INTRODUCTION

Peptic ulcer a breach in the mucosal lining of the digestive tract produced by digestion of the mucosa by pepsin and acid has undergone profound changes in frequency and clinical presentation over the last two centuries. Before 1900, duodenal ulcer was rare, while gastric ulcer was described as a disease of young women (Kanga and Majeed, 2004). The prevalence of duodenal ulcer then increased in the beginning of the 20th century. Ulcer results from an imbalance between aggressive factors and the ability of gastroduodenal mucosa to protect and heal itself. Acid and pepsin secreted by the stomach are key ‘aggressive elements’ and are generally required for the development of ulcers. The causes of peptic ulcer disease can be divided into four major categories: H. pylori induced ulcers, non-steroidal anti-inflammatory drugs (NSAIDs), acid hypersecretory conditions (e.g., Zollinger-Ellison syndrome) and idiopathic o herpes simplex infections but they are rare. In addition gastric and duodenal ulcers can be caused infrequently by chronic disease states e.g. Crohn’s disease, systemic mastocytosis, chronic alcoholism, malignancy, herpes simplex cytomegalovirus viral infections and usage of cocaine (Smoot et al., 2001).

1.1. EPIDEMIOLOGY OF PEPTIC ULCER

Duodenal ulcers were quite rare before the twentieth century. The incidence of duodenal ulcers increased progressively reaching a peak in the 1950s. The cause of this rise is unclear, because H. pylori is thought to have been ubiquitous in the human population for thousands of years. The prevalence of peptic ulcers and their complications has fallen steadily in developed countries in the past 30 years following the decrease in prevalence of Helicobacter pylori. This decrease is thought to be due to less crowded living conditions and improvements in hygiene and sanitation. Helicobacter pylori infection is usually acquired in childhood from the mother or from other children. In developing countries, 80% of the population may be infected by the age of 20 years.

1.2. PATHOLOGY OF PEPTIC ULCER

Ninety five percent of duodenal ulcers are located in the first part of the duodenum, usually within 3 cm of the pylorus. They are usually well demarcated with
a base of eosinophilic necrosis and surrounding fibrosis. It is extremely rare for these ulcers to be malignant. Pyloric or pre-pyloric ulcers behave similarly to duodenal ulcers and have a similar pathogenesis. The commonest site of gastric ulceration is the junction between the antrum and corpus. Ulcerated adenocarcinoma or lymphoma may mimic benign ulcers. Suspicion is aroused if the ulcer is at an atypical site. Peptic ulcers due to *H. pylori* infection are associated with a gastritis characterized by infiltration of the gastric mucosa by lymphocytes and neutrophils. The distribution of inflammation within the stomach varies between patients with duodenal and gastric ulcers. Duodenal ulcers are associated with an antral predominant gastritis and there is often gastric metaplasia in the duodenal bulb. Gastric ulcers are associated with a corpus-predominant or pangastritis, the same pattern of gastritis seen with gastric adenocarcinoma. NSAIDs induced ulcers are associated with chemical gastritis. This is not a true gastritis because it is characterized by a paucity of inflammatory cells but other mucosal changes are marked including epithelial hyperproliferation, oedema and increased vascularity (Majumdar and Atherton 2006).

1.3 ORGANIC ETIOLOGIES, CAUSES AND CLASSIFICATION OF PEPTIC ULCER

1.3.1. NSAIDs associated peptic ulcer

NSAIDs reduce inflammation and relieve pain by inhibiting cyclo-oxygenase, thereby suppressing formation of prostaglandins as well as inhibiting the isoform of COX-2 present in inflammed tissues and responsible for the synthesis of mediators of pain and inflammation. They also inhibit cyclo-COX-1 which is constitutively expressed in the stomach and produces gastroprotective prostaglandins. Prostaglandins in the stomach help preserve mucosal integrity because they enhance production of mucin and bicarbonate and increase mucosal blood flow but inhibition of prostaglandins results in ulcers. Aspirin and some NSAIDs are also weak acids so they may also exert a local effect on the mucosa. The gastrototoxicity of NSAIDs is directly related to the extent to which they suppress cyclo-oxygenase-1. Cyclo-oxygenase-2-selective NSAIDs have lower gastrototoxicity but their cardiovascular side effects limit their uses. Epigastric pain is the most common presenting complaint for
duodenal or gastric ulcers. The pain is usually burning or gnawing and may radiate to the back if a posterior duodenal ulcer is present. Duodenal ulcers pain classically occurs 1-3 hrs after meals and is relieved by antacids, milk or food. Nocturnal pain in waking patient is common. Patients with duodenal ulcers may also point to a discrete site of epigastric pain. In gastric ulcers pain is often precipitated by food and is often associated with anorexia, nausea, vomiting and weight loss and epigastric tenderness is usually the only sign.

NSAID-induced ulcers in particular may initially present with complications usually bleeding, sudden onset of severe pain of the abdomen, haematemesis, melaena followed by rigidity and peritonism. Persistent severe pain and dyspepsia not relieved with food and radiating to the back may indicate an ulcer penetrating into the pancreas.

1.3.2. *Helicobacter Pylori* infection associated peptic ulcer

Around 40% of patients over 40 years age and with peptic ulcer disease are infected with *Helicobacter pylori* infection. *H. pylori* is a gram-negative rod shaped bacterium and has clearly been associated with gastritis, peptic ulcers, gastric adenocarcinoma, and gastric β cell lymphoma. The 80–90% of ulcers may be associated with *H. pylori* infection of stomach. This infection may lead to impaired production of somatostatin by D cells which results into increased gastric acid secretion along with impaired duodenal bicarbonate production. *H. pylori* infection is now proven to be a risk factor for gastric cancer and the organism was classified as carcinogen by WHO (Unge, 1998). *H. pylori* gastritis underlies duodenal and gastric ulceration. Some strains of *H. pylori* cause more inflammation than others and the most important determinant is possession of the cytotoxin-associated gene (cag) pathogenicity island, a collection of 30 genes found in some strains but not others. Cag positive strains interact more closely with epithelial cells and induce release of pro inflammatory cytokines thereby increasing inflammation. Other bacterial virulence factors also increase disease risk e.g. a vacuolating cytotoxin called Vac-A. Whether a duodenal ulcer or a gastric ulcer develops depends on the pattern of gastric inflammation and this is thought to be largely determined by host genetics. Patients with genetic polymorphisms leading to high expression of cytokines (e.g. interleukin-1β) in response to infection are at risk of pangastritis, gastric adenocarcinoma and
gastric ulceration. The genetic determinants of antral predominant gastritis and duodenal ulcers if any are unknown. Antral predominant gastritis underlies the pathogenesis of duodenal ulceration. The inflammation results in reduced production of somatostatin by the antral D cells with subsequent hypergastrinaemia because somatostatin normally exerts a negative feedback effect on gastrin. Hypergastrinaemia leads to increased production of gastric acid in response to stimulation e.g. eating. The increased acid load on the duodenum is thought to lead to the formation of protective gastric metaplasia. *H. Pylori* can not colonize normal duodenal mucosa but can colonize these areas of gastric metaplasia causing inflammation and ulceration. Gastric ulcers arise in patients with a corpus predominant or pangastritis, identical hormonal changes occur but acid production from the damaged corpus is reduced or normal. Ulceration usually occurs in the heavily inflammed transition zone between the corpus and the antrum (Majumdar and Atherton, 2006).

![Peptic Ulcer Causes Diagram](image)

**Fig. 1.1. CAUSES OF PEPTIC ULCER**

A peptic ulcer may arise at various locations:

- Stomach (called gastric ulcer).
- Duodenum (called duodenal ulcer).
- Esophagus (called esophageal ulcer).
- Meckel's Diverticulum (called Meckel's Diverticulum ulcer). The comparative features of gastric ulcer and duodenal ulcer are given in Table 1.1.
1.4. PHYSIOLOGICAL MECHANISMS OF GASTRIC ACID SECRETION

The acute physiological stress is associated with increased gastric acid secretion, decreased mucosal blood flow, gastric mucin production and gastroduodenal ulceration. The influence of stress on gastric acid secretion, visceral blood flow, and gut motility has long been acknowledged. Acute mental stress in the form of arithmetic has been shown to alter interdigestive motility by increasing the duration of the migrating motor complex, this equates to a slowing of the normal motility cycle (Jain et al., 2006).

![Figure 1.2: Mechanism of Gastric Acid Secretion](image)

**Fig. 1.2. MECHANISM OF GASTRIC ACID SECRETION**

Cheng et al., 2000 reported induction of anxiety leads to decreased gastric acid secretion whereas post hypnosis amnesia was associated with an increase in gastric acid secretion. Bresnick et al., 1993 reported that a dichotomous listening task increased gastric acid secretion and that this increase was greater in duodenal ulcer patients than in control subjects. The increase in gastric acid secretion with the dichotomous listening task was paralleled by increases in blood pressure and self-report measures of anxiety, anger and tension.
1.5. DISORDERS ASSOCIATED WITH ELEVATED SECRETION OF GASTRIC ACID SECRETION AND THEIR TREATMENT

(a) Peptic ulcers: Neuropeptide Y, corticotropin releasing factor, calcitonin, neurotensin, interlukin L along with somatostatin, prostaglandins, bicarbonates, and mucin act as mucosal defense factors. Imbalance between these mucosal defense factors and aggressive factors (acid and pepsin) is involved in peptic ulcers. Their rationale treatment is aimed at restoring this balance. In case of duodenal ulcers there is increase in basal acid secretion. However in gastric ulcers there is weakening of mucosal defense mechanism that can lead to injury inspite of low acid secretion. *H. pylori* and non-steroidal anti-inflammatory drugs play important role in ulcer induction, particularly NSAIDs inhibit production of prostaglandins from arachodonic acid by inhibiting enzyme cyclooxygenase (COX). Chronic NSAIDs users are at 2–4% risk of developing a symptomatic ulcer, gastrointestinal bleeding or associated perforation. Coadministration of misoprsotol, the synthetic prostaglandin analogue or acid suppression drug may be beneficial. Proton pump inhibitors are superior to H₂-receptor antagonist in promoting healing and preventing recurrence of both gastric and duodenal ulcer.

(b) Zollinger–Ellison syndrome: In this disease a non β cell tumor of the pancreatic islets may produce gastrin in a large quantity to stimulate the secretion of gastric acid to life threatening levels. This can lead to severe gastroduodenal ulcerations and other consequences of the uncontrolled hyerchlorhydria. In this disease the proton pumps inhibitors being surely the drugs of choice.

(c) Gastroesophageal reflux disease (GERD): It is a disorder of defense mechanism at the esophageal junction caused by regurgitation of the gastric contents especially of gastric acid. GERD is associated with decreased gastric emptying with increased incidence of transient lower esophageal relaxation. Smoking and obesity increase the incidence of GERD symptoms like heartburn, belching and bloating. It has also been linked to tracheopulmonary symptoms like laryngitis and asthma. Besides disturbed gastrointestinal motility, injurious effects of the acid peptic refluxate on the esophageal epithelium are also responsible for GERD symptoms.
Hence along with prokinetic drugs suppression of gastric acid is the current pharmacotherapeutic approach for its treatment. *H. pylori* infection does not necessarily correlate with GERD, although a reduction in acid secretion reduces chances of reflux.

### 1.6. **PROBLEM INVOLVED IN THE TREATMENT OF *H. PYLORI* CAUSED ULCER** (Nagesh *et al.*, 2011)

*H. pylori* gastroduodenitis is a chronic infectious disease but differ from many others in that once established, the organism persists usually for the whole natural life of its host unlike syphilis, tuberculosis and many other chronic infections it does not have a dormant period but causes continuous inflammation. This unusual parasite-host relationship explains perhaps why difficulties have been experienced in identifying a satisfactory treatment. In most infections the antibiotics therapy has merely to tilt the balance in favor of the host to enable it to eliminate the parasites where *H. pylori* gastritis is concerned. However the powerful and continued immunological reaction evoked is evaded or modified by the organism in such a way as to render it impotent or it may perhaps even benefit the parasite. It follows that a successful chemotherapy must be capable of eradicating the organism completely will receive little assistance for the host immune mechanism.

Most antibacterial agents have low minimum inhibitory concentration (MIC) against *H. pylori* culture. However single antibiotic therapy is not effective for eradication of *H. pylori* infection. In vivo treatment of *H. pylori* infection is difficult for three main reasons:-

- Once resistance acquired, *H. pylori* penetrate the gastric mucus layer and the organism exclusively resides on the luminal surface of the gastric mucus under the mucus gel layer and access of antimicrobial drugs to the site is restricted both from the lumen of the stomach and from the gastric blood supply.

- *H. pylori* may have acquired resistance to the commonly used antimicrobial agents (Bayerdorfter *et al.*, 1993)

- The antimicrobial agents may degrade in acidic environment.
The antibiotics are not delivered to the site of infection in effective concentrations and in fully active form from the conventional drug delivery systems. Difficulties are encountered in the localization of the drug when conventional drug delivery systems are used since they fall and localize to the base of stomach from where they are subsequently emptied and as a result little if any drugs delivered to the body or fundus of the stomach. Systemic administration followed by local secretions in the gastric juice has been considered as an option for drug delivery to bacteria. Unfortunately only strong bases diffuse into the stomach while the antibiotics used in *H. pylori* treatment are weak acids and bases, therefore fail to enter the acid environment. *H. pylori* infections are treated by multidrug regimens that are vulnerable to more incidences of side effects, poor patient compliance and inconvenience to the patients while administration.

**1.7. STRATEGIES TO IMPROVE *H. PYLORI* THERAPY** (Umamaheshwari et al., 2004)

- It is necessary to design drug delivery system which could not only curtail and alleviate the short coming of conventional delivery vehicle but also place the antimicrobial to the infected cell lines.
- The drug must diffuse into the mucus layer and the bacterial glycocolyx to reach sufficient concentrations of antibiotics for antibacterial activity.
- The contact time of antibacterial agents with the organism needs to be sufficiently long.
- Better stability and longer residence time will allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori*.
- Drug delivery system should serve to optimize antibiotic monotherapy of *H. pylori* based infections.
- Drug delivery system should inhibit the specific potential biochemical mechanisms by which *H. pylori* established and maintained the gastric infection.
- These systems should suppress the instability of the drugs in the gastric environment, improve safety profile of drugs and enhance the activity and duration for drugs exhibiting short half life.
• Drug delivery system should interact with gastric glycoproteins to form a complex that creates a barrier to hydrogen ion diffusion.

• Drug delivery system should block *H. pylori* adhesion to glycerolipid receptor in gastric epithelium.

• Drug delivery system should permit localized release as well as continuous release of antibiotics at the infection site in effective concentration and in their active form.

• Drug delivery system should have the ability to plug and seal the infected and inflamed mucosal cell lines selectively and eradicate the organism completely.

It is expected that if local delivery of antimicrobial agents from the gastric lumen into the mucous layer can be achieved then *H. pylori* eradication rate will be increased. With this understanding it comes to the realization that successful ulcer therapy must involve gastroretentive drug delivery system and targeted drug delivery system against *H. pylori*. They can be a panacea for all problems involved in the conventional drug delivery system in *H. pylori* treatment. It will be instrumental in revealing the effective management of *H. pylori* caused ulcer. These formulations administrated once daily in (optimized therapy) could lead to improved patient compliance also. (Umamaheshwari et al., 2004)

1.8. BACTERIAL CELL SURFACE RECEPTORS OF *H. PYLORI*

Fucose

Biopsy specimens from histologically normal human gastric mucosa were examined histochemically with the use of lectins. Two lectins (Ulex Europaeus agglutinin I and Ricinus Communis agglutinin I) specific for fucose and galactose residues stained the apical membrane and the intracellular perinuclear region of the antral mucosa more strongly than body (Doz et al., 1995). Among the factor responsible for the bacterial adhesion are adhesions which are surface associated antigens capable of recognizing and binding to specific carbohydrate receptors of mucosal cells. Particularly fucosylated glycoconjugate are more often terminal sugars of glycoprotein in the antral than in the body mucosa of human stomach. Lewis B blood group antigens mediate *H. pylori* to SMCs also confirms the essential role of fucose containing receptor structures in the bacterial binding (Boren et al., 1993;
Benaissa et al. (1996). The Le\textsuperscript{b} and Le\textsuperscript{a} antigens were fucosylated carbohydrate structures unique for the \textit{H. pylori} adherence and could possibly be \textit{H. pylori} receptors. The branched fucose residue on the Le\textsuperscript{b} chain is important for optimal receptor bacterium interaction. The Le\textsuperscript{a} chain lacking the terminal fucose is totally devoid of the receptor activity.

**Lectin**

Lectins have the ability to bind to a wide variety of microbial substances containing simple or complex carbohydrates. They have been used to detect cell wall modifications, elucidate complex cell wall carbohydrates and detect intra strain variations in cell wall carbohydrates or carbohydrate linkages. The specificity of lectin binding to bacterial surface carbohydrate has been reported and exploited as a tool for typing microorganisms such as \textit{Neisseria}, \textit{Staphylococcus}, \textit{Legionella}, \textit{Bacillus campylobacter}. Helicobacter and streptococcus species many bacteria including \textit{H. pylori} were found to have cell wall associated lectins which allow them to bind selectively to mucous and epithelial cells. Khin et al., 2000 applied lectin agglutination assay to characterize the carbohydrate residues on the cell wall of \textit{H. pylori}. Among the 5 lectins tested, strong agglutination patterns were observed with mannose specific Conconavalin A (Con A), Fucose specific \textit{tetragonolobus purpureas} (Lout’s A) and (GlcNAc)\textsubscript{2}, Specific tetravulgaris (WGA) lectins. It is therefore possible that cell wall surface of \textit{H. pylori} having mannose and fucose residues which might mediate its adherence to the corresponding mannose and fucose specific lectin receptors exist in the gastric mucous cells for its colonization.

Lectin histochemistry studies showed that the staining for mannose and glucose (bound by succinylated ConA) was negative in normal mucosa but was positive in \textit{H. pylori} infected mucosa (Baczako et al., 1995). It is therefore possible that \textit{H. pylori} having stronger affinity for mannose, fucose and glucose residue might mediate its adherence to the corresponding mannose, fucose and glucose receptors existing in the gastric mucous cell for its colonization. \textit{H. pylori} bearing different affinities for carbohydrates receptors on the cell surface may account for the variation in the adhesive prosperities of the organism for antral mucous cell in the stomach.
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**Sulphatides**

Kamisago *et al.* (1996) demonstrated the clinical isolate of *H. pylori* prefentially bind to sulphatides ($\Gamma^3$ SO$_3$–Galcer) and GM3 gangliosides ($\Pi^3$ Nell A$_C$Laccer) the two predominant acidic glycosphingolipids in the human gastric mucosa. Sulphated glycol conjugates such as heparin and gastric mucin significantly inhibited *H. pylori* adhesion to KATO III cells suggesting the importance of sulphated moiety as a receptor for *H. pylori* adhesion. Finally a monoclonal antisulphatides antibody markedly reduced *H. pylori* adhesion to KATO III cells.

These results suggest that sulphatides and possibly related sulfated compounds serve as major receptors for cell adhesion by *H. pylori*.

Glycoaminoglycans having long repeating sulfated glycol conjugates may also serve as major receptors for *H. pylori* adhesion. The specific localization and quantitative analysis of glycoaminoglycans in gastric mucosa remain however to be elucidated. Sulphatides are highly enriched in gastric mucosa, moreover immunoflouran staining using a monoclonal anti-sulphatides antibody revealed that sulphatides were significantly localized in the epithelial lining of gastric mucosa. Therefore includes the sulphatides can serve as a major receptor or for cell adhesion by *H. pylori* in the gastric mucosa.

**Phosphotidylethanolamine (PE)**

The prevention of bacterial adherence *in vivo* is complex and may be achieved by targeting a ligand or receptor. This ligand receptor recognition or interaction is a major mechanism for attachment of *H. pylori* to host cells *in vitro* as well as *in vivo* (Lingwood *et al.*, 1989). *H. pylori* was shown previously to bind to a specific alkylacyl glycerolipid derived from human erytocytes HEP2 cells and human antral epithelium (Lingwood *et al.*, 1993). The PE is more strongly bound with *H.Pylori*. *H. pylori* glycerolipid receptor was originally isolated from human erythrocytes, stomach antrum, pig stomach and human epithelial cells (HEP2) and it was characterized as PE (Lingwood *et al.*, 1992). Furthermore cultured human cells with less PE show minimal attachmen of *H. pylori in vitro* (Bitzan *et al.*, 1998) emphasing the importance of PE-*H. pylori* interaction in bacterial adhesion. PE is a predominant
lipid in the antrum of the human stomach and function as receptors for *H. pylori* adhesion. Correlation of the ability of *H. pylori* to adhere to eukaryotic cells with the detected presence of PE receptors however underscores the importance of this lipid as major receptors in promoting *H. pylori* adhesion to intact cells. PE bacterial adhesion exists as a cell surface associated ligand (Gold *et al.*, 1993; Bukhalm *et al.*, 1997).

1.9. **NOVEL DRUG DELIVERY SYSTEMS**

Oral delivery of drugs is by far the most preferable route of drug delivery due to the ease of administration, patient compliance and flexibility in formulation. From immediate release to site specific delivery and mucosal targeted delivery. Oral dosage forms have greatly progressed. It is evident from the recent scientific and patent literature that an increased interest in academic and industrial research groups for effective management of peptic ulcer in novel particulate and vesicular dosage forms that have delivered drugs in the stomach for a prolonged and predictable period of time. The basic rational for novel drug delivery is to alter the pharmacokinetic and pharmacodynamics of drug by enclosing them or harboring them into carrier system.

1.9. 1. **Characteristics of an ideal Carrier**

- It must be able to cross anatomical barrier.
- It must be recognized specially and selectively by target cells.
- The linkage of drug and directing unit should be stable in plasma, intestinal and other biological fluids.
- After reconization and internalization, the carrier system should release the drug moiety inside the target organs tissues or cells.
- Carrier should be nontoxic, nonimmunogenic and biodegradable in nature.

1.9.2. **Various carriers investigated for their targeting potential as a drug delivery system**

(I) **Colloidal carrier**

(a) Vesicular system: liposomes, niosomes, pharmacosomes, virosomes, immunoliposomes etc.

(b) Microparticulate system: microparticles, nanoparticles, magnetic microspheres, albumin microspheres, etc.
(II) **Cellular carrier**: resealed erythrocytes, serum albumin antibodies, leucocytes, platelets etc.

(III) **Supramolecular drug delivery system**
(a) Low aggregation number: reverse micelles, mixed micelles, polymeric micelles.
(b) Intermediate aggregation number: liquid crystals
(c) High aggregation number: lipoprotein (VLDL, LDL, chylomicrons) modified semisynthetic lipoproteins, synthetic LDL mimicking particles (SMBV)

(IV) **Polymer based system**
Signal sensitive, mucoadhesive, biodegradable, bioerodable polymeric system

(V) **Macromolecular carriers:**
(a) Proteins: Serum albumin (human bovine), glycoproteins, neoglycoproteins, artificial viral envelop (AVE)
(b) Glycosylated water soluble polymers: poly (l-lysine)
(c) Monoclonal antibodies: immunological Fab fragment, Ab enzyme complex, biospecific antibodies.
(d) Toxins: immunotoxin, rCD4 toxin conjugates
(e) Lectins: polysaccharides.

1.10. **LIPOSOMES A VESICULAR DRUG DELIVERY SYSTEM**
Liposomes were discovered in early 1960 by Bangham and colleagues and subsequently became the most extensively explored drug delivery system. Structurally liposomes are concentric bilayer vesicles in which an aqueous volume is entirely enclosed by a membraneous lipid bilayer mainly composed of natural or semisynthetic phospholipid. The building block of these bilayerd vesicles are glycerol based amphiphatic phospholipids mainly lecithin, sterols mainly cholesterol and its derivatives, all included as fluidity buffer to stabilize the bilayer membrane in the presence of biological fluids. Liposomes are self assemblages of amphiphiles into closed bilayer structures. Liposomal contents can undergo chemical degradation such...
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as oxidation and hydrolysis. Furthermore the method of formulation, nature of amphiphiles and encapsulated drugs are considered in order to manipulate membrane fluidity, rigidity and permeability characteristics. Various possibilities to promote stability of liposomes include the use of polysaccharide which can be immobilized on outer half of the bilayer with the help of hydrophobic anchors. Adapting palmitaylation and cholesterol esterification capped liposomes were challenged against harsh environment and physico-chemical stimuli to mimic the biological stress to be encountered in biological fluids.

Liposomes have received much attention as potential drug carriers for improvement of intestinal absorption of drugs when taken orally (Freund, 2001) and for delivering of drugs into pathological sites such as tumors and inflammatory sites via i.v. administration by encapsulating drugs (Desormeaux and Bergeron, 1998). The use of liposomes as drug carrier by i.v. injection is limited by their low stability in blood stream. Firstly phospholipids exchange and transfer to lipoproteins mainly HDL destabilize and disintegrates liposomes with subsequent loss of content and the pain associated with injection.

1.10.1. CLASSIFICATION OF LIPOSOMES

Table: 1.2. Classification of liposomes based on structural parameter

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Type of vesicles</th>
<th>Term used</th>
<th>Size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Multilamellar vesicles</td>
<td>MLV</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>2.</td>
<td>Oligolamellar vesicles</td>
<td>OLV</td>
<td>0.1-1</td>
</tr>
<tr>
<td>3.</td>
<td>Unilamellar vesicles</td>
<td>UV</td>
<td>All size range</td>
</tr>
<tr>
<td>4.</td>
<td>Small unilamellar vesicles</td>
<td>SUV</td>
<td>0.025-0.1</td>
</tr>
<tr>
<td>5.</td>
<td>Medium size unilamellar vesicles</td>
<td>MUV</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Large unilamellar vesicles</td>
<td>LUV</td>
<td>&gt; 0.1</td>
</tr>
<tr>
<td>7.</td>
<td>Giant unilamellar vesicles</td>
<td>GUV</td>
<td>&gt; 1</td>
</tr>
<tr>
<td>8.</td>
<td>Multi vesicular liposomes</td>
<td>MVL</td>
<td>5-50</td>
</tr>
</tbody>
</table>
Table: 1.3. Classification of liposomes based on methods of preparation

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Type of vesicles</th>
<th>Term used</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Small unilamellar vesicles</td>
<td>SUV</td>
<td>Sonication, French pressure cell, Ether injection, Ethanol injection</td>
</tr>
<tr>
<td>2.</td>
<td>Single or oligolamellar reverse phase vesicles</td>
<td>REV</td>
<td>Reverse phase evaporation,</td>
</tr>
<tr>
<td>3.</td>
<td>Fat- MLV</td>
<td>MLV</td>
<td>Frozen and thawed</td>
</tr>
<tr>
<td>4.</td>
<td>Vesicles extension technique</td>
<td>VET</td>
<td>Vesicles prepared by extension technique</td>
</tr>
<tr>
<td>5.</td>
<td>Dehydration rehydration vesicles</td>
<td>DRV</td>
<td>Vesicles prepared by dehydration followed by rehydration</td>
</tr>
</tbody>
</table>

Table: 1.4. Classification of liposomes based on composition and applications.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Type</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Conventional liposomes (CL)</td>
<td>Neutral or negatively charged phospholipids and cholesterol</td>
</tr>
<tr>
<td>2.</td>
<td>Fusogenic liposomes (RSVE)</td>
<td>Reconstituted sendai virus envelop</td>
</tr>
<tr>
<td>3.</td>
<td>pH sensitive liposomes</td>
<td>Phospholipids such as PE or DOPE</td>
</tr>
<tr>
<td>4.</td>
<td>Cationic liposomes</td>
<td>Cationic lipid with DOPE</td>
</tr>
<tr>
<td>5.</td>
<td>Long circulatory (stealth) liposomes</td>
<td>Neutral high phase transition temperature cholesterol and 5-10% PEG or DSPE.</td>
</tr>
</tbody>
</table>

1.10.2. Advantages of liposomes as drug delivery system

- Liposomes are biocompatible, completely biodegradable, non-toxic, flexible and nonimmunogenic for systemic and non-systemic administrations.
- Liposomes supply both a lipophilic environment and aqueous “milieu interne” in one system and are therefore suitable for delivery of hydrophobic, amphipathic and hydrophilic drugs and agents.
- Liposomes have the ability to protect their encapsulated drug from the external environment and to act as sustained release depots (Propranolol, Cyclosporin).
- Liposomes can be formulated as a suspension, as an aerosol, or in a semisolid form such as gel, cream and lotion, as a dry vesicular powder (proliposome)
for reconstitution or they can be administered through many routes of administration including ocular, pulmonary, nasal, oral, intramuscular, subcutaneous, topical and intravenous etc.

- Liposomes could encapsulate not only small molecules but also macromolecules like superoxide dismutase, haemoglobin, erythropoietin, interleukin-2 and interferon.
- Liposomes are able to reduce the toxicity and increase the stability of entrapped drug via encapsulation (Amphotericin B, Taxol).
- Liposomes have increased the efficacy and therapeutic index of drug (Actinomycin-D).
- Liposomes help to reduce exposure of sensitive tissues to toxic drugs.
- Liposomes are able to alter the pharmacokinetic and pharmacodynamic property of drugs (reduced elimination, increased circulation life time)
- They possess the flexibility to couple with site specific ligands to achieve active targeting (Anticancer and Antimicrobial drugs).

1.11. DOUBLE LIPOSOME AS A POTENTIAL DRUG DELIVERY SYSTEM

Conventional liposomes (unilamellar and multilamellar) have certain drawbacks like low entrapment efficiency, stability and release of drug after single breach in external membrane have led to the formation of new type of liposomal systems. The challenges have been successfully met in the form of double liposomes. Double liposome is a recently developed type of liposomes consisting of smaller liposomes enveloped in lipid bilayer (Kim et al., 1983; Talsma et al., 1987; Walker et al., 1997). Double liposomes consist of several small liposomes encapsulated in large liposomes i.e. multivesicular vesicles (MUVs). Therefore double liposomes should be discriminated from ordinary classification of multilamellar vesicles (MLVs), large unilamellar vesicles (LUV) and small unilamellar vesicles (SUV). However for these liposomes, the volume of inner phase is large and loading volume of water soluble drug is high. Double liposomes have been estimated to be more effective as a drug carrier for oral delivery as compared with conventional liposomes.
because the outer liposomes were suggested to protect the inner liposome against several enzymes. Therefore double liposomes was thought to be more effective than ordinary liposomes. This concept was also supported by in vitro release characteristic i.e. double liposomes formation inhibited the release of drug encapsulated in inner liposomes (Katayama et al., 2002 a). Other clinical trials are evaluating the ability of double liposomes to deliver intravenous immunomodulating drugs (MTP-PE) to macrophages. Interferon and peptides hormones calcitonins have been given in the form of i.m. depot. These trials and animals studies provide evidence for versatility of double liposomes.

The vary unique feature of such liposomes is that inside each liposomes discontinuous internal aqueous chamber bounded by a continuous nonconcentric network of lipid membrane render a high aqueous volume to lipid ratio and much larger particle diameter compared with traditional liposomes. The nature of double liposomes also provides sustained release of encapsulated drug since in unilamellar vesicles (ULV) a single breach in the external membrane will not result in total release of internal content and such liposomes allow higher efficient incorporation of hydrophilic drugs, slow release and higher stability. The structural characteristic also makes them ideal vehicles for localized regional drug delivery. Their unique vesicles size of 15-50μm is large enough so that the particles cannot pass into the capillaries and thus remain where they are placed. Double liposomes prepared using phosphatidyl choline (PC) with a high transition temperature (53°C) or the addition of cholesterol result in the better stability against gastric juice, bile acid and lipase enzyme.

1.11.1. Methods used for the preparation of double liposomes

**Glass filter method** (Katayama et al., 2002 b)

In this method phosphatidyl choline was dissolved alone or with stearylamine (SA) or phosphatidyl serine as lipid with positive and negative electrical charge in chloroform. Brillant blue or erythrosine in ethanol was poured into lipid solution. The mixture was passed through a G4- glass filter (pore size 10-16 μm) and chloroform was evaporated with help of gentle stream of nitrogen gas at room temperature. The
l lipid layer formed on glass filter was hydrated with phosphate buffer saline (pH 7.4) for 10 min and the glass filter was soaked in water bath and sonicated. Then buffer solution was passed through the filter repeatedly by alternately pressing syringe connected to the both sizes of the filter to form the liposomes. Double liposomes were prepared by filtering a suspension of liposomes prepared using a G4 filter into a G3 filter (pore size 40-100 μm) coated with a similar lipid layer.

**Glass beads method** (Yamabe et al., 2003 a)

In this method the lipid dissolved in chloroform was poured into the Kjeldahl flask with glass beads and the organic solvent was evaporated using a gentle stream of nitrogen gas at room temperature to form a lipid layer on glass beads. The lipid layer formed on glass beads was hydrated with suspension of inner liposomes at temperature above the phase transition temperature of the lipid employed and was agitated. The resultant double liposomes were separated from glass beads by aspiratory filtration.

**Reverse Phase Evaporation Method** (Shinsuke et al., 2006)

A lipid mixture in a different molar ratio was dissolved in chloroform and poured into a Kjeldahl flask. The film was deposited on the inner wall of flask by removal of the organic solvent by rotatory evaporation. The lipid was then redissolved in 3 ml of chloroform and the inner liposomal suspension was added. The mixture was sonicated for 5 min at 20°C to form a w/o emulsion and the organic solvent was slowly removed by rotatory evaporation until the suspension became a gel, followed by brief vortex mixing. Then the residual organic solvent was evaporated until a homogeneous suspension was obtained. The resultant liposomal suspension was centrifuged at 3000 rpm for 10 min, and supernatant was removed. The suspension of double liposomes was obtained by resuspending the residue in PBS.

**Double emulsification method**

This method involves two steps (a) Preparation of w/o phase. (b) W/O emulsion is subsequently emulsified with second aqueous solution resulting in w/o/w double emulsion.
1.11.2. Advantages of double liposomes as drug delivery system:

- High drug loading.
- Possibility to encapsulate large quantity of hydrophobic drugs.
- High encapsulation efficiency.
- Low content of free drug in suspension
- Little chemical change in free drug caused by formulation process.
- Narrow particle size distribution.
- Spherical shape.

1.12. HYDROGEL NANOPARTICLES AS A DRUG DELIVERY CARRIER

Hydrogels are gaining increasing popularity in the area of controlled release drug delivery. These polymers are generally glassy in dehydrated state but swell to become an elastic gel upon water penetration. The entrapped drug within the swollen matrix concomitantly dissolve and diffuse through the swollen network into the surrounding aqueous environment. The rate of drug release from hydrogel is further regulated by controlling the cross linking density and the extent of water swelling. Their affinity to absorb water is attributed to the presence of hydrophilic groups such as –OH, –CONH–, –CONH₂–, and –SO₃H in polymers forming hydrogel structures (Peppas and Khare, 1993). Hydrogels are polymeric networks with three-dimensional configuration capable of imbibing high amounts of water or biological fluids. The contribution of these groups and domains in the network is thus hydrated to different degrees depending on the nature of the aqueous environment and polymer composition (Bouwstra and Jungiger, 1993).

Hydrogel nanoparticles (nanogels) as a family of nanoscale particulate materials have been used as with drug delivery approach which demonstrate the features and characteristics of hydrogels and NPs separately possess at the same time. Therefore it has the advantages of hydrophilicity, flexibility, versatility, high water absorptivity, and biocompatibility. The other advantages of the NPs are mainly long life span in circulation and the possibility of being actively or passively targeted to the desired biophase e.g. tumor sites. The crosslinks in the polymer network are provided
by covalent bonds, hydrogen binding, vander waals interactions, or physical entanglements (Kamath and Park., 1993).

1.12.1. Poly (vinyl alcohol) based hydrogel nanoparticles

PVA hydrogel is a linear polymer synthesized by free radical polymerization of vinyl acetate with subsequent hydrolysis of acetate groups to hydroxyl moieties -[CH₂-CH(OH)]ₙ- resulting in a wide molecular weight distribution. The molecular weight distribution is an important characteristic due to its roles in determining polymer properties including crystallizability, adhesion, mechanical strength, and diffusivity.

The physical properties of PVA such as strength, water solubility, gas permeation and thermal characteristic vary with the degree of crystallinity which are dependent on the degree of hydrolysis and the average molecular weight of the polymer. The actual amount of crystalline material present in the compounded PVA is further dependent on the amount of plastizier, water incorporated into the compound, production process (acid or base catalyzed), degree of hydrolysis and molecular weight. Partially hydrolyzed grades contain residual acetate groups, which reduce the degree of crystallinity and the resulting material with lower strength and increased water solubility than the fully hydrolyzed grades is produced. The partially hydrolyzed grades may be considered as copolymer while those that are fully hydrolyzed may be considered as homopolymer (Mallapragrda et al., 2001).

PVA is hydrophilic semi crystalline polymer and its biocompatibility makes it an excellent material for use in biomedical applications such as drug delivery system, contact lenses and artificial organs (Noguchi et al., 1991). The co-polymeric nature of PVA provides the polymer with unique gelling characteristic which is responsible for its adhesive properties, this makes it suitable for transmucosal and transdermal delivery applications. The polymer solubility and gelling characteristic can be manipulated to control the rate of diffusion of the drugs from PVA particles.

Heterogeneously structured composites involving PVA have been investigated in the field of hydrogel nanoparticles. The cross linking can be carried out either before or after drug loading. The cross linking can be done by chemical cross linking method generally bifunctional group including formaldehyde, glutaraldehyde,
dicarboxylic acids and ketone are used for chemical cross linking (Chandy and Sharma, 1992). Chemical cross linking method has a tendency to leave the residual material and causes toxic behavior to the system. Additionally the cross linking agent added may permanently destroy or alter the substance to be released. The problem associated with chemical cross linking method can be overcome by using physical method of cross linking i.e. freeze thaw technique. When PVA is exposed to number of freeze thaw cycles (FT cycles) crystallites are formed and system behaves as if it has been chemically cross linked. FT cross linking method provides free hydroxyl (-OH) group of PVA for further linkage with other groups. Freezing rate and time is critical parameter in the preparation process of PVA hydrogel nanoparticles which determine its strength and elasticity (Suzuki et al., 1990). It has been reported that the size and density of the crystallites formed significantly decreased with an increase in the freezing time.

Irradiation is another method by which poly (vinyl alcohol) can be physically cross linked through exposure to UV radiation or thermal energy.

![Diagram showing effect of molecular weight and % hydrolysis on the physical properties of PVA](image)

**Fig. 1.3.** Diagram showing effect of molecular weight and % hydrolysis on the physical properties of PVA
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The method producing PVA hydrogel nanoparticles which have the necessary biocompatibility. Three separate methods of production of PVA polymer including salting-out, emulsification diffusion and nanoprecipitation have been used by Galindo-Rodriguez *et al.* (2005). Blends of PVA with other polymers can also be used to control the release mechanisms (Korsmeyll *et al*., 1983). In order to prolong the drug release from such an inherently hydrophilic polymer network, PVA is often modified by cross linking to reduce the macromolecular mesh size available for drug diffusion. By changing the degree of hydrolysis it is possible to control the polymer swelling and dissolution characteristics in water and finally the release rate of drug. The cross linked density was also found to predominantly control the release rate but other characteristics such as ionic strength, pH and particle size were also found to affect the release rate to some degree. As the ionic strength was increased rate of release decreased due to the decrease in swelling of hydrogel particles. The release rate increased as the size of the particles decreased and increased with increasing pH and degree of hydrolysis of PVA.

1.13. NANO HYDROGEL ANCHORED LIPID VESICLE (NANOLIPOBEADS) AS A DRUG DELIVERY SYSTEM

A new hybrid system with structural similarity to natural cells that combines the mechanical stability of polymer beads and unique spectroscopic properties and biocompatibility of liposomes is called lipobeads (Jin *et al*., 1996). Lipobeads consist of a lipid bilayer shell anchored on the surface of hydrogel polymer cores that act as a cytoskeleton. Artificial particulate systems such as polymeric beads and liposomes are finding a variety of biomedical applications in the drug delivery, drug targeting, protein separation, enzyme immobilization and blood cell substitution (Rolland, 1993). Liposomes have a flexible cell like lipid bilayer surface which act as a permeability barrier such that compounds can be entrapped in their aqueous interior. However liposomes can be mechanically unstable and their loading capacity is limited by the water solubility of material to be loaded. Polymeric beads although mechanically more stable and having a larger loading capacity than the liposomes but they lack many of the surface properties of a lipid bilayer shell.
Lipid bilayer supported on various solid surface such as glass, plastic, and metal as well as modified polymers have previously been shown to provide a stable and well defined cell membrane like environment that has found a number of basic applications (Sackman, 1996; Mcconnell et al., 1986). It was therefore reasonable to expect that assembly of lipid bilayers on spherical hydrogel surface could be useful approach for preparing an artificial cell analogue. Gao and Hung (1987) reported that encapsulation of hydrogel particles into liposomes enhanced the loading capacity and overall mechanical strength of liposomal structure however in that system unanchored bilayer is still somewhat unstable and the system could only be formed with specific lipid mixture and only with the polymer cores of certain size and shapes.

Jin et al., 1996 have developed hydrogel anchored liposomes in which these limitations have been overcome. This approach has been to create hydrophobic anchors for the bilayer by attaching lipid molecules to the surface of preformed PVA xerogel (dry hydrogel beads). The surface modified xerogel is then hydrated and treated with a liposome suspension. The hydrophobic lipid molecules and other intrinsic membrane component of liposomes associate spontaneously with the hydrophobic fatty acid anchor on the surface of the hydrated polymer and self organize into a homogenous membrane over the surface through hydrophobic interactions. These anchors add mechanical stability to the bilayer shell and also promote its self assembling. The acyl anchors and the polymer which they are attached not only act as cytoskeleton but self assembly properties allows the bilayer coating to be established on cores ranging in the shape from sheets to spheres.

**1.13.1. Methods of Preparation of Lipobeads**

**Vesicle fusion method** (Jin et al., 1996): Lipobeads preparation was carried out by combining equal parts of suspension of acylated PVA beads to liposomes suspension. PE liposomes (composed of PE and oleic acid 7:3 molar ratio) were prepared by rotating flask evaporation method and the resulting thin lipid film was hydrated with 5 ml of phosphate buffer (pH 7.4). Small vesicles were prepared by sonication for (30sec) of liposomal suspension until it became transparent (Domingo et al., 1993). The suspension of surface modified PVA beads was then combined with equal part of suspension of liposomes. The fusion of liposomes on the acylated PVA beads was then accomplished by lowering pH of the mixture below 6.5 (Fig. 1.4).
Fig. 1.4. Formation of bilayer on modified PVA beads (solid support)

Film formation method: Kim et al., (1997) have originally described the lipobeads synthesis by modified film formation method. For this, a weighed quantity of surface modified beads was suspended in 1:1 (v/v) ethanol/hexane solution. PE: cholesterol (3:1) molar ratio solution was prepared in chloroform containing drug. Phospholipids mixture was then added to the suspension of acylated beads and vortexed for 5 min at
low speed. The suspension was incubated at room temperature for 2 hr and air dried to evaporate the solvent. During the coating steps there may be simultaneous formation of liposomes in addition to lipobeads. The formed liposomes and uncoated particles were washed 3 times in a buffer solution using low speed centrifugation at 500 rpm for 5 min. The lipobeads were stored in glass vials covered with aluminium foil at 4°C in a refrigerator until use.

1.13.2. Potential application of lipobeads:-

The lipid bilayer supported nanobeads *in vitro* mimic the natural biological membrane and lipobeads may also provide a model environment for cell-cell interactions, various biotechnological applications, purification and reconstituted transmembrane protein such as ion channels transporters (Boxer 2000). Lipobeads has many attractive properties and are useful tools for studying the processes at and across the membrane surface. Phospholipids supported on a hydrophilic solid substrate are extensively used in the study of interaction between the model membranes and proteins/polypeptides (Saccani et al, 2003). In addition, lipid coated particles can be exploited as a novel approach to fix the membrane bound enzyme onto the carrier surface (Rothe and Awich, 1989). Another potential application of lipobeads is that it acts as a red cell substitute. By combing the structural features of liposomes and hydrogels lipobeads provide a more realistic red cell replacement while at the same time they have much higher encapsulating efficiency than liposomes. In addition to these applications the fluorescent lipobeads are used for intracellular oxygen measurement in murine macrophage and fluorescence sensing lipobeads are used for intracellular pH measurement. Lipobeads are as biocompatible as liposomes and much more stable mechanically. They can thus serve as a depot delivery formulation. We expect that it will be possible to discover novel polymer cores that are biodegradable or at least recoverable making injectable formulations safer.

**The mechanism of lipid bilayer coating on the surface of hydrogel beads**

The lipobeads modifies the surface of hydrogel beads in such a way that lipid bilayer is formed spontaneously on their surface. This coating protects the interior
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compartment of the bead and modify the release of substances loaded into the bead. This process can be applied to the manufacture of products for drug and vaccine delivery, as hemoglobin carriers in artificial blood and for creating devices for predicting drug absorption and distribution. The process consists of a surface modification that covalently links amphipathic long-chain fatty acids or phospholipids via their polar ends to surface reactive groups of the hydrogel (e.g. ester linkages via surface hydroxyls). As a result, upon exposure to liposomes or other sources of lipids, the new surface hydrophobic groups will direct the spontaneous formation of a lipid bilayer coating. The assembly continues until the surface is completely coated and a continuous lipid vesicle now engulfs the bead creating a permeability barrier around the bead that has the same properties of a cell membrane. The hydrogel core acts as a scaffold, mimicking the role played by a cells cytoskeleton. The naked lipobeads precursor (acylated bead) can be stored in a stable xerogel (dried) state, while the lipids can be stored in a lyophilized state. Addition of water is required to drive the assembly of the product. This unique material system is expected to be potentially useful in several novel applications.

controlled release drug delivery

A number of particulate systems have been explored as potential drug delivery vehicles. As a drug delivery system the non-ionic, water swelling, hydrogel microparticulates are superior to other particulate systems in that they are both biocompatible and have inert interior and important properties for carrying reactive therapeutic agents. The lipid bilayer anchored on the surface of hydrogel particles is well packed (does not have defects or holes) and has all of the permeability properties of other lipid bilayers. It entraps the ions such as calcium despite a 1000 fold concentration gradient but releases that calcium on demand when the lipobead is exposed to a calcium carrier (Schmitt et al., 1994). Many other combinations of controlled release strategies can be used. Indeed the lipid coating of lipobeads will have the same permeability properties as liposomes and amenable to the same technology developed to control release from liposomes. For example the release rate across the lipid bilayer permeability barriers can be controlled by selecting the types
of lipids used in generating the bilayer. These lipids can vary according to their charge, chain length and phase transition temperature. Likewise all of the technology applied to engineering the interior of hydrogel drug carriers can be incorporated into a lipobead device. Moreover, the naked lipobeads can be loaded just like any other hydrogel and then stored in a dried form. Because chemical modification is restricted to the bead surface, this will not interfere with the interior properties of the hydrogel particles. Also since water is the only reagent needed to drive the formation of lipobeads.

**Delivery of synthetic polypeptide vaccines**

Synthetic vaccines are relatively small and as hydrophilic polypeptides tend to be expensive and much effort is being applied to ensure their efficient delivery to the immune system. Optimum effects are found with slow delivery of small amounts. Much has been learned about the movement of small peptides across lipid bilayers from bioavailability studies. This information can be applied directly to designing peptides and lipid coatings on the lipobeads with optimized release properties.

**Red cell substitution**

The safety of the blood supply for operations is of major concern to the surgeons. There is much interest in developing artificial red cell substitutes or supplements. The problem is to develop an infusible preparation of an oxygen carrier with properties similar to hemoglobin. As hemoglobin is readily available, a number of polymer conjugates, polymer micro-capsules and liposomes are currently being studied as hemoglobin carriers (Chang, 1992). Liposomes can encapsulate hemoglobin together with cofactors such as 2, 3-DPG. They also have similar oxygen permeability properties to red cells. However the amount of hemoglobin that can be delivered by tolerated doses of liposomes is inadequate due to their low loading capacity and poor mechanical stability as well as loss of more quantity of hemoglobin. Polymer microcapsules are more stable but they lack the cell like permeability properties of liposomes. However the lipobeads will combine the advantages of both existing strategies in a synergistic manner. Hemoglobin can be
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loaded at high capacities in the core and stored in the xerogel form until needed. The cell-like coating mimics both liposome and red cell surfaces but is more stable than liposomes. In addition the shape and flexibility of the hydrogel core can be engineered to match that of red cells. The larger size of lipobeads will reduce clearance by the reticulo-endothelial system which is a major limitation of liposomes formulations.

Assessment of drug absorption and distribution

The oral bioavailability of most therapeutic agents is limited by the rate of passive diffusion across the intestinal mucosa. An artificial system which offers a fast, simple, and reliable assay and at the same time only uses a small amount of material is therefore highly advantageous. Two possible rate limiting factors must be taken into account in drug absorption by passive diffusion through these mucosal walls: interaction of drug molecules with polar head groups of bilayer lipids and partition of the drugs into the hydrophobic matrix of the bilayer (Kalant and Roschlau, 1989). The currently used systems, including octanol/ water partitioning system and octadecyl silica (ODS) chromatography can model only the hydrophobic interaction with the membrane matrix, and therefore often lead to serious over estimation of hydrophobic drug candidates. Liposomes and immobilized artificial membranes (IAM) based chromatography has been used to model both of the mechanisms. However the liposomal method is experimentally tedious and time consuming as well as has low reproducibility because of its lack of stability. The IAM method on the other hand over estimates significantly for very soluble molecules because what is being measured is related to the ease of entry into a hydrophobic environment and not on the trans membrane transport. For this reason, the drug retention with IAM, which is the sole measurable parameter, reflects not only the membrane partition but also the hydrophilic adsorption onto the head groups. On the other hand lipobeads potentially can be designed to measure trans membrane flux. Moreover the flux can be measured across a single bead thereby minimizing the amount of substance used for analysis. Furthermore beads coated with membrane fragments from the tissue of interest will allow the incorporation of transporters into the assessment system.
Reconstitution of trans-membrane proteins to study cell surface phenomena

Supported lipid bilayers on electrode and other solid substrate surfaces have been used as well defined model bio-membranes to study the membrane protein functions in bio-sensors and other bioassays (Spinke et al., 1992). Applications of this type of supported membranes however are limited to partially associated membrane proteins. In the case of lipobeads and liposheets, the bilayers are anchored on a hydrated polymeric network thus many trans-membrane proteins, such as ion channels and transporters may be reconstituted. This may promote understanding of the mechanistic details of many cell surface phenomena by using fluorescent, electrochemical and spectroscopic techniques.

1.14. OBJECTIVE OF RESEARCH WORK AND RESEARCH ENVISAGED

The specificity of lipid phosphatidylethanolamine (PE) to bind H. pylori surface receptor has been reported and can be explored as a tool for development of localized and targeted drug delivery system. Bitzan et al., have emphasized the importance of PE -H. pylori interaction in vitro in bacterial adhesion. PE is more strongly bound with H. pylori glycerolipid receptor and was originally isolated from human erythrocytes, stomach antrum, pig stomach and human epithelial cells (HEP2). The prevention of bacterial adherence in vivo may be achieved by targeting a PE to H. pylori glycerolipid receptor. This ligand receptor recognition or interaction is a major mechanism for prevention of attachment of H. pylori to host cell in vitro as well as in vivo (Lingwood et al., 1993).

PE is a predominant lipid in the antrum of the human stomach and function as a receptor for H. pylori to adhesion. Correlation of the ability of H. pylori to adherence of eukaryotic cells with the detected presence of the PE receptor underscores the importance of this lipid as a major receptor in promoting H. pylori adhesion to stomach antrum cells. PE bacterial adhesion exists as a cell surface associated ligand. Adhesions play a major role in H. pylori colonization. As with other human pathogens it has been proposed that Helicobacter species express multiple adhesions that would allow bacterial interaction with various target cell
epitopes and mucin components including fucosylated glycoconjugates, phosphotidylethanolamine, fucose and mannose specific lactins etc.

The rational for site specific targeted drug delivery may be appreciated as a set of desirable events including an exclusive delivery of drug to specific compartment with maximal potential intrinsic activity and concomitantly reduced access of drug to irrelevant non target cells. The targeted delivery to previously inaccessible domains such as *H. pylori* offers distinctive therapeutic potential in the eradication of any strain of *H. pylori*. There are two approaches for development of effective drug delivery systems (a) The drug delivery system may be targeted to *H. pylori* i.e. if the drug delivery system has a ligand that is specific for *H. pylori* adhesion receptors, it will target and kill the microorganism. (b) The drug delivery system having adhesion that is similar to *H. pylori* receptor will target the drug to the infection site and hence such drug delivery system can compete the binding of *H. pylori* with gastric mucosa receptors and inhibit the adhesion of *H. pylori* with stomach. It is expected that oral delivery of broad spectrum antibiotics and gastric mucosal protection through a bacterial specific targeted drug delivery system may result in complete removal of micro-organism in the fundal area of the gastric mucosa due to high concentration of antibacterial drug being attained in this area which may lead to better treatment of peptic ulcer diseases.

Very few drug delivery systems have been designed that have specially targeted the drug to the infected gastrointestinal cell linings. The majority of oral dosage forms are intended simply to achieve controlled gastrointestinal absorption since the stomach is rarely a useful site of absorption and efficient targeting to it has been neglected. Conventional drug delivery systems have not been promising as they address some of the clinical problem of bacterial resistance to antibiotics originally either from impermeability of bacterial envelope of antibiotics or from hydrolysis through β lactamases or exogenous enzymes. Recently several vesicular and particulate carriers have been reported for the oral delivery of drugs with gastrointestinal stabilization and localization. *viz.* polymerized liposomes, microencapsulated liposomes, polysaccharide coated liposomes, surface modified liposomes, surface modified nanoparticles, lipid coated nanoparticles ect.
A growing amount of literature describes the development and application of novel targeting and control release of antibiotics to improve their therapeutic index by encapsulation within liposomes and particulate drug delivery system. Among these novel drug delivery systems, the double liposomes (DL) and nano sized hydrogel anchored lipid vesicles system i.e. nanolipobeads are the recent approaches which have been reported to specifically target the drugs to \textit{H. pylori} at stomach site. Double liposomes can be defined as liposomes within liposomes and have been investigated and found to be effective as drug carrier for oral delivery of compound because the outer liposomes were suggested to protect the inner liposomes against several enzymes present in GIT.

A new nanosized hybrid vesicle system that combine the complementary advantage of liposome and polymeric nano particles i.e. nanolipobeads has been developed. This consists of a PE lipoid bilayer shell that is anchored on the surface of hydrogel polymer core. Therefore it reasonable to expect that assembly of lipid bilayer on spherical hydrogel surface could be useful for the development of dual drug delivery system for receptor mediated targeting. The drugs are incorporated in double liposomes and nanolipobeads having phosphorylethanolamine (PE) on surface which permit target specific delivery of drugs to surface of \textit{H. pylori}.

Although \textit{H. pylori} is highly sensitive to many antibiotics, its eradication from patients requires high concentration of drug to be maintained within the gastric mucosa for a longer duration. Thus it can be expected that oral delivery of broad spectrum antibiotic along with an antisecretory mucosal protectant through a targeted drug delivery system i.e. double liposomes and nanolipobeads to \textit{H. pylori} may result in complete removal of the organism in the fundal area of the gastric mucosa due to ‘plug and seal’ effect of the system and bactericidal drug level being attained in the area for longer period of time (Fig 1.5). With these concepts it was proposed to develop two type of targeted drug delivery system based on PE- \textit{H. pylori} interaction which would be better and effective treatment of peptic ulcer :-

(a) Phosphatidylethanolamine based double liposomes entrapping broad spectrum antibiotic system.
(b) PVA hydrogel nanoparticles anchored lipid vesicles system (nanolipobeads).
Fig. 1.5. Recognition of nanolipobeads by PE surface receptor of *H. Pylori*. 
For the present study two drugs i.e. amoxicillin trihydrate (AMOX) having antibacterial activity and ranitidine bismuth citrate (RBC) having mucosal protective and antisecreatory activity were selected. Further, the dual therapy approach was selected because of possibility of development of resistance toward single antibiotic.

**Rational for selecting Ranitidine bismuth citrate (RBC)**

Ranitidine bismuth citrate is a histamine H₂ receptor antagonist with mucosal protective activity. It is a complex of ranitidine and bismuth citrate which together produce *H. pylori* suppressive activity. After oral administration, ranitidine bismuth citrate dissociate in intragastric fluid giving rise to ranitidine and soluble or insoluble forms of bismuth. It inhibits basal and simulated volume of gastric acid and reducing pepsin content of the secretion. Bismuth containing compound ranitidine citrate is bactericidal to *H. pylori* in vitro and has gastro cytoprotective action. It dose not alter plasma pepsinogen I and II concentration or pepsin activity and has no clinically relevant effect on fasting or postprandial plasma gastrin.

Ranitidine bismuth citrate requires frequent dosing due to its short biological half-life and absorbed only in the stomach and in the initial part of small intestine. It has 50% absolute bioavailability. The traditional oral sustained release formulation releases most of the drug in intestine and colon. Thus the drug has a narrow absorption window and the colonic metabolism of it is partly responsible for poor bioavailability from the colon. Therefore ranitidine bismuth citrate is the best candidate for development of *H. pylori* targeted and stomach site specific drug delivery system. Moreover effectiveness of ranitidine bismuth citrate in eradicating *H pylori* in vivo has been shown to be enhanced by the addition of antibiotic such as amoxicillin trihydrate.

**Rational for selecting Amoxicillin trihydrate (AMOX)**

Amoxicillin trihydrate is known to interfere with the synthesis of peptidoglycan which is part of the cell wall material of the *H. pylori*. Several cell wall enzymes are reversibly inhibited by the amoxicillin and most important being D, D carboxypeptidase which also function as a transpeptidase due to the amino group side chain attached to the basic penicillin structure.
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Amoxicillin is better able to penetrate the outer membrane of some gram (-) bacteria and has broad spectrum activity. *H. pylori* eradication requires high concentration of amoxicillin trihydrate to be maintained within the gastric mucosa for a longer duration. Therefore sustained release drug delivery system of amoxicillin with H₂ receptor antagonist may be very successful and clinically acceptable.

The major objective of the present research work was to develop and characterize double liposomes and nanolipolipobeads based stomach specific and *H. pylori* targeted dual drug delivery systems for effective management of peptic ulcer. It was also proposed to study the receptor mediated targeting propensity of these delivery systems on *H. pylori* through bacteria cell line interaction study. Further it was aimed to compare the bioavailability, antisecretary ulcer protective activity and in vivo % bacterial clearance studies of optimized double liposomal and nanolipobeads formulations. These investigations were carried out under the following plan of work:-

**PLAN OF WORK**

► Exhaustive literature survey.
► Selection of drugs for management of peptic ulcer (Ranitidine bismuth citrate and Amoxicillin trihydrate).
► Physicochemical characterization of drugs and preformulation studies:-
  • Identification
  • Solubility
  • Partition coefficient
  • FTIR study
  • Preparation of standard curve for the quantitative estimation of drug in simulated gastric fluid (pH 1.2), simulated intestinal fluid (pH 6.8) and phosphate buffer saline (pH 7.4).
  • Determination of interference of additives in the estimation of ranitidine bismuth citrate and amoxicillin trihydrate.
► Development of a spectrophotometric method for the determination of amoxicillin trihydrate and ranitidine bismuth citrate in PBS (pH 7.4).
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- Preparation of Phosphatidylcholine (PC) inner liposomes and their characterization for vesicle shape, vesicular size distributions, zeta potential, polydispersity index, vesicles counts (number of vesicles/ mm³), % drug entrapment efficiency and in vitro ranitidine bismuth citrate release study from optimized inner liposomes formulation in SGF (pH 1.2), SIF (pH 6.8) and PBS (pH 7.4).

- Preparation of double liposomes by glass beads method and their characterization for vesicular shape, vesicular size distributions, zeta potential, polydispersity index, vesicles counts (number of vesicles/ mm³), % drug entrapment and in vitro release of amoxicillin trihydrate and ranitidine bismuth citrate from double liposomes formulation in PBS (pH 7.4).

- Preparation of PVA hydrogel nanoparticles by emulsification freeze thaw cycling method and optimization of formulation variables like PVA concentration, ranitidine bismuth citrate (drug) concentration, Surfactant concentration (Span 80) and PVA: Oil phase ratio. Process variables like stirring speed, stirring time and number of freeze thaw cycles.

- Characterization of optimized PVA hydrogel nanoparticles for shape and surface morphology, average particle size, zeta potential and polydispersity index, % drug entrapment efficiency and in vitro drug release study in SGF (pH 1.2), SIF (pH 6.8) and PBS (pH 7.4).

- Preparation of acylated PVA hydrogel nanoparticles and their characterization by FTIR.

- Preparation of nanolipobeads formulations by vesicular fusion method and their characterization for surface morphology by (SEM), fluorescent microscopy, confocal laser scanning microscopy (CLSM), average particle size, surface charge, polydispersity index, degree of swelling, % drug entrapment efficiency and in vitro release study of AMOX and ranitidine bismuth citrate from optimized nanolipobeads formulation in PBS (pH 7.4).
Stability study of optimized double liposomes and nanolipobeads formulation for

- Effect of storage on vesicles size, structural integrity, vesicles count and% residual drug content of double liposomes.
- Effect of storage on particle size, structural integrity and % residual drug content of nanolipobeads formulation.

*In vitro* *H. pylori* bacterial cell line interaction study (*Ex vivo* study) of optimized double liposomes and nanolipobeads for

- Determination of the minimum inhibitory concentration (MIC) of AMOX and RBC by disk diffusion method and Epsylometer strip test (E test)
- Determination of the targeting propensity by agglutination assay and *in situ* adherence assay
- *In vitro* % growth inhibition study of *H. pylori* strain.

*In vivo* study of optimized double liposomal and nanolipobeads formulation in albino rats for

- Blood serum level study of drug(s)
- Antisecretory and ulcer protective activity.
- % *H. pylori* clearance study

Compilation of results, statistical treatment and interpretation of data.