Chapter 1

Introduction

1.1 ACUTE BACTERIAL EXACERBATIONS OF CHRONIC BRONCHITIS (ABECB)

Exacerbations were associated with decreased lung function and increased morbidity and mortality. Environmental factors such as airborne irritants and allergens, as well as viral and bacterial infections, play a key role in acute exacerbations of chronic bronchitis. The majority of infective exacerbations are presumed to be bacterial in origin. Acute bacterial exacerbations of chronic bronchitis (ABECB) are characterized by increased cough, sputum production, dyspnea and sometimes fever which are responsible for a significant amount of patient morbidity.

Furthermore, approximately one in five patients with ABECB will require hospitalization (Fuso et al, 1995). The major pathogens in ABECB include *Haemophilus influenzae*, *Streptococcus pneumonia* (Monso et al, 1995) and *Moraxella catarrhalis*. Patients experiencing multiple exacerbations per year, not only with these three core organisms but also due to Gram-negative bacilli (e.g. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) become more probable to cause multiple exacerbations. In all the above said events, the presence of pathogenic bacteria in the bronchial airways can precipitate progressive airway deterioration and ultimately lead to deterioration in lung function. As such, the major goals of therapy are to provide rapid clinical resolution, eradicate the causative pathogens, and to promptly return the patient's respiratory function to their pre-exacerbation baseline.

A decade ago, Anthonisen et al (1987) reported that antimicrobials appeared to be beneficial in patients with AECB. During last two decades beta-lactams have been commonly prescribed for patients experiencing an acute exacerbation (Saint et al, 1995). Cefditoren pivoxil is a third generation semi-synthetic cepholosporin antibiotic exhibiting bactericidal action by inhibiting cell wall synthesis and used to treat uncomplicated skin and skin structure diseases, *Haemophilus influenza*, *Klebsiella pneumonia* and *Staphylococcus aureus*, pharyngitis, tonsillitis, community-acquired pneumonia and acute bacterial exacerbation of chronic bronchitis (Alvarez et al, 2005).
1.1.1 RESPIRATORY TRACT INFECTIONS

Respiratory tract system is an important system for the gaseous exchange in our body composed of upper respiratory tract and lower respiratory tract organs. It is therefore constantly exposed to the gaseous environment, including particulate organic material, such as bacteria, viruses and spores. Extensive findings on respiratory tract infection includes two types of infections are upper respiratory tract infection and lower respiratory tract infection.

1.1.1.1 UPPER RESPIRATORY TRACT INFECTIONS

Upper respiratory tract infections (URTIs) are defined as acute febrile illnesses presenting with cough, coryza, sore throat, or hoarseness, which are very common in the community and are one of the major reasons for visiting primary care physicians, particularly during the winter season. The vast majority of URTIs cases are benign, so additional investigation to identify the precise etiology is not justified in routine practice.

Data in the literature regarding these etiologies are based on studies that did not utilize advanced diagnostic techniques. Prospective studies were conducted to identify the etiological agents of URTIs in adults. Viruses such as influenza A and B viruses, adenovirus, respiratory syncytial virus, para-influenza viruses, and Epstein Barr virus are some of the main etiologies for URTIs. The main bacteria that are responsible for causing URTIs include *Chlamydia pneumoniae*, *Legionella spp.*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. Upper respiratory tract infections are usually caused by viruses and are mostly self-limiting. Exposure to many upper respiratory pathogens occurs at a young age. The sections of the upper respiratory tract commonly affected are the pharynx, epiglottitis and the sinuses (Slotar et al, 2002).
1.1.1.1 PHARYNGITIS

Pharyngitis was the most common upper respiratory tract infection involves inflammation of the oropharynx or the nasopharynx (Braun et al, 2000). The main symptom was sore throat and difficulty during swallowing. Pharyngitis was caused by a range of viruses and bacteria. Viral pharyngitis is generally more common than bacterial pharyngitis and less likely to cause complications. It was quite difficult to distinguish between viral and bacterial cause of sore throat (Der Mar, 1992). The most common bacteria was Group A *streptococcus* Corynebacterium diphtheria, Neisseria gonorrhoeae, Chlamydophila pneumonia, Mycoplasma pneumoniae, (Bisno, 2001).

1.1.1.2 LOWER RESPIRATORY TRACT INFECTIONS

Causative agents of lower respiratory infections are viral, fungal, parasitical and bacterial. Symptoms include cough, fever, chest pain, and sputum production.

1.1.1.3 BRONCHITIS

Bronchitis is a lower respiratory tract infection that causes bronchial inflammation which causes a cough. Most commonly about 95% of cases were virus originated Bronchitis. During bronchitis the cough will lasts about a week to ten days. Occasionally around 25% of cases had cough for months (Falck et al, 1994).

1.1.1.4 EMPHYSEMA

Emphysema was a complication of bronchopleural fistula caused by tuberculosis and actinomycosis. It was a, progressive disease of the pulmonary disorder that primarily causes shortness of breath due to over-inflation of the alveoli. Most commonly this disease needs a surgery to the oesophagus (Light, 2002).

1.1.2 COMMUNITY ACQUIRED PNEUMONIA (CAP)

CAP is the most common lower respiratory tract infection usually occurs when bacteria from the upper respiratory system or any undigested material in the stomach are aspirated into the lung. Infection also spreads through the growth of microorganisms in the lungs and also by inhalation of aerosolized material. According to the recent survey of Infectious Disease Society of America (IDSA), only in USA it was found that greater than 4 million adults were diagnosed with CAP and it was reported that 1.5 million were lead to hospitalization of 14% mortality. Pneumonia is the sixth leading cause of death in
USA. Recently, it was reported that Asian countries - Nepal, Bangladesh, India and Indonesia account for 40% of global CAP.

1.1.3 TREATMENT OF RESPIRATORY TRACT INFECTION

Based on the type of causative organisms the pneumonia treatment will be variable. Bacterial originated pneumonia was treated with antibiotics (Moussaoui et al, 2006). Common anti infective agent used to treat bacterial pneumonia includes, third and fourth-generation cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides (Gonzales et al., 2001). These antibiotics are usually given intravenously. Treatment for acute bronchitis in HIV-infected patients does not varies when compared to normal acute bronchitis patient (McGuinness et al., 1997). Thus Cefditoren pivoxil is a third generation semi-synthetic cephalosporin antibiotic exhibiting bactericidal action by inhibiting cell wall synthesis and used to treat the above mentioned infections including uncomplicated skin and skin structure diseases, Haemophilus influenza, Klebsiella pneumonia and Staphylococcus-aureus, pharyngitis, tonsillitis, community-acquired pneumonia and acute bacterial exacerbation of chronic bronchitis (Alvarez et al, 2005). Therefore, Cefditoren pivoxil was selected for the present investigation.

1.2 GASTRO RETENTIVE DRUG DELIVERY SYSTEMS

Oral administration is still niche among the many routes of drug delivery and it is a common and convenient method for introducing drugs in to the systemic circulation. Novel drug deliveries like Gastro-retentive dosage forms were the topic of interest for the delivery of drugs to the upper GI tract and also for drug release, prolongation and absorption (Kenneth and Waterman, 2007). However, this approach is engaged with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract (GIT) due to variable gastric emptying and motility. Furthermore, the relatively brief gastric emptying time (GET) in humans which normally averages 2-3 h through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose (Rouge, 1996). Therefore, control of placement of a drug delivery system (DDS) in a specific region of the GI tract offers advantages for a variety of important drugs characterized by a narrow
absorption window in the GIT or drugs with a stability problem (Singh, 2000). These considerations have led to the development of a unique oral controlled release dosage form with gastro-retentive properties.

Fig1.1. Flowchart of gastro retentive drug delivery systems.

1.2.1 FACTORS CONTROLLING GASTRIC RETENTION OF DOSAGE FORMULATIONS

The anatomy and physiology of stomach contain various parameters to be considered in the development of gastroretentive dosage forms.

- Density, size and shape of the dosage form, nature of food intake and its frequency of intake.
- Posture, gender, age, sex, sleep, body mass index, physical activity and diseased states of the individual (e.g. chronic disease, diabetes etc.)
- The molecular weight and lipophilicity of the drug depending on its ionization state are also important parameters (Larhed, 1997).

1.2.2 APPROACHES TO ACHIEVE GASTRIC RETENTION

FLOATING DRUG DELIVERY SYSTEMS (FDDS):

Floating FDDS was an effective technology to increase the gastric residence time in order to improve the bioavailability of the drug. FDDS are low-density systems that have sufficient buoyancy to float over the gastric contents and remain in the stomach for a prolonged period (Chikawa, 1991). Based on the mechanism of buoyancy FDDS was classified into effervescent and non-effervescent system.

I) Effervescent systems

These floating delivery systems utilize gas generating agents, carbonates and other organic acids to generate carbon dioxide gas which reduces the density of the formulation and makes the formulation to float in gastric fluid. Recently an alternative approach for these type of systems was designed by incorporating of matrices prepared with swellable polymers such as Methocel or polysaccharides, containing chambers of liquid that generates gas at body temperature which produces an upward motion of the dosage form and maintains its buoyancy (Chikawa, 1991).

These effervescent systems further classified into two types:

1) Gas generating systems.
2) Volatile liquid or Vacuum containing systems.

II) Noneffervescent systems

Non-effervescent systems contains one or more gel-forming (Carbopol), highly swellable, cellulosic hydrocolloids (e.g., hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose [HPMC], and sodium carboxymethylcellulose), polysaccharides, or matrix-forming polymers (e.g., polycarbophil, polyacrylates, and polystyrene) into tablets or capsules (Hilton, 1992). Upon coming into contact with gastric fluid, these gel formers, polysaccharides, and polymers get hydrate and form a colloidal gel barrier and controls the rate of fluid penetration into the dosage form and
amount of drug release (Sheth, 1979 and 1984). The air was entrapped by the swollen polymer and reduces the density of dosage form and enhances its buoyancy.

**NON-FLOATING DRUG DELIVERY SYSTEM**

This approach involves formulation of dosage forms with high density than the normal stomach content (~ 1.004 gm/cm\(^3\)). These formulations was prepared by coating drug on a heavy core or mixed with inert materials such as ferrous powder, barium sulphate, zinc oxide and titanium oxide etc (Vyas, 2006). These inert materials increase the density of dosage formulation by up to 1.5- 2.4 gm/cm\(^3\). The dosage formulations with a density of 2.5 gm/cm\(^3\) were suitable for significant prolongation of gastric residence time (Clarke, 1993). But, till now the effectiveness of this system was not observed in human beings (Moes, 2003).

Based on different mechanisms non-floating drug delivery systems were classified in to the following methods.

**A) Bio Mucoadhesive systems**

These systems was designed to bind to the bind to the gastric epithelial cell surface and increase the gastro retention time by increasing the contact and duration between the dosage form and the biological membrane (gastric-wall).Thus the bioavailability of the formulation will be improved (Wilding, 1994).

On the basis of polymers used for binding to the mucin-epithelial surface, bio muco adhesive systems were classified into two categories (Park, 1984).

**a. Hydration-mediated adhesion**

These systems contain polymers which are hydrophilic in nature and absorbs large amount of water and attain bio-adhesive properties. Certain hydrophilic polymers tend to imbibe large amount of water and become sticky, thereby acquiring bioadhesive properties (Amit, 2010).

**b. Bonding-mediated adhesion**

These systems attain bio-adhesion by various bonding mechanisms like physico-mechanical bonding and chemical bonding. These chemical bonds include either covalent (primary) or ionic (secondary) in nature. The secondary chemical bonds will have Vander Waals interactions and stronger hydrogen bond interactions between the hydroxyl or
carboxyl groups of polymers used in the formulation and epithelial cell surface (Amit, 2010).

B) Receptor-mediated adhesion

Certain polymers like phosphotidyl cholines of plant origin can bind to specific receptor sites on the surface of cells, thereby enhancing the gastric retention of dosage forms. Some phosphotidyl cholines such as tomato lecithins will show more affinity towards the sugar groups present in mucus or on the glycocalyx.

C) Expandable, swellable and unfoldable Systems

These systems increase gastroretentive capacity of the pharmaceutical dosage form mainly by increasing its size above the diameter of the pylorus of the stomach (Fig.1.2). If the dosage form can attain the larger size than pylorus, then the gastro-retentive time of that dosage form will be possible for long time. This size enlargement of the dosage form should be achieved very quickly; otherwise the pharmaceutical dosage form will be evacuated through the pylorus. Thus, the following configurations were required to develop an expandable system to prolong GRT.

i. Initial small size of dosage form for oral intake,

ii. The formulation should be expanded for gastroretention.

iii. A final small form enabling evacuation following drug release from the device.

In addition the developed unfoldable systems are available in various shapes as shown in (Fig.1.2). The concept was to make a carrier, such as a capsule, which extends in the stomach. (Caldwell et al,1988). Caldwell et al was proposed different geometric forms like tetrahedron, ring or planar membrane (4-lobed, disc or 4-limbed cross form) of bioerodible polymer compressed within a capsule (Clarke, 1993).

D) High-density systems

Sedimentation was employed as retention mechanism for this type of high density systems. Normal density of gastric contents was (1.004 g/cm³). A density of ~3g/cm³ seems necessary for significant prolongation of gastric residence time. When high density pellets was given to the patient, it will sink to the bottom of the stomach and are entrapped in the folds of the antrum and withstand the peristaltic waves of the stomach wall (Shweta, 2005). Commonly used excipients are barium sulphate, zinc oxide,
titanium dioxide and iron powder, etc. These materials increase density of formulation by up to 1.5–2.4 g/cm³ with their high density. The only major drawbacks with this systems is that it is technically difficult to manufacture them with a large amount of drug (>50%) and to achieve the required density of 2.4–2.8 g/cm³.

E) Magnetic systems

This approach was developed to increase the gastro retention time based on the simple principle that the dosage form contains a small internal magnet, and a magnet was placed on the abdomen over the position of the stomach. Ito et al, used the technological approaches in rabbits with bio-adhesive granules containing ultra-fine ferrite (α-Fe₂O₃). They guided them to oesophagus with an external magnet (~1700G) for the initial 2 minutes and almost all the granules were retained in the region after 2 hours (Abubakr, 2000). The main discomfort with these systems was, the placement of external magnet.
should be positioned with a degree of precision which compromise patient’s compliance (Huang, 2000).

**F) Raft systems**

Raft systems was developed to increase floating behavior of the pharmaceutical dosage form by incorporating alginate gels which had a carbonate component that interact with gastric acid secretions and generate bubbles in the gel matrix, which enhances the floating of dosage forms (Patel Geeta, 2007).

**G) Super porous hydrogel systems**

Super porous hydrogels were originally developed as a controlled drug delivery system to retain drugs in the gastric medium (Chen et al, 2000). These systems should instantly swell in the stomach and maintain their integrity in the stomach environment and release the pharmaceutical active ingredient. For years, the synthetic features and properties of these super porous hydrogel materials have been modified and improved to meet the requirements for gastric retention applications (Peppas et al, 2000). Recent developments include use of super porous hydrogels that expand dramatically (hundreds of times their dehydrated form within a matter of seconds) when suspended in water. With pore size ranging, 10 nm to 10 µm, absorption window by conventional hydrogel is a very slow process and several hours may be needed to reach an equilibrium state during which, parameter evacuation of the dosage form may occur (Bardonnet, 2006). Super porous hydrogels, average pore size less than 100 µm, swell to equilibrium size within a minute, due to rapid water uptake by capillary wetting through numerous interconnected open pores.

1.3 LIPOSOMES

Liposomes are microscopic vesicles consisting of phosphor-lipid bilayers which enclose aqueous compartments and are utilized as drug delivery systems for both hydrophilic and lipophilic drugs (Nesr et al, 2010). Liposomes are one of the most suitable drug delivery systems to deliver the drug to the target organ and minimize the distribution of the drug to non-target tissues. The importance of liposomes as drug delivery vehicle is now becoming more established. This applies particularly to the ability of liposomes to buffer the toxicity of entrapped drugs while maintaining efficacy,
the area in which liposomes display therapeutic promise as carriers for anti bacterial agents, anticancer, anti parasitics, anti viral, anti fungal and ocular liposomes (Jia et al, 2008). These findings make it increasingly necessary to develop liposomes to satisfy pharmaceutical considerations.

Table 1.1 Various types of Gastro-retentive products available in the market (Chawla, 2004)

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Active Ingredient(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cifran OD ®</td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td>Madopar ®</td>
<td>L-DOPA and Benserazide</td>
</tr>
<tr>
<td>Valrelease ®</td>
<td>Diazepam</td>
</tr>
<tr>
<td>Topalkan ®</td>
<td>Aluminum -magnesium antacid</td>
</tr>
<tr>
<td>Almagate FlatCoat ®</td>
<td>Aluminum -magnesium antacid</td>
</tr>
<tr>
<td>Liquid Gavison ®</td>
<td>Aluminium hydroxide,</td>
</tr>
<tr>
<td>Conviron®</td>
<td>Ferrous sulfate</td>
</tr>
<tr>
<td>Cytotec®</td>
<td>Misoprostal</td>
</tr>
</tbody>
</table>

Liposomal size was directly related to the method of preparation and size ranges from 50 nm to several microns. They form spontaneously when the phospholipids are dispersed in aqueous media. Vesicles can be constructed of natural constituents such that the vesicle membrane forms a bilayer structure which was the principal identical to the lipid portion of natural cell membrane (Barani et al, 2008). Their ability to mimic the behavior of natural membranes and also to be degraded by the same pathways makes vesicles a very safe and efficacious vehicle for medical applications (Mozafari, 2008). Vesicles can be composed even of entirely artificial components, chosen for their improved chemical properties (e.g. fatty acids, double chain secondary amines, and
cholesterol derivates). Moreover, liposomes were used to entrap both hydrophilic and lipophilic molecules; and be used as drug carrier for both types of drug molecules (Gomez-Hens, 2006).

Cholesterol was included in most of the liposomal formulations to improve bilayer characteristics of vesicles, increase micro viscosity of the bilayers, to reduce permeability of the membrane to water soluble molecules, stabilize the membrane and increase the rigidity of the vesicles.
Methods of liposomes preparations

- Passive loading techniques
- Active loading techniques

Mechanical dispersion methods
- Lipid film hydration by hand shaking, non-hand shaking or freeze drying
- Micro-emulsification
- Sonication
- French pressure cell
- Membrane extrusion
- Dried reconstituted vesicles
- Freeze-thawed liposomes

Solvent dispersion methods
- Ethanol injection
- Ether injection
- Double emulsion
- Reverse phase evaporation vesicles
- Stable pluri lamellar vesicles

Detergent removal methods
- Detergent (cholate, alkylglycoside, triton x-100) removal from mixed micelles by:
  - Dialysis
  - Column chromatography
  - Dilution
  - Reconstituted sendai virus enveloped

Fig. 1.3. Methods of liposomal preparations
1.3.1. CLASSIFICATION OF VESICLES

The most common index of characterization in current use of liposomes was according to the size:

- **Multilamellar vesicles (MLVs):** Vesicles cover a wide range of sizes (100 – 1000 nm) and consist of five or more concentric lamellae.

- **Small unilamellar vesicles (SUVs):** These vesicles are at or close to the lower size limit (15 -25 nm), so they will be a relatively homogeneous population in terms of size.

- **Intermediate-sized unilamellar vesicles (IUVs):** Vesicles with diameter of the order of magnitude of 100 nm.

- **Large unilamellar vesicles (LUVs):** Vesicles with diameter of the order of 1000 nm.

Very often the preparations of liposomes are meta-stable which states that the state of free enthalpy is not in equilibrium with the environment. As a result, the vesicles change their lamellarity, size distribution and shape with time. For example small vesicles tend to form larger ones and large vesicles smaller ones. Nevertheless, the stability seems to be the optimal in a range of about 100-300 nm.

1.3.2. PREPARATION METHODS OF LIPOSOMES

1) **Multi lamellar vesicles (MLV):**

(a) **Lipid hydration method:** In this method a solution of lipid was taken and evaporated which leaves a film in the vessel on complete evaporation of the solvent. The film is hydrated with the solvent. The solution so resulted was subjected to centrifugal force which produce liposomes. This method is not as advantageous as it involves very low loading of dose. The drug content can be increased by the use of immiscible solvent (petroleum ether or diethyl ether) to it.

(b) **Solvent spherule method:** This method produces liposomes of uniform size distribution. It was achieved by the use of lipid dissolved hydrophobic solvent dispersed in the aqueous solvent.
2) Small unilamellar vesicle:
   (a) **Sonication method:** In this method the multi laminar liposomes are taken and they are subjected to sonication by bath type sonicator or probe type sonicator. But the major drawback was that as we have selected the MLV which posse’s very small internal volume with in SMV so formed will also be having a small internal diameter.
   (b) **French pressure cell method:** In this method the MLV are allowed to pass through a small orifice at a pressure of 20000 psi and a temperature of $4^\circ C$ there is a reduction in the outer layers during the passage and would result in SULV.

3) Large unilamellar vesicles: It has very high encapsulation efficiency and most of the drugs are used to get liposome by these techniques.
   (i) **Solvent injection method:**
      (a) **Ether infusion method:** A lipid solution is prepared by dissolving it in diethyl ether or ethanol / ether. This solution was injected into the aqueous solution of material to be incorporated under reduced pressure and at a temperature of 55 to 60 $^\circ C$.
      (b) **Ethanol injection method:** The ethanolic solution of lipid was injected rapidly into excess of buffer solution. But so formed liposomes will have a wide range of heterogeneity of 30-110 nm.
   (ii) **Detergent removal method:** Detergents are prepared at their critical miceller concentration to solubilize the lipids. Once the lipids are solubilized the detergent is evaporated by dialysis or by gel chromatography or other methods.
   (iii) **Reverse phase evaporation method:** A w/o emulsion is prepared by a brief sonication of the two medium. The organic phase is subjected to high pressure to remove it. It results in the formation of a viscous gel. The liposomes will be formed when the residual solvent is evaporated with a reduced pressure under continuous rotary evaporation. We can achieve appreciably high encapsulation efficiency of about 65%. And with modified reverse phase evaporation method the encapsulation efficiency can be increased to 80%.
(iv) Calcium induced fusion method: Calcium addition to SUV will cause fusion of layers and will result in multi laminar spiral configuration. When EDTA is added to it that will result in the formation of LUV.

(v) Micro fluidization method: MLV are allowed to pass through the micro fluidizer at an inlet pressure of 40 psi they are allowed to repeat the cycling for 25 times or more that will result in the formation of small lamellar vesicles.

(vi) Freeze-thaw Method: SUV are subjected to rapid freezing and then allowed for slow thawing. This leads to formation of LUV due to fusion of the SUV.

Production of liposomes in industries

Most of the methods proposed for the preparations of liposomes are not suitable for scale up purpose as the complications like the nature of solvent, reproducibity, sterility plays a major role in ruling out many of the above proposed methods.

Most commonly used methods at industrial level are:

1. Detergent dialysis method:
3. Proliposomes
4. Freeze drying method
Table 1.2 Marketed liposomal and lipid-based formulations.

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>DRUG</th>
<th>INDICATIONS</th>
<th>YEAR APPROVED</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approved products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AmBisome (Gilead)</td>
<td>Amphotericin B</td>
<td>Fungal infections, Leishmaniasis</td>
<td>1990 (Europe), 1997 (USA), 2000</td>
<td>(M. R. Hann et al., 2001)</td>
</tr>
<tr>
<td>DaunoXome (Galen)</td>
<td>Daunorubicin</td>
<td>Kaposis's sarcoma</td>
<td>1995 (Europe), 1996 (USA)</td>
<td>(P. Muggia et al., 1996)</td>
</tr>
<tr>
<td>Doxil/Caelyx (Johnson &amp; Johnson)</td>
<td>Doxorubicin</td>
<td>Kaposis's sarcoma, Ovarian cancer</td>
<td>1999 (Europe), 2000 (USA)</td>
<td>(C. E. Peete et al., 2007)</td>
</tr>
<tr>
<td>DaunoXome (Galen)</td>
<td>Daunorubicin</td>
<td>Multiple myeloma + Volcade</td>
<td>2002 (Europe), 2007 (USA)</td>
<td>(R. Rodin et al., 2001)</td>
</tr>
<tr>
<td>Myocet (Cephalon)</td>
<td>Doxorubicin</td>
<td>Breast cancer + cyclophosphamide</td>
<td>2000 (Europe), 2000 (USA)</td>
<td>(J. Walsh et al., 1998)</td>
</tr>
<tr>
<td>Amphotec (Intermenu)</td>
<td>Amphotericin B</td>
<td>Invasive aspergillosis</td>
<td>1996 (Europe), 2000 (USA)</td>
<td>(T. J. Walsh et al., 2001)</td>
</tr>
<tr>
<td>Ablavar (Eizoon)</td>
<td>Amphotericin B</td>
<td>Aspergillosis</td>
<td>1995 (Europe), 2000 (USA)</td>
<td>(T. J. Walsh et al., 2001)</td>
</tr>
<tr>
<td>Vitodysne (QLT)</td>
<td>Vincristine</td>
<td>Testicular cancer</td>
<td>1996 (Europe), 2000 (USA)</td>
<td>(T. J. Walsh et al., 2001)</td>
</tr>
<tr>
<td>DepoCidr (Takara)</td>
<td>Morphine sulfate</td>
<td>Pain following surgery</td>
<td>2004 (Europe), 2000 (USA)</td>
<td>(T. J. Walsh et al., 2001)</td>
</tr>
<tr>
<td>DepoCyt (Pacira)</td>
<td>Vincristine</td>
<td>Lymphoma</td>
<td>1995 (Europe), 2000 (USA)</td>
<td>(T. J. Walsh et al., 2001)</td>
</tr>
<tr>
<td>Digipriyan (AstraZeneca)</td>
<td>Propofol</td>
<td>Anesthesia</td>
<td>1995 (Europe), 2000 (USA)</td>
<td>(T. J. Walsh et al., 2001)</td>
</tr>
<tr>
<td>Estrabu (King)</td>
<td>Estrogen</td>
<td>Menopausal therapy</td>
<td>2003 (Europe), 2000 (USA)</td>
<td>(T. J. Walsh et al., 2001)</td>
</tr>
<tr>
<td>Lipo-Dox (Taiwan Liposome)</td>
<td>Doxorubicin</td>
<td>Kaposis's sarcoma</td>
<td>2004 (Europe), 2000 (USA)</td>
<td>(T. J. Walsh et al., 2001)</td>
</tr>
<tr>
<td>Marqibo (Talon)</td>
<td>Vincristine</td>
<td>Acute lymphoblastic leukemia</td>
<td>2004 (Europe), 2000 (USA)</td>
<td>(T. J. Walsh et al., 2001)</td>
</tr>
<tr>
<td>Products in clinical trials</td>
<td></td>
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</tr>
<tr>
<td>SPI-077 (Alza)</td>
<td>Cisplatin</td>
<td>Solid tumors</td>
<td>Phase II (development terminated)</td>
<td>(M. S. Newman et al., 1999) (N. Soethausen et al., 2010)</td>
</tr>
<tr>
<td>CPX-151 (Celator)</td>
<td>Cytarabine-daunorubicin</td>
<td>Acute myeloid leukemia</td>
<td>Phase II (development terminated)</td>
<td>(K. Riviere et al., 2011)</td>
</tr>
<tr>
<td>CPX-1 (Celator)</td>
<td>Irinotecan HCl-bexoridine</td>
<td>Colorectal cancer</td>
<td>Phase II (development terminated)</td>
<td>(A. Dicker et al., 2010)</td>
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<tr>
<td>MM-308 (Merrimack)</td>
<td>CPT-11</td>
<td>Gastric and pancreatic cancer</td>
<td>Phase II (development terminated)</td>
<td>(A. Dicker et al., 2010)</td>
</tr>
<tr>
<td>MM-302 (Merrimack)</td>
<td>ErbB2/Erbb3-targeted doxorubicin</td>
<td>ErbB2-positive breast cancer</td>
<td>Phase II (development terminated)</td>
<td>(A. Dicker et al., 2010)</td>
</tr>
<tr>
<td>MIP-456 (Medipi)</td>
<td>Topotecan</td>
<td>Gastric cancer and gastro-esophageal junction</td>
<td>Phase II (development terminated)</td>
<td>(A. Dicker et al., 2010)</td>
</tr>
<tr>
<td>Alcos resin (Talon)</td>
<td>Vinorelbine</td>
<td>Newly diagnosed or relapsed solid tumors</td>
<td>Phase II (development terminated)</td>
<td>(A. Dicker et al., 2010)</td>
</tr>
<tr>
<td>Lipoctic (Regolux)</td>
<td>Cisplatin</td>
<td>Non-small cell lung cancer</td>
<td>Phase II (development terminated)</td>
<td>(A. Dicker et al., 2010)</td>
</tr>
<tr>
<td>L-amantadine (Callisto)</td>
<td>Annamycin</td>
<td>Adult relapsed ALL</td>
<td>Phase II (development terminated)</td>
<td>(A. Dicker et al., 2010)</td>
</tr>
<tr>
<td>ThermaDox (Celsion)</td>
<td>Thermodoxensitive doxorubicin</td>
<td>Primary hepatocellular carcinoma</td>
<td>Phase II (development terminated)</td>
<td>(K. Riviere et al., 2011)</td>
</tr>
<tr>
<td>Endo-Tag-1 (Medigene)</td>
<td>Cationic liposomal paclitaxel</td>
<td>Pancreatic cancer</td>
<td>Phase II (development terminated)</td>
<td>(M. Kast et al., 2012)</td>
</tr>
<tr>
<td>ALN-TTR ALN-PCS (Alnylam)</td>
<td>siRNA targeting transthyretin (TTR)</td>
<td>Triple negative breast cancer</td>
<td>Phase II (development terminated)</td>
<td>(M. Kast et al., 2012)</td>
</tr>
<tr>
<td>ALN-VSP (Alnylam)</td>
<td>siRNA targeting PCSH9 RNA targeting liver cancer</td>
<td>Hyperplthesis</td>
<td>Phase II (development terminated)</td>
<td>(M. Kast et al., 2012)</td>
</tr>
<tr>
<td>TKM-PLX1 TKM-ApoB (Takara)</td>
<td>RNAi targeting polo-lowe kinase 1 (POLQ) RNAi targeting apoB</td>
<td>Liver tumors</td>
<td>Phase II (development terminated)</td>
<td>(M. Kast et al., 2012)</td>
</tr>
<tr>
<td>Stimuvax (Oncotargets/Merck)</td>
<td>Anti-MUC1 cancer vaccine</td>
<td>Non-small cell lung cancer</td>
<td>Phase II (development terminated)</td>
<td>(M. Kast et al., 2012)</td>
</tr>
<tr>
<td>Exapar (Pacira)</td>
<td>Bupivacaine</td>
<td>Nerve block</td>
<td>Phase II (development terminated)</td>
<td>(M. Kast et al., 2012)</td>
</tr>
</tbody>
</table>

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1.4 LITERATURE REVIEW ON GASTRO RETENTIVE DRUG DELIVERY SYSTEMS

1.4.1 HYDROGELS

A novel sponge hydrogel of chitosan and silk cross linked by genipin was developed by Silva et al (2008). It was reported that the prepared hydrogels were used for cartilage tissue engineering because of its proliferation, adhesion and matrix production of chondrocyte type of cells.

PH-sensitive chitosan based tri poly phosphate hydrogel beads were developed by Sun et al (2011). In this study glipizide was formulated with crosslinking of chitosan hydrogels. The hydrogel matrix was influenced with the ionization of functional groups along with the polymer chains present in the polymers and ionization of crosslinking agents to achieve controlled glipizide release.

1.4.2 SUPER POROUS HYDROGELS

Super porous hydrogels (SPH) was first developed by Chen et al (1999), with fast swelling kinetics and super absorbent properties. In this study SPH were prepared by polymerization and cross linking different vinyl monomers in the presence of a foaming agent, a foam stabilizer and a foaming aid. To preserve the porous structure of SPH and for dehydration alcohol were used. In this study SPH rate of water absorption was rapidly increased by interconnecting the pores inside the hydrogel structure.

Park et al (2001) for the first time introduced SPH composites by modifying conventional SPHs through the addition of super disintegrants into the formulation. Crosslinked sodium carboxymethylcellulose (Ac-Di-Sol), cross linked Sodium starch glycolate (Primojel) and crosslinked polyvinyl pyrrolidone (Crosovidone) have been tried for these preparations.
Park et al (2005) developed SPH-based gastro retentive platforms by using chitosan and glycol chitosan. After optimization of gelation and foaming kinetics in different acidic conditions it was concluded that glycol chitosan hydrogels offer better swelling properties over the chitosan hydrogels. However, the hydrogel swelling property was found significantly dependent on the foaming/drying method, pH and crosslink density.

The SPH gastro retentive tablets were developed by Omidian et al (2005b) using particles of acrylic acid/sulfopropyl acrylate copolymers mixed with gelatin and tannic acid. In this study the results revealed that the hydrogen bonding between gelatin and tannic acid, as well as the carboxyl groups on the polymeric carrier, create an integrated matrix, which was shown to be stable after swelling. In a 40-min period, the gastro-retentive tablet was swelled up to 30 times of its initial volume. Furthermore, it was reported that the swollen tablet could withstand up to 16 KPa compression force before breaking apart. Thus SPH gastro retentive tablets attained its gastro retentive capacity.

Omidian et al (2006) stated that the major requirements for a pharmaceutical dosage form to had gastro retentive capacity was as follows: the dosage form should have good swelling rate (within minutes), swelling capacity (preferably 8-15% v/v), shape, flexibility, a controlled disintegration, ease of drug loading, stability and mechanical strength (resist pressures in the range 0.5–2.0 N cm\(^2\), preferably in the fed state.

1.4.3 DIFFERENT TYPES OF FLOATING DRUG DELIVERY SYSTEMS

Kawashima et al (1991), Prepared multiple unit hollow microspheres (micro balloons) with acrylic resin containing tranilast and their drug release characteristics were studied. The enteric coated polymer containing the drug in the polymeric shell showed controlled drug delivery by floating mechanism.

The use of mucoadhesive microspheres consisting of a drug and carbopol 934P dispersed within a waxy matrix of polyglycerol esters of fatty acids were developed by
Akiyama and Nagahara (1999). These systems were found to adhere to the stomach mucosa in rats and Mongolian gerbils and prolong the drug’s gastrointestinal residence time after oral administration. It was reported that the adherence was attributed due to the hydration and swelling of carbopol in the microspheres upon contact with water.

Floating and swelling features of gastro retentive ofloxacin formulation was developed by Chavanpatil et al (2005). Psyllium husk, HPMC K100M, crospovidone and their combinations were used as polymers in this study. Results revealed that psyllium husk and HPMCK100M were found to increase the dimensional stability of the formulations. It was also found that In-vitro drug release rate increases with increasing amount of crospovidone due to the increased water uptake, and hence increased driving force for drug release. In-vivo studies were carried out in 24 healthy human volunteers and results showed relative bioavailability of 97.55% compared to the marketed product Zanocin.

Hydrodynamically balanced capsules were prepared by physical mixing of various grades of HPMC and poly (ethylene oxide) (PEO) alone as well as in combinations (Ali et al 2006). In this study a combination of various grades of Eudragit and PEO and two different solvents were used in combination for formulating floating microspheres using solvent diffusion technique for preparation of multiple unit system.

A new approach of mucoadhesive-floating effervescent tablets were developed using polyacrylic acid (AA), polymetacrylic acid (MAA), citric acid, sodium bicarbonate, sodium carboxymethyl cellulose (CMC) and hydroxypropyl methylcellulose (HPMC) by Varshosaz et al (2006). In this study the formulated tablets with 10% effervescent base, 80% CMC/20% HPMC, or 80% AA /20% MAA showed desirable lengthening of the stay of drug in its absorption area for 23-24 h and achieved gastro retention.

propyl methyl cellulose were used. Sodium bicarbonate was used as a gas forming agent and achieved buoyancy and controlled drug release from the prepared microspheres.

The effect of blending alginate with gellan, hydroxypropyl methylcellulose, starch, and chitosan on the bead properties was studied by Srinath A et al (2008). A multi-unit floating alginate system was developed and *In-vitro* drug release was studied in simulated gastric fluid (SGF). It was reported that the release of the drug from the beads was influenced significantly by the properties and concentration of additives.

Arza et al (2009) developed swellable and floating sustained release tablets of ciprofloxacin hydrochloride by wet granulation method. In this method combination of hydrophilic polymer (different grades of hydroxypropyl methylcellulose), different swelling agents (crospovidone, sodium starch glycolate, and croscarmelose sodium) and gas generating agent (sodium bicarbonate) were used. *In-vivo* studies were conducted on healthy volunteers supported prolonging of the gastric residence time, where the mean gastric retention was found to be 48 min (n=6). The result indicated the increase in mean residence time of the ciprofloxacin HCl in the stomach.

Bomma et al (2009) prepared floating matrix tablets by wet granulation technique. In this study three different polymers hydroxypropyl methylcellulose (HPMC K4M, HPMC K100M) and xanthan gum were used. *In-vivo* radiographic studies were performed by incorporating barium sulfate. These studies revealed that the tablets remained in the stomach for 30 min in fasting human volunteers indicating prolongation of gastric residence time.

1.4.4 MISCELLANEOUS

Nagahara et al (1998) prepared amoxicillin mucoadhesive microspheres by spray chilling method. In this study the microspheres formulated with carboxyvinyl and curdlan as adhesive polymers dispersed in melted hydrogenated castor oil. The ability of microspheres to reside in the gastrointestinal tract for an extended period was studied.
Modified positively charged biodegradable mucoadhesive microspheres of amoxicillin were prepared by Wang et al (2000). The microspheres were prepared using aminated gelatin by surfactant free emulsification in olive oil, followed by a cross-linking reaction with glutaraldehyde. The influence of glutaraldehyde concentration, cross-linking reaction time, drug-loading patterns, and type of release media on the In-vitro release characteristics of amoxicillin from the microspheres was investigated.

Gellan based floating in situ gelling system for controlled delivery of amoxicillin was developed by Rajinikanth et al (2007). The systems were prepared by dissolving varying concentrations of gellan gum in deionized water containing sodium citrate, to which varying concentrations of drug and calcium carbonate as gas-forming agent was added. The in situ gelling solutions were subjected to In-vitro gellation, floating and drug release studies in 0.1 N HCl (pH 1.2). The results revealed that the concentration of gellan gum and calcium carbonate significantly affected the In-vitro drug release.

A novel method of “tablets-in-capsule” of clarithromycin was developed by Go´mez-Burgaz et al (2008) to attain gastro-retention. In this system the minimatrices containing clarithromycin was composed of chitosan (CS) and carboxymethyl cellulose (CMC) in different proportions were prepared. In this study the influence of the molecular weight of chitosan and the proportion CS/CMC on physical properties and drug release were studied. Swelling was found to be dependent on CS molecular weight, medium pH and proportion of CS/CMC. The drug release rates have shown to be dependent on pH and on polymer proportion. Based on these results it was reported that the controlled-release gastro-retentive system was obtained by the “tablets-in-capsule” method.
1.5 LITERATURE REVIEW ON LIPOSOMAL DRUG DELIVERY SYSTEMS

Campbell et al. (1983) showed that liposomes are biologically inert in nature, devoid of any antigenic, pyrogenic or allergic reactions and their components can be utilized as the component of biological membrane. Drugs incorporated in liposomes are not inactivated under physiological conditions and do not cause unfavorable side effects as well.

Sharma and Sharma (1997) studied the reason for some liposomal products in the market or in clinical trials are provided as a lyophilized powder. In this study authors stated that lyophilization increases the shelf-life of the finished product by preserving it in a relatively more stable dry state especially if the drug is not stable in aqueous systems.

Patel and Misra (1999) studied the critical parameters controlling the formulation and stabilization of liposomes with encapsulated clofazimine. The study revealed that the entrapment efficiency of clofazimine in liposomes was increased by altering the proportion of phosphatidyl choline (PC) and cholesterol (CHOL) in liposomes. In this study the stability of liposomal suspensions and the liposomal gels (HPMC K4M) in terms of retention of clofazimine was measured at refrigeration temperature (2-8°C) for three months.

Law and Shih (2001) developed calcitonin-containing liposome formulations for intranasal delivery. In this study the parameters of liposomal charge characteristics, charge inducing agent concentration, calcitonin concentration and pH of the medium on the loading efficiency and leakage behaviour, and the chemical stability of calcitonin in liposomes were investigated.

Venuri et al. (1991) studied the effect of sugars on freeze-thaw and lyophilization of liposomes. In this study the authors stated that to maintain the same particle size distribution after freeze-drying-rehydration cycle, a cryo-protectant needs to be added. Various sugars were investigated for their ability to protect liposomes against fusion and
leakage during lyophilization process. The authors concluded that the protective ability of cryo-protectants can extend to both prevention of vesicle fusion and retention of encapsulated compound within the liposome.

Arkadiuszkozubek et al (2000) studied that according to their size, liposomes are known as either small unilammellar vesicles SUV (10-100nm) or large unilamellar vesicles LUV (100-300nm). If more than one layer is present, then they are referred to as multilamellar vesicles (MLV). These are ideal drug delivery systems because of their high degree of biocompatibility and their ability to encapsulate a large amount of material inside the vesicle.

Seth et al (2002) developed Acyclovir loaded liposomes by Reverse Phase evaporation method by using a new mathematical modeling approach for the design and preparation of liposomes and achieved controlled drug delivery when compared to conventional dosage forms.

Mozafari et al (2005) constructed a stable anionic liposee-plasmid particles using heating technique. In this study liposomes were developed by coating the surface of vesicles with the polymers like poly-ethylene glycol and chitosan or by using cationic or anionic charge ingredients into the vesicles. Thus they maximize the repulsive forces between the vesicles and prevent to segregate and settle down together. In this study the authors achieved more stable liposomal formulations.

Clares et al (2009) formulated and characterized the stability of triamcinolone multi lamellar liposomes. In this study after varying composition and storage conditions, and assessing encapsulation efficiency and loss of active principle the authors developed a standardized preparation method for a liposomal delivery vehicle. Further storage conditions effect on stability of liposomes were studied and results revealed that stability of liposomes were improved under refrigeration (4-6 °C) in comparison with samples stored at room temperature.
El-Nesr et al (2010) developed flucanazole multi lamellar liposomes using thin film hydration technique and studied the effect of cryo-protectant activity of sugars on liposomes during lyophilization. In this study the effects of cholesterol molar ratio, charge-inducing agents, and α-tocopherol acetate on encapsulation efficiency values and \textit{In-vitro} drug release of multi lamellar liposomes were studied. All the data were statistically analyzed with Minitab 16 software.

1.6 SCOPE AND OBJECTIVES

The main objectives of the present study are as follows;

- To design and develop novel drug delivery systems, namely, gastro retentive oral hydrogels and liposomes of a selected anti infective drug, Cefditoren pivoxil, using different polymeric systems.

- To carryout \textit{In vitro} evaluation tests for the developed gastro retentive oral hydrogel formulations such as tablet thickness, hardness, content uniformity, friability, disintegration studies, dissolution studies and stability studies.

- To carryout \textit{In vitro} evaluation tests for the developed liposomal formulations such as entrapment efficiency, particle size distribution, zeta potential, diffusion studies and stability studies.

- To carryout pharmacokinetic studies in rabbits and to establish various pharmacokinetics parameters, namely, the peak height concentration (C\textsubscript{max}), time of the peak concentration (t\textsubscript{max}), elimination half-life (t\textsubscript{1/2}) and elimination rate constant (k\textsubscript{e}). The areas under the plasma concentration time curves (AUC) will also be calculated for the selected best formulations.

The work was proposed to be carried out in three stages.

\textbf{Stage 1}: Preformulation studies of the drug such as calibration curve development, solubility, compatibility of the drug with the selected excipients using FTIR and DSC. A
method was developed for estimation of Cefditoren pivoxil using FTIR (TQ analyst software).

**Stage 2:** Formulation of gastro–retentive oral hydrogel tablets by optimizing the polymers and their concentration, plasticizers and penetration enhancers and evaluation of the formulated hydrogel tablets by *In vitro* and *In vivo* methods.

**Stage 3:** Formulation of liposomes by optimizing the polymers and their concentrations and evaluation of the formulated liposomes for their physical properties, surface studies and *In vitro* diffusion studies and *In vivo* pharmacokinetic studies using rabbits.