UNIT-1

CONTROLLED RELEASE DRUG DELIVERY SYSTEM

Controlled drug delivery is one, which delivers the drug at a predetermined rate, for locally or systemically, for a specified period of time. It maintains constant level of drug in blood and tissue for extended period of time.

Advantages

- CRDDS Improve absorption, utilization and there by enhancing bioavailability.
- Decreased local and systemic side effects reduced gastrointestinal irritation.
- Reduction in dosing frequency.
- Better patient acceptance and compliance.
- Reduced fluctuations in circulating drug levels.
- Reduction in the health care cost.
- Bioavailability of certain drugs can be increased.

Disadvantages

- Dose dumping.
- Dose adjustment is difficult.
- Patient education is required for successful therapy.
- Patient need to substantial additional information as to the proper used sustained release product.
- Poor *Invitro-Invivo* correlation..
- Higher cost of single unit as compared to cost of single conventional unit.
- Stability problems.

Differences between Conventional Drug Therapy and Controlled/ Sustained Release Drug Therapy

Sl No	Conventional Drug Therapy	Sustained Release Drug Therapy
1	Rapid and complete release of drug immediately after administration.	Slow/controlled release of drug over an extended period of time.
2	Absorption is the rate-limiting step (kr >>> ka).	Drug release from the dosage form is the rate-limiting step (ka >>> kr).
3	Blood level fluctuates (Peak and Valley).	Constant blood level is maintained over a prolonged period (Reduced fluctuation).
4	Frequent dosing.	Reduced frequency of dosing.
5	Patient non compliance.	Improved patient compliance.
6	More total dose over the entire course of therapy.	Less total dose over the entire course of therapy.
7	More side effects.	Minimize/eliminate incidence of local/systemic side effects.
8	Health care cost ↑.	Health care cost ↓.
9	Incidence of severity of GI side effects due to dose dumping of irritant drugs ↑.	Incidence of severity of GI side effects due to dose dumping of irritant drugs ↓.
10	More flexibility for physician in adjusting dosage required.	Less flexibility.

Differences between Controlled Release Drug Therapy and Sustained Release Drug Therapy

Sl No	Controlled Release Drug Therapy	Sustained Release Drug Therapy
1	Constitutes dosage form that maintains constant drug levels in blood or tissue	Constitutes dosage form that provides medication over extended period of time
2	Maintains constant drug levels in the blood target tissue usually by releasing the drug in a zero order pattern.	SRDF generally do not attain zero order release kinetics
3	Controlled dosage forms contain methods to promote localization of the drug at active site.	Usually do not contain mechanisms to promote localization of the drug at active site.

FUNDAMENTAL STUDY OF DIFFERENT TYPES OF ORAL CONTROLLED RELEASE SYSTEM

Oral route has been most popular & successfully used route for controlled delivery of drugs because of following reasons-

- Convenience & ease of administration.
- Greater flexibility in dosage form design.
- Ease of production & low cost of such a system.

An oral CDDS can be designed as-

CONTINUOS RELEASE SYSTEM– release drug continuously over an extended period of time.

PULSATILE RELEASE SYSTEM– is characterized by a time period of no release followed by a rapid & complete or extended drug release.

CONTROLLED RELEASE ORAL FORMULATION

Continuous Release Systems

1. Continuous transit system

matrix type
reservoir type
oral osmotic pressure type
ion-exchange resins type

2. Gastro retentive system

low density system
high density system
modified shape system
mucoadhesive system

Pulsed Release System

1.Time specific system

osmotic pressure
rupturable coating
swellable coating
diffusive coating pulsincap

2. Site/ Colon specific system

time dependent
pH dependent
time/pH dependent
enzyme dependent
colonic pressure dependent
osmotic pressure dependent

Continuous release system: Release drug continuously over an extended period of time.

Pulsatile release system: These are characterized by a time period of no release (lag time) followed by a rapid and complete or extended drug release.

1. CONTINUOUS- TRANSIT SYSTEMS : These systems release the drug for a prolonged period of time along the entire length of GIT with normal transit of the dosage form.

Matrix type oral CDDS: These are possibly the most common of monolithic devices for controlling the release of drugs for following reason-

1. Easy of fabricate compared to reservoir
2. No danger of accidental dose dumping compared to monolithic reservoir.

In such device active agent present as dispersion within polymer matrix & formed by compression of polymer/ drug matrix or by melting. The drug release properties of monolithic device are dependent upon:

- Initial concentration of drug in the matrix.
- Solubility of the drug.
- Presence of water- soluble additives that create pore and facilitate water permeation in the matrix
- Porosity and tortuosity of matrix as influenced by compression force.
- Polymer system forming the matrix.

Reservoir type oral CDDS: These are the systems where the drug crystal, particle, granule, pellet, minitablet or tablet is present as core encapsulated with a rate-controlling wall, film/membrane having a well defined thickness. Microcapsules also fall under this category. Drug release occurs predominantly by diffusion.

Advantage of such system-

- Zero order delivery is possible.
- Release rate modulated by polymer type, polymer membrane thickness, & membrane porosity.

Disadvantage-

- Higher cost of formulation.
- Possibility of dose-dumping in the event of membrane failure.

Oral osmotic (OROS) CDDS: Based on diffusion & erosion, osmotic system is more complex in design but provide better zero- order drug delivery. They work on principle of osmotic pressure to release the drug at a constant zero-order rate.

In design OROS system comprise of 4 basic components-

1. A rigid shape retaining Semipermeable membrane (SPM) that surrounds drug/ osmogent core.
2. A drug layer.
3. Osmogent that imbibes water & generates osmotic pressure that drives dissolved or dispersed drug through delivery orifice. Commonly used osmogent are sodium chloride, dextrose, and mannitol. Swellable osmopolymers include PEO, HPMC etc.
4. Delivery orifice which is generally laser-drilled into semipermeable membrane.

Some factors in OROS are-

- Drug solubility: Release rate is directly proportional to drug solubility within the core.
- Osmotic pressure: Release rate of drug is directly proportional to osmotic pressure of the core formulation.
- Delivery orifice: It should be within the desired range (0.3mm to 1.0 mm) to control the drug release.
- Coating membrane: Drug release is affected by type and nature forming polymer, thickness of the membrane and presence of other additives that influence membrane permeability and strength.

Ion Exchange Resin-Drug Complexes: The polymeric and ionic properties of IER enable drug release more uniformly than that possible with simple matrices. The various types of resinates in oral CDD are-

- ***Simple resinate:*** These are the simplest form of CRDDS. They can be suspended in liquids, filled directly in a capsule or compressed into tablets.
- ***Microencapsulated or coated resinate:*** Microencapsulation of resinate provide better control over the drug release because of presence of a rate-controlling membrane of polymers like ethyl cellulose or waxes.
- ***Pennkinetic systems:*** In this system, drug resinate is pretreated with PEG 400 to maintain the geometry and improve the coating process. The pretreated resinates are then coated with ethyl cellulose or other water insoluble polymer. PEG helps in controlling the rate of swelling of resinate matrix in water, while outer ethyl cellulose coating modifies the diffusion pattern of ions in and out of the system.
- ***Hollow fibre systems:*** In this system, resinates are filled into hollow fibres made from suitable polymers, to obtain a controlled- release profile.

2. GASTRORETENTIVE DRUG DELIVERY SYSTEM: Some drugs are absorbed at specific site only; these require release at that specific site. Gastro retentive drug delivery (GRDDS) is one of the site specific drug delivery in stomach. It is obtained by retaining dosage form into stomach and drug is being released at controlled manner at specific site.

Advantages

- Enhanced bioavailability

- Sustained drug delivery reduced frequency of Dosing
- Targeted therapy for local ailments in the upper GIT
- Reduced fluctuations of drug concentration
- Improved selectivity in receptor activation
- Extended effective concentration
- Minimized adverse activity at colon

Limitations

- The drug substances that are unstable in the acidic environment of the stomach are not suitable candidates to be incorporated in the systems.
- These systems require a high level of fluid in the stomach for drug delivery to float and work efficiently.
- Not suitable for drugs that have solubility or stability problem in GIT.
- Drugs which are irritant to gastric mucosa are also not suitable.
- These systems do not offer significant advantages over the conventional dosage forms for drugs, which are absorbed throughout GIT.

Classification of GRDDS

1. LOW DENSITY SYSTEM/ FLOATING DOSAGE FORM

   a) Effervescent system /Gas generating system
   b) Non-effervescent systems
      - Swelling/expanding system
      - Inherently low density system
2. HIGH DENSITY SYSTEM

3. MODIFIED SHAPE SYSTEM

4. MUCOADHESIVE SYSTEM

1. LOW DENSITY SYSTEM/ FLOATING DOSAGE FORM: These are prepared by incorporating high level (20-75% w/w) gel-forming hydrocolloids. E.g.:- Hydoxyethylcellulose, hydroxypropylcellulose, HPMC & Sodium CMC into the formulation and then compressing these granules into a tablets or capsules. It maintains the bulk density less than 1. They Have a bulk density less than gastric fluid & so remain buoyant in stomach called as HYDRODYNAMICALY BALANCED SYSTEM (HBS).

a. **Effervescent / Gas generating system:** It increases size of drug delivery system as well as decreases its density & provides floating properties. This system formulated as matrices / resinates. Matrices are prepared with swellable polymers like HPMC, chitosan,

effervescent components like sodium bicarbonate, citric acid and tartaric acid or chambers containing a liquid that gasifies at body temperature.

b. **Non-effervescent system swelling / expanding systems:** These systems use a gel forming / swellable hydrocolloid such as polysaccharides like guargum, cellulosics like HPMC, synthetic polymers like PEO, carbomer. It consist of embedding drug powder/ pellets in gel forming hydrocolloids. After oral administration dosage form swell on contact with gastric fluids and owing to air-entrapment attains a bulk density of less than 1 and a size large enough to prevent its exit from the pyloric sphincter of stomach. The formed swollen gel-like structure acts as reservoir & allows sustained release of drug through gelatinous mass.

c. **Inherently low density systems:** These systems can be provided in one of the two ways-

I. Entrapment of air (hollow microspheres/microballoons):
   - Emulsion solvent diffusion method
   - Modified solvent evaporation method
   - Dehydration of swollen hydrogel
   - Hollow chamber system

II. Incorporation of low density materials

I. HOLLOW MICROSPHERES: Polymers used commonly: Polycarbonates, Cellulose acetate, Calcium alginate, Eudragit S, agar and methoxylated pectin etc

- Emulsion- solvent diffusion method: A solution of polymer & drug in ethanol/ methylene chloride is poured in aq. Solution of PVA. The ethanol partitions into external aq. Phase & polymer participates around methylene chloride droplets. Then subsequent evaporation of entrapped methylene chloride leads to formation of internal microcavity within microparticles.

- Modified solvent evaporation method: Drug powder is dispersed into solution of cellulose acetate butyrate & eudragit RL 100 in acetone. The dispersion is pressurized under CO_2 gas, which dissolves & forms bubbles following the release of pressure. Generated CO_2 bubbles are entrapped within dispersed drug-polymer droplets & leads to formation of internal cavities within hardened microspheres.

- Dehydration of Swollen Hydrogel: System consists of hydrated drug loaded calcium alginate core, which is coated with PVA membrane. Drying of hydrogel result in formation of air compartment owing to shrinkage of hydrated core.

- Hollow chamber system: These system prepared by coating drug on hollow core such as

poprice, empty gelatin capsules / polystyrene beads followed by coating the drugs with rate- controlling membrane

II. LOW DENSITY MATERIALS: Fats & low density polymers used to prepare floating drug matrices. e.g. like Polypropylene foam powder ,matrix-forming polymers ,drug & an optional filler.

2. HIGH DENSITY SYSTEM: Density of system is larger than gastric juice (>1.4 g/ml), the device settles down to bottom of stomach, remaining located below the pylorus. Iron oxide, titanium dioxide & barium sulphate used to increase density of drug pellets. The drug is coated on heavy core & then covered by diffusion controlled membrane. The approach is not very successful.

3. MODIFIED SHAPE SYSTEM / UNFOLDING SYSTEM: This system consist of atleast one erodible polymer, one non-eroidible polymer & drug that is dispersed within polymer matrix. Drugs incorporated in several geometric shapes such as tetrahedron, ring, disc, spiral, pellet/ sphere which can be packed into gelatin capsule & after dissolution of capsule shell unfold to a large size that limits the passage through pyloric sphincter.

4. BIOADHESIVE SYSTEMS / MUCOADHESIVE SYSTEM: Bioadhesive polymer e.g. carbomer, chitosen used to coat dosage form so that it adheres to gastric mucosa. Advantage is – in stomach its intimate contact with mucosa leading to short pathways for locally acting drugs such as antibiotics against H. pylori.

3. TIME-SPECIFIC SYSTEM/ PULSATILE DRUG DELIVERY SYSTEM: It is defined as the rapid and transient release of a certain amount of drug molecules within a short time-period immediately after a predetermined off-release period. In various diseases in which we can recommend the pulsatile drug delivery system such as duodenal ulcer, cardiovascular diseases, arthritis, asthma, diabetes, neurological disorder, cancer, hypertension and hypercholesterolemia. Pulsatile drug delivery systems (PDDS) are gaining importance as they deliver a drug at time and site specific manner resulting in improved therapeutic efficacy as well as compliance.

Necessity of PDDS

- Chronopharmacotherapy of disease which shows circadian rhythms in their pathophysiology like asthmatic attack during early morning, heart attack in middle of night, morning stiffness in arthritis.
- Avoiding first pass metabolism ex. Protein & peptide.
- For targeting specific site in intestine ex. Colon (sulphasalazine).
- For programmed administration of hormone & drug.

- For drug having short half-life ex. ß-blocker

Pulsatile DDS classified as-

I. Osmotic pressure based system
II. Reservoir system with rupturable coatings
III. Reservoir system with swellable/soluble/erodible coating
IV. Capsular system with polymeric plugs (Pulsincap)

I. OSMOTIC PRESSURE RELEASE SYSTEM

a. Capsule/ tablet composed of a large number of pellet- Each pellet has a core that contains therapeutic drug & water soluble osmotic agent. A water-permeable but insoluble polymer film encloses each core. On exposure to water, it's penetration into pellets, osmotic agents dissolves, which causes pellets to swell & drug release. In case of Capsule based systems a single-unit systems are mostly developed in capsule form. The lag time is controlled by a plug (ex. Polymethacrylate), which gets pushed away by swelling or erosion, and the drug is released as a "Pulse" from the insoluble capsule body.

b. PORT (Programmable oral release technology): System composed of gelatin capsule coated with SPM (ex. Cellulosic acetate) that contain immediate release drug, an insoluble plug (ex. Lipids) & osmotic agent with second release of drug for timed release. Upon contact with aqueous media, immediate release drug is delivered, water enter into capsule through SPM , which increase osmotic pressure & result in ejection of plug after lag time , following which second dose is delivered.

II. RESERVOIR PULSATILE WITH RUPTURABLE COATINGS

These system consist of 3 layers- 1) Drug containing core, 2) Pressure generating layer- effervescent excipients (mixture of citric acid/ tartaric acid & sodium bicarbonate),swelling agents or osmagents), 3) Semipermeable polymer coating. Upon contact with GI fluids, water penetrates through polymer coating & generates pressure due to effervescence, hydration of swelling polymer or osmosis, then ruptures polymer coating leading to rapid drug release.

III. RESERVOIR SYSTEM WITH SWELLABLE/SOLUBLE/ERODIBLE COATING:

In this system barrier swells, erodes / dissolves after a specific lag period & drug is subsequently released rapidly. Lag time depends on thickness of coating layer.

Examples –

a. Press-coated / multilayered tablets: This system is based on swelling, disintegration or erosion mechanism for pulsatile drug delivery. A release pattern with two pulses obtained from a three layered tablet conatining two drug layers separated by a drug free gellable polymeric barrier layer.

b. Hydrophilic sandwich (HS) capsule: Based on a capsule- within a capsule, in which the inter-capsular space is filled with a layer of hydrophilic polymer (HPMC). This effectively creates a hydrophilic sandwich between the two gelatin capsules. When outer capsules dissolves, sandwich of HPMC forms a gel barrier layer & cause drug delay release.

c. Time clock system: It is made up of a solid dosage form, coated with a hydrophobic surfactant layer to which a hydrosoluble polymer is added to improve adhesion to the core. The outer layer redisperses in aqueous environment in a time proportional to the thickness of film.

d. Chronotropic system: It consists of drug containing core coated with high viscosity HPMC which is responsible for a lag phase in onset of release. The lag time is controlled by the thickness and the viscosity grades of HPMC. The system is suitable for both tablets and capsules

IV. CAPSULAR PULSATILE SYSTEM WITH POLYMERIC PLUGS:

Example of this system is pulsincap which consist of capsule with water soluble cap, an insoluble body filled with drug & sealed with a hydrogel plug. The length of plug decides lag time. On administration, soluble cap dissolves thereby allowing the hydrogel plug to swell & expand**.** After a predetermined lag time, it is swollen to an extent that it is ejected from capsule body thereby releasing the drug. The plug material consists of insoluble but permeable & swellable polymers. ex. Polymethacrylate.

4. SITE SPECIFIC/COLON TARGETED DRUG DELIVERY SYSTEM:

The most useful drug delivery system to treat colonic disorder & colon cancer are failing due to inappropriate concentration of drug that does not get to the site of action. Colon targeted drug delivery system are suitable site for absorption of peptides & proteins. The CDDS is highly desirable for local treatment of variety of bowl diseases such as ulcerative colitis, crohn's disease, colonic cancer.

Approach for colon targeted drug delivery system

1. **By using pH sensitive polymer:** pH sensitive polymers, especially those that contain carboxyl group which make them insoluble at low pH values and soluble at higher pH values are used for colon targeting of drug. Although these pH dependent polymers can protect a drug moiety in stomach from acidic environment. Eudragit S and FS and their combinations, which dissolve in colon/rectum pH are used for colon targeting.

2. **pH and Time dependent drug delivery system for colon:** A typical multilayered bead formulation used which comprises of an outermost coating of an enteric polymer (eg- Euragit L,S or ES), a second barrier coating of pH independent polymer (eg- Ethyl cellulose) that delay drug release and an innermost drug. The disadvantage of these system are- i) Gastric emptying time varies markedly between subjects, ii) Gastrointestinal movement , specially peristalsis or contraction in stomach result in gastrointestinal transit of drug.

3. **Microbially triggered drug delivery to colon:** The microflora of colon consist mainly of anaerobic bacteria e.g. bacteroides , bifidobacteria, enterococci, entrobacteria etc. This vast microflora fulfil it's energy needs by fermenting various types of substrates that have been left undigested in small intestine, e.g. di & tri saccharides , polysaccharides etc for this fermentation, microflra produces a vast number of enzyme like glucoronidase, galactosidase, arabinosidase, nitroreductase etc. Because of presence of biodegradable enzymes only in colon, use of biodegradable polymer for CDDS seems to be more specific approach.

   - ❖ Prodrug approach for drug delivery to colon: Prodrug is a pharmacologically inactive derivative of parent drug molecule that requires spontaneous/enzymatic transformation in vivo to release active drug. For colonic delivery, prodrug is designed to undergo minimal hydrolysis in upper tracts of GIT & undergo enzymatic hydrolysis in colon there by releasing the active drug moiety from drug moiety.

4. **Osmotically Controlled Drug Delivery Systems:** Depend up on the osmotic pressure exerted by Osmogent on drug compartment with which though drug get released slowly through the orifice.

FACTORS INFLUENCING THE DESIGN AND ACT OF CONTROLLED RELEASE PRODUCTS

Physiological factors

(1) Aqueous Solubility's: Most of the active pharmaceutical moiety (API) are weakly acidic or basic in nature that affect the water solubility of API. Weak water soluble drugs are difficult to design the controlled release formulations. High aqueous solubility drug show burst release followed by a rapid increment in plasma drug concentration. These types of drugs are a good candidate for CRDDS. The pH dependent solubility also creates a problem in formulating CRDDS. BCS class-III & IV drugs are not a suitable candidate

for this type of formulations.

(2) **Partition coefficient (P-value):** P-value denotes the fraction of the drug into oil & aqueous phase that is a significant factor that affects the passive diffusion of the drug across the biological membrane. The drugs are having high or low P value not suitable for CR, it should be appropriate to dissolve in both phases.

(3) **Drug pKa:** pKa is the factor that determined the ionization of drug at physiological pH in GIT. Generally, the high ionized drugs are poor candidates for CRDDS. The absorption of the unionized drug occurs rapidly as compared to ionized drugs from the biological membranes. The pKa range for an acidic drug that ionization depends on the pH is 3.0 to 7.5 and for a basic drug it lay between 7 and 11.

(4) **Drug stability:** Drugs that are stable in acid/base, enzymatic degradation, and other gastric fluids are good candidates for CRDDS. If drug degraded in the stomach and small intestine, it not suitable for controlled release formulations because it will decrease in bioavailability of concern drug.

(5) **Molecular size & molecular weight:** The molecular size & molecular weight are two important factors which affect the molecular infusibility across a biological membrane. The molecular size less than 400 Dalton is easily diffuse but greater than 400 Dalton create a problem in drug diffusion.

(6) **Protein binding:** The drug-protein complex act as a reservoir in plasma for the drug. Drug showing high plasma protein binding are not a good candidate for CRDDS because the protein binding increases the biological half-life. So there is no need to sustain the drug release.

Biological factors

(1) **Absorption:** Uniformity in rate and extent of absorption is an important factor in formulating the CRDDS. However, the rate limiting step is drugged release from the dosage form. The absorption rate should rapid then release rate to prevent the dose

dumping. The various factors like aqueous solubility, log P, acid hydrolysis, which affect the absorption of drugs.

(2) **Biological half-life ($t_{1/2}$):** In general the drug is having short half-life required frequent dosing and suitable candidate for controlled release system. A drug with long half-life required dosing after a long time interval. Ideally, the drugs having $t_{1/2}$ 2-3 hrs are a suitable candidate for CRDDS. Drugs have $t_{1/2}$ more than 7-8 hrs not used for controlled release system.

(3) **Dose size:** The CRDDS formulated to eliminate the repetitive dosing, so it must contain the large dose than conventional dosage form. But the dose used in conventional dosage form give an indication of the dose to be used in CRDDS. The volume of sustained dose should be as large as it comes under acceptance criteria.

(4) **Therapeutic window:** The drugs with narrow therapeutic index are not suitable for CRDDS. If the delivery system failed to control release, it would cause dose dumping and ultimate toxicity.

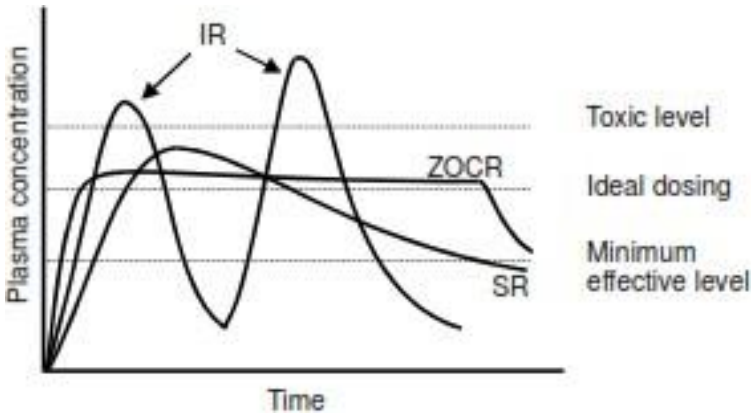
(5) **Absorption window:** The drugs which show absorption from the specific segment in GIT are a poor candidate for CRDDS. Drugs which absorbed throughout the GIT are good candidates for controlled release.

(6) **Patient physiology:** The Physiological condition of the patient like gastric emptying rate, residential time, and GI diseases influence the release of the drug from the dosage form directly or indirectly.

SUSTAINED RELEASE DRUG DELIVERY SYSTEM

Any of the dosage form that maintains the therapeutic blood or tissue levels of drug by continuous release of medication for a prolonged period of time, after administration of a single dose is called sustained release drug release system.

The basic goal of therapy is to achieve steady state blood level that is therapeutically effective and non toxic for an extended period of time.



Graphically representation of plasma concentrations of a conventional Immediate Release (IR), a Sustained Release (SR) and an idealized zero-order controlled release (ZOCR) drug delivery systems

Merits of SRDF

- Reduction in blood level fluctuations of drug, thus better management of the disease.
- Reduction in dosing frequency.
- Enhanced patient convenience and compliance.
- Reduction in adverse effects (both systemic and local), esp. of potent drugs, in sensitive patients.
- Reduction in health care costs.
- Reduces nursing and hospitalizing time.
- Maximum bioavailability with a minimum dose.
- Minimize drug accumulation with chronic dosing.
- Cure or control condition more promptly.
- Make use of special effects, e.g. Treatment of Arthritis.
- Constant blood levels achieve desired effect and this effect is maintained for an intended period of time.
- Drug susceptible to enzymatic inactivation or by bacterial decomposition can be protected by encapsulation in polymer system suitable for SR.

Demerits of SRDF

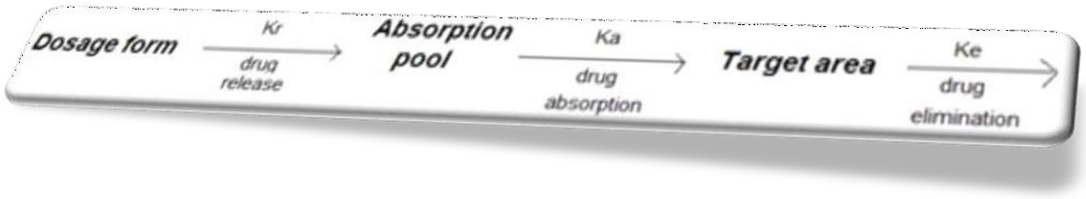
- Administration of sustained release medication dose not permits prompt termination of therapy. Immediate changes in the drug if needed during therapy when significant adverse effects are noted cannot be accommodated.
- The physician has less flexibility in adjusting dosage regimen, as it is fixed by dosage form design.

- Sustained release dosage forms are designed for normal population i.e. on basis of average biologic half-life.
- Consequently, disease states that alter drug disposition, significant patient variation, and so forth are not accommodated.
- More costly process and equipment are involved in manufacturing many sustained release dosage forms.
- Possibility of dose dumping due to food, physiologic or formulation variable or chewing or grinding of oral formulation by the patient and thus increased risk of toxicity.
- Unpredictable and poor in vitro and in vivo relationship.
- Effective drug release time period is influenced and limited by GI residence time.
- Need additional patient education (such as not to chew or crush the dosage form before swallowing).
- Drugs having very short half life or very long half life are poor candidates for sustained release dosage forms. For Ex: diazepam.
- Delayed onset of action, hence sometimes not useful in acute conditions.

CONCEPT OF SUSTAINED RELEASE FORMULATION

The Concept of sustained release formulation can be divided in to two considerations i.e. *release rate & dose consideration*

A) **Release rate consideration: -** In conventional dosage form *Kr>Ka* in this the release of drug from dosage form is *not rate limiting step.*



The above criteria i.e. (Kr>Ka) is in case of immediate release, where as in non immediate (Kr<Ka) i.e. release is rate limiting step. So that effort for developing sustained release formulation must be directed primarily altering the release rate. The rate should be independent of drug removing in the dosage form over constant time.

The release rate should follow zero order kinetics

$$Kr = rate\ in = rate\ out = Ke.Vd.Cd$$

Where,

Ke = overall elimination (first order kinetics).
Vd = total volume of distribution.
Cd = desired drug concentration.

B) *Dose consideration:-* To achieve the therapeutic level & sustain for a given period of time for the dosage form generally consist of 2 part *a) Initial (primary) dose b) maintenance dose* Therefore the total dose 'W' can be

$$W = Di + Dm$$

In a system, the therapeutic dose *release follows zero order process* for specified time period then,

$$W = Di + K^0 r.\ Td$$

Td = time desired for sustained release from one dose.

If *maintenance dose begins to release* the drug during dosing t=0 then,

$$W = Di + K^0 r.\ Td - K^0\ r\ Tp$$

Tp = time of peak drug level.

However a *constant drug* can be obtained by suitable *combination of Di & Dm* that release the drug by first order process, then

$$W = Di + (Ke.Cd\ /Kr)Vd$$

Sustained release, sustained action, prolonged action, controlled release, extended action, time release dosage formed are terms used to identify drug delivery system that are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose .

DESIGN OF ORAL SUSTAINED RELEASE DOSAGE FORM

Formulation methods used to obtain the desired drug availability rate from sustained action dosage form include…….

- Increasing the particle size of the drug.
- Embedding the drug in matrix.
- Coating the drug or dosage form containing drug (microencapsulation).
- Forming complexes of the drug with material such as ion exchange resins.

1) Increasing the particle size of the drug:-

- The purpose of increasing particle size is to decrease the surface to volume ratio slow the rate of drug availability. This method is a single means for obtaining the desired drug availability rate is limited to poorly soluble drug.

2) Embedding the drug in matrix:-

- Matrix may be defined as uniform dispersion of drug in solid which is less soluble than a drug in the dispersion fluid, & which for the continuous external phase of the dispersion effectively impeder the passage of the drug from the matrix to the dispersion fluid.
- One of the least complicated approaches to the manufacture of sustained release dosage form involves the direct compression of drug, materials & additives to form a tablet in which drug is embedded in a matrix core of the retardant.
- Polymers:

  Insoluble, inert - polyethylene, polyvinyl chloride, methyl acrilate, ethyl cellulose.

  Insoluble, erodible – carnauba wax, stearyl alcohol, castor wax.

  Hydrophilic – methyl cellulose, sodium carboxymethyl cellulose, sodium alginate.
- In a matrix system the drug is dispersed as solid particle within a porous matrix formed of a water insoluble polymer, such as poly- vinyl chloride.
- Initially, drug particle located at the surface of the release unit will be dissolved and the drug released rapidly. Thereafter, drug particle at successively increasing distance from the surface of the release unit will be dissolved and release by diffusion in the pores to the exterior of the release unit.
- The main formulation factor by which the release rate from matrix system can be controlled are; the amount of the drug in the matrix, the porosity of the release unit & the solubility of the drug.
- Two types of matrix systems

a) Slowly eroding matrix

b) Inert plastic matrix

a) Slowly eroding matrix: Consists of using materials or polymers which erode over a period of time such as waxes, glycerides, stearic acid, cellulosic materials etc.

Portion of drug intended to have sustained action is combined with lipid or cellulosic material and then granulated - Untreated drug granulated - Both mixed

b) Embedding drug in inert plastic matrix: Drug granulated with an inert, insoluble matrix such as polyethylene, polyvinyl acetate, polymethacrylate.

- Granulation is compressed results in MATRIX
- Drug is slowly released from the inert plastic matrix by leaching of body fluids.
- Release of drug is by diffusion.

3) Coating the drug or a dosage form containing the drug (microencapsulation)

- The method for retarding drug release from the dosage form is to coat its surface with a material (polymers) that retards penetration by the dispersion fluid. Drug release depends upon the physiochemical nature of coating material.
- Microencapsulation is rapidly expanding technique as a process; it is a means of applying relatively thin coating to small particles of solid or droplets of liquids and dispersion.
- The application of microencapsulation might be includes, sustained release or prolonged action medication, taste masked, chewable tablet, powder and suspension, single layer tablets. Containing chemically incompatible ingredient & new formulation concepts for creams, ointments, aerosols, dressing, plasters, suppositories & injectables.
- Polymers: - polyvinyl alcohol, polyacrylic acid, ethyl cellulose, polyethylene, polymethacrlate, poly (ethylene-vinyl acetate), cellulose nitrite, silicones, poly (lactide-co-glcolide)

1) Chemically reacting the drug with material such as an ion-exchange resin:-

- Sustained delivery of ionizing acidic & basic drug can be obtained by complexing them with insoluble non-toxic anion exchanger and cation exchanger resin respectively.
- Here the drug is released slowly by diffusing through the resin particle structure.
- The complex can be prepared by incubating the drug-resin solution or passing the drug solution through a column containing ion exchange resin.
- Principle is based on preparation of totally insoluble ionic material
    - *Resins are insoluble in acidic and alkaline media*

- *They contain ionizable groups which can be exchanged for drug molecules*
- *IER are capable of exchanging positively or negatively charged drug molecules to form insoluble poly salt resinates.*

- Types: There are two types of IER
  - Cationic Exchange resins - $RSO_3^-H^+$ Resins functional groups
  - Anionic Exchange resins – $RNH_3^+ OH^-$
- Structurally made up of a stable acrylic polymer of styrene-divinyl benzene copolymer.
- Mechanism of action: IER combine with drug to form insoluble ion complexes

  $R\text{-}SO_3^- H^+ + H_2N – A = R\text{-}SO_3^- NH_3 +\text{-}A$

  $R\text{-}NH_3 + OH^- + HOOC\text{-}B = RNH_3^+ {}^-OOC – B + H_2O$

  Where A- NH_2 is basic drug

  B-COOH is acidic drug

These resinates are administered orally

⇩

2 hrs in stomach in contact with acidic fluid at pH 1.2

⇩

Intestinal fluid, remain in contact with slightly basic pH for 6hrs.

⇩

Drug can be slowly liberated by exchange with ions present in G.I.T.

RELEASE KINETICS IN CONTROLLED DRUG DELIVERY SYSTEM

- The mathematical models are used to evaluate the kinetics and mechanism of drug release from the tablets.
- The model that best fits the release data is selected based on the correlation coefficient (r) value in various models.
- The model that gives high r value is considered as the best fit of the release data.

Zero order release

- The equation for zero order release is

  $Q_t = Q_0 + K_0 t$

Where,

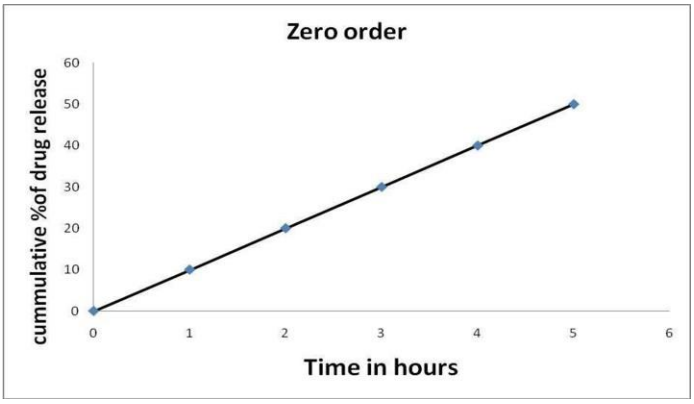
Q_0 = initial amount of drug

Q_t = cumulative amount of drug release

K_0 = zero order release constant

t = time in hours

- It describes the systems where the drug release rate is independent of its concentration of the dissolved substance.
- A graph is plotted between the time taken on x-axis and the cumulative percentage of drug release on y-axis and it gives a straight line.



First order release

- The equation for First order release is

$$\text{Log } Q_t = \text{Log } Q_0 + Kt/2.303$$

Where,

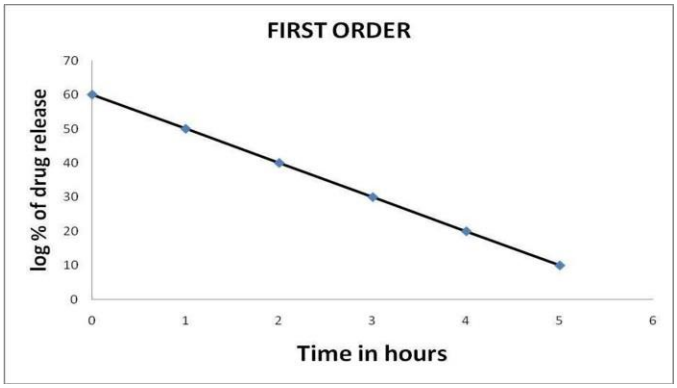
Q_0 = initial amount of drug release at time t

Q_t = cumulative amount of drug

K= first order release constant

t= time in hours

- Here, the drug release rate depends on its concentration.
- A graph is plotted between the time taken on x-axis and the log cumulative percentage of drug remaining to be released on y-axis and it gives a straight line.



DIFFERENT RELEASE TYPE OF ORAL CONTROL DRUG DELIVERY SYSTEM

DISSOLUTION CONTROLLED RELEASE

- Drug with slow dissolution rate is inherently sustained
  E.g. griseofulvin, digoxin, and salycilamide act as natural prolonged release products.
- Others aluminium aspirin, ferrous sulphate and benzamphetamine produce slow dissolving from when it comes to contact with GI fluids.
- Basic principle: If the dissolution process is diffusion layer surface through a unstirred liquid film to bulk solution is rate limiting the flux is 'j' is given by

  J=-D(dc/dx)

  Where,

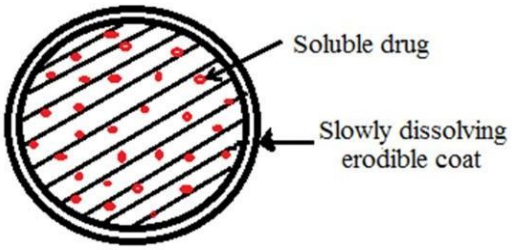
  D= Diffusion coefficient.

  dc/dx = concentration gradient between the solid surface and bulk of solution.

Types of dissolution controlled release products:

- **Encapsulation/ coating dissolution controlled system (reservoir devices)**

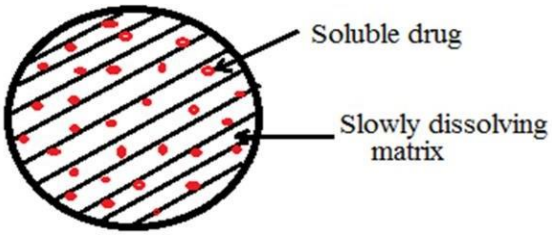
1) Individual particles or granules of drug
2) Coated with slowly dissolving materials like PEG, Wax
3) Coated granules are compressed into tablets or filled on capsules
4) The drug release is controlled by dissolution rate of polymeric coat



- **Matrix (or monolith)/ embedded dissolution**

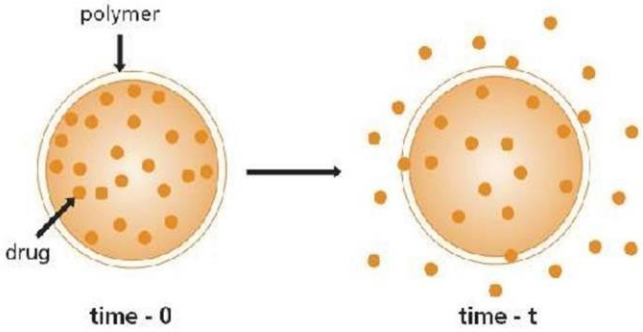
Drug is homogeneously dispersed throughout a rate controlling medium matrix system. Waxes are used beeswax, carnauba wax, hydrogenated castor oil etc.

2) Drug dispersed in molten wax
3) Congealing and granulating
4) The wax controls the dissolution rate by controlling the rate of dissolution fluid penetration into matrix by lathering the porosity of tablet, decreasing its wettability.



DIFFUSION CONTROLLED RELEASE

- Rate controlling step is diffusion of dissolved drug through a polymeric barrier. It is broadly classified into
- **Reservoir type (or laminated matrix devices):**
  - Drug is surrounded by a water insoluble polymer membrane.
  - Coating or microencapsulation is used to apply polymer.
  - Polymers like HPC, EC and polyvinyl acetate are used commonly.



- Mechanism: drug partitioning into membrane with subsequent release into the surrounding fluid by diffusion.

  The rate of drug release explained by Ficks law of diffusion.

  Dm/dt = DSK(AC)/L

  S= active surface area.

  D= diffusion coefficient of the drug across the coating membrane.

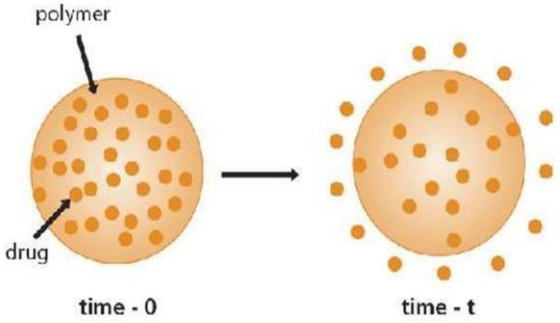
  L = diffusional path length (thickness of polymer coat).

  AC = concentration difference across L.

  K= partition coefficient of drug between polymer and external medium.

- **Matrix diffusion controlled system:**

- Drug is dispersed in insoluble matrix rigid non swellable hydrophobic materials like PVC and fatty materials like stearic acid bees wax
- For swellable hydrophilic substances like (glassy hydrogel) like gums (Natural) – guar gum, tragacanth, Semi synthetic – HPMC, CMC xanthan gum, Synthetic – polyacrylamides.



- Mechanism: initial dehydration of hydrogel – swells – drug diffuses out slowly.

ION EXCHANGE RESIN SYSTEM

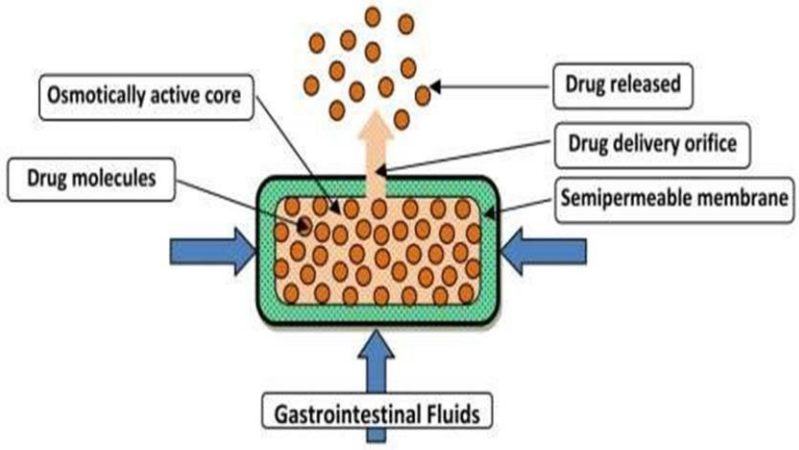
- Ion exchange resins are cross-linked water- insoluble polymers carrying ionizable functional groups. These resins are used for taste masking and controlled release system.
- The formulations are developed by embedding the drug molecules in the ion- exchange resin matrix and this core is then coated with a semi permeable coating material such as Ethyl Cellulose.
- This system reduced the degradation of drug in GIT. The most widely used and safe ion-exchange resin is divinylbenzene sulphonate. In tablet formulations ion- exchange resins have been used as disintegrant.

Ion activated drug delivery system:

- An ionic drug can be delivered by ion activated drug delivery system.
- In electrolyte medium, such as gastric fluid, ions diffuse into system, react with drug resin complex and trigger the release of ionic drug.
- Drugs suitable for resinate preparations:
  - Should have acidic or basic groups.
  - Half-life – 2 to 6 hrs.
  - Should be absorbed from all regions of GIT.
  - Drug should be sufficiently in the gastric juice.

OSMOTIC CONTROLLED RUG DELIVERY SYSTEM

- Osmosis is defined as the movement of solvent from lower to higher concentration through semi permeable membrane.
- Osmotic pressure is the hydrostatic pressure produced by a solution in a space divided by semi permeable membrane due to difference in concentration of solutes.
- Principle: Osmotic pressure is the driving force that generates constant drug release. In this system the drug reservoir can be solution or solid formulation and is placed in within the semi permeable membrane housing with controlled water permeability.
- The drug is activated to release in the solution form at a constant rate through a special delivery orifice.
- Release of drug is activated by osmotic pressure and controlled at a rate determined by water permeability, effective surface area of semi permeable housing and osmotic pressure gradient.



- Basic components are

  Drug

  - Short half life
  - Highly potent
  - Required for prolonged treatment.

Osmotic agent: (osmogents)

- Inroganic water soluble agents: Magnesium sulfate, NaCl, KCl, $NaHCO_3$ etc
- Organic polymer osmogents: Sodium CMC, HPMC, HEMC, MC, PVP.

Semi permeable membrane:

- Must possess sufficient wet strength and wet modulus to retain dimensional integrity.
- Sufficient water permeability so as ot retain water flux rate in the desired range.
- Biocompatible.

pH INDEPENDENT SYSTEMS:

- Most drugs are either weak acids or weak bases. The release from controlled release formulation is pH dependent.
- However buffers such as salts of amino acids, citric acid, phthalic acid phosphoric acid or tartaric acid can be added to formulation to maintain a constant pH thereby rendering pH independent drug release.
- A buffered formulation is prepared by mixing a basic or acidic drug with appropriate pharmaceutical excipient and coating with GI fluid permeable film forming polymer.
- When GI fluid permeates through the membrane, the buffering agents adjust the fluid inside to suitable constant pH thereby rendering a constant rate of drug release.

UNIT-2

- Transdermal Drug Delivery System (TDDS) are defined as self-contained, discrete dosage forms which are also known as "patches", when applied to the intact skin, deliver the drug through the skin at a controlled rate to the systemic circulation.
- Transdermal therapeutic systems have been designed to provide controlled continuous delivery of drugs via the skin to the systemic circulation. In a broad sense, the term transdermal delivery system includes all topically administered drug formulations intended to deliver the active ingredient into the general circulation.

Advantages:

- Hepatic first pass metabolism, salivary metabolism and intestinal metabolism are avoided.
- The ease of usage makes it possible for patients to self-administer these systems.
- In case of an emergency, removing the patch at any point of time during therapy can instantly stop drug input.
- Since the composition of skin structurally and biologically is the same in almost all the humans, there is minimal inter and intra patient variation.
- Drugs showing gastrointestinal irritation and absorption can be suitably administered through the skin.
- Continuous, non-invasive infusion can be achieved for drugs with short biological half-lifes, which would require frequent dosing.
- Due to reduced frequency of dosing there is better patient compliance.
- Therapeutic failures associated with irregularities in the dosing with conventional therapies can be avoided.
- The adverse effects are minimized due to a steady and optimum blood concentration time profile.
- The risks, pain and inconvenience associated with parenteral therapy are evaded.
- The drug release is such that there is a predictable and extended duration of activity.

Disadvantages:

- There is possibility of skin irritation due to the one or many of the formulation components.
- Binding of drug to skin may result in dose dumping.

- It can be used only for chronic conditions where drug therapy is desired for a long period of time including hypertension and diabetes.
- Cutaneous metabolism (e.g.- by Esterase enzymes) will affect therapeutic performance of the system.
- Transdermal therapy is feasible for certain potent drugs only.
- Transdermal therapy is not feasible for ionic drugs.
- It cannot deliver drug in pulsatile fashion.

PRINCIPLE OF TRANSDERMAL PERMEATION

Skin is the most intensive and readily accessible organ of the body as only a fraction of millimeter of tissue separates its surface from the underlying capillary network. The various steps involved in transport of drug from patch to systemic circulation are as follows:

1) Diffusion of drug from drug reservoir to the rate controlling membrane.
2) Diffusion of drug from rate limiting membrane to stratum corneum.
3) Sorption by stratum corneum and permeation through viable epidermis.
4) Uptake of drug by capillary network in the dermal papillary layer.
5) Effect on target organ.

Factors Affecting Transdermal Permeation

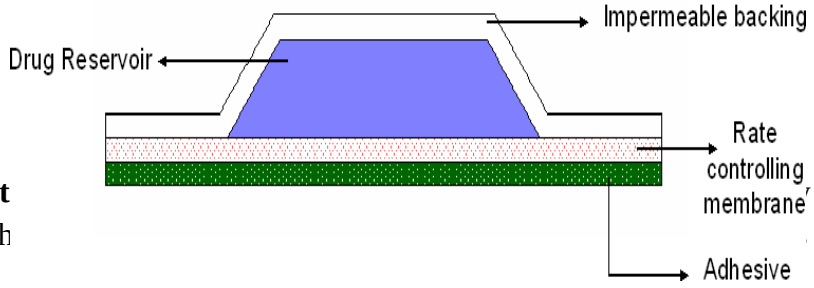
- **Partition coefficient:** Drugs possessing both water and lipid solubilities are favored. Lipid/water partition coefficient of 1 or more is required for optimal transdermal permeability.
- **Molecular size and shape:** Drug absorption is inversely related to molecular weight, small molecules penetrate faster than large ones.
- **pH conditions:** pH conditions of skin and in drug delivery system affect dissociation and permeation of drug molecule.
- **Drug concentration:** permeation is passive diffusion process hence depends on drug conc. on surface of skin layer. More drugs are absorbed through percutaneous absorption when the drug is applied to a large surface area.
- **Composition of drug delivery system:** It has great influence on absorption. It not only affects release rate but also permeability of stratum corneum by means of hydration, mixing with skin lipids or other promoting effects.

- **Release characteristics of drug delivery system:** Generally the more easily the drug is released from delivery system, the higher the rate of permeation. Drug substance should have greater physicochemical attraction to skin than to vehicle in which it is presented in order for the drug to leave in favor of the skin. Mechanism is depends on interfacial partition coefficient of drug from delivery system to skin tissue.
- **Use of permeation enhancers:** Permeation can be improved by use of permeation promoters. Drug absorption enhanced by the vehicles that easily cover the skin surface, mix readily with sebum and bring drug in contact with sebum for tissue absorption.
- **Reservoir effect of horny layer:** Horny layer or its deep layer can act as depot or reservoir. Absorption appears to be greater when drug is applied to skin with thin horny layer than with one that is thick. Thus, site of application affect degree of drug absorption.
- **Vehicles** that increase amount of moisture imbedded by the skin generally favor percutaneous absorption of drug. Oleaginous vehicles acts as moisture barrier through which the sweat from the skin cannot pass and skin therefore remain occluded resulting in increased hydration of skin beneath the vehicle.
- **Skin hydration:** Hydration of stratum corneum appears to increase rate of passage of certain substances that penetrate the skin. Increased absorption is probably due to softening of the tissues and the consequent sponging effect with an increase in size of the pore allowing greater flow of substance, large and small molecules through them. Hydration of stratum corneum can enhance the permeability of skin by as much as eight folds.
- **Skin temperature:** skin permeation of acetyl salicylic acid and glucosteroids was raised ten folds when the environmental temp was raised from 10-37 °C.
- **Skin metabolism:** Skin metabolizes steroids, hormones, chemical carcinogens and some drugs. So skin metabolism determines efficacy of drug permeated through the skin.
- **Skin conditions:** The intact skin itself acts as barrier but many agents like acids ,alkali cross the barrier cells and penetrates through the skin ,many solvents open the complex dense structure of horny layer. Solvents like methanol, chloroform remove lipid fraction, forming artificial shunts through which drug molecules can pass easily.
- **Skin age:** It is seen that the skin of adults and young ones are more permeable than the older ones but there is no dramatic difference .Children shows toxic effects because of the greater surface area per unit body weight. Thus potent steroids, boric acid, hexachlorophene has produced severe side effects.

- **Regional skin site:** Thickness of skin, nature of stratum corneum and density of appendages vary site to site. These factors affect significantly penetration.

Materials employed/ Formulation/ Components of transdermal patch

1. **The drug**
2. **Polymer matrix/ matrices**
3. **Pressure sensitive adhesives**
4. **Permeation enhancers**
5. **Excipients/ other supportive materials.**



1. **Drug subst** system, the drug should be chosen with of a drug for transdermal delivery:

- The drug should have a molecular weight less than 1000 Daltons.
- The drug should have affinity for both lipophilic and hydrophilic phases.
- The drug should have low melting point.
- The drug should be potent, having short half -life.
- The drug should be non-irritant and non-allergic to human skin.
- The drug should be stable when contact with the skin.
- The drug should not get extensively metabolized in the skin.
- Drugs degraded in the GIT or inactivated by the hepatic first pass are suitable candidates for transdermal drug delivery.

2. Polymer matrix: Polymers are the backbone of transdermal drug delivery system. System for transdermal delivery are fabricated as multi layered polymeric laminates in which a drug reservoir or a drug polymer matrix is sandwiched between two polymeric layers, an outer impervious backing layer that prevents the loss of drug through the backing surface and an inner polymeric layer that functions as an adhesive, or rate controlled membrane.

Ideal properties of a polymer to be used in a transdermal system:

- Molecular weight, chemical functionality of the polymer should be such that the specific drug diffuses properly and gets released through it.
- The polymer should be stable, nontoxic, and inexpensive.
- The polymer and its degradation products must be non-toxic or non-antagonistic to the host.
- The mechanical properties of the polymer should not deteriorate excessively when large amounts of active agent are incorporated into it.

Some commonly used polymers for TDD are:

- Natural Polymers: Cellulose derivatives, Gelatin, Proteins, Starch
- Synthetic Elastomers: Polybutadiene, Acrylonitrile, Neoprene
- Synthetic Polymers: Polyvinyl alcohol, Polyethylene, Polyamide

3. Pressure sensitive adhesive: A Pressure Sensitive Adhesive (PSA) is a material that helps in maintaining an intimate contact between transdermal system and the skin surface. It should adhere with not more than applied finger pressure, be aggressively and permanently tacky, and exert a strong holding force. Additionally, it should be removable from the smooth surface without leaving a residue. e.g.: polyacrylamates, polyacrylates, polyisobutylene, silicone based adhesive. PSA should be physicochemical and biologically compatible and should not alter drug release. The PSA can be positioned on the face of the device or in the back of the device and extending peripherally.

4. Penetration Enhancers: These are compounds which promote the skin permeability by altering the skin as barrier to the flux of a desired penetrant (drug).

Ideal properties of penetration enhancers are:

- It should be compatible with all drugs and excipients.

- Upon removal of the material, the skin should immediately and fully recover its normal barrier property.
- The enhancer should not cause loss of body fluids, electrolytes or other endogenous materials.
- The chemical should formulate into all the variety of preparations used topically.
- It should be odorless, tasteless, colorless and inexpensive.

Some commonly used absorption enhancers for TDDS are:

- Surfactants: Na-lauryl sulfate, Na-deoxycholate, Na-glycocholate
- Fatty acids: Oleic acid
- Cyclodextrins: γ- and β-Cyclodextrins
- Chelating agents: EDTA, Polyacrylates
- Positively charged polymer: Chitosan salts, Trimethyl chitosan

5. Excipients/ other supportive materials:

a) **Solvents:** Various solvents such as chloroform, methanol, acetone are used to prepare drug reservoir. In addition plasticizers such as dibutyl pthalate, propylene glycol are added to provide plasticity to the transdermal patch.

b) **Backing laminates:** They should a low moisture vapour transmission rate. They must have optimal elasticity, flexibility and tensile strength. E.g: aluminum vapour coated layer, a plastic film.

c) **Release linear:** During storage release linear prevents the loss of drug that has migrated into the adhesive layer and contamination. However, as the linear is in intimate contact with the delivery system, it should comply with specific requirements regarding chemical inertness and permeation to the drug, penetration enhancer.

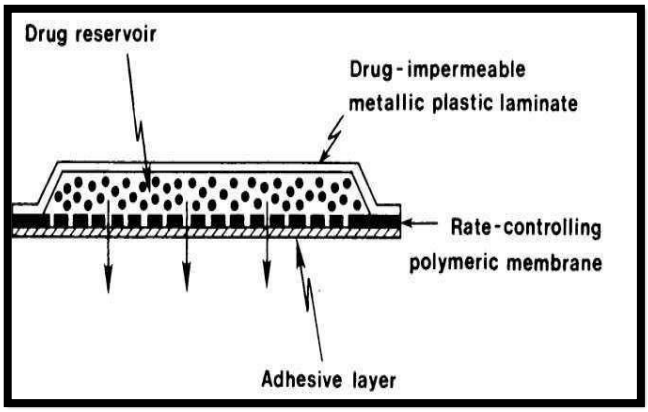
d) **Rate controlling membrane:** These are used to limit the flow of the drug from both reservoir and matrix systems. The function depends on the design of the specific system, the size of the active component and the need to have a rate-limiting factor in order to satisfy the release and absorption characteristics of the system. Ex: Ethylene Vinyl Acetate Membranes (EVA), Microporous Polyethylene Membranes.

Approaches in development of TDDS

1. Membrane permeation controlled systems / Reservoir type systems.
2. Adhesive- dispersion type systems.
3. Matrix diffusion- controlled systems.
4. Microreservoir type/ microsealed dissolution controlled systems.

Membrane permeation controlled systems / Reservoir type systems

- Drug reservoir (homogenous dispersion of drug with polymeric matrix or suspension of drug in un leachable viscous liquid medium such as silicone fluid) is encapsulated within drug impermeable metallic plastic laminate and a rate controlling polymeric membrane (ethylene vinyl acetate co polymer).
- The rate of drug release is determined by the permeability of the rate controlling membrane.
- A layer of adhesive polymer is applied on membrane to secure the device on skin.
- The rate of release of drug is always maintained at constant rate & the type of release is zero order.
- Release rate of this TDDS depends upon the polymer composition, permeability coefficient and thickness of the rate controlling membrane and adhesive.

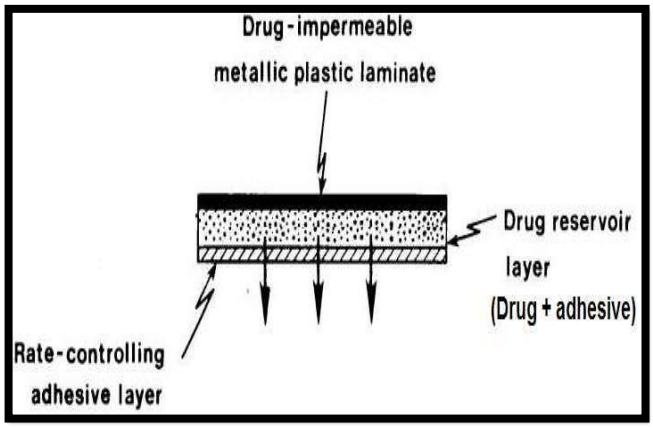


Example of this system: **Scopolamine** releasing TDDS for 72 hrs, prophylaxis of motion sickness.

Adhesive- dispersion type systems

- Drug reservoir is formulated by homogenous dispersion of drug with adhesive polymer poly(isobutylene) or poly acrylate.
- Then spreading of this medicated adhesive by solvent casting/ hot melt on flat sheet of drug impermeable metallic plastic backing to form thin drug reservoir layer.

- On top of the drug reservoir layer, thin layers of rate controlling adhesive polymer of specific permeability and constant thickness are applied to produce an adhesive dispersion- diffusion controlled TDDS.

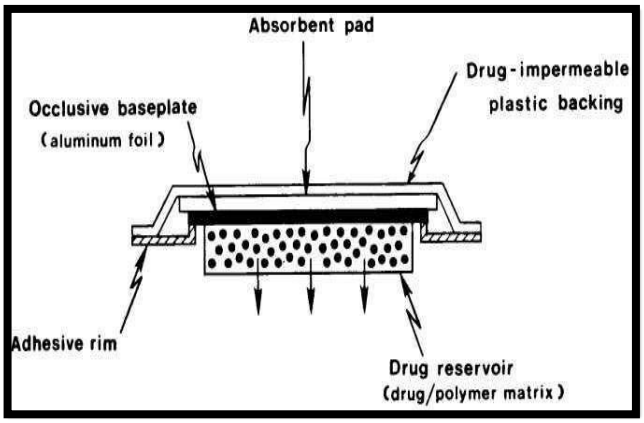


Example of this system: **Verapamil** releasing TDDS

Matrix diffusion- controlled systems

Drug reservoir of homogenous dispersion of drug with hydrophilic or lipophilic polymer is prepared with one of the following methods

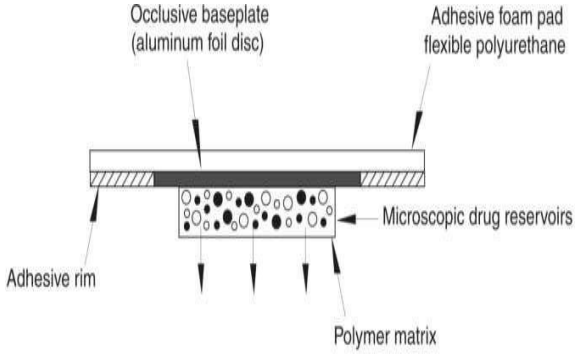
1. Homogenous dispersion of finely ground drug particles with liquid polymer or highly viscous base polymer followed by cross linking of polymer chains
2. Homogenous mixing of drug solid with rubbery polymer at an elevated temperature
3. Dissolving the drug and polymer in a common solvent followed by solvent evaporation in a mould at an elevated temperature or under vacuum.
4. Medicated polymer is moulded in to medicated disc with desired surface area and controlled thickness.
5. This medicated polymer disc is pasted on to an occlusive base plate with impermeable plastic backing.
6. Then the adhesive polymer is spread along the circumference to form a strip of adhesive rim around the medicated disc.



Example of this system: **Nitroglycerin** releasing TDDS deliver daily dose of 0.5mg/cm^2 for therapy of angina pectoris.

Microreservoir type/ microsealed dissolution controlled systems

- It is a combination of reservoir and matrix dispersion system.
- For drug reservoir, the drug is first suspended in aqueous solution of water soluble polymer and then dispersing the solution homogenously in lipophilic polymer to form thousands of unleachable, microscopic spheres of drug reservoir.
- Depending on property of drug and desired rate of drug release disc is coated with a layer of biocompatible polymer.
- This medicated polymer disc is pasted on to an occlusive base plate with impermeable plastic backing.
- Then the adhesive polymer is spread along the circumference to form a strip of adhesive rim around the medicated disc.



Example of this system: Nitroglycerin releasing TDDS deliver daily dose of 0.5mg/cm^2 for once a day therapy of angina pectoris.

UNIT-3

MUCOADHESIVE DRUG DELIVERY SYSTEM

Mucoadhesive drug delivery system interact with the mucus layer covering the mucosal epithelial surface, & mucin molecules & increase the residence time of the dosage form at the site of the absorption. Mucoadhesive drug delivery system is a part of controlled release drug delivery system.

Why are we using Mucoadhesive drug delivery system (MDDS)?

- MDDS prolong the residence time of the dosage form at the site of application or absorption.
- Intimate contact of the dosage form with the underlying absorption.
- Improve the therapeutic performance of drug.
- High drug loading capacity.
- Controlled drug release (preferably unidirectional release).

Advantages-

- MDDS offer several advantages over other controlled oral controlled release systems by virtue of prolongation of residence of drug in GIT.
- Targeting & localization of the dosage form at a specific site.
- High drug flux at the absorbing tissue.
- MDDS will serve both the purposes of sustain release & presence of dosage form at the site of absorption.
- Excellent accessibility.
- Painless administration.
- Low enzymatic activity & avoid of first pass metabolism.

Disadvantages-

- If MDDS are adhering too tightly, it is undesirable to exert too much force to remove the formulation after use; otherwise the mucosa could be injured.
- Some patient suffers unpleasant feeling.

- Unfortunately, the lack of standardized techniques often leads to unclear results.
- Costly drug delivery system.
- Medications administered orally do not enter the blood stream immediately after passage through the buccal mucosa.

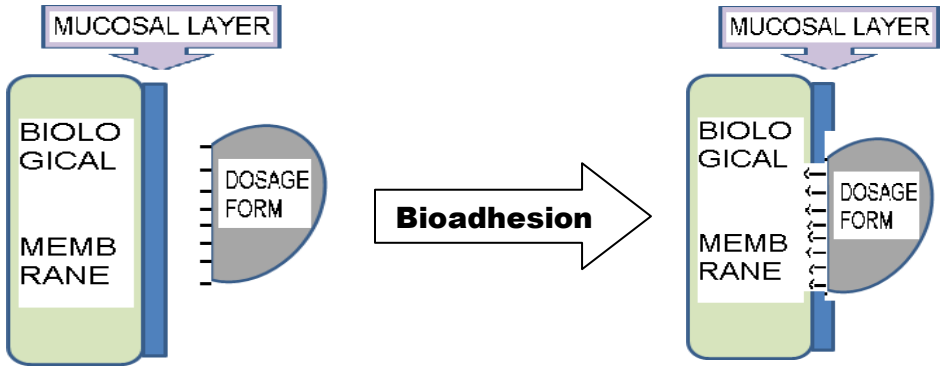
Mucosal membrane are moist membranes that line passageways and structures in the body that lead to the outside environment such as the mouth, respiratory tract, gastrointestinal tract, nose and vagina. They secrete a viscous fluid known as mucus, which acts as a protective barrier and also lubricates the mucosal membrane. The primary constituent of mucus is a glycoprotein known as mucin as well as water and inorganic salts.

Composition of Mucus layer

Mucus is translucent and viscid secretion which forms a thin continuous gel adherent to mucosal epithelial surface.

Water	**95%**
Glycoprotein and lipids	**0.5-5%**
Mineral salts	**1%**
Free proteins	**0.5-1%**

Bioadhesion is used to describe the bonding or adhesion between a synthetic or natural polymer (in a dosage form)and biological substrate such as epithelial cells, which allows the polymer to adhere to the biological surface for an extended period of time, which result in increase residence time, bioavailability & site specificity.

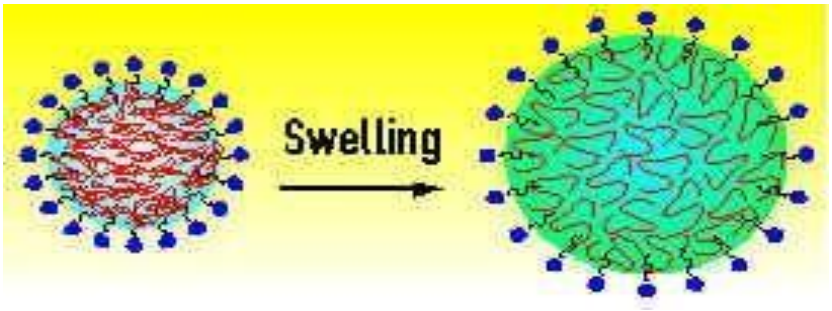


MECHANISMS OF MUCOADHESION

- The mechanism of adhesion of certain macromolecules to the surface of a mucous tissue is not well understood yet. The mucoadhesive must spread over the substrate to initiate close contact and increase surface contact, promoting the diffusion of its chains within the mucus. Attraction and repulsion forces arise and for a mucoadhesive to be successful, the attraction forces must dominate.
- Each step can be facilitated by the nature of the dosage form and how it is administered. For example, a partially hydrated polymer can be adsorbed by the substrate because of the attraction by the surface water.
- Thus, the mechanism of mucoadhesion is generally divided in three steps as follows:

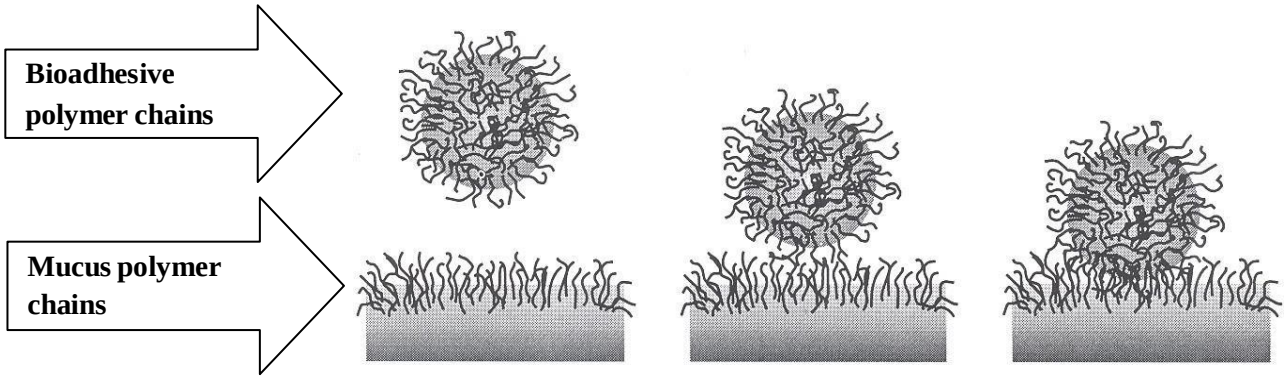
STEP-I:

- The first stage is characterized by the contact between the mucoadhesive and the mucous membrane, with spreading and swelling of the formulation, initiating its deep contact with the mucus layer.
- Swelling of polymers occurs because the components within the polymers have an affinity for water.
- Bioadhesives are able to adhere to or bond with biological tissues by the help of the surface tension and forces that exist at the site of adsorption or contact.
- In some cases, such as for ocular or vaginal formulations, the delivery system is mechanically attached over the membrane.
- On the other hand, in the gastrointestinal tract direct formulation attachment over the mucous membrane is not feasible. Peristaltic motions can contribute to this contact, but there is little evidence in the literature showing appropriate adhesion.



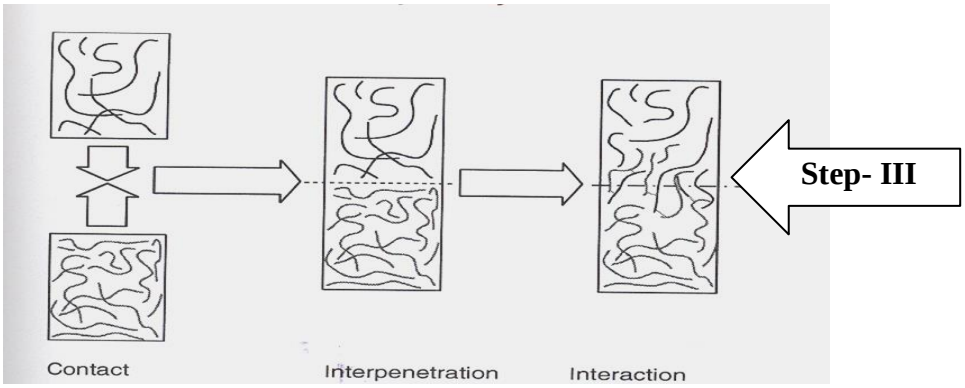
STEP-II:

- The surface of mucosal membrane is composed of high molecular weight polymers known as glycoprotein.
- In this step of the bioadhesive bond formation, the bioadhesive polymer chains and the mucosal polymer chains intermingle and entangle to form semi permeable adhesive bonds. The strength of these bonds depends on the degree of penetration between the two polymer groups.
- In order to form strong adhesive bonds, one polymer group must be soluble in the other and both polymer types must be of similar chemical structure.



STEP-III:

- This step involves the formation of weak chemical bonds between the entangled polymer chains.
- The types of bonding formed between the chains include primary bonds such as covalent bonds and weaker secondary interactions such as Vander Waals Interactions and hydrogen bonds.
- Both primary and secondary bonds are exploited in the manufacture of bioadhesive formulations in which strong adhesions between polymers are formed.



MUCOADHESION THEORIES

Various theories exist to explain at least some of the experimental observations made during the bioadhesion process. However five main theories can be distinguished.

- Wetting theory
- Electronic theory
- Fracture theory
- Adsorption theory
- Diffusion theory

Wetting theory

The wetting theory applies to liquid systems which present affinity to the surface in order to spread over it. This affinity can be found by using measuring techniques such as the contact angle. The general rule states that the lower the contact angle then the greater the affinity. The contact angle should be equal or close to zero to provide adequate spreadability.

Electronic theory

The electronic theory depends on the assumption that the bioadhesive material and the target biological material have different electronic surface characteristics. Based on this, when two surfaces come in contact with each other, electron transfer occurs in an attempt to balance the Fermi levels, resulting in the formation of a double layer of electrical charge at the interface of the bioadhesive and the biologic surface. The net result of such a process is the formation of attractive forces within this double layer.

Adsorption theory

This theory states that the bioadhesive bond formed between an adhesive substrate and the tissue is due to the weak vander waals forces and hydrogen bond formation. For example, hydrogen bonds are the prevalent interfacial forces in polymers containing carboxyl groups. Such forces have been considered the most important in the adhesive interaction phenomenon because, although they are individually weak, a great number of interactions can result in an intense global adhesion. Whilst these interactions require less energy to “break”, they are the most prominent form of surface interaction in mucoadhesion processes as they have the advantage of being semi-permanent bonds.

Fracture theory

This is perhaps the most used theory in studies on the mechanical measurement of mucoadhesion. It analyzes the force required to separate two surfaces after adhesion is established. This force, S_m is frequently calculated in tests of resistance to rupture by the ratio of the maximal detachment force, F_m, and the total surface area, A_0, involved in the adhesive interaction.

$$S_m = F_m/A_0$$

Since the fracture theory is concerned only with the force required to separate the parts, it does not take into account the interpenetration or diffusion of polymer chains. Consequently, it is appropriate for use in the calculations for rigid or semi-rigid bioadhesive materials, in which the polymer chains do not penetrate into the mucus layer.

Diffusion Theory

The concept of the interpenetration and entanglement ob the bioadhesive polymer chains and mucous polymer chains is supported by the diffusion theory. The bond strength increases with the increase in the degree of the penetration. This penetration rate depends on the diffusion coefficient, flexibility and nature of the mucoadhesive chains, mobility and contact time. According to the literature, the depth of interpenetration required to produce an efficient bioadhesive bond lies in the range 0.2- 0.5 μm. This interpenetration depth of polymer and mucin chains can be estimated by equation.

$$L = (tD_b)^{1/2}$$

Where t is the contact time and D_b is the diffusion coefficient of the mucoadhesive material in the mucus. The adhesion strength for a polymer is reached when the depth of penetration is approximately equivalent to the polymer chain size. In order for diffusion to occur, it is important that the components involved have good mutual solubility, that is, both the bioadhesive and the mucus have similar chemical structures. The greater the structural similarity, the better is the mucoadhesive bond.

Mechanical theory

Mechanical theory considers adhesion to be due to the filling of the irregularities on a rough surface by a mucoadhesive liquid. Moreover, such roughness increases the interfacial area available to interactions thereby aiding dissipating energy and can be considered the most important phenomenon of the process.

It is unlikely that the mucoadhesion process is the same for all cases and therefore it cannot be described by a single theory. In fact, all theories are relevant to identify the important process variables.

FACTORS AFFECTING MUCOADHESION

A) POLYMER RELATED FACTORS:-

1) Molecular weight

- Bioadhesion is maximum at certain molecular weight
- High molecular weight- Entanglement is more
- Low molecular weight- interpenetration is more
- Bioadhesive force of polymers is increase up to molecular weight 10000, beyond which there is no further gain.

2) Concentration of active polymer

- When concentration of the polymer is low the number of penetrating polymer chains per unit volume of mucus is small & interaction b/w polymer & mucus is unstable. In general the more concentrated polymer would result in longer penetrating chain length & better adhesion.
- Higher concentration of polymer does not necessarily improve & in some cases, actually diminished mucoadhesive properties. eg- high concentration of the flexible polymeric films based on polyvinyl pyrrolidone as film forming polymer did not further enhance the mucoadhesive properties of the polymer.

3) Degree of hydration

- Depending on the degree of hydration adhesive properties are different. It is maximum at a certain degree of hydration.

- High degree of hydration- formation of slippery, non- adhesive mucilage due to large amount of water leads to loss of adhesiveness.

4) Charge on polymer

- Mucosal surface is negatively charged. So positively charged polymer might facilitate the mucoadhesive process.
- Chitosan have bioadhesion due to electrostatic attraction between positively charged D-glucosamine residue of chitosan and negatively charged sialic acid residues.

5) Hydrogen bonding

- Adhesion of polymers is greatly depend upon the hydrogen bonding that higher the hydrogen bonding stronger is the adhesive strength of the polymers.
- Main functional groups responsible for such type of interactions are hydroxyl, carboxyl and amino groups.

6) Spatial conformation

- Besides molecular weight or chain length, spatial conformation of a polymer is also important. Despite a high molecular weight of 19,50,0000 for dextran, they have adhesive strength similar to that of polyethylene glycol (PEG), with a molecular weight of 2,00,000. The helical conformation of dextran may shield many adhesively active groups, primarily responsible for adhesion, unlike PEG polymers, which have a linear conformation.

B) <u>ENVIRONMENT RELATED FACTORS:-</u>

7) pH

- pH influences the charge on the surface of both mucus and the polymers.
- Mucus will have a different charge density depending on the pH because of the difference in the dissociation of the functional groups on the carbohydrate moiety and amino acids of the polypeptide backbone.
- Polyacrylic acid polymers → degree of hydration increase upto pH 4 to 5 → slightly increases at pH 6 to 7 → decreasing at more alkaline pH.

8) Applied strength

- To place a solid bioadhesive system, it is necessary to apply a defined strength. The adhesion strength increases with the applied strength or with the duration of its application, up to an optimum level.

9) Initial contact time

- The initial contact time between the mucoadhesive and the mucus layer determines the extent of swelling and the interpenetration of the polymer chains.
- The mucoadhesive strength increases as the initial contact time increases.

10) Swelling

- Interpenetration of chains is easier when polymer chains are disentangled and free of interactions.
- When swelling is too great, a decrease in the bioadhesion occurs, such phenomena must not occur too early, in order to lead to a sufficient time for action of the bioadhesive system.

C) PHYSIOLOGICAL FACTORS:-

11) Mucin turnover

- The natural turnover of the mucin molecules from the mucus layer is important for at least two reasons-
- The mucin turn over is expected to limit the residence time of mucoadhesive dosage form on the mucus layer.
- Mucin turnover results in substantial amount of soluble mucin molecules. These mucin molecules interact with mucoadhesive dosage form before they have a chance to interact with the mucus layer.

12) Disease states

- The physiological properties of the mucus are known to change during disease conditions such as the common cold, gastric ulcers etc. The exact structural changes taking place in mucus under these conditions are not yet clearly understood.

MATERIALS USED IN THE FORMULATION OF MDDS

In MDDS, the following **functional agents/ materials** are used-

1) Penetration enhancers
2) Enzyme inhibitors
3) Mucoadhesive agents/ Mucoadhesive polymers

1) **Penetration enhancers (PE):-**

- Penetration enhancers are also required when a drug has to reach the systemic circulation to exert its action.
- Must be inert, non-irritant & have a reversible effect.
- Recently Chitosan & its derivatives, polymers already known for mucoadhesive properties. Chitosan help transportation of drug through paracellular pathway.

Ideal characteristics

- Safe and non toxic, non irritating and non allergenic.
- Pharmacologically and chemically inert.
- They should have no pharmacological activity within the body.
- The penetration enhancers should be compatible with both excipients and drugs

Mechanism

- **Changing mucus rheology:** By reducing the viscosity of the mucus.
- **Increasing the fluidity of lipid bilayer membrane:** Disturb the intracellular lipid packing by interaction with either lipid or protein components.
- **Acting on the components at tight junctions**: Act on desmosomes, a foremost component at the tight junctions by this means enhances drug absorption.
- **Increasing the thermodynamic activity of drugs**: Some enhancers increase the solubility of drug there by alters the partition coefficient

Classification

1. **Surfactants**
   Anionic: E.g. Sodium lauryl sulfate, Sodium dodecyl sulfate
   Nonionic: E.g. Tween80, Sodium glycocholate
   Cationic: E.g. Cetylpyridinium chloride, Chitosan, Trimethyl chitosan

   MOA-
   - Perturbation of intercellular lipids
   - Ionic interaction with negative charge on the mucosal surface
   - Surfactant act by protein denaturation or by swelling of tissue and extraction of lipids component.

2. **Fatty acids and derivatives**

E.g. Oleic acid, Lauric acid, Linoleic acid, Acylcholines

MOA-

- Fatty acid are act by paracellular and transcellular route
- Increase fluidity of phospholipids domains

3. **Bile salts and derivatives**

E.g. Sodium deoxycholate, Sodium taurocholate, Sodium glycocholate

MOA-

- Act by modifying the cell membrane integrity in such a way that the intracellular domain is open up
- At a high concentration it increases absorption of drug through intestinal membrane disruption caused by the solubilization of phospholipids.
- At low concentration absorption of drug increase by formation of reverse micelles and calcium complexation without membrane disruption.

4. **Sulfoxide**

E.g. Dimethyl sulfoxide (DMSO), Declmethyl sulfoxide

MOA-

- Increase fluidity of lipid bilayer and disturb protein component from mucus.

5. **Chelating agents**

E.g. EDTA, Citric acid, Salicylates

MOA-

- Interfere with calcium ion
- Acts by transcellular and paracellular route

6. **Monohydric alcohols**

E.g. Ethanol, Isopropanol, methanol

MOA-

- Increase the partition coefficient of a drug by facilitating the transcellular pathway in a concentration independent manner.

- Disrupt arrangement of intercellular lipids

7. Polyols

E.g. Propylene glycol, Polyethylene glycol, Glycerol, Propanediol

MOA-

- Acts by paracellular route

8. Azone

MOA-

- Azone primarily enhances the transport of lipophilic drug across the keratinized oral mucosa.
- It forms an ion pair with anionic drug thereby promoting their penetration

MATERIALS USED IN THE FORMULATION OF MDDS (Contd..)

2) Enzyme inhibitors-

- Drug + Enzyme inhibitors → improving the buccal absorption of drugs, particularly peptides.
  Ex- Aprotinin, Bestatin, Puromycin.
- Chemical modification of chitosan with EDTA produces polymer conjugate chitosan – EDTA that is a very potent inhibitor of metallopeptidases (carboxypeptidase).

3) Mucoadhesive agents/ Mucoadhesive polymers -

Mucoadhesive drug delivery systems are based on the adhesion of a drug/ carrier to the mucous membrane. To promote this adherence a suitable carrier is required. Mucoadhesive polymers are either water soluble or insoluble, which are swellable networks, connected by cross-linking agents.

Properties of an ideal mucoadhesive polymer:

- The polymer and its degradation products should be nontoxic, non-irritant and nonabsorbable from the GIT.
- Possess high viscosity, proper degree of cross linking and proper spatial conformation of polymer is must.
- It should have site specificity and adhere rapidly to most tissues.
- It must not degrade during the shelf life of the dosage form.
- Strong H-bonding groups (-OH, -COOH) should be present in polymer for bonding with mucous membrane.
- Polymer should have strong anionic charges and high molecular weight.
- It should be sufficiently flexible to interpenetrate the mucus membrane or tissue crevices.
- It should have correct surface tension characteristics suitable for moistening of mucus surface.

PAA derivatives

Poly acrylic acid derivatives are polymers of acrylic acid cross linked with polyalkenyl ethers or divinyl glycol. They are obtained from primary polymer particles of about 1-5 micron diameter. Each primary particle prevails as a network structure of polymer chains interconnected through cross links. Carbopol, a PAA derivative, swells upto 1000 times their original volume in water and gets gelified at a pH range of 4.0 to 6.0. Due to carboxylate group, repulsion occurs between the negative charges which consequently make polymer to swell and hence mucoadhesive strength of the polymer rises.

Chitosan

Chitosan, a cationic semi-synthetic polymer, is obtained from chitin by deacetylation. Studies have shown that chitosan can enhances absorption of hydrophilic molecules by rearrangement of protein structures associated to the intercellular junctions. Chitosan binds to the mucosa via ionic bonds between the amino group and sialic acid residues. Chitosan being linear gives higher polymer chain flexibility.

Collagen

Collagen is a natural protein. It is a tri-helical molecule. Nineteen types of collagen molecules are identified. Collagen has improved biocompatibility, low antigenicity and degrades less on implanting.

Gelatin

Gelatin is a natural water soluble protein which is normally obtained by denaturation of collagen. It has good biodegradability, biocompatibility, and low antigenicity. It is used as support material for gene delivery, cell culture, and more novels is use in tissue engineering. Gelatin-based systems can give zero order release of biologically active agents such as drugs, peptides and proteins. It is possible to entrap bioactive compounds into PEGylated liposome-gelatin gel.

Albumin

Serum albumin was conjugated to polyethylene glycol and cross-linked to form mono-PEGylated albumin hydrogels. These hydrogels can be assessed as drug carrying tissue engineering scaffold materials.

Alginate

Alginate is a naturally occurring linear polysaccharide. Alginate and its derivatives are used for drug delivery and tissue engineering applications due to its enhanced biocompatibility, biodegradability, low toxicity, non-immunogenicity, water solubility, comparatively low cost, better gelling properties, stabilizing properties, and high viscosity in aqueous solutions.

Dextran

Dextran is a linear natural polymer of glucose cross-linked by a 1,6-glucopyranoside, and some branching of 1,3 cross-linked side chains. Its good water solubility, biocompatibility, and biodegradability are responsible for its increasing applications in pharmaceutical field.

Newer second generation polymers

Newer polymers with enhanced mucoadhesive properties are now available. Example like lectins, thiomers and alginate polyethylene glycol acrylate.

Their advantages are:

- Site specificity called cytoadhesiveness.
- Not affected due to high mucus turn-over.
- Targeting of drug.

Lectins

Lectins are natural proteins useful for bio-recognition of cells and proteins. They are structurally varying proteins and glycoprotein which reversibly bind to specific residues of carbohydrates.

After binding to the cell, these might stay on the surface of cell or may be face endocytosis. Thus provide site specific and controlled drug release. The disadvantage is that they are immunogenic.

Thiolated Polymers

Thiolated polymers are derived from water soluble polymers like polyacrylates, chitosan or deacetylated gallan gum. Based on thiol or disulphide exchange reactions or simple oxidation, disulphide bonds are formed between polymers and cysteine rich domains of mucus glycoproteins thus forming the mucus gel layer. Thiomers imitate the natural mechanism of secreted mucus glycoproteins, which are covalently bound in the mucus layer through formation of disulphide bonds. Due to thiol groups, the residence time improves and thus covalent bonding is promoted with the cysteine present in mucus. The disulphide bonds might also alter the mechanism of drug release from the delivery system due to surged rigidity and cross linking.

Water Soluble Resins (WSR)

POLYOX™ polymers are among the fastest-hydrating water soluble polymers used in pharmaceutical systems. This category of high molecular weight polyethylene oxide homo polymers are water soluble, high molecular weight, biocompatible and non-toxic and can also be formulated into tablets, films, gels, microcapsules and syrups.

VARIOUS ROUTES OF MDDS

Oral Mucoadhesive Drug Delivery Systems

- Drug delivery through the oral mucosa has gained significant attention due to its convenient accessibility. The buccal and sublingual routes are considered as the most commonly used rotes. The nonkeratinized epithelium in the oral cavity, such as the mouth floor, the ventral side of the tongue, and the buccal mucosa, offers a relatively permeable barrier for drug transport.
- Drug delivery through the oral mucosa has proven particularly useful and offers several advantages over other drug delivery systems including *bypassing hepatic first-pass metabolism, increasing the bioavailability of drugs, improved patient compliance, excellent accessibility, unidirectional drug flux, and improved barrier permeability compared, for example, to intact skin.*
- The oral cavity has been used as a site for local and systemic drug delivery. Local drug

therapy is used to treat disease states like aphthous ulceration, gingivitis and xerostomia. Different dosage designs include adhesive gels, tablets, films, patches, ointments, mouth washes, and pastes.

- Until now adhesive tablets have been the most commonly used dosage forms for buccal drug delivery. Tablets can be applied to different regions of oral cavity, such as cheeks, lips, gums, and palate. Unlike conventional tablets, buccal tablets allow drinking, eating, and speaking without any major discomfort.
- It was observed that when tablets were prepared by using hydroxyethyl cellulose (HEC) and carbopol 940 in a 1:1 ratio as matrix-forming polymers at varying compression forces the compression forces did not significantly affect the water penetration and polymer chain stretching; however, mucoadhesion performance and drug release were influenced by compression force. Moreover, it was observed that tablets prepared with the lowest force gave the best results, in comparison with tablets prepared with highest forces causing pain during *in vivo* application, needing to be detached by human volunteers.
- Oral mucosal ulceration is a common condition with up to 50% of healthy adults suffering from recurrent minor mouth ulcers. The mucoadhesive patch was found to be more effective than the oral solution in terms of healing time and pain intensity after 12 and 24 h.
- A buccal patch system consists of a matrix patch containing drug, mucoadhesive polymers, and polymeric elastomers surrounded by a backing material. This buprenorphine patch is capable of delivering the drug for a period up to 12 h, with good patient comfort reported.
- Semisolid dosage forms, such as gels and ointments, have the advantage of easy dispersion throughout the oral mucosa. Poor retention of the gels at the site of application has been overcome by using mucoadhesive formulations. Certain mucoadhesive polymers, for example, sodium carboxymethylcellulose, carbopol, and xanthan gum, undergo a phase change from liquid to semisolid. This change enhances the viscosity, which results in sustained and controlled release of drugs. Hydrogels are also a promising dosage form for buccal drug delivery.

- Spray which is capable of delivering large molecules, such as insulin across the oral mucosa. Nitroglycerin is a small molecule that can be rapidly delivered across the sublingual oral mucosa using a spray for angina relief.

Nasal Mucoadhesive Drug Delivery Systems

- The area of the normal human nasal mucosa is approximately 150 cm^2, a highly dense vascular network and relatively permeable membrane structure; all these factors make nasal cavity interesting.
- Drawbacks are local toxicity/irritation, mucociliary clearance of 5 min, presence of proteolytic enzymes, and influence of pathological conditions (cold and allergies). Among the advantages are rapid uptake and avoiding first-pass hepatic metabolism. In addition, bioadhesive application of liquids, semisolids, and solids can significantly increase retention time.
- Nasal delivery of protein and peptide therapeutics can be compromised by the brief residence time at this mucosal surface. Some bioadhesive polymers have been suggested to extend residence time and improve protein uptake across the nasal mucosa.
- A viscosity enhancing mucosal delivery systems was studied for the induction of an adaptive immune response against viral antigen.
- Powder formulations based on spray-dried mixtures of starch (Amioca®) and poly (acrylic acid) (Carbopol® 974P) in different ratios were used as carriers of the viral antigen.

Ocular Mucoadhesive Drug Delivery Systems

- Drug administration to the eye is a challenge because there are several mechanisms (tear production, tear flow, and blinking) that protect the eye from the harmful agents.
- Conventional delivery methods are not ideal. Solutions and suspensions are readily washed from the cornea and ointments alter the tear refractive index and blur vision; so it is a target to prolong the residence time by mucoadhesion.
- Ocular films applied behind the eye lid were found to prolong retention time and precision of dosing. However, films were found to have a tendency to move across the

surface of the eye, thus resulting in irritation, for example, from Ocusert® (Alza). It has been shown that the addition of mucoadhesive polymers to ocular films reduced film movement across the eye, minimizing ocular irritation and burning sensations.

Vaginal Mucoadhesive Drug Delivery Systems

- The vagina is a fibrovascular tube connecting the uterus to the outer surface of the body. The vaginal epithelium consists of a stratified squamous epithelium and lamina propia.
- Dosage forms used for vaginal route are solutions, gels, suspensions, suppositories, creams, and tablets and all have short residence time. Bioadhesive can control the rate of drug release from, and extend the residence time of, vaginal formulations. These formulations may contain drug or, quite simply, act in conjunction with moisturizing agents as a control for vaginal dryness.
- Recent advances in polymeric technology have increased the potential of vaginal gels. Vaginal gels are semisolid polymeric matrices comprising small amounts of solid, dispersed in relatively large amounts of liquid and have been used in systems for microbicides, contraceptives, labor inducers, and other substances.

Rectal Mucoadhesive Drug Delivery Systems

- The rectum is part of the colon, it is 10 cm in length, and has surface area 300 cm^2. The function of the rectum is mostly concerned with removing water. Surface area without villi gives it a relatively small surface area for drug absorption.
- Most rectal absorption of drugs is achieved by a simple diffusion process through the lipid membrane. Drugs that are liable to extensive first-pass metabolism can benefit greatly if delivered to the rectal area, especially if they are targeted to areas close to the anus.

Gastrointestinal Mucoadhesive Drug Delivery Systems

- Oral route is undoubtedly most favored route of administration, but hepatic first-pass metabolism, degradation of drug during absorption, mucus covering GI epithelial, and high turnover of mucus are serious concerns of oral route.

- In recent years, the gastrointestinal tract (GIT) delivery emerged as a most important route of administration. Bioadhesive retentive system involves the use of bioadhesive polymers, which can adhere to the epithelial surface in the GIT. Using bioadhesive would be achieved increase GI transit time and increase in bioavailability.

MUCOADHESIVE DOSAGE FORMS

Tablets

- Tablets are small, flat, and oval, with a diameter of approximately 5–8 mm. Unlike the conventional tablets, mucoadhesive tablets allow for drinking and speaking without major discomfort. They soften, adhere to the mucosa, and are retained in position until dissolution and/ or release is complete.
- Mucoadhesive tablets, in general, have the potential to be used for controlled release drug delivery, but coupling of mucoadhesive properties to tablet has additional advantages, for example, it offers efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio and facilitates a much more intimate contact with the mucus layer. Mucoadhesive tablets can be tailored to adhere to any mucosal tissue including those found in stomach, thus offering the possibilities of localized as well as systemic controlled release of drugs.
- The application of mucoadhesive tablets to the mucosal tissues of gastric epithelium is used for administration of drugs for localized action. Mucoadhesive tablets are widely used because they release the drug for a prolonged period, reduce frequency of drug administration and improve the patient compliance. The major drawback of mucoadhesive tablets is their lack of physical flexibility, leading to poor patient compliance for long-term and repeated use.

Films

- Mucoadhesive films may be preferred over adhesive tablets in terms of flexibility and comfort. In addition, they can circumvent the relatively short residence time of oral gels on the mucosa, which are easily washed away and removed by saliva.
- Moreover, in the case of local delivery for oral diseases, the films also help protect the wound surface, thus helping to reduce pain, and treat the disease more effectively.
- An ideal film should be flexible, elastic, and soft, yet adequately strong to withstand breakage due to stress from mouth movements. It must also possess good mucoadhesive strength in order to be retained in the mouth for the desired duration of action. Swelling

of film, if it occurs, should not be too extensive in order to prevent discomfort.

Patches

- Patches are laminates consisting of an impermeable backing layer, a drug-containing reservoir layer from which the drug is released in a controlled manner, and a mucoadhesive surface for mucosal attachment.

  - Patch systems are similar to those used in transdermal drug delivery. Two methods used to prepare adhesive patches include solvent casting and direct milling. In the solvent casting method, the intermediate sheet from which patches are punched is prepared by casting the solution of the drug and polymer(s) onto a backing layer sheet, and subsequently allowing the solvent(s) to evaporate. In the direct milling method, formulation constituents are homogeneously mixed and compressed to the desired thickness, and patches of predetermined size and shape are then cut or punched out. An impermeable backing layer may also be applied to control the direction of drug release, prevent drug loss, and minimize deformation and disintegration of the device during the application period.

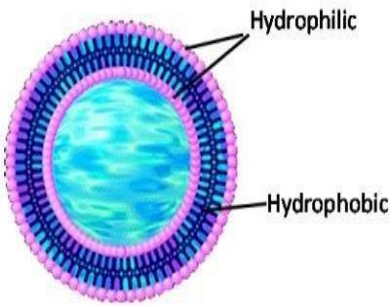
Gels and Ointments

- Semisolid dosage forms, such as gels and ointments, have the advantage of easy dispersion throughout the oral mucosa. However, drug dosing from semisolid dosage forms may not be as accurate as from tablets, patches, or films. Poor retention of the gels at the site of application has been overcome by using mucoadhesive formulations.
- Certain mucoadhesive polymers, for example, sodium carboxymethylcellulose, carbopol, and xanthan gum, undergo a phase change from liquid to semisolid. This change enhances the viscosity, which results in sustained and controlled release of drugs.
- Hydrogels are also a promising dosage form for buccal drug delivery. They are formed from polymers that are hydrated in an aqueous environment and physically entrap drug molecules for subsequent slow release by diffusion or erosion. The application of mucoadhesive gels provides an extended retention time in the oral cavity, adequate drug penetration, as well as high efficacy and patient acceptability.
- A major application of adhesive gels is the local delivery of medicinal agents for the treatment of periodontitis, which is an inflammatory and infectious disease that causes formation of pockets between the gum and the tooth, and can eventually cause loss of teeth. HPMC has been used as an adhesive ointment ingredient. Additionally, a highly viscous gel was developed from carbopol and hydroxypropylcellulose for ointment dosage forms that could be maintained on the tissue for up to 8 hours.

UNIT-4

- Liposomes are colloidal, vesicular structures composed of one or more lipid bilayers surrounding an aqueous compartment.
- The sphere like shell encapsulated a liquid interior which contain substances such as peptides and protein, hormones, enzymes, antibiotic, antifungal & anticancer agents.
- A free drug injected in blood stream typically achieves therapeutic level for short duration due to metabolism & excretion. Drug encapsulated by liposomes achieve therapeutic level for long duration as drug must first be release from liposome before metabolism & excretion.

– Spherical vesicles with a phospholipid bilayer



- Due to their size and hydrophobic and hydrophilic character, liposomes are promising systems for drug delivery.
- Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation.
- Furthermore, the choice of bilayer components determines the 'rigidity' or 'fluidity' and the charge of the bilayer. For instance, unsaturated phosphatidylcholine species from natural sources (egg or soybean phosphatidylcholine) give much more permeable and less stable bilayers, whereas the saturated phospholipids with long acyl chains (for example, dipalmitoylphos phatidylcholine) form a rigid, rather impermeable bilayer structure.
- Generally, liposomes size is ranging from **20 nm to several micrometers**. They

consist of one or more lipid bilayers surrounding aqueous units, where the polar head groups are oriented in the pathway of the interior and exterior aqueous phases.

Advantages of Liposomes

- Liposomes are biocompatible, completely biodegradable, non-toxic and non-immunogenic.
- Suitable for delivery of **hydrophobic, amphipathic and hydrophilic drugs**.
- Protect the encapsulated drug from the external environment.
- Liposome helps in sustained drug release.
- Flexibility to couple with **site-specific ligands** to achieve active targeting.
- Liposomes help reduce the exposure of sensitive tissues to toxic drugs. It also reduces the chances of overall drug toxicity.
- The size, surface charge and other characteristics of liposome can be altered based on the requirement of formulation.

Disadvantages of Liposomes

- Production cost is high
- Leakage and fusion of encapsulated drug/molecules
- Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction
- Short half-life in circulation.

Components

1) **Phospholipids:**

- Glycerol containing phospholipids are most common used component of liposome formulation and represent greater than 50% of weight of lipid in biological membranes.
- These are amphipathic molecule i.e. having **affinity for both aqueous and nonaqueous moieties**, as they have a hydrophilic head and a hydrophobic tail.
- The tail portion consists of 2 fatty acid chain having 10-24 carbon atoms and 0-6 double

bonds in each chain.

- The head or polar portion consists of phosphoric acid bound to a water soluble molecule.

➢ *Commonly used phospholipids are-*

Natural- Phosphatidyl choline (PC), Phosphatidyl ethanolamine (PE).

Synthetic- Dioleoyl Phosphatidyl choline (DOPC), Dioleoyl Phosphatidyl ethanolamine (DOPE), Dilauryl Phosphatidyl choline (DLPC), Distearoyl phosphatidyl choline (DSPC).

2) Cholesterol

➢ Cholesterol & its derivatives are often included in liposomes for

- *decreasing the fluidity or microviscocity of the bilayer*
- *reducing the permeability of the membrane to water soluble molecules*
- *Stabilizing the membrane in the presence of biological fluids such as plasma.*

➢ Liposomes without cholesterol are known to interact rapidly with plasma protein such as albumin, transferrin, and macroglobulin. These proteins tend to extract bulk phospholipids from liposomes, there by depleting the outer monolayer of the vesicles leading to physical instability.

➢ Cholesterol appears to substantially reduce this type of interaction.

➢ Cholesterol has been called the **mortar of bilayers**, because by virtue of its molecular shape and solubility properties, it fills in empty spaces among the Phospholipid molecules, anchoring them more strongly into the structure.

Mechanism of drug release from liposome

a) **Endocytosis:** RES detect the liposome and endocytosis occurs. Then the drug is released.
b) **Adsorption:** Liposome will adsorb to the specific cell membrane and release the drug by diffusion process.
c) **Fusion:** Fusion will occur when cationic liposome come in contact with negatively charged cell membrane. Then drug will be delivered into cell.
d) **Lipid transfer:** There will be no direct contact with cell membrane, but lipid matrix will release into cell membrane, then drug will be release.

Classification:

Based on their structure:

1) MLV (Multilamellar vesicles): Made of series of concentric bilayers of lipid.
2) OLV (Oligolamellar vesicles): Made of 2-10 bilayers of lipid.
3) ULV (Unilamellar vesicles): Made of single bilayers of lipid. They may me different types like-

   Small unilamellar vesicles (20-40 nm)

   Medium unilamellar vesicles (40-80 nm)

   Large unilamellar vesicles (100-1000 nm)

   Giant unilamellar vesicles (>1000nm)
4) MVV (Multivesicular vesicles): Having more than one vesicle.

Based on method of preparation:

1) DRV- Dehydration rehydration method
2) REV- SUV/ OLV made by reverse phase evaporation method
3) MLV-REV- MLVs made by reverse phase evaporation method.
4) VET- Vesicles prepared by Extrusion technique.
5) ATMLV- Frozen and thawed MLV

Based on composition and application:

1) CL (conventional liposomes)- Neutral/ negatively charged phospholipids and cholesterol.
2) Fusogenic liposomes- Reconstituted sendai virus
3) pH sensitive liposomes- Using phospholipids such as PE or DOPE with OA
4) Cationic liposomes- Cationic lipids with DOPE.
5) Immuno liposomes- With attached monoclonal antibody.

APPLICATIONS OF LIPOSOME

- The aim of any DDS is to modulate the pharmacokinetics and distribution of the drug in a beneficial way.
- Among the variety of delivery systems, applications of liposome-based

formulations and products are extremely wide, because of ability of liposomes to carry a wide variety of substances large number of drugs: Antimicrobial agents, drugs against cancer, antifungal drugs, peptide hormones, enzymes, vaccines, their structural versatility and the innocuous nature of their compound. Some of the main applications of liposomes in various fields are-

1) **Drug targeting**

- Liposomes can be incorporated with **opsonins and ligands** (e.g., antibodies, sugar residues, apoproteins or hormones, which are tagged on the lipid vesicles) for site-specific drug delivery system.
- The ligand recognizes specific receptor sites and, thus, causes the lipid vesicles to concentrate at such target sites. By this approach, the otherwise preferential distribution of liposomes into the reticuloendothelial system (liver, spleen, and bone marrow) is averted and reduces the probabilities of drug-related toxicities.

2) **Cancer therapy**

- Liposome-based chemotherapeutics used in the treatment of cancer such as breast cancer can improve the pharmacokinetics and pharmacodynamics of associated drugs.
- Liposome can target a drug to the intended site of action in the body, thus increase its therapeutic efficacy.
- Anthracyclines are drugs which inhibit the growth of dividing target anti-cancer drugs to cells by intercalating into the DNA and, thus, kill mainly rapidly dividing cells. These cells are not only in tumors but are also in hair, gastrointestinal mucosa, and blood cells; therefore, this class of drug is very toxic.
- The encapsulation of **cytotoxic agents** within liposomes allows accumulation at of anti-cancer drugs at the tumor site.
- In addition, the presence of the phospholipids bilayer prevents the encapsulated active form of the drug from being broken down in the body before reaching tumor tissue and also serves to minimize exposure of the drug to healthy sensitive tissue. As a result, reduces the toxicity of anti-cancer drugs.

3) Transdermal drug delivery

- Transdermal DDSs offer a number of potential advantages over conventional methods such as injectables and oral delivery.
- The main problem to the transdermal delivery system is the limitation of the penetration of macromolecules and hydrophilic drugs through the stratum corneum.
- Liposome system is a suitable carrier to improve drug delivery through the skin because of **intercellular lipids of the stratum corneum** and they are predominantly phospholipids bilayer similar to that existence in biological membranes.
- Different forms of liposome preparations such as creams, gels, and ointments can deliver compounds across the stratum corneum. Liposomes have high member fluidity; therefore, they can increase the permeability of skin for various entrapped drugs and deliver drugs to target.
- Liposomes are regularly released from the base in topical administration, and also they tend to accumulate in the stratum corneum of the skin and after entering this layer, slowly out of it and enter the circulatory system, therefore, can act as a depot from which the entrapped compound is slowly released over time across skin.
- As a result, topical drugs are prepared as liposomes compared to traditional local forms, need less drug to create a therapeutic concentration in the local administration site, on the other hand, increase the duration of action and decrease the frequency of administration. As a result, side effects are reduced.

4) Parasitic diseases and infections

- Liposomes can be made in a particular size and used as a viable target for macrophages. These liposomes may be digested while in the macrophage's phagosome, thus releasing its drug.
- For this reason, they are suggested as an ideal carrier for treatment parasitic diseases like **Leishmaniasis**, a group of endemic diseases caused by the leishmania intramacrophagic parasite. The main therapeutic agents against Leishmaniasis are pentavalent antimonials, **amphotericin B,** pentamidine, paromomycin, however, the use conventional chemotherapy for Leishmaniasis due to high toxicity and serious adverse reactions, e.g.,

gastrointestinal disorders and cardiac arrhythmias, long duration of treatment are not appropriate.

- Liposomal encapsulated drugs appear as an option for the treatment of Leishmaniasis, providing greater efficacy for the active and reducing its side effects by accumulate at infected macrophages and releasing drug at the desired location.

5) Treatment HIV infection

- Several new drugs, such as antiretroviral nucleotide, have been developed nowadays for the treatment patients suffering of HIV infections. Liposomes can serve as vehicle for delivery of such oligonucleotides and other antiviral drugs. These potential anti-HIV nanocarriers are concentric lipid bilayers, which can be fabricated to protect molecules and to target the drugs to specific sites.

6) Immunology

- Liposomes rapidly accumulate in macrophages, so this ability can be used in vaccination and activation of macrophages.
- In immunology, antigens encapsulated in liposomes are developed to create antibodies, to activation passive and active immunization and for many other applications.
- Liposomes are used as immunological adjuvant in many cases such as hepatitis B-derived polypeptides, subunit antigens from the influenza virus, adenovirus type 5 hexon, etc.

7) Antibiotic therapy

- Liposomes increase the effect of antibiotics for two reasons:

- *First, they encapsulate hydrophilic antibiotics such as vancomycin and triclosan and their lipid nature increases the entry of antibiotics into the microorganism cells. As a result, the effective dose of the drug and its toxicity decrease.*
- *Second, they protect the entrapped drug against enzymatic degradation. For example, protect the penicillins and cephalosporins from degradation by the beta-lactamase enzyme, which is produced by certain microorganisms.*

8) Diagnosis

- Addition to the therapeutic area, liposomes are also effective in diagnosis cases such as therapeutic **imaging modalities**, liposomes encapsulate contrast agents and

through this are employed in diagnostic X-ray, and nuclear magnetic resonance imaging.

9) Cosmetics

- In the dermatological and cosmetic field, liposomes are used because of their capability of enclosing many different biological materials and of delivering them to the epidermal cells.
- The moisture content of the skin has special significance in cosmetic applications; therefore Cosmetic care is concerned to equilibrate the moisture balance of the skin. Liposomes easily are hydrated and can reduce dry skin, which is on factor aging of the skin.
- In addition, anti-inflammatory agents, immunostimulants, and enhancers of molecular and cellular detoxification within liposomes could prevent age spots, dark circles, wrinkles, and other clinical aspects of skin.
- Liposomes are potent DDSs for treating hair follicle-associated disorders, such as acne. It can increase tretinoin concentration in the epidermis and dermis and protects it from photodegradation and minimize skin irritation compared to conventional cream or gel, and this way enhances the clinical effect.

10) Food and farming industry

- In the food industry, liposomes have been employed for developing new taste, controlling the release of flavor, improving the food color and modifying the texture of food components because they can entrap unstable compound, for example, antimicrobials, antioxidants, flavors, and protect them against a range of environmental and chemical change including enzymatic chemical changes, as well as temperature and releases the ingredients at designate targets when required.
- Research has shown that adding proteases to the cheese mixture reduces the cost and time of the preparation of cheese. As well as liposome-entrapped proteinases reduce the firmness of cheddar cheeses but increase their elasticity and improve their flavor

- In the food production industry, liposomes are used to encapsulate enzymes with the aim of stabilize the enzymes against food manufacture processes and preserving them for a long time and maintain their useful effects in foods.

11) In Arthritis

- Conventional administration of steroids in arthritis results in their shorter duration of action. Therefore, the duration of action of such steroids can be prolonged by encapsulating them in MLVs.

12) In Ophthalmic drug delivery

- Most of the drugs administered by ocular route do not exhibit greater bioavailability. Moreover, the vehicles used for such delivery are also limited due to high sensitivity of the cornea.
- Therefore, liposomes can be used for ocular drug delivery as seen in animal studies wherein liposomal **idoxuridine** was found to be highly effective in the treatment of acute and chronic herpetic keratitis.

METHODS OF LIPOSOME PREPARATION

General methods of preparation: All the methods of preparing the liposomes involve four basic stages:

- Drying down lipids from organic solvent.
- Dispersing the lipid in aqueous media.
- Purifying the resultant liposome.
- Analyzing the final product.

Method of liposome preparation and drug loading

The following methods are used for the preparation of liposome:

I. Passive loading techniques
II. Active loading technique.

I. **Passive loading techniques** include three different methods:

1. Mechanical dispersion method.
2. Solvent dispersion method.
3. Detergent removal method

1) **Mechanical dispersion method:** The following are types of mechanical dispersion methods:

- Sonication.
- French pressure cell: extrusion.
- Freeze-thawed liposomes.
- Lipid film hydration
- Micro-emulsification.
- Membrane extrusion.

Sonication

Sonication is perhaps the most extensively used method for the preparation of SUV. Here, MLVs are sonicated either with a bath type sonicator or a probe sonicator under a passive atmosphere. The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to be encapsulated, elimination of large molecules, metal pollution from probe tip, and presence of MLV along with SUV. There are two sonication techniques:

Probe sonication: The tip of a sonicator is directly engrossed into the liposome dispersion. The energy input into lipid dispersion is very high in this method. The coupling of energy at the tip results in local hotness; therefore, the vessel must be engrossed into a water/ice bath. Throughout the sonication up to 1 h, more than 5% of the lipids can be deesterified. Also, with the probe sonicator, titanium will slough off and pollute the solution.

Bath sonication: The liposome dispersion in a cylinder is placed into a bath sonicator. Controlling the temperature of the lipid dispersion is usually easier in this method, in contrast to sonication by dispersal directly using the tip. The material being sonicated can be protected in a sterile vessel, dissimilar the probe units, or under an inert atmosphere.

French pressure cell extrusion:

French pressure cell involves the extrusion of MLV through a small orifice. An important feature of the French press vesicle method is that the proteins do not seem to be significantly pretentious during the procedure as they are in sonication. An interesting comment is that French press vesicle appears to recall entrapped solutes significantly longer than SUVs do, produced by sonication or detergent removal.

The method involves gentle handling of unstable materials. The method has several

advantages over sonication method. The resulting liposomes are rather larger than sonicated SUVs. The drawbacks of the method are that the high temperature is difficult to attain, and the working volumes are comparatively small (about 50 mL as the maximum).

Freeze-thawed liposomes:

SUVs are rapidly frozen and thawed slowly. The short-lived sonication disperses aggregated materials to LUV. The creation of unilamellar vesicles is as a result of the fusion of SUV throughout the processes of freezing and thawing. This type of synthesis is strongly inhibited by increasing the phospholipid concentration and by increasing the ionic strength of the medium. The encapsulation efficacies from 20% to 30% were obtained.

Lipid film hydration:

Hand Shaken Method: This is the simplest and widely used method. The lipid mixture and charged components are dissolved in chloroform and methanol mixture (2:1 ratio) and then this mixture is introduced in to a 250 ml round bottomed flask. The flask is attached to rotary evaporator connected with vacuum pump and rotated at 60 rpm. The organic solvents are evaporated at about 30 degrees. A dry residue is formed at the walls of the flask and rotation is continued for 15 minutes after dry residue appeared. The evaporator is detached from vacuum pump and nitrogen is introduced into it. The flask is then removed from evaporator and fixed onto lypholizer to remove residual solvent. Then the flask is again flushed with nitrogen and 5 ml of phosphate buffer is added. The flask is attached to evaporator again and rotated at about 60 rpm speed for 30 minutes or until all lipid has been removed from the wall of the flask. A milky white suspension is formed finally. The suspension is allowed to stand for 2 hours in order to complete swelling process to give MLVs.

Non-Shaking Method: This is similar to shaking method except that care is taken in swelling procedure. The solution of lipid in chloroform and methanol mixture is spread over the flat bottom of the conical flask. The solution is evaporated at room temperature by flow of nitrogen through the flask without disturbing the solution. After drying water saturated nitrogen is passed through the flask until the opacity of the dried film disappears. After hydration, lipid is swelled by addition of bulk liquid. The flask is inclined to one side, 10 to 20 ml of 0.2M sucrose in distilled water is introduced down the side of the flask and then flask is slowly returned to up right position. The solution is allowed to run gently over the lipid layer on the bottom of the flask. The flask is flushed with nitrogen sealed and allowed to stand for 2 hours at 37 degrees for swelling. After that the vesicles are mixed to yield a milky suspension. The suspension is centrifuged at 1200 rpm for 10 minutes. The layer of MLVs floating on the surface is removed. From the remaining fluid, LUVs are produced.

Freeze Drying: Another method of dispersing the lipid in a finally divided form prior to addition of aqueous media is to freeze dry the lipid dissolved in a suitable organic solvent. The solvent usually used is tertiary butanol.

Micro-emulsification:

- **"Micro Fluidizer"** is used to prepare small MLVs from Concentrated lipid dispersion.
- The lipids introduced into fluidizers, either as a dispersion of large MLVs or as a slurry of anhydrated lipids in organic medium.
- Micro fluidizer pumps the fluid at very high pressure (10,000psi, 600-700 bar) through a 5um orifice.
- Then it is forced along defined micro channels, which direct two streams of fluid to collide together at right angles at a very high velocity, thereby affecting an efficient transfer of energy.
- The fluid collected can be recycled through the pump and interaction chamber until vesicles of the spherical dimension are obtained.
- After a single pass, the size of vesicles is reduced to a size 0.1 and 0.2um in diameter.

Membrane extrusion:

- It is used to process LUVs as well as MLVs.
- Liposomes prepared by this tech. are called as membrane filter extrusion liposomes.
- The 30% capture volume can be obtained using high lipid conc. The trapped volume in this process is 1-2 litre /mole of lipids.
- It is due to their ease of production, readily selectable vesicle diameter, batch to batch reproducibility & freedom from solvent or surfactant contamination is possible

2) Solvent dispersion method

❖ **Ether injection (solvent vaporization)**

A solution of lipids dissolved in diethyl ether or ether-methanol mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure. The consequent removal of ether under vacuum leads to the creation of liposomes. The main disadvantages of the technique are that the population is heterogeneous (70 to 200 nm) and the exposure of compounds to be encapsulated to organic solvents at high temperature.

❖ **Ethanol injection**

A lipid solution of ethanol is rapidly injected to a huge excess of buffer. The MLVs are at once formed. The disadvantages of the method are that the population is heterogeneous (30 to 110 nm), liposomes are very dilute, the removal of all ethanol is difficult because it forms into azeotrope with water, and the probability of the various biologically active macromolecules to inactivate in the presence of even low amounts of ethanol is high.

❖ **Reverse phase evaporation method**

Liposomes made by reverse phase evaporation method can be made from numerous lipid formulations and have aqueous volume-to-lipid ratios that are four times higher than hand-shaken liposomes or multilamellar liposomes. Briefly, first, the water-in-oil emulsion is shaped by brief sonication of a two-phase system, containing phospholipids in organic solvent such as isopropyl ether or diethyl ether or a mixture of isopropyl ether and chloroform with aqueous buffer. The organic solvents are detached under reduced pressure, resulting in the creation of a viscous gel. The liposomes are shaped when residual solvent is detached during continued rotary evaporation under reduced pressure. With this method, high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl.

3) Detergent removal method:

❖ **Dialysis**

The detergents at their critical micelle concentrations (CMC) have been used to solubilize lipids. As the detergent is detached, the micelles become increasingly better-off in phospholipid and lastly combine to form LUVs. The detergents were removed by dialysis. A commercial device called LipoPrep, which is a version of dialysis system, is obtainable for the elimination of detergents. The dialysis can be performed in dialysis bags engrossed in large detergent free buffers (equilibrium dialysis).

❖ **Detergent removal of mixed micelles (absorption)**

Detergent absorption is attained by shaking mixed micelle solution with beaded organic polystyrene adsorbers such as XAD-2 beads and Bio-beads SM2. The great benefit of using detergent adsorbers is that they can eliminate detergents with a very low CMC, which are not entirely depleted.

❖ **Gel-permeation chromatography**

In this method, the detergent is depleted by size special chromatography. Sephadex G-50, Sephadex G-l00, Sepharose 2B-6B, can be used for gel filtration. The liposomes do not

penetrate into the pores of the beads packed in a column. They percolate through the inter-bead spaces. At slow flow rates, the separation of liposomes from detergent monomers is very good. The swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids; therefore, pre- treatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

❖ **Dilution**

Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer, the micellar size and the polydispersity increase fundamentally, and as the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from polydispersed micelles to vesicles occurs.

II. Active loading technique:

- Weak amphipathic bases accumulate in the aqueous phase of lipid vesicles in response to a difference in pH between the inside and outside of the liposomes (pHin & pHout).
- Two steps process generates this pH imbalance and active (remote) loading.
- Vesicles are prepared in low pH solution, thus generating low pH within the liposomal interiors, followed by addition of the base to extra liposomal medium.
- Basic compounds, carrying amino groups are relatively lipophilic at high pH and hydrophilic at low pH.
- In two chambered aqueous system separated by membrane liposomes, accumulation occurs at the low pH side, under dynamic equilibrium conditions.
- Thus the unprotonated form of basic drug can diffuse through the bilayer.
- The exchange of external medium by gel chromatography with neutral solution.
- Weak base doxorubicin; Adriamycin and vincristine which co-exist in aqueous solutions in neutral and charged forms have been successfully loaded into preformed liposomes via the pH gradient method.

EVALUATION OF LIPOSOMES

- Liposomal formulation and processing for specified purpose are characterized to ensure their predictable in vitro and in vivo performance. The characterization parameters for purpose of evaluation could be classified into three broad categories

which include physical, chemical and biological parameters.

- Physical characterization evaluates various parameters including size, shape, surface features, lamellarity, phase behaviour and drug release profile.
- Chemical characterization includes those studies which establish the purity and potency of various lipophillic constituents
- Biological characterization parameters are helpful in establishing the safety and suitability of formulation for therapeutic application.
- Some of parameters are:

EVALUATION/ CHARACTERIZATION OF LIPOSOMES:

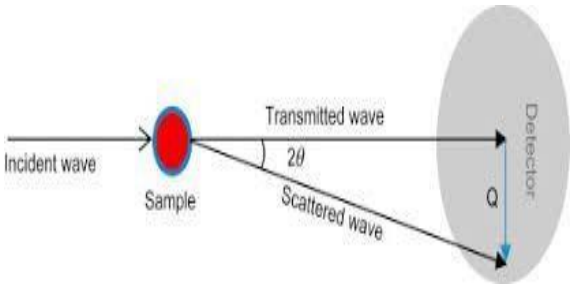
- PHYSICAL PROPERTIES
- CHEMICAL COMPOSITIONS
- BIOLOGICAL PARAMETERS

PHYSICAL PROPERTIES

1. SIZE AND ITS DISTRIBUTION:

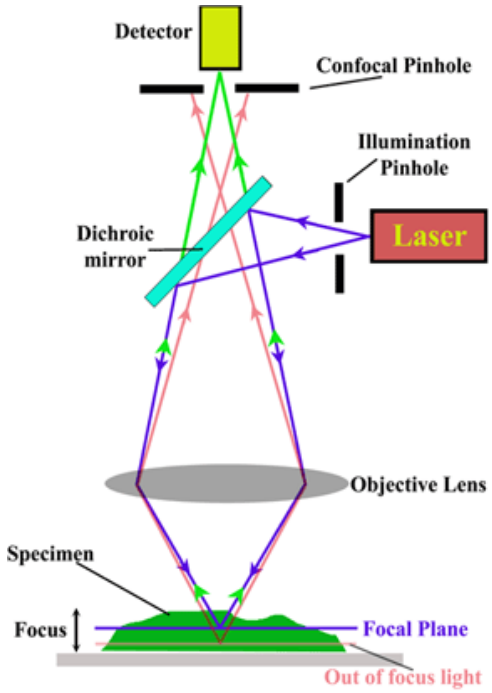
- **Transmission electron microscopy:** The most precise method of determine size of liposome is Electron Microscopy since it permit one to view each individual liposome and to obtain exact information about profile of liposome population over the whole range of sizes. The microstructure of colloidal system can visualized with high magnification power of electron microscope.

- **Negative stain electron microscopy:** Negative Stain Electron Microscopy, visualizes bright areas against dark background (hence termed as negative stain). The negative stains used in TEM analysis are ammonium molybdate or Phosphotungstic acid (PTA) or uranyl acetate.
- **Laser light scattering microscopy:** Laser light scattering method is very simple and rapid to perform but having disadvantage of measuring an average property of bulk of liposomes. Laser light diffraction is applied for particles >1μm in size and refer to the proportionality between the intensity of diffraction and square of the particle diameter. For particles <200nm in size and refer to proportionality between the scattering intensity and to the sixth potency of the particle diameter.
- **X-Ray Scattering:** Small angle x-ray diffraction is most appropriate technique for exact determination of the distance of interlayer spacing of liquid systems. The Q is defined as **$Q = (4\pi/\lambda) \sin\theta$,** where λ is the wavelength and 2θ is scattering angle and it is related as **$Q = 2\pi/d$** where, to the d-inter layer spacing between scattering objects in a material by **Bragg's law.**

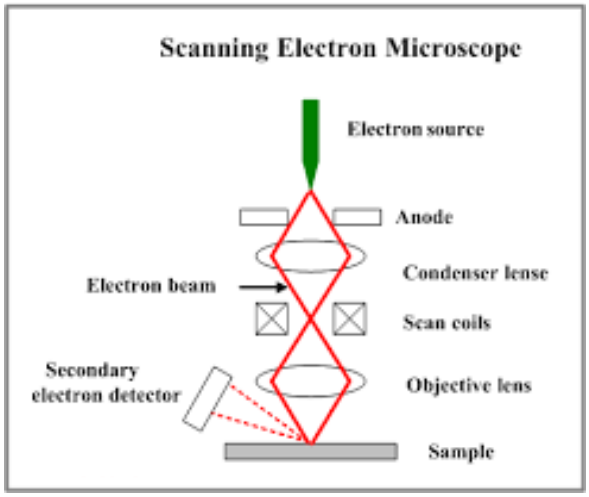


- **Freeze fracture electron microscopy:** The freeze-fracture/freeze etch technique starts with rapid freezing of a cell. Then the frozen cells are cleaved along a fracture plane. This fracture plane is in between the leaflets of the lipid bilayer, the two fractured sections are then coated with heavy metal (etched) and a replica is made of their surfaces. This replica is then viewed in an electron microscope.
- **Confocal laser light scanning microscopy:** Confocal microscopy is an optical imaging technique used to increase optical resolution and contrast of a micrograph by using point

illumination and a spatial pinhole to eliminate out-of-focus light in specimens that are thicker than the focal plane. It enables the reconstruction of three-dimensional structures from the obtained images. Technique for obtaining high- resolution optical images with depth selectivity i.e. Penetration and Permeation Studies.



- **Scanning electron microscopy:** A scanning electron microscope (SEM) is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a faster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface composition and other properties such as electrical conductivity.



2. SURFACE CHARGE:

Depending on head group composition of the lipid and pH forms, liposomes may bear a negative, neutral, positive charge on their surface. The surface charge of liposomes governs the kinetics and extent of distribution in vivo, as well as interaction with and uptake by the target cells. Here the method involves several steps:

a) A cellulose acetate plate dipped in sodium borate buffer of pH 8.8.
b) 5 moles of lipid sample are applied on the plate, and then subjected to electrophoresis at 4°C for 30 mins at 18 V/cm.
c) The plate is dried and then sprayed with molybdenum blue reagent to visualize the phospholipids.

3. ENCAPSULATION EFFICIENCY:

It describes the percent of the aqueous phase and hence percent of water soluble drug that become ultimately entrapped during preparation of liposomes and is usually expressed as % entrapment/mg lipid. Encapsulation efficiency is assessed using 2 techniques including Minicolumn centrifugation method and Protamine aggregation method. Minicolumn centrifugation is generally used both as a mean of purification and separation of liposomes on small scale.

In mini column centrifugation method, the hydrated gel is filled in a barrel of 1ml syringe without plunger which is plugged with **whatman GF/B filter pad**. This barrel is rested in a centrifuge tube. This tube is spun at 2000 rpm for 3 min. to remove excess saline solution from gel. After centrifugation the gel column should be dried and have come away from side of barrel. Then eluted saline is removed from collection tube. Liposome suspension (0.2ml) is applied drop wise to top of gel bed, and the column is spun at 2000 rpm for 3 min. to expel the void volume containing the liposomes into centrifugation tube. The elute is then removed and set aside for assay.

Protamine aggregation method may be used for neutral and negatively charged liposomes. In this method liposome suspension in normal saline and 0.1 ml **protamine sulpahte** are added to a conical centrifuge tube, mixed and allowed to stand for mins. Then 30 ml saline is added and the tube is rotated at 2000 rpm for 20 mins. Then the supernatant liquid is decanted and assayed for the presence of any free drug by standard method.

4. ENTRAPPED VOLUME:

The percent drug encapsulated in liposomes can also be determined by entrapped volume per lipid weight varies for Multilamellar Vesicles and Small Unilamellar vesicles. The trapped volume is determined by dispersing lipid in an aqueous medium containing a radioactive solute. The proportion of solute trapped is determined by removing external radioactivity by centrifugation and subsequently residual activity per lipid is determined.

5. LAMELLARITY:

The average number of bilayers present in a liposome can be found by freeze electron microscopy and by **31P-NMR**. In the latter technique, the signals are recorded before and after the addition of broadening agent such as manganese ions which interact with the outer leaflet of the outermost bilayers. Thus, a 50% reduction in **NMR signal** means that the liposome preparation is unilamellar and a 25% reduction in the intensity of the original NMR signal means that there are 2 bilayers in the liposome.

6. PHASE BEHAVIOUR OF LIPOSOMES:

An important feature of lipid membrane is the existence of a temperature dependent, reversible phase transition, where the hydrocarbon chains of the phospholipid undergo a transformation from an ordered (gel) state to a more disordered fluid (liquid crystalline) state . These changes have been documented by freeze fracture electron microscopy, but most easily demonstrated by differential scanning calorimetry. The physical state of the bilayers profoundly affects the permeability, leakage rates and overall stability of the liposomes. The phase transition temperature (Tc) can give good clues regarding liposomal stability, permeability and whether drug is entrapped in the bilayers or the aqueous compartment.

7. INVITRO DRUG RELEASE:

The in vitro release of drug from conventional and PEGylated liposomes was determined by dialysis method. After reconstituting the freeze dried liposomes in 10ml phosphate buffer solution (PBS) (pH 7.4), an aliquot of each liposomal dispersion was placed in dialysis tube. Then, dialysis tube was immersed in a beaker containing 200 ml of release medium , and stirred with magnetic stirrer at 150 rpm to maintain sink condition. The sample (5ml) were taken at predetermined time intervals from release medium and replaced by same volume of fresh medium. Concentration of drug was determined after filtering the samples through 0.22µm syringe filter and were assayed UV spectrophotometrically.

CHEMICAL PROPERTIES:

1. QUANTITATIVE DETERMINATION OF PHOSPHOLIPID:

Consequently the method most widely used for determination of phospholipid is an indirect one in which the phosphate content of the sample is first measured. The phospholipids are measured either using- 1) Bartlett assay 2) Stewart Assay

- **Bartlett assay:** In the Bartlett assay, the phosphorous in the sample (lipid bilayar) is first hydrolyzed to inorganic phosphate. This is converted to phospho-molybdic acid by the addition of ammonium molybdate. Then phospho-molybdic acid is quantitatively reduced to a blue colored compound by amino-naphthylsulfonic acid. The intensity of the blue color is measured spectrophotometrically and is compared with the curve of standards to give phosphorous and hence phospholipid content.
- **Stewart assay:** In Stewart assay, the phospholipid forms a complex with ammonium ferrothiocyanate in organic solution. The advantage of this method is that the presence of inorganic phosphate does not interfere with the assay. In this method, the standard curve is first prepared by adding 0.1M solution of ammonium ferrothiocyanate (reagent) with different known concentrations of phospholipids in chloroform. Similarly, the samples are treated with the same reagent and optical density of these solutions is measured at 485nm and the absorbance of samples compared with the standard curve of phospholipids to get the concentration.

2. PHOSPHOLIPID OXIDATION:

Oxidation of the fatty acids of phospholipids in the absence of specific oxidants occurs via a free radical chain mechanism. The initiation step is abstraction of a hydrogen atom from the lipid chain that can occur most commonly as a result of exposure to electro-magnetic radiation or trace amount of contamination with the transition metal ions. A number of techniques are available for determining the oxidation of phospholipids at different stages i.e., UV absorbance method, and GLC method.

3. CHOLESTEROL ANALYSIS:

Cholesterol is qualitatively analyzed using capillary column of flexible fused silica whereas it is quantitatively estimated (in the range of 0-8 µg) by measuring the absorbance of purple complex produced with iron upon reaction with a combined reagent containing ferric perchlorate, ethyl acetate and sulfuric acid at 610nm.

BIOLOGICAL PARAMETERS:

1. STERILITY TEST:

In order to ensure the sterility of finished products, the optimized formulations were subjected to sterility test. The sterile formulations were incubated with different culture media like Fluid thioglycolate medium for anaerobic/ aerobic bacteria, Soybean casein digest for fungi, Nutrient agar for bacillus subtilis, Macconkeys agar for E-coli, and Mannitol salt agar for Staphelococcous aureus . The sterility test was performed by spread plate method. Same media for positive control with specific organisms and negative control without any inoculation was incubated for 14 days and results were noted.

2. ANIMAL TOXICITY:

The modification of blood biochemical indexes was evaluated to measure the blood toxicity index of free drug and drug encapsulated in conventional as well as PEGylated liposomes. Four groups each containing 3 albino rats was treated i.v. with different formulations (5 mg/kg) every three days for 30 days. Then blood samples were collected via ocular vein plexus immediately frozen on addition of anticoagulant. Different blood parameters were then measured by biochemical auto analyzer. The blood samples obtained by healthy albino rats were used as control

3. STABILITY IN VITRO:

Stability in vitro mainly covers the chemical stability of constitutive lipid under various accelerated or long-term storage condition. 1) Lipid oxidation and peroxidation 2) lipid hydrolysis

- **Lipid Oxidation and Peroxidation:** Most of the procedure used to measure lipid peroxidation either based on disappearance of unsaturated fatty acid or appearance of conjugated dienes. Later technique is now well established as it accompanied by increase UV absorption in 230 to 260nm range
- **Lipid hydrolysis:** Formation of lyso-phospholipid from phospholipid is chemical instability of lipid. Lyso-PC is usually analyzed by phospholipid extraction followed by separation of PC and lyso-PC by TLC.

Attempts for encapsulating substances in RBC started in **1970**. Ihler & Zimmerman suggested resealed erythrocytes are useful carriers. The term "Carrier RBC" was introduced in 1979.

The RBC membrane encloses cytoplasm and hemoglobin, when erythrocytes are lysed in a controlled manner by different techniques and again resealed, the cells loses some of the properties of normal erythrocytes and referred as **RESEALED ERYTHROCYTES**.

During resealing, exchange of intra cellular and extra cellular solute occurs, some of the hemoglobin is lost and other cellular contents are retained. Resealed erythrocytes that contain no or little hemoglobin are called **ghosts**.

Properties of resealed erythrocyte as novel drug carriers:

1. The drug should be released at target site in a controlled manner.
2. It should be appropriate size, shape and should permit the passage through capillaries and minimum leakage of drug should take place.
3. It should be biocompatible and should have minimum toxic effect.
4. It should possess the ability to carry a broad spectrum of drug.
5. It should possess specific physicochemical properties by which desired target site could be recognized.
6. The degradation product of the carriers system, after release of the drug at the selected site should be biocompatible.
7. It should be physico-chemically compatible with drug.
8. The carrier system should have an appreciable stability during storage.

Advantages:

- They are the natural product of the body which is biodegradable in nature.
- Isolation of erythrocyte is easy and larger amount of drug can be encapsulated in a small volume of cells.
- The entrapment of drugs does not require any chemical modification of the substance to be entrapped.
- They are non-immunogenic, non-toxic in action and can be targeted to disease tissue/ organ.
- They prolong the systemic activity of drug while residing for a longer time in the body
- They protect the premature degradation, inactivation and excretion of proteins and enzymes
- They can target the drugs within reticuloendothelial system.
- They facilitate incorporation of proteins and nucleic acids in eukaryotic cells by cell infusion with RBC.
- A longer life span in circulation as compared to other synthetic carrier.
- Isolation of RBC is easy, collection and storage techniques are well established.

Disadvantages:

- They have a limited potential as carrier to non- phagocytic target tissue & compounds extensively metabolized in liver.
- Being from biological origin, entrapped erythrocytes may present variability and lesser standardization in their preparation, compared to other carrier systems.
- Possibility of clumping of cells and dose dumping.
- Fragility of RBC membrane, their permeability to various drugs and leakiness.
- These are not suitable for highly polar and non-diffusable drugs (heparin, gentamycin).
- Safety and technical concerns related to the storage of the loaded erythrocytes.

Isolation of RBC

1. Erythrocytes may be prepared as carriers from blood taken from human beings and from different animal species, such as rats , mice , rabbits , dogs , etc.
2. Blood is collected into heparinized tubes by vein puncture.
3. Blood is withdrawn from cardiac/splenic puncture (in small animals) and through veins (in large animals) in a syringe containing a drop of anti coagulant.
4. The whole blood is centrifuged at 2500 rpm for 5 min at 4±10 C in a refrigerated centrifuge.
5. The serum and buffy coats are carefully removed and packed cells washed three times with phosphate buffer saline (pH=7.4).
6. Several washes are subsequently performed. This is a process that normally involves repeated centrifugation with a solution to remove other blood components.
7. The washed erythrocytes are diluted with PBS and stored at 4°C until used.

APPLICATION TO RESEALED ERYTHROCYTES

The potential therapeutic applications of resealed erythrocytes as a drug delivery system cover a wide spectrum of pharmacological as well as therapeutic targets which is mainly based on the intravenous slow drug release as well as the targeted drug delivery. Carrier erythrocytes have a number of possible applications in various fields of human as well as veterinary medicine. Such cell could be used as circulating carriers to disseminate (distribute) drug within a prolonged period of time in circulation or in target-specific organs, including the spleen, liver and lymph nodes. A majority of the drug delivery studies using drug resealed erythrocytes are still in the preclinical phase. Though in some cases, the successful clinical trials on this delivery system have been reported.

In-Vitro Application: The most frequent in- vitro application of RBC mediated micro injection, a protein or nucleic acid to be injected into eukaryotic cells by fusion process. Similarly, when antibody molecules are introduced using erythrocytic carrier system, they immediately diffuse throughout the cytoplasm. Antibody RBC auto injected into living cells have been used to confirm the site of action of fragment of diptheria toxin. In-vitro tests include use of erythrocytes carrier to introduce ribosomes inactivating proteins into cells by fusion technique.

In-Vivo Applications: The In-Vivo applications of the resealed erythrocytes include the following application:

1) **Slow drug release:** Slow release dosage forms are mainly formulated with an idea to achieve a prolonged therapeutic effect of the drug by continuously releasing the drug over an extended period of time after administration of single dose. Due to the long life span of resealed erythrocyte in the circulation, they can be used as circulating depots for anti-parasitic, antitumor, antibiotics as well as cardiovascular drugs. This is possible only if the drug and the selected method for the drug loading don't change the morphological and physiological parameters of erythrocytes. For effective treatment of diseases a number of bioactive agents are encapsulated in erythrocytes so as to give sustained release of the drug in the circulation. Resealed erythrocytes serve as an ideal carrier for antineoplastic agents, antimicrobial drugs, vitamins and steroids. Thus it can be concluded that resealed erythrocytes have been used as circulating depots for the sustained delivery of anti-amoebics, anti-parasitics, anti- neoplastics, veterinary, vitamins, steroids, antibiotics and cardiovascular drugs.
2) **Drug targeting:** In drug delivery system the drug should be delivered to the specific site and specific target thus to exhibit maximal therapeutic index with minutest adverse effects. Resealed erythrocytes can act as drug carriers as well targeting tools. Erythrocytes with surface modification are mainly used to target organs of mononuclear phagocytic system/RES as the change in the membrane is recognized by macrophages.
3) **Targeting Reticulo-endothelial system (RES) organs:** Erythrocytes with Surface modification are used to target organs of mononuclear phagocytic systems/ reticulo-

endothelial system because the alterations in membrane are recognized by macrophages. The various methods used include:

- Surface modification with glutaraldehyde.
- Surface modification with antibodies (coating of loaded erythrocytes byanti-Rh or other types of antibodies).
- Surface chemical crosslinking.
- Surface modification with sulphydryl.
- Surface modification with carbohydrates such as sialic acid.

4) **Targeting the liver-deficiency/therapy:** A large numbers of metabolic disorders which arises due to deficient or missing of enzymes can be treated by injecting these enzymes. However, the main complications included in use of exogenous enzyme therapy are the shorter circulation half-life of enzymes, allergic reactions and toxic manifestations. However these problems can be efficaciously overcome by administration of these enzymes as resealed erythrocytes. The enzymes that are used include P-glucoronidase, P-glucosidase and P-galactosidase. The disease aroused due to accumulation of glucocerebrosidaes in the liver and spleen can be treated by glucocerebrosidase-loaded erythrocytes.
5) **Treatment of parasitic disease:** The unique ability of resealed erythrocytes to selectively accumulate within RES organs makes them beneficial in delivery of anti-parasitic drugs. Parasitic diseases involve harboring parasites in the RES organs which can be successfully controlled by use of resealed erythrocytes by this method. The studies were carried on animal model results were favorable for erythrocytes loaded with anti-malarial, anti-leishmanial and anti-amoebic drugs.
6) **Removal toxic agents:** Cannon and his colleagues carried a study on cyanide intoxication using resealed erytrocytes and reported inhibition of cyanide intoxication with murine carrier erythrocyte containing bovine rhodanase and sodium thiosulphate. However antagonization of organo-phosphorus intoxication by released erythrocyte containing a recombinatephosphodiestrase also has been reported.
7) **Treatment of hepatic tumors:** Successful administration of antineoplastic drugs such as

methotrexate (MTX), bleomycin, asparginase and adiramycin by erythrocytes has been documented. For e.g. in a study carried on methotrexate, it showed a preferential drug targeting to liver followed by lungs, kidney and spleen.

8) **Delivery of antiviral agents:** Several reports have been quoted in the literature about antiviral drugs loaded in resealed erythrocytes for effective delivery and targeting of the drugs. Because most of the antiviral drugs are nucleotides or nucleoside analogs, their entrapment in erythrocytes and exit through the membrane requires careful consideration.
9) **Enzyme therapy:** Enzymes can be injected into the blood stream to replace a missing or deficient enzyme in metabolic disorders or to degrade toxic compounds accumulated in the blood due to a disease likewise, environmental, lysosomal storage disorders such as Gaucher's disease, hyperphenyl- alaninaemia and kidney failure are only few examples of metabolic disorders that can be treated by administration of enzymes.
10) **Removal of RES iron overloads:** Erythrocytes loaded with desferrioxamine have been used in treatment of excess iron accumulation due to multiple transfusions to thalassemic patients. Targeting of this drug to the RES is very beneficial as the aged erythrocytes are destroyed in RES organs, which results in an accumulation of iron in these organs.
11) **Targeting Non RES:** Resealed erythrocytes have also been used to target organs other than RES. The various approaches for targeting non-RES organs include as follows:

- Use of ultrasound waves.
- Entrapment of photosensitive material.
- Entrapment of paramagnetic particles along with the drug.
- Antibody attachment to erythrocytes membrane to get specificity of action.

FORMULATION OR DRUG ENTRAPMENT METHODS

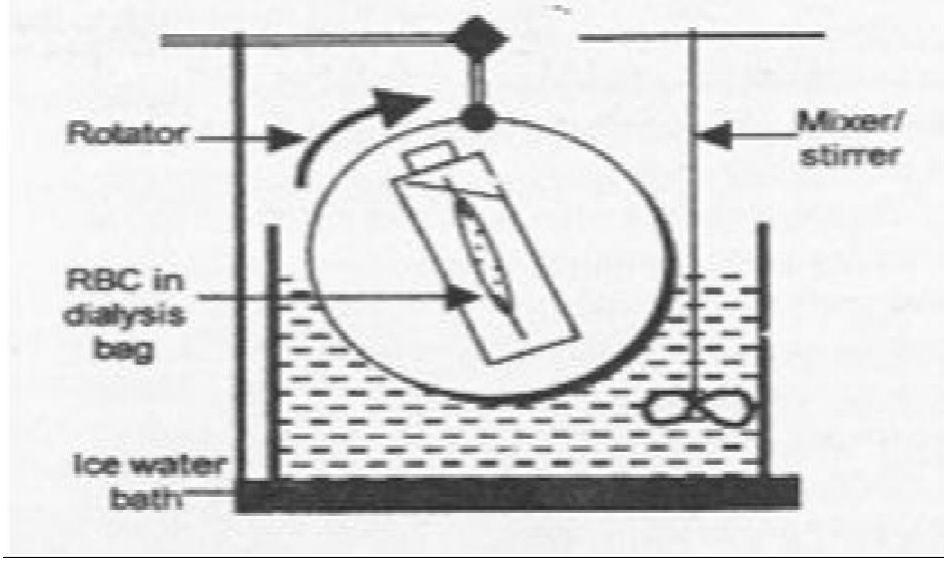
1. **Hypo- osmotic lysis method**
    a. Dilution method
    b. Dialysis method
    c. Pre-swell method

d. Isotonic osmosis lysis method

2. **Electrical breakdown method**
3. **Endocytosis method**
4. **Membrane perturbation method**
5. **Lipid fusion method**
6. **Osmotic pulse method/ Incubation method**

1. **Hypo-osmotic lysis method:** In this process, the intracellular and extracellular solutes are exchanged by osmotic lysis and resealing. The drug present will be encapsulated with in the erythrocytes membrane.
   a) **Dilution method**: In this method, a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug. The RBC'S are exposed to hypotonic solution (corresponding to 0.4 % Nacl), the erythrocytic membrane ruptures permitting escape of cellular contents and equilibrium is achieved with in one minute. The cells swell up to 1.6 times its original volume. The swelling results in the appearance of pores of 200 – 500 A° in size. The length of time for which these pores remain open is not fixed. However at 0oC the opening permits long enough to allow partial resealing of membrane. Increasing the ionic strength to isotonicity and incubating the cells at 37°C causes the pores to close and restore osmotic properties of the RBC'S. This method is simplest and fastest yet the capsulation efficacy is very low i. e. 1 – 8 %. Efficient for of low weight drugs. Examples of encapsulate agents: β-glucosidase, salbutamol.
   b) **Dialysis method:** A desired Haematocrit is achieved by mixing washed erythrocyte suspension and phosphate buffer (pH 7.4) containing drug solution. This mixture is placed into dialysis bag and then both ends of the bag are tied with thread. An air bubble of nearly 25 % of the internal volume is left in the tube. During dialysis bubble serves to blend the content. The tube is placed in a bottle containing 200 ml of lysis buffer solution and placed on a mechanical rotator at 4°C for 2 hrs. The dialysis tube is then placed in 200 ml of resealing solution (isotonic PBS pH 7.4) at

temperature 25 – 30°C for resealing. The loaded erythrocytes thus obtained are then washed with cold PBC at 4°C. The cells are finally resuspended in PBC. Examples of encapsulated agents: gentamicin, adriamycine, erythropoietin, Pentamidine, furamycin A, interleukine-2, IgG.



c) **Pre-swell dilution technique**: This technique is based upon initial controlled **swelling of RBC without lysis** in hypotonic buffered solution.

I. Erythrocyte suspension (2ml, 50% hematocrit) is centrifuged at 1000 rpm for 10 min at 4°C to obtain packed **RBC**.

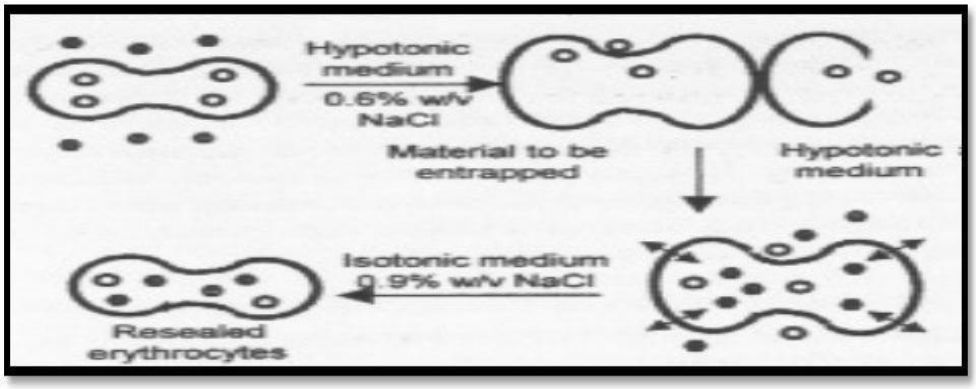
II. Remove supernatant. Collect packed RBC and add 4 ml of 0.65% NaCl (hypotonic pre-swelling solu.) and centrifuge 600 rpm for 5 min to collect **swollen RBC cells**.

III. To swollen RBC, add small volumes of drug solution until they reach the point of lysis. Point of lysis is detected by appearance of thin layer of white ghosts on centrifugation.

IV. After 10 min, hypertonic saline solution is added and suspension is incubated at 37°C for 10 min to restore isotonicity and resealing.

V. Cells are washed thrice with washing buffer to remove hemoglobin and unentraped drug. Cells are finally suspended in PBS buffer.

This techniques result in good retention of cytoplasmic constituents, high drug entrapment (72%) and good in-vivo survival. Examples of encapsulated agents: Thyroxin, Ibuprofen.



d) **Isotonic osmotic lysis technique:** This method, also known as the osmotic pulse method, involves isotonic hemolysis. Erythrocytes are incubated in solutions of a substance with high membrane permeability; the solute will diffuse into the cells because of the concentration gradient. Chemicals such as urea solution, polyethylene glycol and ammonium chloride have been used for isotonic hemolysis. The lysed erythrocytes are resealed under isotonic condition by dilution with a glycol free medium. Examples of encapsulated agents: Inositol hexaphosphate, DMSO, monosaccharides.

2. **Electric breakdown technique:** Electrical break down of cell membrane is observed when the membrane is polarized very rapidly (nano-microseconds) using voltage of 1 volt. This is due to electromechanical compression of membrane which leads to formation of pores. The break down occurs at lipid region / lipid-protein junction in

membrane. The potential difference across the membrane can be built up either directly (inter/ intra cellular electrodes) or indirectly by applying electric field pulse to cell suspension.

1) Firstly, erythrocytes are suspended in an isotonic buffered solution in electric discharge chamber connected to the capacitor and external circuit.
2) The charge is discharged at definite voltage within definite time interval through cell suspension to produce a square-wave potential.
3) The optimum intensity of an electric field is between 1-10 kW/cm and optimum discharge time is 20-160μs.
4) The compound which is to be entrapped was added to the medium.
5) After certain time the cell suspension was transferred to pre-cooled tubes and kept at 4^{o}C.
6) Resealing of electrically perforated erythrocytes membrane is then done by incubation at 37^{o}C in an osmotically balanced medium.

   Examples of encapsulated agent: Sucrose, Urease, methotrexate, interleukine.

3. **Endocytosis method**: Intra cellular vesicles with small molecules, drugs, enzymes, viruses (100 nm) can be induced in erythrocytes. The vesicle membrane separates endocytosed material from cytoplasm thus protecting it from the erythrocytes and vice-versa.

1. In this method, 1vol. of packed erythrocytes + 9 vol. of buffer (containing 2.5mM ATP, 2.5mM $MgCl_2$ and 1mM $CaCl_2$) and incubated for 2min at room temperature.
2. The pores created in this method are resealed by using 154mM of NaCl and incubate at 37^{o}C for 2min.
3. The entrapment of drug was obtained by endocytosis.

   Examples of encapsulated agents: Hydrocortisone, propranolol, vitamin A Primaquine, vinblastine, chlorpromazine.

4. **Membrane perturbation:** This method is based on the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemicals.

- Antibiotics such as amphotericin-B damage micro- organisms by increasing the permeability of their membrane to metabolites and ions.
- This property could be exploited for loading of drug into erythrocytes.
- Amphotericin-B was used to load erythrocytes with anti-leukaemic drug daunomycin.
- Amphotericin-B interacts with the cholesterol of the plasma membrane of eukaryotic cells causing change in permeability of the membrane.

  Examples of encapsulated agents: Daunomycin

5. **Lipid fusion method:** Lipid vesicles containing drug can be directly fused with human erythrocytes leading to exchange of lipid entrapped drug.

- This technique was used for loading inositol hexaphosphate into resealed erythrocytes.
- Cell fusion takes place by cell swelling, followed by cell adhesion.
- This method gives very low encapsulation efficiency (1%).
- Fusion can be induced by carboxylic acids, their esters, retinol, tocopherol.

  Examples of encapsulated agents: Inositol monophosphate.

6. **Osmotic pulse method/ Incubation method:** This method has 4 steps.

- Step-1: DMSO incubation: The RBC suspension can be incubated with different concentration of DMSO with variable hematocrit.
- Step-2: Isotonic dilution with the material to be encapsulated: The drug can be added with constant mixing.
- Step-3: Post dilution incubation with cellular swelling: The RBC swell and drug is encapsulated through pores.
- Step-4: Return to original shape: Drug loaded resealed erythrocytes are obtained.

EVALUATION OF RESEALED ERYTHROCYTES

In-Vivo Characterization: The efficacy of resealed erythrocytes is determined mainly by their survival time in circulation upon reinjection. The various methods used to determine in-vivo survival time include labeling of cells by 51Cr or fluorescent markers such as fluorescinisothiocyanate or entrapment of 14C sucrose or gentamicin. The circulation survival kinetics of resealed erythrocytes show typical bimodal behavior with a rapid loss of cells during the first 24 hrs after injection, followed by a slow decline phase with a half life on the order of days or weeks.

The in-vivo performance of resealed erythrocytes is affected to a great extent by their biological properties. Hence in-vitro characterization forms an important part of studies involving such cellular carriers.

In-Vitro Characterization:

1. **Shape and Surface Morphology:** The life span of erythrocytes after administration is mainly decided by their morphology. The morphological characterization of erythrocytes is carry out by comparing it with untreated erythrocytes using either Transmission electron microscopy (TEM) or Scanning electron microscopy (SEM).

   Scanning electron microscopic studies have revealed that a majority of the cells maintain their biconcave discoid shapes after the loading procedure, and few stomatocytes a form of spherocytes with an invagination in one point are formed. Electron microscopy observation may be made of the morphological changes in the erythrocytes induced by osmosis-based encapsulation methods, when they are subjected to solutions of different osmolality.

   Shape change (deformability) is another factor that affects the life span of the cells. This parameter evaluates the ease of passage of erythrocytes through narrow capillaries and the RES. It decides the rheological behavior of the cells and depends on the viscoelasticity of the cell membrane, viscosity of the cell contents and the cellular surface-to-volume ratio. The deformability of the cells is determined by passage time of definite volume of cells through capillary of 4µm diameter or polycarbonate filter with

average pore size of 45µm. Another indirect method is to evaluate chlorpromazine induced shape changes turbid metrically.

2. **Drug content:** Packed loaded erythrocytes (0.5ml) are first deproteinized with acetonitrile (2ml) and subjected to centrifugation at 2500 rpm for 10 min. The clear supernatant is analyzed for the drug content using specified estimation methodology for entrapped drug.
3. **In-vitro drug release and hemoglobin content**: The drug loading may produce sustained release of the drug that influences the pharmacokinetic behavior in-vivo of the loaded erythrocytes. In-vitro leakage of the drug from loaded erythrocytes is tested using autologous plasma or an iso-osmotic buffer at 37°C with a hematocrit value maintained between 0.5% to 50%. The supernatant is sampled at pre- determined programmed time intervals and replaced by an equal volume of autologous plasma or buffer.

   The content of hemoglobin in erythrocytes may be diminished by the alterations in the permeability of the membrane of the red cells during the encapsulation procedure. Moreover, the relationship between the rate of hemoglobin and the rate of drug release helps in interpreting the mechanisms involved in the release of the drug encapsulated in erythrocytes. The hemoglobin leakage is tested using a red cell suspension and their by recording the absorbance of supernatant at 540 nm on a spectrophotometer.

   In-vitro release of drug(s) and hemoglobin are supervised periodically form drug loaded cells. The cells suspension (5% hematocrit in PBS) is stored at 4°C in amber coloured glass containers. The clear supernatant are withdrawn at pre-determined time interval using a hypodermic syringes equipped with 0.45 m filter and then de-proteinized using methanol and thus are estimated for drug content. The supernatant of each sample after centrifugation is collected and assayed, percentage haemoglobin release may be calculated using the formula

$$\% \text{ hemoglobin release} = \frac{A540 \text{ of sample} - A540 \text{ of background}}{A540 \text{ of 100\% hemoglobin}}$$

(where A540 refers to absorbance at 540nm)

4. **Cell counting and cell recovery:** This consists of counting the number of red blood cells per unit volume of whole blood, usually by automated counting machine. Red cell recovered may be calculated on the basis of the differences in volume of the suspension of erythrocytes before and after loading and the hematocrit. The main objective is to reduce the loss during the encapsulation technique and to maximize cell recovery.
5. **Osmotic fragility and Osmotic shock study:** Osmotic fragility is a carried to determine abnormal fragility of red blood cells. Untreated or loaded erythrocytes are tested by exposing them to hypotonic solutions, thereby making them swell, in order to determine the relative fragility of the red cells. When RBC's are exposed to solution of different tonicities their shape change due to osmotic imbalance. To study the effect of varying tonicities, erythrocytes loaded with drug are incubated separately in normal saline solution at 37± 2°C for 10 minutes, which is further followed by centrifugation at 2000 rpm for 10 min. For osmotic shock study, resealed erythrocyte suspension is dispersed in distilled water and centrifuged at 300 rpm for 15 min. The supernatant is estimated for percent haemoglobin release spectrophotometrically.
6. **Turbulence Fragility:** The turbulence fragility is yet another characteristic that hinge upon changes in the integrity of cellular membrane and reflects resistance of loaded cells against hemolysis resulting from turbulent flow within circulation. Turbulence shock aids in evaluation of the stability of the loaded erythrocytes against the turbulence stress applied by the cells against in-vivo circulation turbulence. It is the degree of simulating destruction of loaded cells during injection.

   The turbulence fragility is determined by the passage of cell suspension through needles with smaller internal diameter (e.g., 30 gauges) at a flow rate of 10 ml/min which is comparable to the flow rate of blood or vigorously shaking the cell suspension. It is followed by collecting of an aliquot and thus centrifugation sample is further estimated. Drug loaded erythrocytes looks to be less resistant to turbulence, probably indicating destruction of cells upon shaking. Thus in both the cases, haemoglobin and drug released after the procedure are determined. The results show that the turbulent fragility of resealed cells is found to be higher.

7. **Erythrocyte sedimentation rate (ESR):** It is an evaluation of the suspension stability of RBC in plasma and is associated to the number and size of the red cells and to relative concentration of plasma protein, especially fibrinogen and α, β globulins. This test is carried out by determining the rate of sedimentation of blood cells in a standard tube. Normal blood ESR is 0 to 15 mm/hr higher rate is indication of active but obscure disease processes.
8. **Determination of entrapped magnetite:** Atomic absorption spectroscopic method is used for determination of the concentration of particular metal in the sample. The HCl is added to a fixed amount of magnetite bearing erythrocytes and content are heated at 60°C for 2 hours, then 20% w/v trichloro acetic acid is added thus the supernatant obtained after centrifugation is used to determine magnetite concentration using atomic absorption spectroscopy.
9. **In vitro stability:** The stability of the loaded erythrocytes is estimated by incubating the cells in the autologous plasma or in an iso-osmotic buffer, setting hematocrit between 0.5% and 5% at temperatures of 4°C and 37°C.
10. **Lipid content:** Lipid content and the spatial arrangement of lipids on the external surface of resealed erythrocytes affects their in- vivo half life. The longer half-life of loaded RBC indicated it maintains cell surface similar to normal RBC which prevents its removal by RE cells.

NANOPARTICLES

- Targeted drug delivery implies for selective and effective localization of pharmacologically active moiety at preidentified targets in therapeutic concentration, while restricting its access to non-target normal cellular linings, thus minimizing toxic effects and maximizing therapeutic index. The colloidal carriers based on biodegradable and biocompatible polymeric systems like liposomes, nanoparticles and micro emulsion have largely influenced the controlled and targeted drug delivery concepts.
- Nanoparticles dimensions between 1 nm and 1000 nm. Nano derives from the Greek word "nanos", which means dwarf or extremely small. Nanoparticles consist of

macromolecular materials in which the active ingredients (drug or biologically active material) is dissolved, entrapped, or encapsulated, or adsorbed.

Advantages of Nanoparticles:

- ✓ Particle size and surface characteristics of Nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
- ✓ They control and sustain the release of the drug during the transportation and at the site of localization.
- ✓ Subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
- ✓ Drug Loading is relatively high and drugs can be incorporated into the systems without chemical reaction.
- ✓ Site-specific targeting can be achieved by attaching targeting ligands to surface of particles.
- ✓ The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.,

Disadvantages of Nanoparticles:

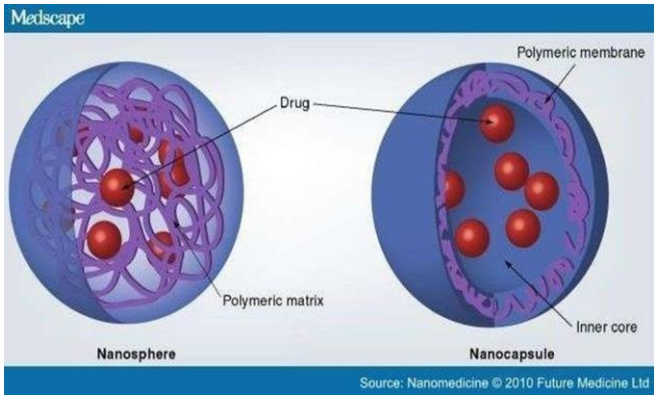
- ✓ They are susceptible to particle-particle aggregation due to their small size and large surface area.
- ✓ They are difficult to handle in liquid and dry form.
- ✓ Limited drug loading.
- ✓ Susceptible to bursting and leakage of contents.

TYPES OF NANOPARTICLES

- ❖ There are various approaches for classification of Nanomaterials.
- ❖ Polymeric nanoparticles are colloidal structures composed of natural, synthetic or semi synthetic polymers. Nanoparticles formulated from those polymers have gained importance. This drug delivery system provides targeted drug delivery, increased bio-

availability, and sustained release of drugs and protects drugs from enzymatic degradation. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix.

- Depending upon the method of preparation of nanoparticles, nanospheres or nanocapsule can be obtained.
  - **Nanocapsules** are vesicular systems in which the drug is confined to a cavity surrounded by a unique polymeric membrane.
  - **Nanospheres** are matrix systems in which the drug is dispersed throughout the particles.



- Nanoparticles are classified based on one, two and three dimensions.
  - One dimension nanoparticles: Example- Thin films (sizes1-100 nm) or monolayer. These thin films are using in different technological applications, including information storage systems, chemical and biological sensors, fibre-optic systems and optical device.
  - Two dimension nanoparticles: Example- Carbon nanotubes (CNTs)
  - Three dimension nanoparticles: Example- Fullerenes

Types of Nanoparticles

- **Fullerenes:** Fullerenes are spherical cages containing from 28 to more than 100 carbon atoms. This is a hollow ball composed of interconnected carbon pentagons and hexagons, resembling a soccer ball. Fullerenes are class of materials displaying unique physical properties. They can be subjected to extreme pressure and regain their original shape when the pressure is released. Fullerenes are offering potential application in the rich area of nanoelectronics. Since fullerenes are empty structures with dimensions similar to several biological active molecules, they can be filled with different substances and find potential medical application.

- **Carbon Nanotubes:** Nanotubes (NTs) are cylindrical fullerenes. Carbon nanotubes are hexagonal network of carbon atoms, 1 nm in diameter and 100 nm in length, as a layer of graphite rolled up into cylinder. CNTs are of two types, single walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). They have a great capacity for molecular absorption and offering a three dimensional configuration. Moreover they are physically and chemically very stable.

- **Solid lipid nanoparticles (SLNs):** SLNs are lipids in nature which remain in solid phase at normal room temperature. The particle size of SLNs ranges from 50 nm to 1,000 nm. SLNs are composed of solid hydrophobic core and a single coating layer of phospholipids. SLNs are stabilized by different surfactants for emulsification and also show many properties such as increased bio-degradability, increased bio- availability and drug targeting in the brain.

Different forms of lipids are used for formulating SLNs. These include a) fatty acids such as palmitic acid, decanoic acid, b) triglycerides such as tri-laurin, tri-myristin, and tri-palmitin, c) cholesterol, d) partial glycerides such as glyceryl mono-stearate and e) waxes such as cetyl palmitate. Different types of surfactants are also used for stabilizing lipid dispersions. These include lecithin, poloxamer 188, and sodium-cholate. SLNs have vast applications in cancer therapy. SLNs have ability to accumulate tumor, and also increase allow anticancer drugs delivery to the brain.

- **Super Paramagnetic Nanoparticles:** These are attracted towards a specific magnetic field. When the magnetic field is removed, these cannot retain their residual magnetism. Particles range in the size of 5 nm to 100nm and used for selective magnetic bio-separations and can be visualized in magnetic resonance imaging (MRI). These work on the principle of magnetic field and heated to trigger the drug release. These have also shown major role in cancer therapy and diagnosis.
- **Nanostructure lipid carriers (NLC):** NLC are prepared by using blend of solid lipids and liquid lipids. The particles remain in solid state at normal room temperature. Nanostructure lipid carriers (NLC) and the lipid drug conjugate (LDC) nanoparticles are prepared in the form of matrices. These matrices increase drug loading capacity and bio-availability. These are used for drug delivery via different routes such as oral, topical and parenteral (subcutaneous, intramuscular, intravenous etc.). These also have applications in the fields of cosmetics and food. These have been extensively used in the delivery of anti-inflammatory drugs, cosmetic preparation.
- **Nanoshells:** Nanoshells, also known as core-shells, are spherical cores of concentric particles which are surrounded by an outer coating of thin layer of another material. Nanoshells have biomedical imaging and therapeutic applications. These exhibit distinct property such as reduced susceptibility to chemical/thermal denaturation.
- **Quantum Dots (QD):** The QD are known as semiconductor nano-crystals and core-shell nano-crystals. These are 2 nm to 10 nm in size. These are used as drug delivery system for various hydrophilic drugs such as small interfering RNA (si-RNA) and anti-sense oligo-deoxy-nucleotide (ODN)) as well as targeting antibodies, peptides etc. QD also

have extensive applications in imaging contrast.

- **Dendrimers:** Dendrimer is derived from a Greek word Dendron which means a tree. Dendrimers are polymeric molecules made up of multiple perfectly branched monomers. Different polymers such as polyamido-amine, poly-L- glutamic acid, poly ethyleneimine (PEI), poly propylene-imine (PPI), and polyethylene glycol, and chitin are used in formulation of dendrimer. These are extensively applied in magnetic resonance imaging and targeting cancerous cells.
- **Ceramic nanoparticles:** Ceramic nanoparticles are porous in nature and particle size is less than 50 nm. These possess distinct properties such as sol-gel process, work in ambient temperature condition and product produced of desired size, shape and porosity as well as effective in hiding the uptake by reticulo endothelial system. These are broad applications such as novel non- viral vector for genes delivery and in diabetic wounds healing. These also play major role in the field of orthopedic and used as orthopedic biomaterial because these provide support to natural bone.
- **XPclad® nanoparticles:** XPclad® nanoparticles are novel carriers for the poor hydrophilic drugs which face significant problem of bio-availability and absorption. These nanoparticles are used for systemic, cutaneous, and oral administration of anti-cancer drugs, vaccines, and therapeutic proteins. These are regarded as useful in tumor therapy because of lower toxicities and cause the destruction of prostate tumor cells.
- **Nanofibers:** Nanofibers are fibers with diameter in nanometer range (less than 100 nm). They are effective carriers for drug delivery and show advantages such as specific surface with small pore size, porosities, reduced toxicity and increased therapeutic level and bio-compatibility. Different polymers such as polyvinyl alcohol (PVA), gelatin, collagen, chitosan and carboxy methylcellulose (CMC) are used. Nanofibers are considered ideal for the preparation of biosensors and biochips, as drug delivery systems. Nanofibers of indomethdacin for colonic drug delivery system found to be very effective.
- **Gold Nanorods:** Gold nanorods were first time prepared in mid- 1990. These exhibit distinct optical and electronic properties and depend on shape, size and aspect ratios. These can be easily stabilized, conjugated to antibodies and show distinctive biological

applications.

- **Nanoerythrosomes:** Nanoerythrosomes are derived from a red blood cell membrane by the process of haemo-dialysis through filter. Nanovesicles are of defined pore size and composed of proteins, phospholipids and cholesterol. These can load a variety of biologically active agents such proteins. They composed of a natural membrane which allows the insertion of recombinant ligands along with better stability. The membrane allows the conjugation by using simple and well known molecule such as monoclonal antibodies.

APPLICATIONS OF NANOPARTICLE

1) Nanoparticle in drug delivery systems

- The use of pharmacological agents is frequently limited by drug resistance at the target level owing to physiological barriers cellular mechanism is encountered. In addition, many drugs have a poor solubility, low bioavailability and they can be quickly cleared in the body by the **reticuloendothelial** system.

a) Gastrointestinal tract:

- It is known that the kinetics of particle uptake in GI tract depends on diffusion and accessibility through mucus initial contact with enterocytes, cellular trafficking and post- translocation events. The smaller the particle diameter is, the faster they could diffuse through GI secretion to reach the colonic enterocytes.
- Following uptake by GI tract, nanoparticles can translocate to the blood stream and distribute all over the body.
- Targeting strategies to improve the interaction of nanoparticles with adsorptive sites (enterocytes and M-cells) in the GI tract utilizes specific binding to ligands or receptors and nonspecific adsorptive mechanism. The surface of enterocytes and M cells shows cell-specific carbohydrates, which can serve as binding sites to nanoparticle drug carriers with appropriate ligands. Certain glycoproteins and lectins bind selectively to this type of surface structure by specific receptor-mediated mechanism.

b) Brain:

- The brain is probably one of the least accessible organs for the delivery of drugs due to the presence of the blood–brain barrier (BBB) that controls the transport of endogenous and exogenous compounds, thus providing the neuroprotective function.
- Drugs normally unable to cross the BBB could be delivered to the brain after binding to the surface-modified poly butyl cyanoacrylate (PBCA) nanoparticles.

c) Tumor cell targeting:

- With the use of nanotechnology, targeting drug molecules to the site of action is becoming a reality resulting in a personalized medicine, which reduces the effect of the drug on other sites while maximizing the therapeutic effect.
- This goal is mainly achieved by the small size of these particles, which can penetrate across different barriers through small capillaries into individual cells.
- In addition, nanoparticles can be prepared to entrap, encapsulate, or bind molecules improving the solubility, stability and absorption of several drugs, as well as avoiding the reticulo-endothelial system, thus protecting the drug from premature inactivation during its transport.
- In fact, it has been shown that nanoparticles have the ability to carry various therapeutic agents including DNA, proteins, peptides and low molecular weight compounds. Among all of them, liposome and polymer-based nanoparticulates are the most widely used nanoparticles as drug delivery systems, as these compounds are generally biodegradable, do not accumulate in the body and they are possibly risk-free.
- For instance, several anticancer drugs, including paclitaxel, 5-fluorouracil, doxorubicin, have been successfully formulated using polymers and liposomes as drug delivery systems.

d) Respiratory tract:

- Nanoparticles could avoid normal phagocytic defences therein respiratory tract and gain access to systemic circulation and may reach to CNS.

- Aerosol therapy using nanoparticles as drug carrier is gaining importance for delivering therapeutic compounds.

- The lung is an attractive target for drug delivery due to non- invasive administration via inhalation aerosols, avoidance of first-pass metabolism, direct delivery to the site of action for the treatment of respiratory diseases and the availability of a huge surface area for local drug action and systemic absorption of drug.
- Colloidal carriers (i.e., nanocarrier systems) in pulmonary drug delivery offer many advantages such as the potential to achieve relatively uniform distribution of drug dose among the alveoli, a sustained drug release which consequently reduces dosing frequency, improves patient compliance and decreases incidence of side effects.

2) For gene delivery

- The key ingredient of polynucleotide vaccines, DNA, can be produced cheaply and has much better storage and handling properties than the ingredients of the majority of protein-based vaccines.
- There is efficient delivery of the polynucleotide to the target cell population and its localization to the nucleus of these cells and ensuring that the integrity of the polynucleotide is maintained during delivery to the target site.
- Nanoparticles loaded with plasmid DNA could also serve as an efficient sustained release gene delivery system due to their rapid escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment. Following the intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in sustained gene expression.

3) For Diagnosis and Bioimaging

- A number of molecular imaging techniques are available, such as optical imaging (OI), magnetic resonance imaging (MRI), ultrasound imaging (USI), and positron emission tomography (PET).
- Two different types of nanoparticles have been widely used for imaging: luminescent nanoprobes for OI and magnetic nanoparticles for MRI.
- Nanobiotech scientists have successfully produced microchips that are coated with

human molecules. The chip is designed to emit an electrical impulse signal when the molecules detect signs of a disease.

- Special sensor nanobots can be inserted into the body under the skin where they check blood contents and warn of any possible diseases. They can also be used to monitor the sugar level in the blood.
- Gold nanoparticles are being used for detection of cancer. Gold nanoparticles have been used as ultrasensitive fluorescent probes to detect cancer biomarkers in human blood. They can be easily prepared and, unlike other fluorescent probes such as quantum dots or organic dyes, don't burn out after long exposure to light.

4) For Tissue repair:

- Tissue repair using iron oxide nanoparticle is accomplished either through welding, apposing two tissue surfaces then heating the tissues sufficiently to join them, or through soldering, where protein or synthetic polymer-coated nanoparticles are placed between two tissue surfaces to enhance joining of the tissues.
- Temperatures greater than 50°C are known to induce tissue union induced by the denaturation of proteins and the subsequent entanglement of adjacent protein chains.
- Gold- or silica-coated iron oxide nanoparticles have been designed to strongly absorb light. The nanoparticles are coated onto the surfaces of two pieces of tissue at the site where joining was desired. This technique affords methods to minimize tissue damage by using the least harmful wavelengths of light and/or lower powered light sources.
- Magnetic nanoparticles can also be used to target the stem cells and activate at required sites of injury and repair in diseases such as diabetes, cancer, heart disease, Alzheimer's and Parkinson's disease.

FORMULATION OF NANOPARTICLES

1. Materials
2. Preparation of Nanoparticles
3. Surface Modification of Nanoparticles
4. Drug Loading into Nanoparticles

1) MATERIALS:

- Polymers:

Methods of Nanoparticles Preparation	Polymers
Monomers polymerization	Poly(alkyl cyanoacrylate), Poly(alkyl methacrylate), Poly(styrene), Poly(vinyl pyridine)
Nanoprecipitation	Poly(ε-caprolactone), Poly(lactic acid), Poly(lactic-co-glycolic acid), Poly(methacrylate)
Solvent evaporation	Poly(ε-caprolactone), Poly(lactic acid), Poly(lactic-co-glycolic acid), Poly(β-hydroxybutyrate), Ethyl cellulose
Salting out	Cellulose acetate phthalate, Poly(alkyl methacrylate), Ethyl cellulose, Poly(lactic acid), Poly(lactic-co-glycolic acid)
Desolvation, denaturation, ionic gelation	Albumin, Casein, Gelatin, Alginate, Chitosan, Ethyl cellulose

- Stabilizer: Generally surfactants are used as stabilizers to reduce high surface free energy of nanosized particles. Generally used stabilizers are Cellulosic, Poloxamers 184, 188, 338, 407, Poloxamine 908, Polysorbates 20, 40, 60, 80, Lecithin, Polyoxyethylene lauryl ether, Povidone, Lecithin is stabilizer of choice for parenteral preparations.
- Organic Solvents:
  Water immiscible organic solvent: Methylene chloride, Chloroform, DCM
  Partially water miscible solvent: Ethyl acetate, Triacetin, Propylene carbonate, Benzyl alcohol
  Water miscible solvent: Ethanol, Isopropanol
- Co-surfactants: Bile salts, Dipotassium Glycerrhizinate, Ethanol, and Isopropylalcohol.
- Other Additives: Buffers, Salts, Polyols, Osmogents, and Cryoprotectants.

2) PREPARATIONS OF NANOPARTICLES:

- Solvent Evaporation:
  i) Polymer is dissolved in organic solvent like acetone, chloroform etc.
  ii) The drug is dissolved or dispersed into the preformed polymer solution.
  iii) Then the mixture is emulsified with aqueous phase to prepare o/w emulsion by using a surfactant.
  iv) After formation of a stable emulsion, the organic solvent is evaporated either by

increasing temperature/under reduced pressure or by continuous stirring.

v) The w/o/w method is also applied to prepare water soluble drug loaded NPs.
vi) Both the above method uses a high speed homogenization or Sonication.

❖ Spontaneous emulsification/Solvent diffusion method: It is a modified version of solvent evaporation method.

i) Here water soluble solvent like acetone along with water insoluble solvent like chloroform are used as an oil phase.
ii) Due to spontaneous diffusion of water soluble solvent, an interfacial turbulence is created between two phases that leads to formation of smaller particles.
iii) As the concentration of water soluble solvent increases, a considerable decrease in particle size can be achieved.

❖ Salting out: Drug and polymer are first dissolved in solvent and then they are subjected to homogenization with aqueous solvent having salting out agent and at last salts are removed by cross-flow filtration.

❖ Monomer polymerization: Here we will see NPs formation using poly (alkyl cyanoacrylate).

i) The cyanoacrylic polymer is added to an aqueous acidic solution of surface active agent (polymerization medium) under vigorous mechanical stirring.
ii) Drug is dissolved in the polymerization media either before the addition of monomer or at the end of polymerization reaction.
iii) The NP suspension is then purified by ultracentrifugation or by resuspending the particles in an isotonic surfactant free medium.
iv) Particle size and molecular mass of NP depend upon the type & conc. of surfactant, pH of the medium, conc. of monomer and stirring speed.

❖ Nanoparticles prepared by hydrophilic polymers:

i) Denaturation: It involves emulsification of an aqueous solution containing a natural polymer and the drug to be entrapped in an oil emulsion. The particles are hardened by heat Denaturation, cooling below the gelation point or by cross-linking with suitable agent.
ii) Desolvation: Commonly known as coacervation (similar to microspheres).
iii) Ionic gelation: Ion induced gelation results into formation of NPs.

❖ Supercritical fluid technology:

i) Rapid expansion of super critical solution (RESS)

- The solute of interest is first dissolved in SCF.
- Then the solution is expanded through a nozzle.
- Thus the solvent power of SCF decreases and so the solute precipitates.
- This technique is clean because the precipitated solute is completely solvent free.
- Unfortunately, most polymers exhibit little or no solubility in SCF, thus making the technique less of practical interest.

ii) Supercritical anti-solvent (SAS)

- Both the solution of solute in a suitable solvent and SCF are charged in the precipitation vessel.
- Because of high pressure, enough antisolvent will enter into the liquid phase, so the solvent power will be reduced and solute precipitates.

iii) Gas anti-solvent technique (GAS)

- It is a modified version of SAS method.
- The solution of solute is rapidly introduced into the SCF through a narrow nozzle.
- The SCF completely extracts the solvent, causing precipitation of solute.

3) SURFACE MODIFICATION OF NANOPARTICLES: Following two methods are useful for surface modification:

- Surface coating with hydrophilic polymers/surfactants: PEG, PEO, Poloxamer, Poloxamine, Polysorbate
- Development of biodegradable co-polymers with hydrophilic segments (PLA-PEG, PLGA-PEG)

These modification lead to change in zeta potential and hydrophobicity of NPs that ultimately affects following properties:

- Stability
- Mucoadhesive properties
- Oral absorption
- Protein adsorption at surface

4) DRUG LOADING IN NANOPARTICLES: Following methods are used for drug loading into NPs:

❖ Entrapment method

- It involves the incorporation of the drug at the time of NP production.
- It depends on the concentration of monomers.
- Large amount of drug can be entrapped by this method as compared to adsorption method.

❖ Adsorption method

- Here, the drug is loaded by incubating the pre-formed NPs in the drug solution.
- The capacity of adsorption depends on the hydrophobicity of the polymer and the specific surface area of NPs.

❖ Chemical conjugation

- New method for drug loading of water soluble drugs.
- In one article, they have utilized this method to prepare conjugated doxorubicin-PLGA NPs. These NPs showed higher loading capacity as compared to unconjugated doxorubicin-PLGA NPs.

EVALUATION OF NANOPARTICLES

The followings are different evaluation parameters of nanoparticles:

1) Particle size

Particle size distribution and morphology are the most important parameters of characterization of nanoparticles. Morphology and size are measured by electron microscopy. The major application of nanoparticles is in drug release and drug targeting. It has been found that particle size affects the drug release. Smaller particles offer larger surface area. As a result, most of the drug loaded onto them will be exposed to the particle surface leading to fast drug release. There are several tools for determining nanoparticle size as discussed below:

a) Dynamic light scattering (DLS)

Currently, the fastest and most popular method of determining particle size is dynamic light scattering (DLS). DLS is widely used to determine the size of Brownian nanoparticles in colloidal suspensions in the nano and submicron ranges. Shining monochromatic light (laser) onto a solution of spherical particles in Brownian motion causes a Doppler shift when the light hits the moving particle, changing the wavelength of the incoming light. This change is related to the size of the particle. It is possible to extract the size distribution and give a description of the particle's motion in the medium and measuring the diffusion coefficient of the particle.

b) Scanning Electron microscopy

Scanning electron microscopy (SEM) is giving morphological examination with direct

visualization. For SEM characterization, nanoparticles sample should be first converted into a dry powder, which is then mounted on a sample holder followed by coating with a conductive metal, such as gold, using a sputter coater. The sample is then scanned with a focused fine beam of electrons. The surface characteristics of the sample are obtained from the secondary electrons emitted from the sample surface. The mean size obtained by SEM is comparable with results obtained by dynamic light scattering. Moreover, these techniques are time consuming, costly and frequently need complementary information about sizing distribution.

c) Transmission electron microscope

TEM operates on different principle than SEM, yet it often brings same type of data. The sample preparation for TEM is complex and time consuming because of its requirement to be ultra thin for the electron transmittance. The nanoparticles dispersion is deposited onto support grids or films. To make nanoparticles withstand the instrument vacuum and facilitate handling, they are fixed using either a negative staining material, such as phosphotungstic acid or derivatives, uranyl acetate, etc, or by plastic embedding. The surface characteristics of the sample are obtained when a beam of electrons is transmitted through an ultra thin sample, interacting with the sample as it passes through.

2) Surface Charge

The nature and intensity of the surface charge of nanoparticles is very important as it determines their interaction with the biological environment as well as their electrostatic interaction with bioactive compounds. The colloidal stability is analyzed through zeta potential of nanoparticles. The measurement of the zeta potential allows for predictions about the storage stability of colloidal dispersion. High zeta potential values, either positive or negative, should be achieved in order to ensure stability and avoid aggregation of the particles. The extent of surface hydrophobicity can then be predicted from the values of zeta potential. The zeta potential can also provide information regarding the nature of material encapsulated within the nanocapsules or coated onto the surface.

3) Surface hydrophobicity

Surface hydrophobicity can be determined by several techniques such as hydrophobic

interaction chromatography, biphasic partitioning, adsorption of probes, contact angle measurements etc. Recently, several sophisticated analytical techniques are reported in literature for surface analysis of nanoparticles. X – Ray photon correlation spectroscopy permits the identification of specific chemical groups on the surface of nanoparticles.

4) Surface area

An instrument called Sorptometer is used to determine the specific surface area of freeze- dried nanoparticles. The specific surface area can be calculated using following equation:

$$A = 6/\rho d$$

Where,

A= Specific surface area of nanoparticles
ρ= Density o the medium
d= Diameter of the nanoparticles

5) Recovery of Nanoparticles (% yield)

Percentage yield of nanoparticles can be calculated by the following equation:

% Nanoparticle yield = [(Conc. Of drug in the nanoparticles) X 100] / Conc. of the recovered nanoparticles

6) Entrapment Efficiency

✓ Centrifugation:

- Nanoparticle dispersion is to be centrifuged at 20,000 rpm for 1hr to collect the supernatant liquid
- The collected liquid was filtered to measure the free drug concentration after suitable dilution with a fresh buffer and can be analyzed using HPLC or UV Spectrophotometer.

Entrapment efficiency = (Wt. of drug incorporated/Wt. of drug initially taken) × 100

7) Drug Release

The drug loading of the nanoparticles is generally defined as the amount of drug bound per mass of polymer (usually moles of drug per mg polymer or mg drug per mg polymer); it could also be given as percentage relative to the polymer. The technique used for this analysis is classical analytical methods like UV spectroscopy or high performance liquid

chromatography (HPLC) after ultracentrifugation, ultra filtration, gel filtration, or centrifugal ultrafiltration. Quantification is performed with the UV spectroscopy or HPLC. Drug release assays are also similar to drug loading assay which is assessed for a period of time to analyze the mechanism of drug release.

Methods of evaluation for release of drugs

- ❖ Dialysis Method:
- ➢ In this method, physical separation of the dosage forms is achieved by usage of a dialysis membrane which allows for ease of sampling at periodic intervals.
- ➢ With the regular dialysis technique, the nanoparticles are introduced into a dialysis bag containing release media (inner media/compartment) that is subsequently sealed and placed in a larger vessel containing release media (outer media/compartment), agitated to minimize unstirred water layer effects.
- ➢ In general, the volume enclosed in a dialysis bag (inner media) is significantly smaller than the outer media. For instance, inner media volumes reported in literature range from 1 to 10 mL, whereas the outer media volume is much greater, typically around 40– 90 mL.
- ➢ Thus, container size will depend on the total volume of release media required for the in vitro release study. In the regular dialysis technique, drug released from the nanoparticles diffuses through the dialysis membrane to the outer compartment from where it is sampled for analysis.
- ❖ Diffusion cell:
- ➢ The apparatus consists of two chamber (donor and receptor), which are separated by means of a millipore membrane. The diffusion cell is placed on a shaker stand.
- ➢ The sample is filled in donor chamber and phosphate buffer is filled in receptor chamber.
- ➢ After specified time interval, aliquot samples are removed from receptor chamber and is assayed to determine the rate of drug release.
- ❖ Ultrafiltration:
- ➢ Nanoparticle suspension is added directly into a stirred ultrafiltration cell containing buffer.

- At regular time intervals, aliquots of buffer are initially filtered through the ultrafiltration membrane and then assayed for the released drug.

UNIT-5

INTRAUTERINE DEVICES

INTRODUCTION

- IUD's are medicated devices intended to release a small quantity of drug into uterus in a sustained manner over prolonged period of time.
- An Intrauterine Device (IUD) is a small object that is inserted through the cervix and placed in the uterus to prevent pregnancy.
- A small string hangs down from the IUD into the upper part of the vagina.
- The IUD is not noticeable during intercourse.
- IUD's can show pharmacological efficacy for about 1-10 years.

Advantages:

- Long time action and highly effective
- Convenient: no action needed before, during or after sex; requires no daily attention
- Easily removed by provider at any time
- Women who are breast-feeding can use this

Disadvantages:

- Doesn't prevent against sexually transmitted infections.
- Small risk of pelvic infection for first few weeks after insertion;
- May increase cramps and bleeding during monthly periods
- Requires health care provider visits for insertion and removal

Contraception: It is the method which results into temporary or permanent loss of capability to reproduce or conceive a young one. There are 2 types of contraception:

- ✓ **Temporary contraception:** It is a method or lifestyle that ensures reversible infertility for stipulated period of time depending on the subject. e.g. IUD's, oral contraceptive pills, condoms etc.

- ✓ **Permanent contraception:** It is the method or technique adopted to give life long acquired inability to reproduce, but it is not the loss of sense or loss of sexual desire. e.g. ovarectomy, uterectomy, vasectomy, etc.

The wall of the uterus consists of 3 layers:

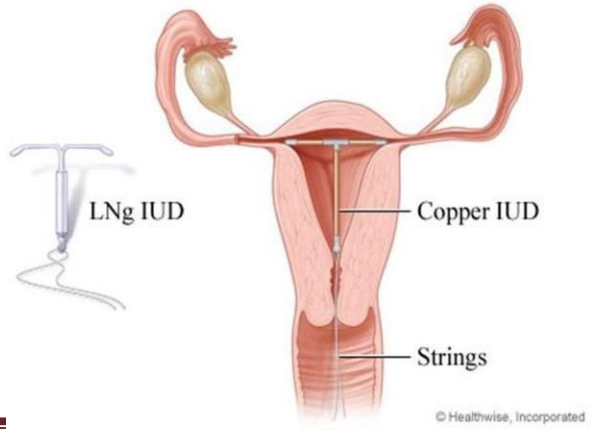
- **Endometrium**- Inner coat of the uterine wall and is a mucous membrane. It consists of epithelium lining and connective tissue. Epithelium consist stratified sqamous epithelium, and lamina propria.
- **Myometrium**- Thick, muscular middle layer made up of bundles of interlaced, smooth muscle fibers emmbeded in connective tissue. It is Sub-divided into 3 ill-defined, intertwining muscular layers containing large blood vessels of uterine walls.
- **Peritoneum**- External surface of the uterus, which is attached to the both sides of the pelvic cavity by broad ligaments through which the uterine arteries cross.

INTRAUTERINE DEVICES

PRINCIPLE:

- ❖ IUDs are small objects that are inserted through the **cervix** and placed in the uterus to **prevent pregnancy**. A small string hangs down from the IUD into the upper part of the vagina.
- ❖ They work by changing the lining of the uterus and fallopian tubes affecting the movements of eggs and sperm and so that fertilization does not occur.

Location of IUDs



Basic Types:

1) **Non-Medicated IUD's:** These IUD's exert their contraceptive action by producing a sterile inflammatory response in the **endometrium** by its mechanical interaction. These do not contain any therapeutic agent. E.g.- ring shaped IUD's plastic IUD's, lippes loop, Dalkon shield, Saf- T-Coil.

2) **Medicated IUD's:** These IUD's are capable of delivering pharmacologically active antifertility agents. E.g.- copper bearing IUD's, progesterone releasing IUD's.

Based on generation they can be classified as follows:

First generation IUDs:

- Non medicated or inert IUDs
- Made up of polyethylene or other polymers.
- Eg- Loops

Second generation IUDs:

- Copper containing device, smaller and easier to fit.
- Incorporation of copper in plastic IUDs
- Eg- Copper 7, Copper T

Third generation IUDs:

- T shaped device filled with hormone like progesterone
- Release slowly hormone in uterus

APPROACHES/FABRICATION:

➢ **NON – MEDICATED IUD'S**

- These IUD's do not contain any therapeutically active agent.
- These prominently make use of metal or plastic rings and coils. e.g. Dalkon shield, Lippes loop, Saf - T- coil.
- Rings of stainless steel have mechanical effects on the uterus leading to contraception.
- Plastic rings also act as mechanical barrier for sperms and eggs so they don't fuse.
- Non medicated IUD's are not used now a day because of one or more reasons like safety and effectiveness, irregularities in menstrual bleeding, cases of pelvic inflammatory diseases (PID) and higher rates of pregnancies.

➢ **MEDICATED IUD'S**

1) Copper bearing IUD'S

- This device made of **T shaped** polyethylene plastic and copper wire (thickness of 0.2-0.4 mm) wound to the stem.
- There are various grades as per the surface area of the Cu-wire such as Cu-T-30, Cu-T-200, Cu-T-380
- At low concentration- Spermatocidal & Spermatodepressive.
- In high concentration copper is cytotoxic. It enhances the spermatocidal and spermato-depressive action of an IUD.
- Cupric ion (Cu++) is a competitive inhibitor of progesterone and to lesser effect estrogen.
- e.g.- cu –T-200, cu-T-30, cu-T-380, Cu-T-220

- Release of Copper from the device: The release is linear by chelation, ionization, and corrosion over the period of 12 years. Release rate is directly proportional to the surface area of exposed Cu. e.g. Cu-T-380A.

2) Hormone releasing IUD'S

- A **T-shaped progesterone** releasing IUD having vertical limb embedded with drug-containing silicone capsule was evolved.
- Coated with polymer for achieving slower release.
- The device has a solid poly EVA (ethyl vinyl acetate) side arms and a hollow core. The microcrystalline **progesterone (pg)** is suspended in the core in the silicone oil with BaSo4.
- Progesterone (pg) is released by diffusion through rate limiting membrane.
- Does not inhibit ovulation but interfere with implantation in endometrium, thickening of cervical mucus.
- Progesterone administered by IUDs show 45 times greater bioavailability than the other oral delivery and subcutaneous injection.
- They diminish sperm transport through the cervix to the oviduct by increasing the thickness of the cervical mucous.
- Steroid releasing devices induce progesteronal changes that result in endometrial gland atrophy and inhibit further development of the ova.

INJECTIONS/ INJECTABLES

INTRODUCTION

- It is defined as "The delivery of drug at predetermined rate, and maintaining optimal and effective drug level for prolonged duration."

- The ingredient used for parenteral controlled release formulation should be **sterile, pyrogen free**, non-irritating, bio-compatible and biodegradable into nontoxic compounds.

- ***Major Routes Of Parenteral Administration:***

✓ **Subcutaneous:** This route is generally limited to non-irritating, water soluble drugs that are well absorbed, e.g: Insulin. To avoid local tissue damage and accumulation of unabsorbed drug injection is rotated for chronically administered drugs. The volume of subcutaneous injection is restricted to 0.5-1.5 ml.

✓ **Intramuscular:** The best sites for intramuscular injection are gluteal, deltoid and vastus Laterals muscles. It is important that the injection is deep in the muscle and away from

the major nerves and arteries. To avoid tissue damage, the volume of intramuscular injection should not exceed 2ml.

- ✓ **Intravenous**: The intravenous route is occasionally use as a route of administration for sustained/ controlled dosage forms such as liposomes, nanoparticles, erythrocytes and polypeptides. When drug particles are administered i.v, larger particles are either trapped in lungs or taken up by spleen or liver and smaller particles accumulate in the bone marrow.

- ✓ **Intraperitoneal:** Macromolecules administered intraperitoneally can gain access to the lymphatic system and return slowly to the vascular compartment, thus it can be used as a carrier to target antineoplastic agents into the lymphatic system.

PRINCIPLE:

- Injectable controlled drug delivery system offer extension of duration of action for days or even months. These depot formulations increase the therapeutic effect by continuously releasing drugs over an extended period of time after administration of single parenteral dose.
- It possess various advantages like ease of application, localized delivery for a site-specific action in the body, e.g. In local anesthesia/analgesia reduced dosing frequency without compromising the effectiveness of the treatment, increased dosing compliance.
- In developing controlled release parenteral dosage forms to have concentrated on the **subcutaneous & intramuscular** routes.
- Resulting in such products as aqueous and oil suspensions, oil solutions etc.
- Drug molecules will be released continuously from the reservoir at a rate determined by the characteristics of each formulation.
- This continuous release of drug molecules will result in a prolonged drug blood level.
- The rate of absorption and hence duration of therapeutic activities will be determined by the nature of the vehicle.
- The Parenteral administration route is the most common and efficient for delivery of active drug substances with poor bio-availability and the drugs with a narrow therapeutic index.
- But through the parenteral route of administration some disadvantages also present, to overcome those problems these **controlled delivery system** of parenteral dosage forms are used.

APPROACHES/ FABRICATION:

- Use of viscous, water-miscible vehicles, such as an aqueous solution of gelatin or polyvinylpyrrolidone.
- Utilization of water-immiscible vehicles, such as vegetable oils, plus water repelling agent, such as aluminum monostearate.
- Formation of **thixotropic** suspensions.
- Preparation of water-insoluble drug derivatives, such as salts, complexes, and esters.
- Dispersion in polymeric microspheres or microcapsules, such as lactide-glycolide homopolymers or copolymers.
- Co administration of vasoconstrictors.

INJECTIONS/ INJECTABLES

❖ **Classification *(Fabrication)* of Depot Preparations:** On the basis of different mechanism, depot formulation categories into four types.

1) **Dissolution controlled depot formulation**: In this depot formulation the rate limiting step of drug absorption is the dissolution of drug particles in the formulation or in the tissue fluid surrounding the drug formulation. The rate of dissolution can be determined mathematically as-

$$Q/t = SDC/h$$

Where, Q/t = rate of dissolution,
S = surface area of drug particle
D = diffusion coefficient
C = saturation solubility,
H = thickness of hydrodynamic diffusion layer.

The dissolution can be controlled by

- Formation of Salt or complexes with low aqueous solubility: E.g.: preparation of penicillin G procaine (C=4 mg/ml) and penicillin G benzathaine (C=0.2mg/ml) from highly water soluble alkali salt of penicillin G.
- Suspension of Macrocrystals: Macrocrystals are known to dissolve more slowly than Microrystals (small crystals). Example is the aqueous suspension of testosterone isobutyrate for intramuscular administration.

2) **Adsorption type depot formulation:** This depot preparation is formed by the binding of drug molecules to adsorbents. In this case only the unbound, free species of the drug is available for absorption.

- As soon as the unbound drug molecules are absorbed a fraction of the bound drug molecules is released to maintain equilibrium.

3) **Encapsulation type depot formulation:** This depot preparation is prepared by encapsulating drug solids within a permeation barrier or dispersing drug particles in a diffusion matrix.

- The release of drug molecule is controlled by the rate of permeation across the permeation barrier and the rate of biodegradation of the barrier macromolecules such as gelatin, dextran, polylactic acid and long-chain fatty acids and glycerides.

4) **Esterification type depot formulation:** This depot preparation is produced by esterifying a drug to form a bioconvertible Prodrug-type ester and then formulating it in an Injectable formulation.

- This chemical approach depends upon number of enzyme (esterase) present at the injection site. This formulation forms a drug reservoir at the site of Injection.
- The rate of drug absorption is controlled by the interfacial partitioning of drug esters from the reservoir to the tissue fluid and the rate of bioconversion of drug esters to regenerate active drug molecules.

❖ **Type II Classification of Controlled Release Parenteral Dosage Forms**

Aqueous solutions

- By increasing the viscosity of the vehicle, the diffusion coefficient of the drug will be reduced which thereby delays the drug transfer. Viscosity building agents like methylcellulose, sodium carboxy methylcellulose, PVP. Delay in drug absorption also occurs if a water-soluble drug undergoes complexation with these macromolecules.
- Complexes formed by decreasing the solubility of the parent drug can also result in controlling the release of the drug from aqueous solutions. Eg: acetaminophen formed 1:1 complexes with theophylline and caffeine, which has lower solubility and therefore lower dissolution rates than the parent compound.

Oil solutions

➢ Oil solutions involve a less elegant mechanism to achieve parenteral controlled release. In this case, the drug release is controlled by partitioning of the drug out of the oil into

the surrounding aqueous medium.

- The number of oils acceptable for intramuscular injection is rather limited. They include sesame oil, olive oil, arachis oil, maize oil, almond oil, cottonseed oil, and castor oil.
- The "oily solution" is limited to those drugs which are appreciably oil soluble and have optimum partition coefficient.

Aqueous suspensions

- A suspension gives longer duration of action than an aqueous solution when given i.m or s.c.
- Factors affecting dissolution rate are examined using modified form of Noyes-Whitney equation: Mean dissolution rate = ADCs/L Where A is the mean surface area available for dissolution, D is drug's diffusion coefficient, Cs is the saturation solubility of the drug, and L is the thickness of the diffusion layer. From the formulation point of view, the parameters subjectable to change are A (particle size), D, and to an extent, Cs.
- In addition to particle size, prolonged release can be achieved by altering the solubility of the drug. The most direct approach is with salt or derivative formation including using polymorphic forms.

Oil suspensions

- Drug release from oil suspensions combines the principles of aqueous suspensions and oil solutions. With suspended particles acting as a drug reservoir, the process of drug availability consists of dissolution of drug particles followed by partitioning of drug from oil solution to the aqueous medium.
- The duration of action obtained from oil suspensions would be longer than that obtained from oil solutions.

Emulsions

- Besides use in topical drug delivery, emulsions have been used as drug vehicles both orally and parenterally. Lipid soluble antineoplastic agents such as methotrexate diester were incorporated into microemulsions which act as readily obtainable synthetic, protein free analogues of LDL.
- Using w/o/w emulsions prepared by dissolving the drug in the internal water phase, prolongation of action of chemotherapeutic agents like methotrexate and vinblastin sulphate were observed.

- It was suggested that the rate of drug release can be controlled by varying three basic parameters of the internal phase of the primary w/o emulsion; namely, internal phase volume, concentration of emulsifier, and the osmolarity of the dispersed phase.

❖ (Type II Classification of Controlled Release Parenteral Dosage Forms) Cotd…..

Microspheres

- Microspheres are solid, spherical particles containing dispersed drug molecules either in solution or crystalline form. Eg: narcotic antagonists and antineoplasic agents. The method consists of suspending the drug in a biodegradable/ bioerodible polymer, followed by reducing the mixture to particles of the order of 600 µm, which are then injected as a suspension in carboxy methylcellulose solution.

Magnetic microspheres

- Magnetic microspheres are developed to decrease the reticuloendothelial clearance and to increase target site specificity. This system has a great potential in the treatment of localized tumours in the regions of well-defined blood supply.
- They are prepared from albumin and magnetite and are about 1.0µm in size, which is small enough to allow them to be injected intravenously without occluding the microvasculature.
- Typically, magnetic microspheres are infused into an artery supplying a given target site. A magnet of sufficient field strength is then placed externally over the target area to localize the microspheres at the capillary bed in this region.

Biocompatible carriers

Erythrocytes

- When erythrocytes are lysed and then resealed, exchange of intracellular and extracellular solutes will occur. A drug present in the medium of lysis procedure will therefore be encapsulated within the membrane envelope of the erythrocyte upon resealing. The various advantages of resealed erythrocytes are: biodegradable, nonimmunogenic since patient's own erythrocytes are used, can circulate intravascularly for prolonged periods.
- Erythrocytes can be coupled with other drug carrier systems to achieve controlled and sustained drug release which include DNA, albumin, polymers or proteins.

Biological & Synthetic macromolecules

- A variety of biological macromolecules can be used as drug carriers. Serum albumin can be polymerised and cross-linked to form microbeads to entrap steroid hormone, anticancer drugs, dyes, and peptides.
- Besides biological macromolecules, a number of synthetic polypeptides and polymers have been studied as carriers for drug delivery which include ethylene glycol, lysine, and glutamic acid.

Liposomes

- Liposomes are hydrated liquid crystals formed when phospholipids are allowed to swell in an aqueous media. When suitably dispersed, they consist of a series of concentric bilayers alternating with aqueous compartments. Water or lipid soluble substances can be entrapped within their aqueous or lipid phase, respectively.
- Liposomes are of great potential in parenteral therapy because of their versatility, ability to encapsulate both lipophilic and hydrophilic drugs.
- The elimination and distribution of liposomes is controlled by the size, surface charge, and composition of the liposomes. Because of their affinity for the phagocytic cells of the liver and spleen, liposomes have been investigated as target drugs to those phagocytic cells which have been infested with parasites.

Nanoparticles

- Nanoparticles are transport carrier compartments for drugs or other active molecules of non- liposomal character in the nanometer size range [10nm - 1μm]. Since they are mainly taken up by the RES following i.v administration, nanoparticles are useful in delivering drugs to the liver and to phagocytically active cells.

Niosomes

- Niosomes are reported to mimic liposomes. A large number of surfactants have been used of which most popular are spans and polyalkylethers. They are generated from self-assembly of hydrated amphiphilic surfactant monomers. The niosomal encapsulation of methotrexate and doxorubicin increases drug delivery to tumor and tumoricidal activity.

IMPLANT

INTRODUCTION:

- Implants are small sterile solid masses consisting of a highly purified drug made by compression or molding or extrusion.
- Implants are intended for implantation in the body **subcutaneous** or **intramuscular** tissue by a minor surgical incision or injected through a large bore needle.
- Implants are developed with a view to provide **continuous release** of the drug into the blood stream over long periods of time without the repeated insertion of needles.

Advantages

- Controlled drug delivery for over a long time.
- Improve patient compliance.
- Targeted drug delivery.
- Bypass first pass metabolism.
- Decrease side effects.
- Improved stability of drugs.
- Improve availability of drugs.

Disadvantages

- Mini-surgery is needed (Painful).
- Uneasy to simply discontinue the therapy.
- Local reactions.
- Inadequate release.

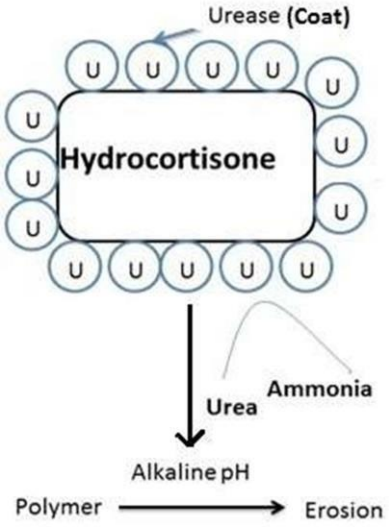
The basic type of Implants are-

Non biodegradable implant

- The drug is dispersed homogeneously, inside the polymeric matrix through which the drug diffuses slowly providing sustained release.
- This type of system has several disadvantages, the outer membrane is **nondegradable.**
- Thus minor surgery is necessary for the removal of the delivery system from the body.
- There is also a possibility that membrane rupture will potentially lead to **"drug dumping"** during therapy.

Biodegradable implant

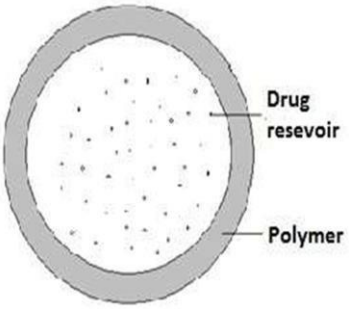
- The inert polymers, used are eventually **absorbed** or **excreted** by the body.
- No need for surgical removal of the implant after the conclusion of therapy.
- Drug is dispersed in to a **biodegradable** polymer matrix like *poly vinyl methyl ether* and is coated with immobilized urease in a neutral PH.
- Urease catalyzes the hydrolysis of urea into ammonia and CO_2.
- The production of **ammonia** causing increase in PH at which polymer degrades leading to drug release.



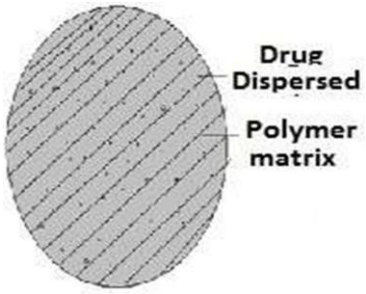
APPROACHES/ FABRICATION:

1) **Rate programmed drug delivery system:**

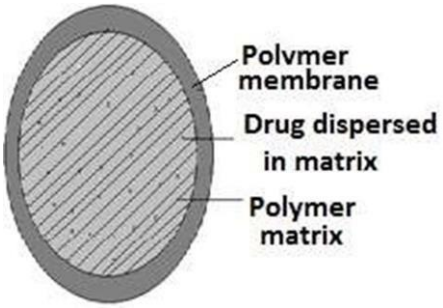
a) **Polymer membrane permeation controlled drug delivery**: Drug reservoir is **encapsulated** within a spherical compartment that is enclosed by a rate controlling polymeric membrane. **Example**: Norplant subdermal implant, Progestasert IUD.



b) **Matrix diffusion controlled drug delivery systems**: It is prepared by homogeneously **dispersing drug particles** at a rate controlling polymeric matrix fabricated from either a lipophilic or hydrophilic polymer.



c) **Matrix Hybrid Type Drug Delivery System**: It is a hybrid of Membrane permeation controlled DDS and Matrix diffusion controlled DDS. It **minimizes the risk of dose dumping** associated with membrane permeation controlled DDS.

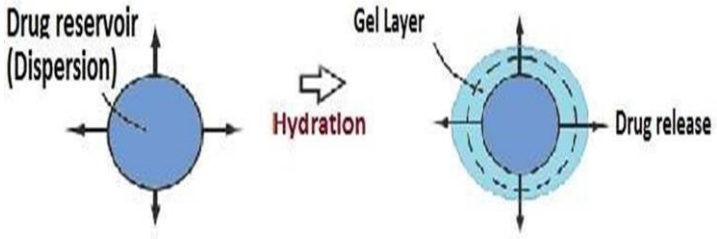


IMPLANT

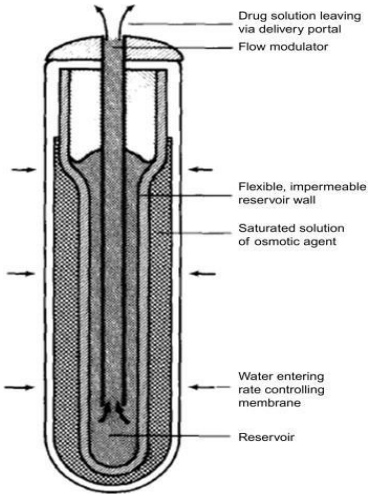
APPROACHES/ FABRICATION: (contd..)

2) **Activation modulated DDS:** This system is activated by some physical, chemical or biochemical process facilitated by an external energy supplier.

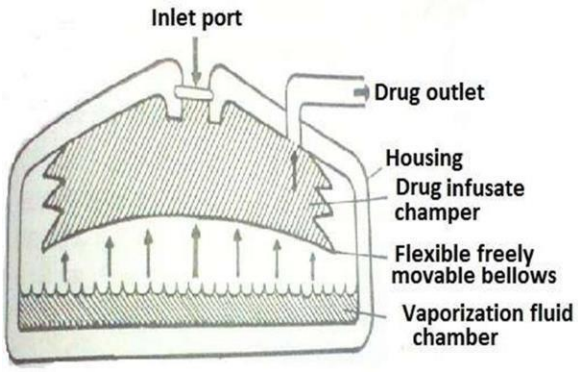
i. **Hydration Activated Drug Delivery System:** Drug reservoir is homogeneously dispersed in a swellable hydrophilic polymeric matrix. The system will get activated and hydrated by biological fluid when it will be injected. Then the drug molecules are released by diffusing through polymeric matrix. Ex: Norgestomet releasing HYDRON implant.



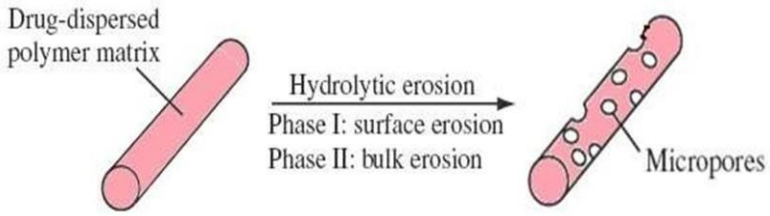
ii. **Osmotic Pressure Activated Drug Delivery Device**: In this type of DDS, the drug in solution is released through a specialized laser drilled delivery orifice at a constant rate under a controlled gradient of osmotic pressure. When it injected in the body, water from surrounding tissue fluids is imbibed through the Semipermeable membrane at a controlled rate the dissolves the osmotic agent which creates a osmotic pressure differential across the membrane. The osmotic sleeve thus expands and since the outer wall is rigid, it squeezes the inner flexible drug reservoir and drug solution is expelled at a constant rate. Ex: ALZET Osmotic Pump.



iii. **Vapor Pressure Activated Drug**: In this system, the drug reservoir in a solution formulation, is contained inside an infusate chamber which is separated from the vapor pressure chamber *(contains a vaporizable fluid)* by a freely movable bellows. It will get activated by body temperature cause vaporizable fluid getting vaporized and create vapor pressure. This pressure causes the bellows move upward & forces the drug solution in the infusate chamber to release, through a series of flow regulators & the delivery cannula into the blood circulation at a constant flow rate. Ex- INFUSAID pump



iv. **Hydrolysis Activated Drug Delivery**: These systems are prepared from a bio-erodible or bio-degradable polymer such as polylactide or poly(lactide-glycolide) copolymer. This device is activated *(by biological fluid or enzymes)* to release the drug upon hydrolysis of polymer base by tissue fluid at the implantation site. Ex: ZOLADEX system.



3) **Feed back regulated process:** The release of drug molecules is activated by a triggering system, such as a biochemical substance in the body, through some feedback mechanisms.

a) **Bioerosion Regulated:** This consists of bio-erodible drug dispersed polymer matrix fabricated from poly (vinyl methyl ether) half ester, which was coated with a layer of immobilized urease.

b) **Bioresponsive Drug Delivery:** The drug reservoir is contained in a device enclosed by a bioresponsive polymer membrane whose permeability to drug molecules is controlled by concentration of biochemical agent in the tissue. Ex: Glucose Triggered Insulin Delivery System.

Implant drug delivery systems (Examples)

a) **Contraceptive implants "Nexplanon®"**: The contraceptive implant is a small flexible tube about 40mm long that's inserted under the skin of your upper arm.

The implant steadily releases the hormone **progestogen** into the bloodstream which leads to stops **ovulation,** thickens the mucus from the cervix, makes the lining of the womb thinner so that it is unable to support a fertilized egg.

Advantages:

- ✓ It works for **three years**.
- ✓ It is an option if anyone **cannot use oestrogen**-based contraception.
- ✓ The implant is **safe to use while you are breastfeeding.**
- ✓ **Fertility will return to normal** as soon as the implant is removed.
- ✓ It may **reduce heavy periods** or painful periods after the first year of use.
- ✓ Offer some protection against pelvic inflammatory disease.

b) **Histrelin implants**: Histrelin implant is a small, thin, flexible tube containing medication that is inserted by a doctor on the inside of the upper arm.

Uses:

- ✓ The Vantas ® **t**reat **symptoms of prostate cancer** in men.
- ✓ The Supprelin LA® treat **precocious puberty** in both male and female children.

c) **Buprenorphine implants "Probuphine®"**: It consists of a small, solid implant made from a mixture of ethylene-vinyl acetate (EVA) and a drug substance and placed subcutaneously, normally in the upper arm. Each implant contains the equivalent of **80 mg of buprenorphine**.

Uses

- ✓ It is indicated for the maintenance treatment of **opioid dependence** in patients who have achieved and sustained prolonged clinical stability on low-to-moderate doses of a transmucosal buprenorphine-containing product.

d) **Naltrexone implants**: Naltrexone implants are small medication pellets that get inserted under the skin and slowly release the medication over varying lengths of time. Naltrexone is a drug belonging to a class of drugs called **opioid antagonists**, which help reduce the desire for drugs such as: Heroin, Morphine, Fentanyl, Oxycontin. But naltrexone **doesn't treat the withdrawal symptoms** that opioid users may experience, including: Anxiety, Agitation, Sleep disturbances, Sweating, Abdominal pain