CHAPTER-I

1. INTRODUCTION

1. CONTROLLED DRUG DELIVERY:

In contempo years, much importance has been given on the placement of medicament at the site of action by developing newer delivery system of medicament.

If anyone has to develop the ideal delivery systems of medicament, two concepts should be noticed, it should deliver the API at a concentration needed by the body during the period of treatment. This may need delivery of API at a constant proportion for API’s that have a clear connection between uniform plasma levels and the resultant pharmacological action or at a capricious rate for which need either a series of peaks and valleys or act on a rhythm. Second, it should route the API solely to the site of action. This may constrain delivery of API to specific receptors, as in the case of H₁ and H₂ antagonists, localization to tumor cells, as it is needed in most of cancer treatments, or to specific areas of the body in diseases like arthritis or gout [Robinson JR (2005)] & [Jantzen GM (1996)].

Currently, there are not any delivery systems that can reach all the required goals, Conventional dosage forms like prolonged & controlled release can prolong & control the rate of release of drugs from the formulation but they lack to control over the consequence of API when it enters into the body.

![Diagram of drug levels](image)

**Figure I.1: Allusive plasma drug levels with different dosage form of administration**
1.1 PER ORAL CONTROLLED RELEASE DOSAGE FORMS

Per oral controlled release dosage forms have been advanced over the past forty years for their advantages such as ease of patient acquiescence, administration and flexibleness in formulation. The short gastric emptying time in human’s ranges from 2-3 hours through the considerable absorption site, i.e., stomach and duodenum leads to fractional release of drug from the delivery system leads to subtherapeutic action of the administered dose. Thus if we control the release of drug in specific area of GIT tract it would be open a new window for the drugs which are unstable in acidic pH & having a small absorption window these contemplation have led to develop an exclusive per oral sustained release dosage form of mucoadhesive properties. After per oral administration, of mucoadhesive dosage form it would be adhered in the stomach and release the drug from dosage forms in a programmed and protract manner, so that the API’S can be kept continuously in contact with the stomach to its absorption sites in the stomach. Mucoadhesive formulation can remain in stomach for prolonged time and hence significantly enhance the GI residence of drugs. Protracted gastric retention improves bioavailability, reduced dosage size [Garg R (2008)].

1.2 ANATOMY OF GASTROINTESTINAL TRACT
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1.3 Progress in Gastro-retentive Drug Delivery system

GRDDS is the feasible approach for achieving a prolonged and anticipated drug delivery model in the GI tract to control the gastric residence of drug.

a) Drugs that can boon from Gastric-retentive Dosage form:

- API’S that act regionally in the stomach
- API’S that are chiefly absorbed in GIT
- API’S with a small window of retention
b) Requirements for gastric retention:

When we want the gastric retention of any dosage form in the stomach the physiological factor should be considered. First we have to design the dosage form in such a way that it should resist the peristaltic movement of stomach followed by constant chumming mechanism.

To formulate a successful gastric retentive dosage form, it must avoid unanticipated gastric emptying. Further, once the release of drug has been completed from dosage form, the dosage form should be taken from the stomach without any difficulty.

1.4 Different approaches for Gastric retention

Over the last two decades numerous gastro retentive dosage forms have been designed to prolong gastric residence time which can be broadly classified as [Bardonnet PL (2006)], [Nangia A (2006)] & [Tao SL (2005)]

a) High density (sinking) systems  e) Magnetic system
b) Low density (floating) system   f) Mucoadhesive system
c) Expandable systems
   d) Superporous hydrogel

1.5 Bioadhesive/ Mucoadhesive Drug Delivery System

A significant achievement in NDDS is the advancement of the mucoadhesive drug delivery system, an advanced and highly versatile drug delivery system. In the year 1980, Prof. JR. Robinson trail-blazed the idea of mucoadhesion as new method to extend residence time of different API'S in the eye. Since then from many years mucoadhesive polymers have proved to act as pharmaceutical glue to the different mucosal membranes present in the body. The capacity to adhere to the mucus layer present on the epithelial tissues makes these polymers as very benificial excipients in drug delivery. Mucoadhesive drug delivery system utilizes the acreage of bioadhesion of polymers which adheres on hydration and hence anchors with the mucosal layer, this property has
been widely exploited for targeting API’S to particular region where mucus layer is present to prolong the residence time of drug [Chowdary KPR (2000)], [Sahlin JJ (1997)], [Vijapur LS (2012)].

The mucoadhesive/bioadhesive drug delivery system targets the following biological sites for mucoadhesion:

- Buccal mucoadhesive delivery system
- Oral mucoadhesive delivery system
- Vaginal mucoadhesive delivery system
- Rectal mucoadhesive delivery system
- Nasal mucoadhesive delivery system
- Ocular mucoadhesive delivery system

While developing per oral mucoadhesive delivery system one should keep in mind that GIT tract contains a coating of protective gel layer known as mucosa. Keeping this in mind one should develop a mucoadhesive/bioadhesive delivery system which can anchor into the deep layer of mucosa & should mimic as a pharmaceutical glue, i.e it should adhere to mucosa of GI tract.

1.5.1 Bioadhesive Layers: The epithelium of the gut is protected by continuous coating of mucus which is secreted by number of different cells:

1. Mucus neck cell: From the necks of the gastric glands in the stomach produces soluble mucus.
2. Surface epithelial gobalt cells: produce visible mucus in the stomach & intestine.
4. Crypts of liberkuhn: produce mucus in small and large intestine.

However, it has the following general composition:
Table No 1.2: Composition of Mucosa layer

<table>
<thead>
<tr>
<th>Contents of Mucus</th>
<th>Concentration %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>90 %</td>
</tr>
<tr>
<td>Glycoprotein’s and lipids</td>
<td>0.6-5 %</td>
</tr>
<tr>
<td>Mineral salts</td>
<td>0.9 %</td>
</tr>
<tr>
<td>Free proteins</td>
<td>0.6-1 %</td>
</tr>
</tbody>
</table>

Role of mucosa:

- Protection: resulting particularly from its hydrophobicity.
- Barrier: blockade in tissue absorption of API.
- Adhesion: strong adherence property and firm binding to the epithelial cells.
- Lubrication: to maintain mucosal layer moist.

1.5.2 BIOADHESION:

Bioadhesion is the state in which the bioadhesive polymer & mucosal layer are held together by adhesive forces [Mathiowitz E (2006)], [Khar RK (2002)] & [Khanna R (1998)]. In biological systems, four types of bioadhesion can be distinguished they are:

i. Bioadhesion of cell on another cell.
ii. Bioadhesion of a cell with a distinct substance.
iii. Bioadhesion of a normal cell to another cell.
iv. Bioadhesion of a mucoadhesive polymer to mucosal layer.

For delivery of API’S bioadhesion means clamping of API to delivery system to particular biological system. The biological system means epithelial cells layer or mucosal layer. If the adhesion is to mucosa, then it is called as mucoadhesion. Leung & Robinson told mucoadhesion is reciprocal action between mucosal layer & mucoadhesive polymer.
The term “bioadhesion” means a substance which has ability to interact with biological substrate and retains over biological substrates for longer time. Bioadhesion is categorized mainly three types on their phenomenon of observation.

**Type I:** Adhesion in between biological substance without involving counterfeit material. E.g. cell collection and cell melding.

**Type II:** Bioadhesion can be resembled by cell adherence onto culture or adherence to a variety of substrates including lustrous chemical element (alloy) and other artificial material.

**Type III:** Bioadhesion can be demonstrated as adhesion of synthetic/natural substrate to epithelial cells or mucosal layer such as adherence of polymers to stomach or ocular surface.

1.5.2 A) **Mechanisms of Bioadhesion:** To develop ideal BDDS, it is important to try to describe and understand the forces that are responsible for adhesive bond formation [Smart JD (2005)] & [Andrew GP (2009)]. The theory involved in the bioadhesion can be explained by three ways:

1. Dewy and abscess of polymer for contact between biological substrate.
2. Penetration of bioadhesive polymer and anchoring of polymer to mucosal layer.
3. Establishment of weak bonds between polymer & biological substrate.

These characters help in the formation of bonds which may be mechanical bond or chemical bond between mucoadhesive polymer & biological substrate.

**i) Mechanical Bonds:** They involve the artificial entanglement of mucin string with flexible bioadhesive polymer chains and the penetration of mucin string into the porous structure of polymer.

**ii) Chemical Bonds:** Chemical bond includes strong covalent bond as well as weak forces such as ionic bonds, Vander Waal’s forces of interaction and hydrogen bond.

Thus, polymers with high molecular weights and greater concentration of reactive polar groups likely intensify mucoadhesive bonds.
1.5.2 B) Hypothesis on Bioadhesion:

Several hypothesis have been evolved to explain the process in formation of bioadhesive bond. These hypothesis have been used as tool in developing possible BDDS. Some are based on the establishment of mechanical bonds while others focus on chemical bond formation [Smart JD (2005)] & [Andrew GP (2009)].

i) The electronic hypothesis

ii) Absorption hypothesis

iii) Wetting hypothesis

iv) Diffusion hypothesis

v) Fracture hypothesis

1.5.2 C) Factors Important to Mucoadhesion:

Factors which influence mucoadhesion are:

a) Polymer related factors

b) Environmental related factors

a) Polymer Related Factors:

According to the hypothesis of mucoadhesion, different functional groups and polymer structures effect on the degree of Polymer/mucosal interaction.

i) Functional Group Contribution

ii) Degree of Hydration

iii) Polymer Molecular Weight, Chain Length, Conformation & Degree of Cross-linking

iv) pH and Charge

v) polymer Concentration

vi) spatial conformation
vii) Carboxylic acid group: These are important for the covalent bonding of the polymer with mucus glycoprotein.

\[
\text{polymer} - \text{OH} + \text{NH}_2 - \text{SH} \xrightarrow{\text{EDAC}} \text{polymer} - \text{NH} - \text{SH}
\]

**Figure I.4: Synthetic pathway of Polymer–Cysteamine conjugate**

Synthesis of polymer–cysteamine conjugates shows that covalent bonding was done by the formation of NH bond between the COOH functional group of the polymer and the NH\(_2\) group of cysteamine intercede by EDAC polymer, sodium carboxymethylcellulose or polycarbophil.

**b) Environmental related factors:**

i. pH
ii. Applied Strength
iii. Initial Contact Time
iv. Selection of the Model Substrate Surface
v. Swelling

**1.5.3 Mucoadhesive Polymers for Drug Delivery:** Their no limitation for the polymers which adhere to the gastric mucosa. Polymers which are dissolved in pH 4 and have groups which are positively charged are preferred.

Polymers commonly applied for the formulation of mucoadhesive delivery system which cohere to mucosa-epithelial cell surface may be characterized into three categories:
1. Polymers which become tacky when immersed in aqueous media and demonstrate their bioadhesion.

2. Polymers that stick by unspecific, non covalent bonds those are primarily adhere by electrostatic forces.

3. Polymers that adhere to particular receptor present on cell.

**i) Traditional unspecific First Generation Mucoadhesive Polymers:**

First generation non-specific mucoadhesive polymers are:

1. Anionic polymers  
2. Cationic polymers

1) **Anionic Polymers:** ex: polyacrylic acid, sodium cmc.

2) **Cationic Polymers:** ex: chitosan.

**ii) Innovative Mucoadhesives**


**1.5.4 Drugs for Gastrointestinal Mucosa:**

The active ingredients are absorbed from gastrointestinal mucosa or express its efficacy directly or indirectly in the gastrointestinal tract [Akiyama (1999)]. For example antibiotics/anti-bacterial and anti-protozoals intended to be absorbed from the GI tract, this class includes:

- **Penicillins:** Amoxacillin, Benzylpenicillin, Pipercillin.
- **Cephalosporins:** Erythromycin, Clarithromycin, Roxithromycin.
- **Tetracycline:** Minocycline.
- **Amino glycoside:** Gentamycin, Anikacin.
- **Bismuth salts:** Bismuth acetate, Bismuth citrate.
- **Imidazole:** Tinidazole, Metronidazole.
- **Quinolones:** Ofloxacin.
1.5.5 Thiomers:

Since the concept of mucoadhesion has been trail blazed in 1980s, number of efforts has been made to promote the mucoadhesive property of polymers. These venture involve the use of straight chain polymers like PEG as adhesion booster [Sahlin JJ (1997)], the negation of ionic mucoadhesive polymers [J.D. Smart (1984)], adhesion by a slow hydration process [Sahlin JJ (1997)] and by developing consolidated polymer-adhering substrate conjugates [Bologna WJ (1998)] & [B. Naisbett (1994)] contribute to characteristic binding to epithelia. All these approaches are established on non-covalent bonds such as keesom forces, intermolecular bonds such as hydrogen bonding & ionic interaction which demonstrates weak adhesion in numerous cases they failed to localize the drug at required site. This will lead to a weak mucoadhesion which is insufficient for localization of delivery system at a given target site & thus failed to prove as efficient pharmaceutical adhesive. The new engenderment of adhesive polymers is thiolated conjugate polymers widely called as thiomers.

In compared to well exist mucoadhesive polymers these innovative polymers are having the ability to form covalent bond. The connecting structures are commonly found in the biological systems these disulfide bonds have demonstrated the strong covalent attachment of polymers to the mucus gel layer of the mucosa. Thiolated polymers are mucoadhesive base of polymers, which has –SH in the side chain. Once these thiomers enter into the biological system they form a S-S bond with glycoprotein’s of mucosa which contains a very rich cysteine subdomains

Thus, thiolated conjugates of polymer act as the naturally secreted glycoprotein’s of mucosa which are present in mucosal layer by forming S-S bond [Andreas. BS (1995)], [R. Khosla (1987)], [Lehr Cm (1994)], [Andreas BS (1999)] & [Takeuchi H (2001)].

CHITOSAN

The biodegradable polymer chitosan is prepared from removing acetyl group in alkaline condition from chitin, which is widely found polysaccharide in nature. Carapace waste of shrimp, crab and lobster are the sources of chitin. The -NH₂ group of chitosan
helps in preparation of various chitosan conjugates with acids. At lower pH, the amine group is protonated which helps in solubility but in higher & neutral pH chitosan remains insoluble because of these favorable properties such as non-toxic, biodegradable and bioactivity [Felt O (1998)]. Chitosan has gained attention as an innovative excipient in delivery systems, and thus it has been incorporated in the European Pharmacopoeia from 2002. As far chitosan is considered it has been used in various pharmaceutical formulations including local (ocular), peroral (tablets), parenteral (dispersion system) & gene delivery [Takeuchi H (2001)], [Senel S (2000)], [Andreas BS (1998)], [Andreas BS (2001)], [Kushwaha Swatantra KS (2010)] & [SA Sreenivas (2008)].

**THIOLATED CHITOSAN**

Polymers containing -SH groups demonstrated higher mucoadhesive property than the polymers which are generally used for mucoadhesive delivery system. Higher mucoadhesion of thiomers on mucosal layer can be explained by the formation of covalent bond between the glycoprotein’s present on mucosal layer & thiomer, bond formed between thiomer & mucosal layer will be stronger than any non-covalent bond [SA Sreenivas (2008)]. To increase the miscibility of chitosan, to enhance the mucoadhesive property for enhancing the penetration properties different conjugates have been synthesized such as trimethylated chitosan [Thanou M (2000)], N-carboxy methyl derivative of chitosan [Thanou M (2001)], N-sulfochitosan [Baumann H (2001)] and chitosan- Ethylenediaminetetraacetic acid derivative [Kushwaha Swatantra KS (2010)] were synthesized.

Further derivatization of chitosan was done with thiolating agent like thioglycolic acid, 4-thio-butyl-amide was done by immobilizing the –SH group containing chemical entities [Hornof MD (2003 [Andreas BS (2003)]. The so called thiomers have many advantages compared to parent molecule such as enhanced mucoadhesion, solubility & permeation property [Roldo M (2004)] & [Langoth N (2004)]. Strong adhesive property of thiolated chitosan, because of its covalent bonding to mucus layer makes this as a valuable polymer for designing mucoadhesive delivery system for drugs [Hornof MD (2003)] &
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[Roldo M (2003)]. Liquid dosage form of thiolated chitosans demonstrates *in situ* gelling properties at physiological in biological system at particular pH [Hornof MD (2003)].

1.5.6 Merits of mucoadhesive delivery system

- Extends the residing time of delivery system at the locus of absorption.
- Provides a superior path for the efficient delivery of drug which has lower bioavailability due to enzymatic degradation in liver.
- Profound reduction in the size of dose can be accomplished, dose related side effects.
- It provides a passive system for drug absorption.
- Adjustability in size, shape and surface of dosage form.

1.6 REVIEW OF LITERATURE

1.6.1 LITERATURE REVIEW ON CHITOSN

- **Sevda S et al [2004]**, reviewed on application of chitosan in veterinary medicine & concluded chitosan & its derivative are biocompatible polymers which has numerous applications such as mucosal vaccines, other vaccines, delivery of hormones to animals etc.
- **Hua Z et al [2001]**, showed that a almond emulsion contains chitinase enzyme which is capable to hydrolyze chitin substrates. Results showed that higher mol wt and chitosans with low acetylation have lesser affinity for the rate of enzymatic degradation.
- **Hiroshi U et al [2001]**, evaluated chitosan for its wound healing property on L929 mice fibroblast culture, chitosan did not directly involved in production of extracellular matrix (ECM) but it accelerated m-RNA protein synthesis platelet derived growth factor. Results indicated that chitosan will not involve directly in extracellular matrix formation by proliferation or formation of fibroblast, and extracellular matrix formation may be enhanced by growth factors.
- **Eugen K et al [2003]**, surveyed on implantable application of chitosan & chitin in various departments of medicinal sciences such as periodontal, tissue engineering,
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wound healing, API delivery & sterilization issues of the prepared material as implants, found that chitosan & chitin is a good biocompatible material.

- **Mingyu C et al [2003]**, studied chitosan & gelatin composite films for its nerve cell generation at different concentration on PC12 cell lines. Results showed that gelatin with chitosan produced soft & elastic complex with good affinity to cell, PC12 cells were grown more rapidly on Chitosan-gelatin conjugate than alone chitosan & concluded that Complex of chitosan & gelatin at optimum ratios is a promising material for nerve regeneration.

- **Yan H et al [2005]**, studied Chitosan & gelatin composite in tissue generation, adding gelatin to chitosan affected the firmness of two dimensional & three dimensional scaffolds & maintained integrity in existence of lysozyme. Where alone chitosan showed poor adhesion property in presence of human umbilical vein endothelial cells where in combination with gelatin showed good 3D adhesion & showed that combination of chitosan & gelatin is a excellent material for tissue grafting.

- **Kean T et al [2010]**, revived on chitosans biodegradation, biodistribution & toxicity of chitosan, in his review he concluded that chitosan is a nontoxic biocompatible material & said that care should be taken to its purity, metals may be likely cause harmful effect in conjugate synthesis and in delivery system.

- **Shirui M et al [2010]**, formulated chitosan delivery for deoxyribonucleic acid & si-Ribosenucleic acid where he showed that higher molecular weight & deacetylation of chitosan leads to highly stable polyplex in biological fluids which leads to low delayed transfection thus he concluded that transfection ability of chitosan delivery systems can be improved by replacing formulation linked parameters.

- **Prashant A et al [2010]**, reviewed molecular image using chitosan based Nanoparticle, which is being used in preclinical studies because of chitosans biocompatible nature with different molecular weight of chitosan & its derivative, where he found that chitosan & its derivative are potential toolkit for advanced molecular imaging applications
• **Yoshshiko H et al [2007]**, studied on the chitosans used as chewing gum for inhibiting the cariogenic bacterial growth, by their formulation containing chitosan they successfully abolished the growth of bacteria present in the saliva. In their findings they suggested the presence of chitosan in gum is an efficient way method to control the growth of bacteria in oral cavity.

• **Shaoyun Y et al [2004]**, developed a chitosan solution containing insulin for nasal delivery with penetration enhancers like EDTA, Tween-80, \( \beta \)-CD & HP-\( \beta \)-CD. Were he found the formulation containing HP-\( \beta \)-CD showed maximum hypoglycemic effect & thus concluded that chitosan can be used as vehicle for delivery of insulin.

• **Yang G et al [2008]**, used chitosan oligomers for enhanced absorption of insulin in intestine of rats, in their experiment they found FD4 & insulin with chitosan & hexamar increased the uptake of insulin from jejunum which showed chitosan hexamar is safe and good penetration enhancer for peroral delivery.

• **Noha N et al [2009]**, studied the cytotoxicity profile of chitosan/PLGA Nanoparticle where he used COS-1 & A549 cell lines for toxicity studies with the formulation chitosan/PLGA with different ratios. From the study, they found that toxicity of chitosan & PLGA is a function of the cell line.

• **Mia S et al [2002]**, studied microcrystalline chitosan (MCCh) as gel forming excipient using paracetamol (class-I drug) & ibuprofen (class-II drug) as a model drug. Where they found that class-II drug release was controlled by using MCCh, while class-I drug release was not controlled in the pH 1.2 & 5.8 & concluded that drug displacement from the formulation can be maintained by using different molecular weight & acetylation of MCChS, results were satisfactory in acidic pH indicating MCCh is useful for targeting stomach specific drugs.

• **Seneel S et al [2000]**, studied by using 2% chitosan gel across buccal mucosa utilizing its mucoadhesive & permeabilizer property for absorption of transforming growth factor-\( \beta \) (TGF-\( \beta \)). The localization of transforming growth factor-\( \beta \) within the buccal mucosal layer was found by longitudinal sections and numbering. Chitosan enhanced permeability effect in buccal mucosal layers for protein drug.
• **Sania M et al [2006],** used chitosan for gene therapy, where he used folic acid (FA) for internalization of DNA. They compared Chitosan-DNA & FA-Chitosan-DNA nanoparticles for DNA transfection on HEK293 cell lines for their toxicity & condensation of DNA, results showed that FA-Chitosan-DNA nanoparticles is less toxic, has good condensation of DNA, positive zeta potential makes a promising candidate for non viral gene vector.

• **Shu XZ et al [2002],** cross linked chitosan with anions like sulfate, citrate & tripolyphosphate (TPP) & formulated beads of riboflavin using above mentioned cross linked chitosan, evaluation of the formulated beads showed that chitosan/TPP better mechanical property & controlled the release of riboflavin compared to sulfate/chitosan & citrate/chitosan at the same time release of chitosan/TPP was not affected in acidic pH & thus concluded that cross linked beads could be utilized for stomach specific delivery.

• **Tانية B et al [2002],** studied on biodistribution of ultrafine chitosan nanoparticles containing $^{99m}$Tc. On intravenous injection of nanoparticles to mice showed nanoparticles invades RES system followed by deposition in different organs, biodistribution studies also confirmed that small amount of nanoparticles entered in bone marrow thus implying that nanoparticles of $^{99m}$Tc can be used for bone imaging.

• **Ko JA et al [2002],** formulated chitosan microparticles utilizing TPP by cross linking using felodopine, microparticles particles prepared in acidic media at higher amount of TPP resulted in slow release of drug. Thus concluded that TPP-chitosan microparticles could be utilized for controlled release of API.

• **Lian YW et al [2006],** formulated prepared microspheres of insulin using chitosan by combination of membrane emulsification & stepwise solidification method. By this method they obtained uniform size microspheres with 70% entrapment efficiency, *in vitro* results indicated there was no burst effect thus concluded that prepared microspheres by the above method can be used orally & mucosal delivery of drugs & proteins with increased bioavailability.

• **Anil KA et al [2006],** prepared ampicillin microspheres with chitosan using spray dry & by emulsification using tripolyphosphate (TPP), where results indicated
emulsification method was superior method compared to spray drying technique for preparation of microspheres. Release of drug was 4-8 hours without TPP & with TPP more than 24 hours drug release was observed. Thus by this method a precise release of ampicillin can be done, this method can be used for controlled application for antibiotics locally or systemic.

- **Gen M et al [2006]**, designed chitosan capsule with tripolyphosphate(TPP) for the release of drug by osmotic mechanism through orifice for water soluble & moderate water soluble API. Drug release studies revealed that delivery of drug from the unsymmetrical coated layer can control by using osmotic pressure generated in the capsule.

- **Wanlop W et al [2008]**, prepared nano sized particles for the delivery of DNA in CHO-K1 cells using the salts of salts of chitosan including chitosan HCL, chitosan acetate (CAc), chitosan lactat, chitosan glutamate (CGl) and chitosan aspartat (CAs), The introduction of DNA into the cell efficiency of the chitosan-DNA composite was depended on the mol wt and chitosan’s salt. All CS/DNA complexes were low toxic to CHO-K1 cells. Thus study suggested CS & its salts can be utilized as most efficient safe method for delivery of genes.

- **Juliane H et al [2009]**, formulated chitosan solution & particles for permeation enhancement on the cells of MDCK, mol wt based permeation was verified & different sized particles was formulated using chitosan. As the particles of chitosan was reduced for low viscosity, there was increased permeation enhancement of low permeable FITC dextran4.

- **Sudhir SC et al [2011]**, demonstrated that chitosan-PLGA microparticles of paclitaxel are more cytotoxic to cell rather chitosan microparticles towards 4T1 cells, this was due to cellular cytotoxicity and association of paclitaxel was increased with chitosan-PLGA particles.

- **Liu L et al [2011]**, prepared in situ gelation system of chitosan for parentral delivery of drug with the help of NaHCO₃. Where he found that NaHCO₃ plays very important role in gelation. *In vivo* gelation was tested by posterior sc injection of chitosan/NaHCO₃ mixture in rat. *In-situ* gel formation suggested that these systems have promising applications in injectable delivery system.
• **Driton V et al [2012]**, studied the absorption enhancing effect of chitosan on Calu-3 & Caco-3 *in-vitro* lines of cell, where they observed that Caco-3 cell lines where less permeable compared to former. Based on the above result suggested a different amount of toxicity on lines of Calu-3 cells by chitosan. As an excipient chitosan can increase the absorption from mucosa for macromolecules but optimization of dosage form is required.

• **Deirdre OC et al [2006]**, formulated spray dried chitosan microparticles of salbutamol using HCl & acetic acid as dissolution medium for chitosan which affected the amount of acetyl group present on chitosan. Release of drug from microparticulates was found to be rapid. Twin impinging examination showed more repairable amount for co-sprayed dried particles, which are having greater inherent for delivering by pulmonary route.

### 1.6.2 LITERATURE REVIEW ON CHITOSN CONJUGATE/THIOMER

• **Jae HP et al [2010]**, reviewed on targeting low molecular drug using chitosan & its derivatives, in their review they found that primary amino & hydroxyl group of chitosan gives an opportunity for chitosan modification, this modification can be used for enhancing mucoadhesion & producing prodrug delivery. On review of various articles of chitosan they found that chitosan derivates can be used for targeting low molecular drug to various organs.

• **Suzanne MB et al [2011]**, synthesized N-trimethyl chitosan-ovalbumin conjugates for intracutaneous immunization using immunized mice transcutaneously, intradermally and intranodally with microneedles. In conclusion they found that after transcutaneous administration, trimethyl chitosan–ovalbumin were more immunogenic amongst all the formulations, this was due to (TMC-OVA) penetrated the skin more efficiently than nanoparticles, they also demonstrated the higher uptake by dendritic cell than trimethyl chitosan–ovalbumin mixtures.

• **Sarah D et al [2012]**, synthesized S-protected thiolated chitosan using aromatic molecule like 6-mercaptonicotinamide & also dimer 6,6-dithionicotinamide & characterized for swelling, mucoadhesion and disintegration behavior, cytotoxic
effects and controlled release. Based on the results concluded that protected thiol bearing chitosan, has benefit of not oxidizing, this will create a new different uses for the controlling the drug release.

- **Jianing Q et al [2010]**, fabricated bovine serum albumin–dextran–chitosan nanoparticles using Maillard reaction for doxorubicin targeting. Doxorubicin nanoparticles were subjected for antitumor activity evaluated for survivability and tumor inhibition of murine ascites hepatoma tumor mice. Loaded nanoparticles largely decreased the doxorubicin toxicity and increased the life span of tumor mice.

- **Deni R et al [2012]**, synthesized hydroxyethyl cellulose-cysteamine conjugates & investigated for their biocompatibility and out flow pump inhibitory property using rodamine and fluoresce in isothiocyanate-dextran-4(FD4). Derivative with less thiolation showed insitu gelling property due to S-S bonding between themselves & other atom. Activity of derivative became less with substitution of more number of -SH groups, all derivatives showed less biological toxicity, thus these derivative would be used as tool for delivering protein to the GIT.

- **Ylenia Z et al [2009]**, synthesized quaternary ammonium chitosan (QAC) for enhanced intestinal absorption QAC carries free ammonium groups which has the ability to open tight junction of Caco-2 cells, this effect is reversible & integrity of cell is reversible on discontinuation of QAC. Thus based on this QAC can be used of enhancement of absorption for the macromolecules in intestine.

- **Marata R et al [2004]**, synthesized thiolated chitosan from 4-thio-butyl-amidine(TBA) mediated by for orally administered drug. As the concentration of TBA substructures increased on the polymer, mucoadhesion was more as observed. In their observation they found that intermediate mol wt thiolated chitosan the most mucoadhesive polymer. Thus result suggests that chitosan-TBA/thiomer polymer exhibited higher mucoadhesion compared to other chitosan derivatives.

- **Andreas BP et al [2004]**, prepared poly methacrylic acid (PMMA)-starch conjugates. Due to -SH groups on poly methacrylic acid the mucoadhesive
properties of the polymeric was enhanced this resulted showed the reason for increased residence time for per oral API delivery.

- **Alexander KH et al [2004]**, synthesized chitosan-TBA (chitosan-4-thiobutylamidime) conjugate for delivering insulin tablet orally in non-diabetic rats with enzyme inhibitors, they prepared the tablets of insulin using synthesized polymer & then they were coated with eudragit L100-55 & they were administered for the rats. Results after evaluation indicated that glucose level was decreased in non diabetic rats for the tablets prepared from chitosan-TBA compared to unmodified chitosan.

- **Verena ML et al [2004]**, synthesized polycarbophil-cysteine (PCP-Cys) conjugates for targeting human growth hormone as microparticles through nasal route using glutathione as permeation mediator. Formulated microparticles improved the bioavailability of growth hormone (GH), thus *in vivo* results showed that the PCP-Cys/GH nasal microparticulates can open a new door for delivery of GH and peptide drugs.

- **Federica S et al [2010]**, synthesized thiolated hydroxy ethyl cellulose (HEC-SH) for in-situ gelling where rhodamine-123(Rho-123) a model drug, results indicated that polymer possessed good mucoadhesion, swelling & gelled when HRC-SH came in contact with biological system. Based on above observation they concluded that synthesized polymer claims the potential excipient for development of delivery of API’s.

- **Ylenia Z et al [2010]**, derivatized chitosan by using quaternary ammonium compound for increased absorption of dexamethasone from ocular route, quaternary ammonium ions increased the permeability of cornea while –SH group enhanced polymer adherence. This combined action is the base for polymer adhesion & for permeation enhancement. Thiomers showed good compatibility with ocular tissue which was confirmed by *in-vivo* experiments. Thus thiomers can be used in ophthalmic drops is because of their aqueous miscibility and doesn’t increase the viscosity, hence thiolated quaternary ammonium–chitosan conjugates may prove a good vehicle for ophthalmic delivery.
• **Xueqing W et al [2012]**, prepared a series of poly acrylic acid-cysteine-2-mercaptonicotinic acid derivatives to check the effect of mol wt with 2-MNA for its permeability enhance property. Activated –SH polymer improved the permeation of sodium fluorescence on Caco2 cell lines & rat intestine mucosal layer of rat. Based on the above observation activated –SH polymer could be consider as a good large molecular permeation enhanced polymer for per oral delivery of drugs.

• **Javed I et al [2012]**, Synthesized polyacrylicacid cysteine 2-mercaptonicotinic acid of different mol wt. Prepared conjugates showed highest swelling property and disintegration when compared to unmodified polymers and -SH polymers. All polymers found to be untoxic to Caco2 cell lines. Based on the results the previously activated –SH polymer may be promising mucosal adhesive polymers which can be utilized for increased residence in biological system for various mucosal surface present in human body.

• **Timo K et al [2008]**, tried to demonstrate paracellular uptake mannitol on Caco-2 cell lines using N-piperazine and N-betainate derivatives of chitosan which showed excellent solubility in aqueous medium. Where the substitution was less biological pH, these derivatives possessed same biological activity as of parent molecule displayed at lower pH. Thus it was concluded that chitosan nitrogen-bentainates must contain less amount of substituent’s to assure water miscibility for achieving the highest activity.

### 1.6.3 LITERATURE REVIEW ON MUCOADHESIVE DRUG DELIVERY

• **Ravindra S et al [2008]**, formulated nanoparticles of cefuroxime axetil for enhancing oral bioavailability of cefuroxime axetil. The authors prepared the nanoparticles by three methods i.e., sonoprecipitation, precipitation and spray drying and also compared their sizes and nature of particles, cefuroxime axetil with sonofication pttion resulted same sized nano sized particles. The authors also concluded that nanoparticles obtained by sonoprecipitation showed increased dissolution & oral absorption in the rats of wistar this was due to enhanced solubility & elevated absorption area.
• **Carretero PR et al [2004]**, observed the mechanism involved in the elimination of cefuroxime in rodents such as rats. Drug concentration was measured by using High performance chromatography. Elimination of drug from body and plasma concentration of cefuroxime of rats were tested after by injecting drug in tail vein of rat in a dose between 1.78-17.8 mg, followed by oral administration in dose of 2.02-8.9 mg of cefuroxime. They found that concentration maximum for drug was smaller compared to larger dose.

• **Chavan MD et al [2006]**, evaluated oral sustain release gastro retentive dosage forms for Ofloxacin with rate controlling polymers HPMC K100M as swelling agent. Crosspovidone was used with these polymers in combination to release upto twenty four hours. These dosage form were checked for their release, swelling ability. The bioadhesion was more for the prepared formulation in combined state when compared alone.

• **Ramana MV et al [2007]**, fabricated mucoadhesive buccal tablet of metoprolol tartarate with mucoadhesive polymers like carbopol934, hydroxypropyl methylcellulose, hydroxyethylcellulose and sodium carboxymethylcellulose. Prepared dosage form were evaluated for their physical & chemical properties, *in vitro* release and *in vivo* placebo results. The excellent adhesion & release of drug was in the order hydroxyethylcellulose was better than carbopol 934.

• **Owens ST et al [2005]**, formulated fluoride tablets for oral administration developed localized fluoride delivery to the oral cavity. PEG, Sodium CMC, C934 on the *in vitro* drug release & *in vitro* bioadhesion of fluoride tablets. Results revealed showed that tablets of fluoride have both the property of adhesion to mucosa at the same time it can control the fluoride release.

• **Takahashi Y et al [2007]**, prepared mucoadhesive tablet of bovine lactoferrin an iron binding glycoprotein which has ability to act as antibiotic & regulation of immune system. Bovine lactoferrin mucoadhesive tablets having gum of tamarind, pectin were formulated by direct compression. Formulation containing carboxymethylcellulose and tamarind gum was unacceptable due to insufficient bioadhesive force and unpleasant taste respectively. The formulation having pectin demonstrated having higher bioadhesion and acceptable taste by the
addition of an appropriate amount of xylitol. The medicinal effect was checked by using ulcerated rodent such as rat on the mucosa of buccal cavity which subsided subsequently.

- **Madhusmita M et al [2012]**, formulated mucoadhesive microparticles of doxycycline hyclate using carboxymethyl cellulose, sodium alginate & PVP-K30 by spray drying. Cytotoxicity of prepared microparticles was tested on H 1299 human alveolar cell lines. Mucoadhesion was greater for the microparticles prepared from carboxymethyl cellulose amongst all polymers used. Thus concluded that carboxymethyl cellulose microparticles may act as ideal carrier for inhalation delivery of doxycycline hyclate.

- **Hyo KH et al [2012]**, developed chitosan based mucoadhesive liposomal delivery system for alendronate to minimize the irritability of alendronate in the intestines DSPC & DSPG salt of sodium. Chitosan coated liposomes indicated enhanced uptake by the cell lines of caco2 for alendronate increased 2.6 times absorption for alendronate by oral route. Thus for oral delivery of liposomes which have mucoadhesive property had small sized dispersion and found to be more efficient for increased bioavailability for rats.

- **Shanker G et al [2009]**, formulated and evaluated mucoadhesive drug delivery of tizanidine hcl for buccal adhesion, it has got a first pass metabolism in liver. Formulation was done by direct compression method using mucoadhesive polymers such as CMC, hydroxypropyl methylcellulose K4M, and in combination of two polymers. The tablets were evaluated for mucoadhesive strength, residence time by *ex vivo*, non fickain *in vitro* release was observed for optimized formulation.

- **Pluta J et al [2008]**, evaluated insulin availability for bioadhesive tablets formulated from MC & sodium alginate, using hyaluronic acid as peptide carrier. Study demonstrated that sugar level of rabbit was lowered by the use insulin tablets which were mucoadhesive. *In vivo* release study indicated that, tablets with methylcellulose showed longer hypoglycemic activity as compared to alginate tablets.
• Mirkka SP et al [2012], fabricated mucoadhesive porous silicon nanoparticles for oral drug delivery using $^{18}$F using thermally-hydro carbonized porous silicon (THCPSi) using class II hydrophobin (HFBII). When prepared nanoparticles were administered to the rats orally, they found that class II hydrophobin & thermally-hydro carbonized porous silicon nanoparticles in the GI of rat adhered to stomach for three hrs after oral administration. On passage through intestine mucoadhesive property was lost. Based on the above observation they concluded that double function nanoparticle can increase bioavailability by per oral route.

1.6.4 LITERATURE REVIEW ON METHOTREXATE (MTX)

1. Structure:

![Chemical Structure of Methotrexate]

2. IUPAC : (S)-2(4-((2,4-diaminopteridin) methylamin benzamid pentandioic acid
3. Mol.Wt: 454.46g/mol
4. Mol formula: C$_{20}$H$_{22}$N$_{8}$O$_{5}$
5. CAS number: 59-05-2
6. Description: Methotrexate is a bright yellow to orange, odorless powder
7. Half Life: 3-15 hours dose dependent
8. Dose: 10-15mg
9. Bioavailability: 17-90 %
10. Excretion: Renal 48-100 %
11. Solubility: Soluble in slight in dilute alkali & acidic pH,
12. Storage: Store in a well closed amber colored bottle.

Pharmacokinetics

Absorption: Absorbed well in GI tact for dose 25mg/ml, at larger doses absorption becomes erratic
CHAPTER-I

INTRODUCTION

**Distribution:** When MTX is administered by intra venous route, drug distributes in three phases. In first phase there will be rapidly distribution of drug, in second phase there will be MTX clearance from kidney & at third phase elimination of MTX from kidney. If there is renal failure there will be precipitation of toxic effect like bone marrow depression etc

**Metabolism:**

Around 50% of MTX is reversible bound to albumin a protein present in plasma. Methotrexate is metabolized to polyglutamate derivatives. This property is very important, because the polyglutamates, which also inhibit Dihydro folate reductase (DHFR), remain in the cell even in the absence of extracellular drug. High doses of methotrexate undergoes hydroxylation at 7-position. This derivative is much less active as a antimetabolite.

**Excretion:**

90% of administered dose is eliminated from the kidney without any change as urine within the first 8 to 12 hrs. Very less amount of MTX is excreted in stools. Metabolism of Methotrexate in humans is usually minimal. After high doses, however, metabolites are readily detectable; these include 7-hydroxy-methotrexate, which is potentially nephrotoxic. Excretion takes form kidney through filtration of glomuleras and tubular secretion actively.

**Clinical Applications:**

It is used in single or in combination in chemotherapy of various kinds of cancer. It is still the mainstay for the treatment of many kinds of neoplastic disorders including acute lymphoblastic leukemia. In the treatment of severe Psoriasis, remission of rheumatoid arthritis, Acute lymphoblastic leukemia (ALL) in children, Meningeal leukemia, Choriocarcinoma. Leucovorin may be administered to counteract the toxicity of methotrexatethat escapes into the systemic circulation, although this is generally not necessary.
Pharmacodynamics

Mechanism of action: MTX is a class of antimetabolite used for treating variety of cancers it mainly interacts competitively with the enzyme dihydrofolate reductase enzyme reversible, this enzyme plays a predetermined step in folate synthesis, this interaction of MTX with the dihydrofolate reductase enzyme is due to its higher affinity towards this enzyme than folate. MTX is highly effective in S phase of cell cycle because folic acid is a precursor for the synthesis of DNA & RNA components like thymidine thus by inhibiting the thymidylates stop the uncontrolled growth of cells.

Adverse Effects:

The common adverse effects are aplastic anemia, Low WBC count, increased risk of bursting of blood vessels and revulsion. MTX is teratogenic should not be administered to pregnant women.

Available Dosage:

Oral, i.m, s.c, i.v infusion, Liquids, Powders, Tablets and Injections (5mg, 10mg & 25mg)

Drug interactions:

MTX interacts mainly with weak organic acids like NSAID’S which slows the MTX excretion & leads to bone marrow suppression. [Bruce A (2006)], [Klaseskog LD (2004)] & [Johnston A (2004)].

1.6.5 DATA OF CEFURUXIME AXETIL(CFA)

Chemically, CFA is 1-acetyloxy ethylester of cefuroxime which is (RS)-1-hydroxyethyl(6R,7R) 7-[2-(2-furyl)glyoxyl-amid]-3-(hydroxymethyl)-8-oxo-5-thia-1 azabicyclo[4.2.0] oct-2-ene-2-carboxylate, 7-(Z)-(O-methyl-oxime), 1-acetate 3-carbamate.
CHAPTER-I

INTRODUCTION

Structure:

![Structure Diagram]

1. Molecular formula: C_{20}H_{22}N_{4}O_{10}S
2. Molecular weight: 510.5
4. Description: White or almost white powder.
5. Storage: Stored in a cool, air tight container, preserved from direct light.
6. Stability: Cefuroxime axetil suspension in three different vehicles containing sucrose was found to be stable for at least 28 days at 5^{0}{C} in amber bottles. There was no change in either pH or physical appearance [Indian Pharmacopoeia (2007)], [British Pharmacopoeia (2007)].
7. Dose: 250-500mg.

Chemistry:

Cephalosporin C contains a side chain derived from D-β-aminoacidipic acid, which is condensed with a dihydrothiazine β-lactam ring system (7-aminoccephalosporanic acid). Compounds containing such as cephalosporins & pencillin are relatively stable in dilute acid and highly resistant to penicillinase regardless of the nature of their side chains and their affinity for the enzyme.

Mechanism of Action: Bacterial cell wall consists of peptidoglycon made up of two repeating units of glycon with a linear chain consisting of two repeating amino sugars where these amino sugars are linked by a chain of protein. β-lactams inhibit the
peptidoglycon synthesis i.e. cell wall synthesis & thus vanishes the bacteria [William AP (2006)], [Satoskar RS (2003)] & [Martindale (2007)].

**Adverse Effects and drug Interactions:** Gastrointestinal disturbance like nausea, vomiting, anaphylaxis, subcutaneous edema, itching of skin etc.

**Contraindications:** Patients having allergy to cephalosporins are contraindicated

**Table No 1.3: Pharmacokinetic Data of Cefuroxime Axetil [Sader HS (2007)]**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral availability</td>
<td>32 (21-44) % Food</td>
</tr>
<tr>
<td>Urinary Excretion</td>
<td>96 ± 10 %</td>
</tr>
<tr>
<td>Bound in plasma</td>
<td>33 ± 6 %</td>
</tr>
<tr>
<td>Clarence</td>
<td>0.94 CL ± 0.28 ml.min⁻¹.kg⁻¹.</td>
</tr>
<tr>
<td>Vd</td>
<td>0.20 ± 0.04 liters/ kg</td>
</tr>
<tr>
<td>Half life</td>
<td>1.7 ± 0.6 hours</td>
</tr>
<tr>
<td>Peak – time</td>
<td>2-3 hours</td>
</tr>
<tr>
<td>Peak concentrations</td>
<td>7-10 µg/ml</td>
</tr>
<tr>
<td>Serum protein Binding</td>
<td>50 %</td>
</tr>
<tr>
<td>Dosing Interval</td>
<td>12 hr.</td>
</tr>
</tbody>
</table>
### Table No 1.4: Doses of Cefuroxime Axetil According to Diseases

<table>
<thead>
<tr>
<th>Infection</th>
<th>Dosage</th>
<th>Duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharyngitis or tonsillitis</td>
<td>250 mg b.i.d.</td>
<td>10</td>
</tr>
<tr>
<td>Acute maxillary sinusitis</td>
<td>250 mg b.i.d.</td>
<td>10</td>
</tr>
<tr>
<td>Acute bacterial exacerbations of chronic bronchitis</td>
<td>250 or 500 mg b.i.d.</td>
<td>10</td>
</tr>
<tr>
<td>Secondary bacterial infections of bronchitis</td>
<td>250 or 500 mg b.i.d.</td>
<td>6-10</td>
</tr>
<tr>
<td>Uncomplicated skin and skin-structure infections</td>
<td>250 or 500 mg b.i.d.</td>
<td>10</td>
</tr>
<tr>
<td>Uncomplicated urinary tract infections</td>
<td>250 mg b.i.d.</td>
<td>6-10</td>
</tr>
<tr>
<td>Uncomplicated gonorrhea</td>
<td>1,000 mg once</td>
<td>single dose</td>
</tr>
<tr>
<td>Early Lyme disease</td>
<td>500 mg b.i.d.</td>
<td>20</td>
</tr>
</tbody>
</table>

### 1.6.6 DATA OF CHITOSAN

- Nonproprietary names: Polycationic Polymer.
- Structure:

![Chemical Structure of Chitosan](image)
• Synonyms: Chitopearl, Chitosan
• Functional category: Pharmaceutical Excipient, Biodegradable Polymer, Wound Dressing, Haemostatic material, Fibres for sutures. For non medical use it is used as Fat binder lowers cholesterol and is added in various slimming formulations.
• Structural Class: Polysaccharide.
• CAS Reg no: 9012-76-4 ;57285-05-0
• Description: A creamy white crystalline powder
• Solubility: Soluble in 1% organic acid solutions
• Temperature: With concentrated acetic acid at higher temperature leads to depolymerization.
• Stability and Storage Conditions: Store in closed containers to protect from moisture.
• Safety: It is safe, as is a biodegradable Polymer.
• Handling precautions: Nil

1.6.7 DATA OF THIOGLYCOLIC ACID

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>THIOGLYCOLIC ACID</strong></td>
<td><img src="image" alt="Thioglycolic Acid Structure" /></td>
</tr>
<tr>
<td><strong>Molecular Formula</strong></td>
<td>( \text{C}_2\text{H}_4\text{O}_2\text{S} )</td>
</tr>
<tr>
<td><strong>Molar mass</strong></td>
<td>92.11 g/mol</td>
</tr>
<tr>
<td><strong>Density</strong></td>
<td>1.32 g/cm(^3)</td>
</tr>
<tr>
<td><strong>Melting Point</strong></td>
<td>-16°C</td>
</tr>
<tr>
<td><strong>Boiling Point</strong></td>
<td>96°C at 5 mmHg</td>
</tr>
</tbody>
</table>
### 1.6.8 DATA OF L-CYSTEINE

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Formula</td>
<td>$C_3H_7NO_2S$</td>
</tr>
<tr>
<td>Molar mass</td>
<td>121.17 g mol$^{-1}$</td>
</tr>
<tr>
<td>Appearance</td>
<td>White Crystals</td>
</tr>
<tr>
<td>Melting Point</td>
<td>240ºC</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water</td>
</tr>
</tbody>
</table>

L-CYSTEINE

![Chemical Structure of L-Cysteine](image_url)