Fig. 3). The main source of its production is the sucrose-rich environment of Lactobacillus, Leuconostoc, and Streptococus. Commercially, it is available with different molecular weights. The degree of branching and molecular weight are known to affect the physicochemical properties of dextran.Dextran is known to have a wide range of therapeutic applications. Clinically it has been used in plasma volume expansion, peripheral blood flow enhancement, thrombosis prophylaxis, and as artificial tears. Low molecular dextrans have short biological half-life (8 h) and are secreted from the kidneys, whereas high molecular weight dextrans exhibit longer half-lives and are subsequently degraded by reticuloendothelial system. Moreover, dextrans are also metabolized by enzymes (a-1-glucosidases) in various parts of the body. Dextran-based carrier system has gained significant interest over the recent decadesin which therapeutic proteins can be incorporated in a variety of ways. Dextran-based car-rier systems can be obtained either by chemical and/or chemical cross-linking. Till now ,a large number of therapeutic proteins have been successfully incorporated in dextran-based carrier systems and significant therapeutic outcomes have been obtained either from in vitro or in vivo experimental studies. Most of the studies conducted on dextran-based carrier systems for delivery of therapeutic proteins have shown that dextran is biocompatible with incorporated proteins.

Alginates.

Alginate is also known as align and/or alginic acid and isanionic polysaccharide that is widely distributed in the cell walls of brown algae. It is mainly extracted from three different species of brown algae (Laminaria hyperborean, Ascophyllum nodosum, and Macrocystis pyrifera) and is composed of alternating blocksof 1–4 linked a-L-guluronic and b-D-mannuronic acid residues (Fig. 5). Alginate has attained considerable attention due to its excellent mucoadhesive property, biocompatibility, and biodegradability.64Recently it has been used as a component of a carrier system for efficient delivery of therapeutic proteins and peptides.25,65Alginate requires only amild condition for fabrication in aqueous solutions, which is favorable for heat-sensitive therapeutic proteins. Moreover, alginate have shown to protect the labile proteins and peptides from gastric environment and delivers them safely to the intestine.16Despite its wide range of pH-sensitivity for labile proteins, alginate has some limitations as a protein carrier system including drug loss during preparation of beads and/or leaching of drug through the pores in beads. To cope with this problem, many modifications have been made in the structure of alginate.

Pectins. Pectin is another important polymer having distinct mucoadhesiveproperty on the intestinal epithelium. These are linear polysaccharides that are extracted from the plant cell walls. These are mainly composed of a-(1-4)- linked D-galacturonic acid residues interrupted by 1,2- linked L-rhamnose residues . The carboxylic groups present in pectin are responsible for showing a mucoadhesive property by interact-ing with functional groups present on the mucus layer and remain intact in the physiological environment of GIT. The mucoadhesive strength of pectin depends upon its molecular weight and degree of esterification. Despite the molecular weight and degree of esterification of pectin, the mucoadhesive property of pectin also depends on the site ofintestine. These are non-toxic and generally considered inert for physiological fluids. Pectin prevents the enzymatic proteolysis of incorporated proteins and significantly increases the intestinal absorption of several therapeutic proteins and peptides. It has also been found that pectin delivers a variety of therpaeutic proteins to the target sites via different routes

Poly (N-isopropylacrylamide-co-propylacrylic acid) copolymers. Poly (N-iso-propylacrylamide-co-propylacrylic acid) copolymers (PNIPAAM) and its derivatives arethe most invasively investigated polymers for delivery of therapeutic substances.183–186Though, PNIPAAM has a great potential for delivery of therapeutic proteins and pepti-des, 187but the clinical use of PNIPAAM and its derivatives is limited as these are non-biodegradable and upon contact with blood, they activate the platelets.

Poly(lactic-co-glycolic acid). Poly (lactic-co-glycolic acid) (PLGA) is a copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA). PLGA is an acronym for poly D,L-lactic-coglycolic acid in which D- and L-lactic acid forms an equal ratio. PLGA is biodegraded by hydrolysis of the ester linkages. The biodistribution of PLGA follows a non-linear and dose-dependent profile. It belongs to the family of FDA-approved biodegradable polymers that has excellent biocompatibility and change-able biodegradability. A considerable amount of research has been conducted on PLGA for the delivery of therapeutic proteins and peptides since PLGA has exhibited immense potential as therapeutic substance carrier. PLGA has been extensively studied for delivery of many therapeutic proteins, peptides, and other macromolecules such as DNA and RNA via different routes.166–168There are certain limitations including low entrapment efficiency, initial burst release, instability of entrapped protein, and incomplete release profile that limit PLGA to be an ideal polymer. These shortcomings can be overcome by copolymerizing other polymers with PLGA .Various types of block copolymers of PLGA with PEG have been developed including PLGA-PEG, PLGA-PEG-PLGA, PEG-PLGA-PEG. These block copolymers of PLGA behave as thermoreversible block copoly-mers. They have flowing properties at or below room temperature and rapidly convertinto the gel at body temperature. These block copolymers undergo gel-sol transition in water; therefore, triblock of PEG-PLGA-PEG are used for the encapsulation of therapeutic substance. PEG-PLGA-PEG triblock copolymer forms gel as the temperature increases and works as sustained drug delivery depot in vivo. The critical gelation concentration is controlled by changing the composition of PEG-PLGA-PEG copolymers. It has been found that in situ gel formed after subcutaneous administration of PEG-PLGA-PEG into the rats can persist more than one month.These block copolymers are biodegradable in nature and their biodegradability has been well documented.

They are biodegraded into non-toxic small molecule monomers. These block copolymers havebeen extensively studied for efficient delivery of therapeutic proteins and peptides

DRUG RELEASE

Drug release is an important property of a therapeutic system, constituting a prerequisite to absorption of the therapeutic agent and one that contributes to the rate and extent of active availability to the body. Drug release refers to the process by which the drug loaded in or on the NMs is released in the body through diffusion or dissolution of the NMs matrix releasing the drug in solution. Biodegradation refers to the process by which the drug delivery system is broken down inside the body.

Both drug release and biodegradation are important to consider when developing an NMs drug delivery system. Ordinarily, effectiveness of drugs is dependent not only on its active components but also on its solubility and diffusion. When the drug is delivered using an NMs delivery system, effectiveness is affected by parameters such as the particle size, release process which is in turn affected by the biodegradation of the particle matrix. The smaller the particles, the larger the surface area-to-volume ratio; therefore, most of the drug associated with small particles would be at or near the particle surface which leads to faster drug release. In contrast, larger particles have large cores, which allow more drugs to be encapsulated per particle and give slower release. Thus, control of particle size provides a means of tuning drug release rates.

5.7.1 Factors Affecting Drug Release

In general, the drug release rate depends on: (1) drug solubility, (2) desorption of the surface-bound or adsorbed drug, (3) drug diffusion out of the NM matrix into the body, (4) NM matrix erosion or degradation, and (5) the combination of erosion and diffusion processes.[118] The method of drug incorporation into the NM delivery system also affects the release profile.

Drug release when loaded by covalent attachment on the particle system is affected almost solely by drug–NM diffusion. This system has a relatively small burst effect and sustained release characteristics.[119] When the drug is encapsulated inside a NM, the release is controlled by diffusion of the drug from the NM interior.

The polymer coating acts as a drug release barrier; hence, the drug solubility and diffusion in or across the polymer membrane becomes a determining factor in drug release. The release rate can also be affected by ionic interactions between the drug and secondary ingredients. In the event

that polymer-encapsulated drug interacts with auxiliary ingredients, a less water-soluble complex may form causing a slower drug release that almost has no burst release effect.[62] On the other hand, addition of auxiliary ingredients, e.g. ethylene oxide–propylene oxide block copolymer (PEO-PPO), to chitosan (CS) reduces the interaction of the drug with the matrix material via competitive electrostatic interaction of PEO-PPO with CS, and an increase in drug release could be achieved.[61]

In an encapsulated drug where the drug is uniformly distributed inside the NM matrix, drug release occurs by diffusion and/or erosion of the matrix. When the diffusion of the drug is faster than matrix erosion, diffusion largely controls the mechanism of release. The rapid, initial release, or "burst," is mainly attributed to weakly bound or adsorbed drug to the relatively large surface of NMs.[120]

There are several in vitro methods that can be used to study the release of drugs loaded in an NM. These include: (1) side-by-side diffusion in cells with artificial or biological membranes, (2) diffusion through a dialysis bag, (3) reverse dialysis bag diffusion, (4) agitation followed by ultracentrifugation or centrifugation, (5) ultrafiltration, or (6) pH change. In general, drug release study is carried out by controlled shaking to allow the drug to ooze out of the NM into a release media followed by centrifugation to separate the NM from the drug in solution. However, the difficulties in the separation of NMs from the release media favor the use of the dialysis technique. Exception to these is the use of IOMNPs where loading with drugs is performed at high pH and drug is released at low physiological pH. After the drug release, the IOMNPs can easily be separated from the media with the use of magnets (SuperMag, Ocean NanoTech) and the concentration of drug in the release media can be established depending upon the properties of the drug (unpublished paper, Ocean Nanotech).

Only a few studies have been published on drug release,especially on the release mechanisms. The mechanisms on in vitro drug release were generated by studying the model drugs tetracaine, etomidate, and prednisolone. However, lipid NMs exhibited burst release when incorporating tetracaine and etomidate. A prolonged drug release was obtained first with prednisolone that demonstrated the suitability of the solid lipid NMs for prolonged drug release. Drug release can be controlled as a function of the lipid matrix, surfactant concentration, and production parameters such as temperature[122] achieving as long as 5–7 weeks. The formulation can be

modulated for prolonged release without any burst or with different percentages of burst followed by prolonged release.[67] The burst can be used to deliver an initial dose when desired

DRUG RELEASE KINETICS

Drug dissolution is important test used to evaluate drug release of solid and semisolid dosage forms. This test is developed for quantification of the amount and extent of drug release from dosage forms. The values that are obtained from the dissolution study can be quantitatively analyzed by using different mathematical formulae. Because qualitative and quantitative changes in a formulation may alter release of drug and in vivo performance, developing tools that facilitate product development by reducing the necessity of bio-studies is always desirable. Thus mathematical models can be developed. This development requires the comprehension of all phenomena affecting drug release kinetics and this has a very important value in the formulation optimization. The model can be simply thought as a 'mathematical metaphor of some aspects of reality'. For this generality, mathematical modeling is widely employed in different disciplines such as genetics, medicine, psychology, biology, economy and obviously engineering and technology . Model dependent methods are based on different mathematical functions, which describe the dissolution profile. Once a suitable function has been selected, the dissolution profiles are evaluated depending on the derived model parameters. To compare dissolution profiles between two drug products model dependent (curve fitting), statistic analysis and model independent methods can be used.

Zero order model: Dissolution of the drug from pharmaceutical dosage forms that do not disaggregate and release the drug slowly can be represented by the following equation:

$W0 - Wt = Kt$ ---------- (1)

Where, W0 is the initial amount of drug in the pharmaceutical dosage form Wt is the amount of drug in the pharmaceutical dosage form at time t and K is proportionality constant. Dividing this equation by W0 and simplifying:

$ft = K0t$ ----------(2)

where $ft = 1- (Wt /W0)$ and ft represents the fraction of drug dissolved in time t and K0 the apparent dissolution rate constant or zero order release constant. In this way, a graphic of the drug-dissolved fraction versus time will be linear if the previously established conditions were

fulfilled [7]. The pharmaceutical dosage forms following this profiles release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. The following relation can, in a simple way, express this model: Qt = Q0 + K0t ----------(3) Where, Qt is the amount of drug dissolved in time t, Q0 is the initial amount of drug in the solution and K0 is the zero order release constant.

To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time . Applications: This relation can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs, coated forms, osmotic systems, etc .

DRUG RELEASE-FIRST ORDER RELEASE APPROXIMATION,MULTIPLE DOSING

First order model:

The application of this model to drug dissolution studies was first proposed by Gibaldi and Feldman (1967) and later by Wagner (1969). This model has been also used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualise this mechanism in a theoretical basis . The dissolution phenomena of a solid particle in a liquid media imply a surface action, as can be seen by the Noyes–Whitney Equation:

dc/dt = K (CS - C) ----------(4)

Where C is the concentration of the solute in time t, Cs is s order the solubility in the equilibrium at experience temperature and K is first order proportionality constant. This equation was altered by Brunner et al. (1900), to incorporate the value of the solid area accessible to dissolution, S, getting:

dc/dt = K1 S (CS - C) ----------(5)

Where, k1 is a new proportionality constant. Using the Fick first law, it is possible to establish the following relation for the constant

k1: k1 = D/Vh ----------(6)

Where, D is the solute diffusion coefficient in the dissolution media, V is the liquid dissolution volume and h is the width of the diffusion layer. Hixson and Crowell adapted the Noyes–Whitney equation in the following manner:

dW/ dt = KS(Cs -C) ---------- (7)

Where, W is the amount of solute in solution at time t, dW/dt is the passage rate of the solute into solution in time t and K is a constant. This last equation is obtained from the Noyes–Whitney equation by multiplying both terms of equation by V and making K equal to k1V. Comparing these terms, the following relation is obtained:

K = D/h ----------(8)

In this manner, Hixson and Crowell equation (eq.7) can be written as:

dW/ dt = KS/V (VCs-W) = k (VCs-W)-------(9)

Where, k = k1S. If one pharmaceutical dosage form with constant area is studied in ideal conditions

(sink conditions), it is possible to use this last equation that, after integration, will become:

W = VCs (1 – e -kt) ----------(10)

This equation can be transformed, applying decimal logarithms in both terms, into:

Log (VCs- W) = log VCs- (kt/2.303)--------(11)

The following relation can also express this model: Qt = Q0e -K 1 t or ln (Qt /Q0) = K1t or ln Qt = ln Q0 K1t Or in decimal logarithms:

log Qt = logQ0 + (K1/2.303)----------(12)

Where, Qt is the amount of drug released in time t, Q0 is the initial amount of drug in the solution and K1 is the first order release constant. The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of-K/2.303. Applications: This relationship can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water soluble drugs in porous matrices . N. Ahuja, Om Prakash Katare, B. Singh, was studied dissolution enhancement and mathematical modelling of drug release of a poorly water-soluble drug using water-soluble carriers. They studied dissolution profile of drug by using zero order, first order and Hixson- Crowell model and they found that first order model fitted well at early time periods.

QUESTION	OPTION A	OPTION B	OPTION C	OPTION D	ANSWERS
Pharmacokinetics is:	The study of biological and therapeutic effects of drugs	The study of absorption, distribution, metabolism and excretion of drugs	The study of mechanisms of drug action	The study of methods of new drug development	The study of absorption, distribution, metabolism and excretion of drugs
What does “pharmacokinetics” include?	Complications of drug therapy	Drug biotransformation in the organism	Influence of drugs on metabolism processes	Influence of drugs on genes	Drug biotransformation in the organism
What does “pharmacokinetics” include?	Pharmacological effects of drugs	Unwanted effects of drugs	Chemical structure of a medicinal agent	Distribution of drugs in the organism	Distribution of drugs in the organism
What does “pharmacokinetics” include?	Localization of drug action	Mechanisms of drug action	Excretion of substances	Interaction of substances	Excretion of substances
The main mechanism of most drugs absorption in GI tract is:	Active transport (carrier-mediated diffusion)	Filtration (aqueous diffusion)	Endocytosis and exocytosis	Passive diffusion (lipid diffusion)	Passive diffusion (lipid diffusion)
What kind of substances can’t permeate membranes by passive diffusion?	Lipid-soluble	Non-ionized substances	Hydrophobic substances	Hydrophilic substances	Hydrophilic substances
A hydrophilic medicinal agent has the following property:	Low ability to penetrate through the cell membrane lipids	Penetrate through membranes by means of endocytosis	Easy permeation through the blood-brain barrier	High reabsorption in renal tubules	Low ability to penetrate through the cell membrane lipids
What is implied by «active transport»?	Transport of drugs trough a membrane by means of diffusion	Transport without energy consumption	Engulf of drug by a cell membrane with a new vesicle formation	Transport against concentration gradient	Transport against concentration gradient
What does the term “bioavailability” mean?	Plasma protein binding degree of substance	Permeability through the brain-blood barrier	Fraction of an uncharged drug reaching the systemic circulation following any route administration	Amount of a substance in urine relative to the initial doze	Fraction of an uncharged drug reaching the systemic circulation following any route administration
The reasons determing bioavailability are:	Rheological parameters of blood	Amount of a substance obtained orally and quantity of intakes	Extent of absorption and hepatic first-pass effect	Glomerular filtration rate	Extent of absorption and hepatic first-pass effect
Pick out the appropriate alimentary route of administration when passage of drugs through liver is minimized:	Oral	Transdermal	Rectal	Intraduodenal	Rectal
Which route of drug administration is most likely to lead to the first-pass effect?	Sublingual	Oral	Intravenous	Intramuscular	Oral
What is characteristic of the oral route?	Fast onset of effect	Absorption depends on GI tract secretion and motor function	A drug reaches the blood passing the liver	The sterilization of medicinal forms is obligatory	Absorption depends on GI tract secretion and motor function
Tick the feature of the sublingual route:	Pretty fast absorption	A drug is exposed to gastric secretion	A drug is exposed more prominent liver metabolism	A drug can be administrated in a variety of doses	Pretty fast absorption
Pick out the parenteral route of medicinal agent administration:	Rectal	Oral	Sublingual	Inhalation	Inhalation
Parenteral administration:	Cannot be used with unconsciousness patients	Generally results in a less accurate dosage than oral administration	Usually produces a more rapid response than oral administration	Is too slow for emergency use	Usually produces a more rapid response than oral administration
What is characteristic of the intramuscular route of drug administration?	Only water solutions can be injected	Oily solutions can be injected	Opportunity of hypertonic solution injections	The action develops slower, than at oral administration	Oily solutions can be injected
Correct statements listing characteristics of a particular route of drug administration include all of the following EXCEPT:	Intravenous administration provides a rapid response	Intramuscular administration requires a sterile technique	Inhalation provides slow access to the general circulation	Subcutaneous administration may cause local irritation	Inhalation provides slow access to the general circulation
Biological barriers include all except:	Renal tubules	Cell membranes	Capillary walls	Placenta	Renal tubules
What is the reason of complicated penetration of some drugs through brain-blood barrier?	High lipid solubility of a drug	Meningitis	Absence of pores in the brain capillary endothelium	High endocytosis degree in a brain capillary	Absence of pores in the brain capillary endothelium
The volume of distribution (V relates:	Single to a daily dose of an administrated drug	An administrated dose to a body weight	An uncharged drug reaching the systemic circulation	The amount of a drug in the body to the concentration of a drug in plasma	The amount of a drug in the body to the concentration of a drug in plasma
For the calculation of the volume of distribution (V one must take into account:	Concentration of a substance in plasma	Concentration of substance in urine	Therapeutical width of drug action	A daily dose of drug	Concentration of a substance in plasma

The term “biotransformation” includes the following:	Accumulation of substances in a fat tissue	Binding of substances with plasma proteins	Accumulation of substances in a tissue	Process of physicochemical and biochemical alteration of a drug in the body	Process of physicochemical and biochemical alteration of a drug in the body
Biotransformation of the drugs is to render them:	Less ionized	More pharmacologically active	More lipid soluble	Less lipid soluble	Less lipid soluble
Tick the drug type for which microsomal oxidation is the most prominent:	Lipid soluble	Water soluble	Low molecular weight	High molecular weight	Lipid soluble
Pick out the right statement:	Microsomal oxidation always results in inactivation of a compound	Microsomal oxidation results in a decrease of compound toxicity	Microsomal oxidation results in an increase of ionization and water solubility of a drug	Microsomal oxidation results in an increase of lipid solubility of a drug thus its excretion from the organism is facilitated	Microsomal oxidation results in an increase of ionization and water solubility of a drug
Stimulation of liver microsomal enzymes can:	Require the dose increase of some drugs	Require the dose decrease of some drugs	Prolong the duration of the action of a drug	Intensify the unwanted reaction of a drug	Require the dose increase of some drugs
Metabolic transformation (phase 1) is:	Acetylation and methylation of substances	Transformation of substances due to oxidation, reduction or hydrolysis	Glucuronide formation	Binding to plasma proteins	Transformation of substances due to oxidation, reduction or hydrolysis
Biotransformation of a medicinal substance results in:	Faster urinary excretion	Slower urinary excretion	Easier distribution in organism	Higher binding to membranes	Faster urinary excretion
Conjugation is:	Process of drug reduction by special enzymes	Process of drug oxidation by special oxidases	Coupling of a drug with an endogenous substrate	Solubilization in lipids	Coupling of a drug with an endogenous substrate
Which of the following processes proceeds in the second phase of biotransformation?	Acetylation	Reduction	Oxidation	Hydrolysis	Acetylation
Conjugation of a drug includes the following EXCEPT:	Glucoronidation	Sulfate formation	Hydrolysis	Methylation	Hydrolysis
In case of liver disorders accompanied by a decline in microsomal enzyme activity the duration of action of some drugs	Decreased	Enlarged	Remained unchanged	Changed insignificantly	Enlarged
Half life (t ½) is the time required to:	Change the amount of a drug in plasma by half during elimination	Metabolize a half of an introduced drug into the active metabolite	Absorb a half of an introduced drug	Bind a half of an introduced drug to plasma proteins	Change the amount of a drug in plasma by half during elimination
Half life (t ½) doesn’t depend on:	Biotransformation	Time of drug absorption	Concentration of a drug in plasma	Rate of drug elimination	Time of drug absorption
Elimination is expressed as follows:	Rate of renal tubular reabsorption	Clearance speed of some volume of blood from substance	Time required to decrease the amount of drug in plasma by one-half	Clearance of an organism from a xenobiotic	Clearance of an organism from a xenobiotic
Elimination rate constant (Kelim) is defined by the following parameter:	Rate of absorption	Maximal concentration of a substance in plasma	Highest single dose	Half life (t ½)	Half life (t ½)
Systemic clearance (CLs) is related with:	Only the concentration of substances in plasma	Only the elimination rate constant	Volume of distribution, half life and elimination rate constant	Bioavailability and half life	Volume of distribution, half life and elimination rate constant
Pharmacodynamics involves the study of following EXCEPT:	Biological and therapeutic effects of drugs	Absorption and distribution of drugs	Mechanisms of drug action	Drug interactions	Absorption and distribution of drugs
Pharmacodynamics involves the study of following?	Mechanisms of drug action	Biotransformation of drugs in the organism	Distribution of drugs in the organism	Excretion of drug from the organism	Mechanisms of drug action
Pharmacodynamics involves the following?	Information about main mechanisms of drug absorption	Information about unwanted effects	Information about biological barriers	Information about excretion of a drug from the organism	Information about unwanted effects
Pick out the answer which is the most appropriate to the term “receptor”	All types of ion channels modulated by a drug	Enzymes of oxidizing-reducing reactions activated by a drug	Active macromolecular components of a cell or an organism which a drug molecule has to combine with in order to elicit its specific effect	Carriers activated by a drug	Active macromolecular components of a cell or an organism which a drug molecule has to combine with in order to elicit its specific effect
What does “affinity” mean?	A measure of how tightly a drug binds to plasma proteins	A measure of how tightly a drug binds to a receptor	A measure of inhibiting potency of a drug	A measure of bioavailability of a drug	A measure of how tightly a drug binds to a receptor
Target proteins which a drug molecule binds are:	Only receptors	Only ion channels	Only carriers	All of the above	All of the above

An agonist is a substance that:	Interacts with the receptor without producing any effect	Interacts with the receptor and initiates changes in cell function, producing various effects	Increases concentration of another substance to produce effect	Interacts with plasma proteins and doesn't produce any effect	Interacts with the receptor and initiates changes in cell function, producing various effects
If an agonist can produce maximal effects and has high efficacy it's called:	Partial agonist	Antagonist	Agonist-antagonist	Full agonist	Full agonist
If an agonist can produce submaximal effects and has moderate efficacy it's called:	Partial agonist	Antagonist	Agonist-antagonist	Full agonist	Partial agonist
An antagonist is a substance that:	Binds to the receptors and initiates changes in cell function, producing maximal effect	Binds to the receptors and initiates changes in cell function, producing submaximal effect	Interacts with plasma proteins and doesn't produce any effect	Binds to the receptors without directly altering their functions	Binds to the receptors without directly altering their functions
A competitive antagonist is a substance that:	Interacts with receptors and produces submaximal effect	Binds to the same receptor site and progressively inhibits the agonist response	Binds to the nonspecific sites of tissue	Binds to one receptor subtype as an agonist and to another as an antagonist	Binds to the same receptor site and progressively inhibits the agonist response
The substance binding to one receptor subtype as an agonist and to another as an antagonist is called:	Competitive antagonist	Irreversible antagonist	Agonist-antagonist	Partial agonist	Agonist-antagonist
Irreversible interaction of an antagonist with a receptor is due to:	Ionic bonds	Hydrogen bonds	Covalent bonds	All of the above	Covalent bonds
Mechanisms of transmembrane signaling are the following EXCEPT:	Transmembrane receptors that bind and stimulate a protein tyrosine kinase	Gene replacement by the introduction of a therapeutic gene to correct a genetic effect	Ligand-gated ion channels that can be induced to open or close by binding a ligand	Transmembrane receptor protein that stimulates a GTP-binding signal transducer protein (G-protein)	Gene replacement by the introduction of a therapeutic gene to correct a genetic effect
Tick the second messenger of G-protein-coupled (metabotropi receptor:	Adenylyl cyclase	Sodium ions	Phospholipase C	cAMP	cAMP
Tick the substance which changes the activity of an effector element but doesn't belong to second messengers:	cAMP	cGMP	G–protein	Calcium ions	G–protein
The increase of second messengers' (cAMP, cGMP, Ca2+ concentration leads to	Inhibition of intracellular protein kinases and protein phosphorylation	Proteinkinases activation and protein phosphorylation	Blocking of interaction between a receptor and an effector	Antagonism with endogenous ligands	Proteinkinases activation and protein phosphorylation
Tick the substances whose mechanisms are based on interaction with ion channels	Sodium channel blockers	Calcium channel blockers	Potassium channels activators	All of the above	All of the above
All of the following statements about efficacy and potency are true EXCEPT:	Efficacy is usually a more important clinical consideration than potency	Efficacy is the maximum effect of a drug	Potency is a comparative measure, refers to the different doses of two drugs that are needed to produce the same effect	The ED50 is a measure of drug's efficacy	The ED50 is a measure of drug's efficacy
Give the definition for a therapeutical dose:	The amount of a substance to produce the minimal biological effect	The amount of a substance to produce effects hazardous for an organism	The amount of a substance to produce the required effect in most patients	he amount of a substance to accelerate an increase of concentration of medicine in an organism	The amount of a substance to produce the required effect in most patients