2. REVIEW OF LITERATURE

2.1. PEPTIC ULCER

Rarely mentioned as a cause of death, disability, or hospitalization in the late 1800s, peptic ulcer disease burst onto the medical scene in the first half of the 20th century, touted as a disease of epidemic proportions (Friedenwald, 1912; Mendeloff, 1974; Ivy, 1946, & Morris and Titmuss, 1944). This was followed by an inexorable decrease in the incidence and prevalence of peptic ulcer disease during the last 4 decades (Sturdevant, 1976; Vogt and Johnson, 1980, & Wylie, 1981)

2.1.1. Pathophysiology of peptic ulcer

Although it is now recognized that the large majority of duodenal and gastric ulcers are caused by *Helicobacter* infestation and or NSAID use, the final common pathway to ulcer formation is acid-peptic injury of the gastro-duodenal-mucosal barrier (Spechler, 2002). Thus, the adage “no acid, no ulcer” is as true today as it ever was. Elimination of *H. pylori* infection or NSAID use is also important for optimal ulcer healing and, perhaps even more important, in preventing recurrence and complications. A variety of other diseases are known to cause peptic ulcer, including Zollinger-Ellison (ZE) syndrome (gastrinoma), antral G-cell hyper function and/or hyperplasia, systemic mastocytosis, trauma, burns, and major physiological stress. Other “causative agents” are drugs (eg: all NSAIDs, aspirin, and cocaine), smoking, alcohol, and psychological stress. It is reported convincingly that there is a synergism between *Helicobacter* infection and NSAID use for the development of peptic ulcer as well as ulcer bleeding (Huang et al., 2002).

In a meta analysis, Kurata and Nagawa (1997) concluded that 89 to 95 % of serious peptic ulcer complications can be attributed to *Helicobacter* infection, NSAID use, and cigarette smoking. Svanes et al., (1997) showed a strong association between cigarette smoking and peptic ulcer perforation.

An important focus of investigation in the pathophysiology of peptic ulcer has been compromised mucosal defense mechanisms (Mertz and Walsh, 1991). Important mucosal defense mechanisms include an intact layer of surface epithelial cells, rapid restitution of this layer when surface epithelial cells are lost, mucus, luminal
bicarbonate, and an alkaline tide of bicarbonate on the serosal side of the mucosa to buffer back diffusing hydrogen ions, mucosal blood flow, growth factors, angiogenesis factors and GI motility. Important mediators of this complex process of “cytoprotection” include prostaglandins (particularly PGE2), nitric oxide, calcitonin gene related peptide, and a variety of gastrointestinal (GI) hormones. Some important protective reflexes are mediated by gastric afferent sensory neurons. In addition to these local factors, there are important protective factors in swallowed saliva, duodenal secretions, and pancreaticobiliary secretions. It is generally accepted that *Helicobacter* predisposes to ulceration both by acid hypersecretion and by compromise of mucosal defense mechanisms. NSAIDs are thought to lead to peptic ulcer predominantly by compromise of mucosal defenses (Mertz and Walsh, 1991).

Duodenal ulcer (DU) has historically been viewed as a disease of increased acid or peptic aggression on the duodenal mucosa, whereas gastric ulcer has been viewed as a disease of weakened mucosal defenses in the face of relatively normal acid or peptic aggression. However, increased understanding of the pathophysiology of peptic ulcer has blurred this distinction. Clearly weakened mucosal defenses play a role in many duodenal and most gastric ulcers like DU in a *Helicobacter* negative patient receiving NSAIDs; or typical type I gastric ulcer with acid hyposecretion; whereas acid or peptic aggression may result in a duodenal or gastric ulcer in the setting of normal mucosal defenses (Mertz and Walsh, 1991).

### 2.1.2. Epidemiology of peptic ulcer

The modern pathophysiologic tenets discussed above should be consistent with epidemiologic observations. Peptic ulcer disease remains a common outpatient diagnosis, but the number of patients admitted to hospital with this diagnosis has decreased steadily over the past three decades in Sweden (Gustavsson and Nyren, 1989). The incidence of perforated and bleeding peptic ulcer unchanged from 1974 to 1984 in Denmark (Christensen et al., 1988). These epidemiologic trends can be attributed to several factors including decreased prevalence of *Helicobacter* infection, better medical therapy, increase in outpatient management, and ulcer prophylaxis in NSAID patients. Although most patients with chronic *Helicobacter* infection (usually acquired in childhood) never develop clinically significant peptic ulcer disease, it is
clear that peptic ulcer disease is more common in populations with a high prevalence of *Helicobacter* infection. As this prevalence decreases in this country, probably as a result of improving socioeconomic conditions and better hygiene, it makes sense that the overall prevalence of peptic ulcer disease in the population should decrease.

Interestingly, this hypothesis is consistent with a strong epidemiological cohort phenomenon as described by Susser and Stein (1962). These investigators demonstrated that the risk of developing gastric or duodenal ulcers differs for individuals born in different decades and that the risk incurred in childhood persists throughout the life of the individuals. The risk of death from peptic ulcer has decreased dramatically through last decades of the 20th century (Sonnenberg, 1995; El-Serag and Sonnenberg, 1998) as has the risk of becoming a chronic carrier of *Helicobacter* (Graham *et al*., 1991). Finally, the number of NSAID users has increased as has the number of elderly patients. Both of these facts would undoubtedly increase the incidence of peptic ulcer complications, but the recognition that high risk patients requiring NSAIDs need prophylactic anti ulcer medication may blunt this anticipated increase in these patients. Peptic ulcer disease is increasingly a disease of the elderly, debilitated, and poor. Elderly cohorts are more likely to be *Helicobacter* positive and to use NSAIDs (Cryer and Feldman, 1994, & Hernandez-Diaz and Rodriguez, 2000). Other factors contributing to the development of ulcer are wrong eating habits, stress, empty stomach, medication and food.

Vitamin C or vitamin E supplementation leads to some short-term protective effects on *H. pylori*-induced gastritis by reduced mucosal 3-nitrotyrosine concentrations to normal levels, decreased mucosal protein carbonyls and TBARS in short-term gastritis. Vitamin C supplements cause attenuated mucosal oxidative DNA damage and milder mucosal inflammation in short-term gastritis (Sun *et al*., 2005).

### 2.1.3. Factors associated with peptic ulcer

#### 2.1.3.1. Acid secretion in ulcer disease

Clinically significant peptic ulceration is caused by autodigestion of the gastroduodenal mucosa by acid and or pepsin. The paradigm of peptic ulcer disease caused by acid peptic injury is ZE syndrome. Suppression of acid secretion to levels
below which pepsinogen is inactive allows ulcers to heal and prevents new ulcers from forming. Ulceration can also be caused by direct injury of the mucosa by hydrochloric acid without pepsin. It has long been recognized that DU patients as a group have a higher basal acid output and also a higher maximal acid output than normal controls (Blair et al., 1987, & Feldman and Richardson, 1986). Acid output correlates closely with gastric parietal cell mass (Cox, 1952).

Many patients with DU also have increased rates of gastric emptying due to increased motility, which delivers an increased acid load per unit of time to the duodenum (Amdrup et al., 1979). Finally, the buffering capacity of the duodenum in many patients with DU is compromised due to decreased duodenal bicarbonate secretion (Isenberg et al., 1987).

In patients with gastric ulcer, acid secretion is variable. Patients with the most common Johnson type I gastric ulcer (lesser gastric curvature, around angularis incisura) or type IV (juxtaesophageal) gastric ulcer have normal or below normal levels of acid secretion, whereas patients with type II (gastric and duodenal) or III (prepyloric) ulcer have acid secretion resembling that of patients with DU. Patients with type I gastric ulcer may have weak mucosal defenses, which permit an abnormal amount of injurious acid back-diffusion into the mucosa. Duodenogastric reflux may also play a role in weakening the gastric mucosal defenses in patients with gastric ulcer (Grossman et al., 1963; Fisher and Cohen, 1973 & Ritchie, 1975).

2.1.3.2. Medical management of peptic ulcer disease by acid suppression

Approximately 80 to 90% of patients with chronic duodenal or gastric ulcer disease can be healed by the diligent use of antacids or acid suppressive medication (Wolfe and Sach, 2000; Walan et al., 1989; Gitlin et al., 1987; Dekkers et al., 1999; Poynard et al., 1995, & Yeomans et al., 1998). In Helicobacter positive patients, endoscopic recurrence is the rule unless acid suppression is continued indefinitely or unless Helicobacter is eradicated (Cohen, 2000; Van der Hulst et al., 1997). A good antacid regimen is equally effective as H₂ receptor blocker treatment but is much more cumbersome and associated with more disturbing side effects, particularly diarrhea (McArthur, 1993). Although 80 to 90% of peptic ulcers will heal with H₂
receptor blockers or PPIs, healing is a little faster and the percentage of ulcers healed a little higher with PPIs (Tunis et al., 1997, & Holt and Howden, 1991).

Although gastrin and acetylcholine bind to receptors on the parietal cell and activate the proton pumps, the majority of acid secretion from parietal cells is controlled by histamine release from mucosal enterochromatin like cells, a plentiful endocrine cell throughout the gastric mucosa (Feldman, 2002; Zeng et al., 1999, & Li et al., 2000).

Histamine binds to H$_2$ receptors on the parietal cell, activating adenyl cyclase, increasing cyclic adenosine monophosphate and activating the H$^+/K^+$-ATPase (proton pump). This explains the effectiveness of H$_2$ receptor antagonists in blocking the majority of acid secretion. PPIs are converted to their active moiety in the acidic environment of the secretory canaliculus and irreversibly inactivate the acid secretory protein, H$^+/K^+$-ATPase (Holt and Howden, 1991).

2.1.3.3. NSAIDs in peptic ulcer disease

NSAIDs are inextricably linked to peptic ulcer disease (Laine, 2001; Wolfe et al., 1999, & Lanza, 1998). Patients with rheumatoid arthritis and osteoarthritis who take NSAIDs have a 15 to 20% annual incidence of peptic ulcer, and the prevalence of peptic ulcer in chronic NSAID users is approximately 25%. Complications of peptic ulcer disease are much more common in patients taking NSAIDs. More than one half of patients who present with peptic ulcer hemorrhage or perforation report the recent use of NSAIDs including aspirin. Many of these patients remain asymptomatic until these life-threatening complications develop (Laine, 2001).

Factors that clearly put patients at increased risk for NSAID induced GI complications include age over 60, prior GI event, high NSAID dose, concurrent steroid intake, and concurrent anticoagulant intake (Lanza, 1998).

2.1.3.4. Smoking and cocaine and peptic ulcer disease

Epidemiologic studies suggested that smokers are approximately twice as likely to develop peptic ulcer disease as non smokers (Kurata and Nogawa, 1997). Smoking increases gastric acid secretion and duodenogastric reflux. Smoking decreases both gastroduodenal prostaglandin production and pancreaticoduodenal
bicarbonate production (Cryer et al., 1992, & Ainsworth et al., 1993). These observations may be related, and any or all could explain the observed association between smoking and peptic ulcer disease. Recently, the use of crack cocaine has been linked to juxtapyloric peptic ulcers with a propensity to perforate (Feliciano et al., 1999).

2.1.3.5. Role of stress in the development of peptic ulcer

Although difficult to measure, both physiological and psychological stress undoubtedly play a role in the development of peptic ulcer in some patients. In 1842 Curling described 12 burn patients with DU and/or duodenitis (Stabile and Passaro, 1984). Cushing described the appearance of acute peptic ulceration in patients with head trauma (Cushing ulcer). Levenstein et al., (1999) have pointed out the undeniable links, even recognized by the ancients, between peptic ulcer disease and stress. They contend that attempts to explain the etiology of peptic ulcer disease solely on the basis of *H. pylori* and/or NSAIDs are inadequate.

2.1.3.6. *H. pylori* infection predisposes to peptic ulceration


The microorganism, now known as *H. pylori*, which resides mainly in the gastric mucosa or at the interface between the mucous layer and the epithelial cells of the antral region of the stomach is considered to be an important etiological factor in gastritis, peptic ulcers and there is evidence that the risk of gastric cancer is increased by infection with the bacterium (Marshall and Warren, 1984; Peterson, 1991, & Beil and Kilian, 2007).

*H. pylori* are spiral-shaped microaerophilic gram negative bacteria that become coccoid in unfavorable growth conditions (Catrenich and Makin, 1991). The organism’s multiple flagellae (Geis et al., 1993) and large amounts of urease enzyme
are essential for colonization. Once *H. pylori* have colonized the mucus layer and epithelium of the stomach they tend to persist for the lifetime of the host. *H. pylori* reduce gastric acidity by producing ammonia catalyzed by its urease (Lee, 1994). Flagellae enable the organism to burrow into the mucus layer, within which the bacteria are relatively protected from the harsh acidic environment of the stomach (Dunn *et al*., 1997). The host response leads to inflammation of the gastric mucosa, is not able to eradicate the organism (Blaser, 1992).

The incidence of *H. pylori* is higher in developing countries (3 to 10 %) than in developed countries (Parsonnet, 1995). Ethnicity appears to be a risk factor for *H. pylori* independent of socio-economic status (Fraser *et al*., 1996).

The relative risk of *H. pylori* infection significantly increases with age, lower socioeconomic status, and lower household income. Approximately 90 to 95 % of duodenal ulcers and 70 to 75 % of gastric ulcers are attributable to infection with *H. pylori*. Duodenal ulcers in children are unlikely to occur in the absence of *H. pylori* infection, unless they are on nonsteroidal antiinflammatory drugs. *H. pylori* infection increases the risk of developing gastric adenocarcinoma later in life (Uemura *et al*., 2002).

Although *H. pylori* colonization of gastric mucosa causes chronic gastritis in children, it may remain asymptomatic (Nowicki and Coyle, 2001).

2.1.4. Pathogenic determinants of *H. pylori*

Several pathogenic determinants of the organism *H. pylori* have been proposed, including adhesins, cytotoxins, and different enzymes: urease, catalase, lipase, protease, etc. One of the most striking enzymatic characteristics of *H. pylori* is its very potent urease activity (Clyne *et al*., 1995; Crabtree *et al*., 1992; Borchlace *et al*., 2008; Eaton *et al*., 1991, & Sidebotham *et al*., 1991). *H. pylori* urease hydrolyses the urea present in the gastric juices to generate ammonia and bicarbonate, which effectively neutralize the acidic pH of its environment (Marshall, 2002, & Skouloubris *et al*., 2000) and the pH of the stomach is possibly elevated (to a pH of about 4.5–7.0) (Bardonnet *et al*., 2006, & Eslick, 2004). *H. pylori* strains mainly reside in the gastric mucosa or at the interface with the mucus layer (Fedwick *et al*., 2005).
2005, & Hazell et al., 1989), and produce the vacuolating cytotoxin (Vac A), which modulates the integrity of the tight junctions of the epithelium and reduces the stability of the cytoskeleton (Borlace et al., 2008; Fedwick et al., 2005, & Falk et al., 1993).

The over-expression of COX-2 (Cyclooxygenase 2) protein has been reported to play a key role in the incidence and development of *H. pylori* associated gastric cancer. Recently it has been reported that the COX-2 mRNA and proteins expression level and the activity of COX-2 promoter increased remarkably with *H. pylori* stimulation in the MKN45 gastric cancer cells (Yamac et al., 2008). *H. pylori* also stimulated phosphorylation of p38MAPK and ATF-2, which is the downstream kinase of p38MAPK. Moreover, the expression levels of COX-2 were suppressed with p38MAPK inhibitor treatment and *H. pylori* induced activation of p38MAPK/ATF-2-mediated signal pathway is necessary for the expression of COX-2 (Li et al., 2009).

![Fig. 1.1. *H. pylori* lodged on the gastric epithelium](image)

**Fig. 1.1. *H. pylori* lodged on the gastric epithelium** (Marshall and Warren, 1998)

There are 4 strong lines of evidence supporting the central role of *H. pylori* in the pathophysiology of peptic ulcer disease (Cohen, 2000). These include the natural history of *H. pylori* infection, epidemiologic data, and the outcome of peptic ulcer disease after the cure of *Helicobacter* infection, and animal models of *H. pylori* infection. In the United States, patients with *H. pylori* infection and antral gastritis are three and one half times more likely to develop peptic ulcer disease than patients
without *Helicobacter* infection (Nomura et al., 1994). Ninety percent of patients with DUs, and 70 to 90% of patients with gastric ulcers, have *H. pylori* Infection (Cohen, 2000).

It is clear that curing *H. pylori* infection dramatically alters the natural history of peptic ulcer disease, decreasing the recurrent ulcer rate from more than 75% in patients treated with a course of acid suppressive therapy alone to less than 20% in patients treated with a course of anti *Helicobacter* therapy (Graham et al., 1992; Hentschel *et al.* 1993; Hopkins *et al.*, 1996, & Van der Hulst *et al.*, 1997). Leodolter *et al.*, (2001) concluded, from an analysis, that eradication of *H. pylori* infection produces similar cure rates of both duodenal and gastric ulcers.

In another analysis, Laine *et al.*, (1998) found the same result with regard to cure rates, but puzzlingly found that 20% of patients studied had recurrent ulcer in 6 month despite successful eradication of *Helicobacter* and reported abstinence from NSAIDs. Obviously other factors are involved in the etiology of peptic ulcer disease, since everyone who is infected with *H. pylori* does not get peptic ulcer disease. Only approximately 15 to 20% of patients colonized with *H.pylori* will develop peptic ulcer disease over their lifetime (Blaser, 1997, & Peek and Blaser, 1997).

Many patients receiving aspirin and NSAIDs develop peptic ulcer disease without *Helicobacter* infection (Laine, 2001). In animal models, Koch’s postulates have been substantially fulfilled for peptic ulcer when it comes to the role of *H. pylori* infection (Nomura *et al.*, 1994).

The *H. pylori* bacteria are uniquely equipped for survival in the hostile environment of the stomach (Peterson and Graham, 2002; Fennerty, 1994, & Gerata and Graham 1996). It possesses the urease enzyme, which converts urea into ammonia and bicarbonate. This creates an environment around the bacteria which buffers the acid secreted by the stomach. Mutant strains of *H. pylori* that do not produce urease are unable to colonize the stomach, (Blaser, 1997) emphasizing the importance of this enzyme in stabilizing the microenvironment for the *Helicobacter* organism. The organism lives in the mucus layer atop the gastric surface epithelial cells, propelled by its flagellum, and some attach to the surface epithelial cells. There
are a variety of possible mechanisms whereby *H. pylori* produces mucosal injury (Kauffman, 2000).

### 2.1.5. Mechanisms of gastroduodenal mucosal damage by *H. pylori*

The following are the mechanisms of mucosal damage (Kauffmann, 2000)

#### 2.1.5.1. Local effects

- Elaboration of toxins
  - vacA
  - cagA

#### 2.1.5.2. Effect on immune response

- Elaboration of cytokines
- Elaboration of IL-8
- Recruitment of inflammatory cells
- Release of inflammatory mediators
- Production of immunoglobulins

#### 2.1.5.3. Effect on acid secretion

- Initial hypochlorhydria
- Subsequent hyperchlorhydria
- Elevated serum gastrin levels
- Reduced gastric antral somatostatin levels
- Increased levels of gastric fundic *N*-methylhistamine
- Hypergastrinemia may contribute to greater parietal cell mass

#### 2.1.5.4. Effect on duodenal *HCO₃⁻* secretion

- Reduced secretion of duodenal *HCO₃⁻* in patients colonized with *H. pylori*
Colonization with *H. pylori* results in chronic superficial gastritis, which increases the risk for the development of duodenal and gastric ulcers, gastric adenocarcinoma, or non-Hodgkin's gastric lymphoma (Parsonnet *et al*., 1995).

One fundamental mechanism appears to be a disturbance in acid secretion. When patients are first infected by *H. pylori* there is an initial period of hypochlorhydria or achlorhydria, followed by the development of acid hypersecretion and hypergastrinemia. This is due at least in part to the inhibitory effect that *H. pylori* exerts on antral D cells, which secrete somatostatin, a potent inhibitor of antral G cell gastrin production. In the gastric mucosa, *H. pylori* infection is associated with decreased levels of somatostatin, decreased somatostatin mRNA production, and fewer somatostatin producing D cells. These effects are probably mediated by *Helicobacter* induced local alkalinization of the antrum and *Helicobacter* mediated increases in other local mediators and cytokines. The end result is hypergastrinemia and acid hypersecretion (Harris *et al*., 1996).

It has been suggested that this hypergastrinemia leads to the parietal cell hyperplasia found in many patients with DU. The acid hypersecretion and the antral gastritis are thought to lead to antral epithelial metaplasia in the postpyloric duodenum (Peek and Blaser, 1997). This duodenal metaplasia allows *H. pylori* to colonize the duodenal mucosa and in these patients the risk of developing a DU increases 50-fold. When *H. pylori* colonize the duodenum, there is a significant decrease in acid stimulated duodenal bicarbonate release. When *Helicobacter* infection is treated successfully, the physiology of acid secretorion tends to normalize (Harris *et al*., 1996).

Other mechanisms whereby *H. pylori* can induce gastroduodenal mucosal injury include: the production of toxins (e.g., vacA, cagA), local elaboration of cytokines, particularly interleukin-8 (IL-8), by infected antral mucosa, recruitment of inflammatory cells and release of inflammatory mediators, and production of immunoglobulins (Blaser and Berg, 2001).

Several studies have been carried out focusing bacterial factors in gastric diseases and it has been assumed that *H. pylori* strains having cagA+/vacAs1 genotype are more virulent than other genotypes. Some studies have reported that the vacAs1
strain is usually toxigenic and tends to be cagA⁺ (Atherton, 1997, & Atherton et al., 1995).

*H. pylori* infection induces an inflammatory response that is also oxidative. The gastric epithelium and the bacteria induce production of interleukin-8 (IL-8) that contributes to the generation of great amounts of toxic reactive oxygen species (ROS), with marked infiltration of inflammatory cells, and can elicit induction of interleukin-1β (IL-1β), interleukin-6 (IL-6), IL-8, interleukin-12 (IL-12), tumour necrosis factor-α (TNF-α), (Noach et al., 1994; 1997; Bauditz et al., 1999; Hida et al., 1999, & Meyer et al., 2000) and interferon-γ (IFN-γ) (Noach et al., 1994).

Increased pathogen inducible nitric oxide synthase (iNOS) activity has also been observed in the gastric mucosa of patients with duodenal ulcer (Rachmilewitz et al., 1994), gastric cancer (Rieder et al., 2003) and gastritis (Antos et al., 2001) caused by *H. pylori* infection. iNOS is induced by a variety of stimuli, including bacterial lipopolysaccharides (pyrogens) produced by gram negative bacteria, cytokines and products from the bacterial wall, and its expression contributes to oxidative stress (Lim et al., 2001).

Another oxidative enzyme induced by *H. pylori* in gastric disease is NADH oxidase 1 (Kawahara et al., 2001). It constitutively produces both superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$). Increased expression of NADH oxidase 1 mRNA moderately increases O$_2^-$ generation, which leads to a reduction in aconitase activity, making NADH oxidase 1 a good marker of oxidative stress (Arnold et al., 2001).

*H. pylori* also induces ROS production by gastric epithelial cells, contributing to increased damage in the mucosa (Bagchi et al., 1996), and *H. pylori* itself generates great amounts of O$_2^-$ to inhibit bactericidal action of nitric oxide (NO) produced by inflammatory cells (Nagata et al., 1998).

Gastritis was found to be associated with significant oxidative stress marker expression of TNF-α and IL-8 that was also related to *H. pylori* virulence, suggesting that they are the main oxidant stress markers responsible to trigger an increase in ROS
level that contributes to decrease the expression of the antioxidant markers such as manganese superoxide dismutase and glutathione peroxidase (Augusto et al., 2007).

2.1.6. Tests for H. pylori infection

A variety of tests help to determine whether or not the patient has active Helicobacter infection (Graham et al., 1999). A positive serology is presumptive evidence of active infestation if the patient has never been treated for Helicobacter. The gold standard test is considered to be the histologic examination of an antral mucosal biopsy by using special stains. Other sensitive tests include commercially available rapid urease tests, which assay for the presence of urease in mucosal biopsies. From late nineties the labeled carbon urea breath test has become available (Walsh and Peterson, 1995). This has become the standard test to confirm eradication of Helicobacter following appropriate treatment. In this test the patient ingests urea labeled with radioactive $^{14}$C or nonradioactive $^{13}$C. The labeled urea is acted on by the urease present in the H. pylori and converted to ammonia and carbon dioxide. The radiolabeled carbon dioxide is excreted from the lungs and analyzed in the expired air. It can also be detected in a blood sample. The fecal antigen test is also quite sensitive and specific for active H. pylori infection and may prove useful in confirming a cure (Manes et al., 2001).

2.1.6.1. Serologic test

Test of choice when endoscopy is not indicated and is not an option and when the patient has not received antimicrobial therapy for H. pylori infection. It is noninvasive; sensitivity of 80 %, specificity of about 90 %. It does not confirm eradication, because serologic “scar” remains for indefinite period after microbiologic cure.

2.1.6.2. Urea breath test

Preferred for confirming cure of H. pylori infection, but no sooner than 4 week after completion of therapy Simple; sensitivity and specificity of 90 to 99 % and false negatives possible if testing is done too soon after treatment with proton pump inhibitors, antimicrobials, or bismuth compounds; small radiation exposure with $^{14}$C method; this test is expensive.
2.1.6.3. **Histologic test**

To directly ascertain presence of *H. pylori* when endoscopy is being used; also used when determination of neoplastic status of lesion is necessary. Sensitivity of 80 to 100 %, specificity of 95 %; hematoxylineosin and Diff-Quik stains are simplest; Genta stain has sensitivity of 95 % and specificity of 99 %, requires laboratory facilities and experience; when hematoxylineosin stain is nondiagnostic, another staining method is employed.

2.1.6.4. **Rapid urease test**

Simplest method when endoscopy is necessary. Simple; rapid (once biopsy specimen has been obtained); sensitivity of 80 to 95 %, specificity of 95 to 100 %. Invasive; false negatives possible if testing is done too soon after treatment with proton pump inhibitors, antimicrobials, or bismuth compounds.

2.1.6.5. **Bacterial culture method**

After repeated failure of appropriate combination antibiotic therapy; when antimicrobial resistance is suspected or high level of resistance exists in the population allows the determination of antibiotic susceptibility. It is time consuming; expensive; usually not necessary unless resistance is suspected.

2.1.7. **Epidemiology of *H. pylori* Infection**

2.1.7.1. **Helicobacter in the developing world**

Numerous studies have demonstrated that *H. pylory* is ubiquitous; approximately 50 % of the world’s population is infected with the organism (Torres *et al*., 2000). In contrast with industrialized nations, *H. pylori* infections occur earlier in life and with a higher frequency in the developing world. Also, while the prevalence of the infection has dropped significantly in many parts of North America, Western Europe and Asia (especially Korea), no such decline has been noted in the developing world (Torres *et al*., 2000). However, as in industrialized nations, gender does not appear to be related to infection with *H. pylori*. In many developing countries, the prevalence of infection with *H. pylori* exceeds 50 % by 5 years of age, and by
adulthood, infection rates exceeding 90% are not unusual (Dunn et al., 1997; Go, 2002, & Bardhan, 1997).

In a study of 569 Bangladeshi children between 2 and 10 years of age, the prevalence of *H. pylori* was already 42% by 2 years of age and quickly rose to 67% by 10 years of age (Rahman et al., 1998, & Clemens et al., 1996). Similar findings were reported from studies performed on children from many parts of the developing world including Peru, the Gambia and China (Klein et al., 1994; Thomas et al., 1999, & Mitchell et al., 1992).

The results of epidemiological studies of *H. pylori* in Egypt, of 200 mothers tested from a project in Alexandria, Egypt, 90% were infected with *H. pylori*, and 15% of their infants had become infected by 9 month of age, with the prevalence increasing to 25% by the time the children reached 18 month of age (Bassily et al., 1999). A subsequent survey using children participating in a 3 years study found that *H. pylori* infection increased with age and reached 30% by 3 years of age (Naficy et al., 2000).

In a recent prospective double-blind study from France, 100 children with non-ulcer dyspepsia referred for upper gastrointestinal endoscopy for epigastric pain were evaluated. There were 26 *H. pylori* infected and 74 non-infected children. No specific characteristics of symptoms in non-ulcer dyspeptic *H. pylori* infected children as compared with non-infected children were found (Kalach et al., 2005).

### 2.1.7.2. The Asian scenario

The Indian scenario is that 80% of the adult population is infected with *H. pylori*. Among children 22% at the age group of 0–4 years and 87% at the age group of 10–19 years (Graham et al., 1991b).

*H. pylori* infection is more common and contracted earlier in Asian countries such as India, Pakistan, Bangladesh, and Thailand. Seroprevalence of *H. pylori* infection in adults in these countries varies from 55 to 92% compared with about 50% in China and Japan. However, the frequency of gastric cancer is low in these so-called Asian enigma countries compared with that in Japan and China (Sharma, 2008).
H. pylori infection is strongly linked with upper abdominal pain in children. Infection rate as demonstrated directly from antral biopsy specimens increases with age substantiating increasing seroprevalence rates with age. H. pylori infected children without treatment remain chronically infected. H. pylori infection was significantly higher in children with upper abdominal pain than controls (Singha et al., 2006).

2.1.8. Treatment of H. pylori Infection

There are triple and quadruple therapies available for H. pylori infection.

- **Triple therapy**: Proton pump inhibitor, clarithromycin (500 mg/250 mg), metronidazole (500 mg) or amoxicillin (1 g), twice a day for two weeks. Tetracycline (500 mg) can be substituted for amoxicillin.

- **Quadruple therapy**: Proton pump inhibitor (twice a day), metronidazole (500mg) (three times daily), bismuth subsalicylate (525 mg) and tetracycline (500 mg) (three times daily) or H₂ bismuth subsalicylate (525 mg), metronidazole (250mg), tetracycline (500 mg) (four times daily). The proton pump inhibitors used are omeprazole (20mg), lansoprazole (30mg), rabeprazole (20mg), pantoprazole (40mg), esomeprazole (40mg) and the H₂ receptor antagonists are cimetidine (400mg), famotidine (20mg), nizatidine (150mg), and ranitidine (150mg).

To remove H. pylori, a triple therapy over a period of two weeks is usually required. Unfortunately, when administered in vivo, no single antibiotic is effective in the eradication of H. pylori (Fontana et al., 2001, & Peterson et al., 1996). The failure of single antibiotic therapies could be attributable to the poor stability of the drug in the gastric acid and the poor permeation of the antibiotic across the mucus layer, which is followed by their resecretion into the lumen, where sufficient drug must diffuse to the bacteria (Endo et al., 2001; 2002). Although the microorganism is highly susceptible to many antimicrobial agents in vitro, clinical trials with a single antimicrobial agent have resulted in a low eradication rate of H. pylori (Umamaheshwari et al., 2003c, & Labenz, 2001).
Triple therapies (one or two antibiotics combined with a proton pump inhibitor) are proved effective in clinical application. However, some other reports and clinical trials indicated that the therapies cannot bring out complete eradication of *H. pylori* and suggested that the therapeutic effect needs more investigation (Lin *et al.*, 2002, & Kawabami *et al.*, 2001).

There are two major reasons for the failure of *H. pylori* eradication with conventional dosage forms of antibiotics. One of the reasons for incomplete eradication may be the degradation of antimicrobial agents such as amoxicillin and clarithromycin by gastric acid (Lin *et al.*, 2002). Therefore, the administration of high doses of antimicrobial agents on a daily basis is necessary for *H. pylori* eradication, but they are usually accompanied by adverse effects and poor patient compliance (Kawabami *et al.*, 2001). Another reason for incomplete eradication is probably that the residence time of antimicrobial agents in the stomach is so short that effective antimicrobial concentrations cannot be achieved in the gastric mucous layer or epithelial cell surfaces where *H. pylori* exists (Cuna *et al.*, 2001, & Hirayama *et al.*, 1996).

Eradication of *H. pylori* infection is possible in approximately 90% of patients with a 10 to 14 days course of triple therapy, given twice daily (Calvet *et al.*, 2000, & Hoffman and Cave, 2001). Eradication is most easily assessed with the C\(^{13}\) or C\(^{14}\) urea breath test. Quadruple therapy is used in areas with high metronidazole resistance or in triple therapy failures. Infectious disease consultation may be helpful in intractable cases of *Helicobacter* infection (Dore *et al.*, 2000). Patients failing standard triple or quadruple therapy have been given a variety of novel regimens with variable results. One regimen is rifabutin based, another is furazolidone based, and yet another includes a novel capsule that has 3 drugs, i.e. bismuth, tetracycline, and metronidazole (Hoffman and Cave, 2001, & Graham *et al.*, 2000).

Resistance to both clarithromycin and metronidazole is increasing. Easier and more reliable tests of antibiotic resistance in *H. pylori* may improve the results of treatment (Trebesius *et al.*, 2000). This is likely to occur as there is an increase in the understanding of the genetic basis of antibiotic resistance in these omnipresent bacteria. Research continues on a vaccine for *H. pylori* (Ikewaki *et al.*, 2000).
A review of 36 studies found that the incidence of persistent *Helicobacter* infection following *Herpes simplex* virus (HV) was 71 to 95% due to the reduced immune response (Danesh *et al*., 1998).

*H. pylori* infection and NSAID use are both independent risk factors for the development of peptic ulcer disease. The preponderance of data suggests that concomitant *H. pylori* infection does not potentiate the risk of peptic ulcer formation in most NSAID users. However, in high risk patients, particularly the elderly, the combination of *H. pylori* infection and NSAID use can represent a dangerous combination (Treiber and Lambert, 1998, & Butler *et al*., 2001).

Currently, the first line treatment remains clarithromycin, amoxicillin or metronidazole and a proton pump inhibitor twice daily, but a number of recent studies have shown low eradication rates with this regimen (Egan *et al*., 2007).

Bismuth based triple therapy and PPI based triple therapies have been the most widely recommended. PPI based regimens are superior to H₂ antagonist based ones. Bismuth based quadruple therapy is effective, but the complexity of the regimen and its associated adverse effects limit the compliance (Gisbert *et al*., 2007).

Some studies have shown that *H. pylori* decreases vitamin C levels in gastric juice, and *in vitro* vitamin C has been shown to inhibit the growth of *H. pylori*. Considering these effects, it is possible that addition of vitamin C to the eradication regimen may increase the eradication rate. A daily high dose of vitamin C in *H. pylori* infected patients with chronic gastritis resulted in apparent *H. pylori* eradication in 30% of treated patients (Jarosz *et al*., 1998). Yet another study has proved that the addition of vitamin C to *H. pylori* treatment regimen of amoxicillin, metronidazole and bismuth can significantly improved *H. pylori* eradication rate significantly *H. pylori* eradication rate from 49 to 78% (Zojaji *et al*.,2009).

The third Maastricht Consensus Conference reported that the urea breath test, stool antigen tests and serological kits with a high accuracy are non invasive tests, which should be used for the diagnosis of *H. pylori* infection. Triple therapy using a PPI with clarithromycin and amoxicillin or metronidazole given twice daily remains the recommended first choice treatment. Bismuth containing quadruple therapy, if
available, is also a first choice treatment option. Rescue treatment should be based on antimicrobial susceptibility (Malfertheiner et al., 2007).

2.1.9. Novel formulations for H. pylori therapy

The classical way to cure H. pylori infection is to use a 7 days triple therapy based on two antibiotics (amoxicillin, clarithromycin) and one proton pump inhibitor (omeprazole, lansoprazole, and pantoprazole). However, because of the high level of antibiotic resistance to H. pylori and the poor patient compliance in a lesser measure (Mc Loughlin et al., 2004).

Katayama et al., (1999) focused on designing and evaluating a sustained release antibiotic (ampicillin) prepared by sodium alginate. The gastroretentive property of the antibiotic was provided by sodium alginate to form a firm gel by adding divalent metal ions (Ca\(^{2+}\)). The gel is used for spreading out, adherence of the antibiotic to gastric mucosa, and also sustaining the release.

In order to localize antibiotics at the H. pylori infection site on the gastric epithelium for improving the efficacy of anti H. pylori agents, a novel cationic nanoparticle delivery system composed of chitosan and heparin have been reported recently (Lin et al., 2009). Chitosan is a polycationic, nontoxic, mucoadhesive polymer, which has been proven to be safe (Mansouri et al., 2006, & Jin et al., 2004). It allows a prolonged interaction between the delivered drug and the membrane epithelia, facilitating more efficient drug diffusion into the mucus/epithelium layer (Thanou et al., 2001, & Hejazi and Amiji, 2004).

2.1.9.1. Floating and gastroretentive drug delivery systems for H. Pylori

In 1994, a patent assigned to Reckitt and Colman Products described a raft-forming formulation using triclosan. The drug was mixed with alginic acid, sodium bicarbonate, calcium carbonate and mannitol. The mixture was granulated, citric acid added, and then packed into sachets or compressed to tablets. In contact with the acid conditions of the stomach, carbonate or bicarbonate salts produced effervescence which aerated the raft structure formed by the alginates, causing it to float. However, the authors noticed that, in some patients with H. pylori infections, the pH of the stomach contents had possibly been elevated (possibly to as high as pH 6) reducing
effervescence and, consequently, reducing the ability of the rafts to float. For this reason, they had added citric acid to their formulation (Dettmar and Lloyd-Jones, 1994).

Yang _et al._, (1999) proposed a gas generating system consisting of an expandable asymmetric triple layer tablet. One layer was the swellable gas generating layer (poly ethylene oxide), HPMC and sodium bicarbonate or calcium carbonate. The second one was the expandable and sustainable drug containing layer (poly ethylene oxide), tetracycline hydrochloride and metronidazole. The third one was a rapidly dissolving drug layer (bismuth salts). According to the authors, the aim of such a device was to obtain a simple regimen for a standard triple therapy. Indeed, they obtained _in vitro_ a duration of buoyancy and sustained release of metronidazole and tetracycline over 6–8 h, with buoyancy lag time in the range of 17–28 min. The rapid effect of the device would be due to the rapidly dissolving layer containing the bismuth salt, which disintegrated within 10–15 min _in vitro_. However, no _in vivo_ data are available concerning the floating characteristics of the drug delivery system or its effect against _H. pylori_.

In another studies, scientists developed several drug delivery systems especially designed to improve efficiency against _H. pylori_. In all of them, they used an antiurease drug, acetohydroxamic acid, as an active agent against the bacterium (Umamaheshwari _et al._, 2003b, & Umamaheshwari and Jain, 2004). _H. pylori_ urease hydrolyses urea present in the gastric juice and extracellular fluid to generate ammonia and bicarbonate, which effectively neutralize an acidic pH in its environment (Marshall, 2002, & Skouloubris _et al._, 2000). Thus, urease inhibitors hinder the bacterium to protect itself against low pH and avoid thus the problem of treatment of antibiotic-resistant strains (Umamaheshwari _et al._, 2003b, & Umamaheshwari and Jain, 2004).

There was a report on polycarbonate microballoons prepared by by an emulsion solvent evaporation technique. _In vitro_ (in simulated gastric fluid), 74 to 85 % of microballoons stayed buoyant up to 12 h and exhibited a sustained drug release profile. _In vitro_ and _in vivo_ growth inhibition studies were performed using cultures of _H. pylori_ and _H. pylori_ infected Mongolian gerbils, respectively.
Microballoons showed 10 times higher anti *H. pylori* activity compared with acetohydroxamic acid solution (Umamaheshwari et al., 2003b).

The authors of the latter study also formulated floating bioadhesive microspheres. The microballoons (made by a quasi emulsion solvent diffusion method) were coated with 2 % w/v solution of polycarbophil by an air suspension coating method. *In vitro* floating studies, detachment force measurements and *in vivo* growth inhibition studies demonstrated the potential of this device, which combines bioadhesive and floating properties (Umamaheshwari et al., 2002).

Besides, cellulose acetate butyrate coated cholestyramine microcapsules were also proposed as gastroretentive drug delivery systems by Umamaheshwari et al. (2003c). Indeed, they used CO$_2$ generation to provide floatability, and cholestyramine for a mucoadhesive effect. Ion exchange resin particles were loaded with bicarbonate followed by acetohydroxamic acid and coated with cellulose acetate butyrate by an emulsion solvent evaporation method. *In vitro* (drug release, buoyancy) and *in vivo* gastric mucoadhesion studies in the rat stomach led the authors to conclude that this drug delivery system possessed both floating and bioadhesive properties, and may be successful in the treatment of *H. pylori* (Umamaheshwari et al., 2003c).

### 2.1.9.2. Mucoadhesive gastroretentive drug delivery systems

Nagahara et al., (1998) formulated mucoadhesive microspheres containing amoxicillin. They dispersed the drug and bioadhesive polymers (carboxyvinyl polymer and curdlan, a polysaccharide) in melted hydrogenated castor oil. Microspheres of 250 to 335 μm in diameter were obtained by a spraychilling method followed by sieving. They compared these microspheres with an amoxicillin suspension in infected Mongolian gerbils under feeding conditions. The microspheres with an amoxicillin dose of 1.0 mg kg$^{-1}$ provided the same clearance rate (20 %) as the amoxicillin suspension with a dose of 10 mg kg$^{-1}$. Moreover, 47 % and 20 % of microspheres remained in the stomach wall after 2 and 4 h, respectively due to mucoadhesion. The authors concluded that these mucoadhesive microspheres containing an appropriate antimicrobial agent should be useful for the eradication of *H. pylori*. 
Recently, there was a report on mucoadhesive microspheres containing amoxicillin, prepared by an emulsification/evaporation method, using ethylcellulose as matrix and carbopol 934P as a mucoadhesive polymer and demonstrated that free amoxicillin was rapidly degraded in acidic medium; however, amoxicillin entrapped in the microspheres kept stable. The in vitro release test showed that about 90% of the amoxicillin was released in the pH 1.0 HCl solution within 4 h, while in vivo evaluation of mucoadhesiveness showed that, during the same time, 63.6 and 21.9% of the microspheres still remained in the rat stomach. Furthermore, they found a higher amoxicillin concentration in gastric tissue of rats after oral administration of mucoadhesive microspheres vs. amoxicillin powder at the same dose of 43 mg kg\(^{-1}\). Finally, studies on the in vivo clearance of H. pylori revealed that, in a single-dosage administration, the mucoadhesive microspheres had a better effectiveness compared to amoxicillin powder (Liu et al., 2005).

Katayama et al., (1999) proposed a sustained release liquid preparation using sodium alginate. The gastroretentive property of the device was provided by the ability of sodium alginate to form a firm gel when an acid or di or trivalent metal ions (Ca\(^{2+}\), Ba\(^{2+}\), Sr\(^{2+}\)) were added. In vitro ampicillin release was retarded by calcium pretreatment due to gel formation. To evaluate the gastric retention time of the preparation, the authors compared, in isolated perfused rat stomach, the remaining percent of ampicillin when an aqueous ampicillin solution vs. the sodium alginate preparation were administrated. With calcium pretreatment, the total remaining percent of ampicillin at 120 min was 0.3% and 8% for the aqueous ampicillin solution and the sodium alginate preparation, respectively. Moreover, it was observed that the sodium alginate preparation remained mainly on the gastric mucus. In vivo studies were also performed with administration of aqueous ampicillin solution or a sodium alginate preparation through a gastric tube to fasting rats. Because sodium alginate is insoluble in acidic conditions, the authors pre administrated ranitidine to rats just before the calcium solution. Under these conditions, the total remaining percent of ampicillin at 60 min was near zero for aqueous ampicillin solution and 87% for the sodium alginate solution.
Hejazi and Amiji, (2003) published several papers about tetracycline loaded cross linked chitosan microspheres. They used different cross linking methods. First, they prepared chitosan microspheres by ionic cross linking and precipitation with sodium sulfate. However, it was observed that such chitosan microspheres did not provide a longer retention time in the fasting gerbil stomach. Moreover, the tetracycline concentration profile in the stomach, following administration in microsphere formulation, was similar to that of the aqueous solution. They then tried a second cross-linking method which used a chemical cross-linker such as glyoxal. A radioiodinated ($^{125}$I) glyoxal cross linked chitosan microsphere suspension was administered to fasted gerbils, and the animals were sacrificed at different time points to assess the radioactivity in tissues and fluids. After 2 h in the fasting stomach, 17% of the cross-linked chitosan microspheres were still present, and the tetracycline concentration profile in the stomach from the cross-linked microsphere formulation was higher AUC than that of the aqueous solution and the non-crosslinked microsphere formulation. Ten h after administration, 11% of cross linked chitosan microspheres remained in the stomach.

2.1.9.3. Drug delivery systems with specific interaction

Many scientists explored the possibility to use a specific ligand (e.g. a lectin) coupled with the dosage form, to target specifically a site in the gastrointestinal tract. Similarly, Umamaheshwari et al., (2003c) proposed drug delivery systems with a specific targeting of H. pylori. To reach this goal, the authors proposed two different drug delivery systems. First, they developed nanoparticles bearing AHA coated with fucose. Indeed, it is well known that some strains of H. pylori express an adhesin, BabA2, which interacts with the fucosylated histo blood group antigen Lewis b (Leb) (Suerbaum and Michetti, 2002; Skouloubris et al., 2000; Ilver et al., 1998, & An and Friedman, 2000). This Leb blood group antigen is expressed on the surface of gastric cells and the BabA2 adhesin is essential for the bacterial adhesion (Suerbaum and Michetti, 2002). Hence, by using fucose as ligand on the surface of nanoparticles, Umamaheshwari et al., (2003a) accomplished a specific targeting of H. pylori. Chitosan glutamate nanoparticles were prepared by an ionotropic gelation method, and the L-fucose was covalently bound to nanoparticles. The interactions between (I)-
fucose conjugated chitosan glutamate nanoparticles and \textit{H. pylori} were characterized in situ by an adherence assay with FITC (fluorescein isothiocyanate) labelled strains on sections of human stomach. A “plug and seal” effect between \textit{H. pylori} and nanoparticles was observed. Furthermore, \textit{in vitro} growth inhibition studies showed that L-fucose conjugated chitosan glutamate nanoparticles exhibited 2 fold inhibitory efficacy compared to chitosan-glutamate nanoparticles and the plain drug (Umamaheshwari \textit{et al.}, 2003a). By combining a bioadhesive effect (provided by chitosan and glutamate) and targeting capacity (provided by fucose), this drug delivery system presented interesting properties. However, not all strains of \textit{H. pylori} express BabA2 adhesin (Suerbaum and Michetti, 2002; Skouloubris \textit{et al.}, 2000; Ilver \textit{et al.}, 1998, & An and Friedman, 2000). Consequently, such a system cannot be used in all \textit{H. pylori} infections and will never provide for a 100 \% eradication rate. Then, Umamaheshwari’s research group proposed to develop a receptor mediated drug delivery system, based on phosphatidyl ethanolamine (PE) containing lipid. PE seems to be a major receptor promoting \textit{H. pylori} adhesion to intact cells (Lingwood \textit{et al.}, 1992; Lingwood, 1993, & Huesca \textit{et al.}, 1996). They used as a carrier, a new hybrid vesicle, called lipobead, combining complementary advantages of liposomes and polymeric beads. This system consists of a lipid bilayer shell that is anchored on the surface of a hydrogel polymer (polyvinyl alcohol xerogel) core. The specific binding between lipobeads and a specific surface receptor of \textit{H. pylori} was confirmed by in situ adherence and radiolabelling assays with human stomach cells and KATO-III cells, respectively.

2.2. A REVIEW ON GASTRORETENTIVE DRUG DELIVERY SYSTEMS

Limited gastrointestinal (GI) transit time often restricts the complete absorption of oral drugs, or limits the duration of absorption. In general, the transit time from mouth to cecum can vary from 3 to 16 h (Davis \textit{et al.}, 1987; Feely and Davis, 1989; Khosla and Davis, 1989, & Khosla \textit{et al.}, 1989). The transit time in the small intestine ranged from 3 to 4 h under both fasted and fed conditions (Wellman, 1984; Davis \textit{et al.}, 1987, & Khosla \textit{et al.}, 1989). Thus the time for absorption from the GI tract is limited for most drugs (Rao and Suresh, 2011).
Thus dosage of a few times a day is generally needed (Khosla and Davis, 1989). Prolonged gastric residence time (GRT) and controlled release of drugs within the GI tract helps to reduce dosing frequency and total dose, improve patient compliance and convenience, maintain a less fluctuating plasma level, as well as reduce GI side effects (Gupta and Robinson, 1992). Variable and short gastric emptying time can result in incomplete drug release from the drug delivery system (DDS) above the absorption zone (stomach or upper part of small intestine), leading to a diminished efficacy of the administered dose (Chueh et al., 1995, & Iannuccelli et al., 1998).

Prolonging the GRT of therapeutic agents is thought to be beneficial especially under several circumstances such as for drugs acting topically on the gastric region, for drugs with a narrow therapeutic window or for drugs with the major absorption site in the upper GI tract (Klausner et al., 2003). Many attempts were made to control gastric retention time by altering the size (Meyer et al., 1985; Itoh et al., 1986; Park et al., 1987; Khosla et al., 1989; Khosla and Davis, 1990, & Sirois et al., 1990), shape (Meyer et al., 1985, & Park et al., 1987), density (Bechgaard et al., 1985; Meyer et al., 1985; Davis et al., 1986; Inganni et al., 1987, & Sirois et al., 1990), and surface properties (Ch’ng et al., 1985, & Harris et al., 1990a,b) of oral devices. It has been proved for the first time that objects could be retained in the stomach for 24 h under fasted conditions if they possess certain tetrahedral or ring-like geometries with their combined effects of size, shape and flexibility were important in gastric retention. The mechanisms of gastric retention of those devices, however, are not understood (Cargill et al. 1988, 1989).

Various approaches have been proposed to retain the dosage form in the stomach. These methods include bioadhesive systems (Deshpande et al., 1996, & Santus et al., 1997), swelling and expanding systems (Deshpande et al., 1996, 1997) and floating systems (Menon et al., 1994, & Whitehead et al., 1998). Various gastro retentive drug delivery systems reported are:

- High-density systems
- Floating systems
2.2.1. Floating drug delivery system (FDDS)

Floating drug delivery system (FDDS) is one of gastroretentive dosage forms which could prolong GRT to obtain sufficient drug bioavailability (Whitehead et al., 1998; Singh and Kim, 2000; Arora et al., 2005, & Bardonnet et al., 2006). The system basically floats in the gastric fluid because of its lower bulk density compared to that of the aqueous medium. FDDS is desirable for drugs with an absorption window in the stomach or in the upper small intestine (Rouge et al., 1996, & Sato et al., 2004a). It is also useful for drugs that act locally in the proximal part of gastrointestinal (GI) tract such as antibiotic administration for H.pylori eradication in the treatment of peptic ulcer (Cooreman et al., 1993; Yang et al., 1999; Umamaheshwari et al., 2003b, & Bardonnet et al., 2006) and for drugs that are poorly soluble or unstable in the intestinal fluid (Singh and Kim, 2000, & Jain et al., 2005). These systems also offer various pharmacokinetic advantages like maintenance of constant therapeutic levels over a prolonged period and thus reduction in fluctuation in therapeutic levels minimizing the risk of resistance especially in case of antibiotics. Gastrointestinal retention depends on many factors such as density of the dosage form, size of the dosage form, fasting and fed condition, nature of the meal taken, sleep, posture, etc. It also depends strongly on a complicated and unpredictable gastric emptying with migrating myoelectric complex motility of the stomach (Talukder and Fassihi, 2004a). In fact, the buoyant dosage unit enhances gastric residence time (GRT) without affecting the intrinsic rate of emptying (Stithit et al., 1998).
The concept of floating microparticles can also be utilized to minimize the irritant effect of weakly acidic drugs on the stomach by avoiding direct contact with the mucosa and providing a mean of getting low dosage for prolonged periods (Thanoo et al., 1993).

2.2.2. Single vs. multiple unit FDDS

Most of the floating systems previously reported are single unit systems such as tablets and capsules. A drawback of these systems is the high variability of the GI transit time due to their all-or-nothing emptying processes (Ichigawa et al., 1991; Kawashima et al., 1991; Streubel et al., 2003b; Umamaheshwari et al., 2003a; Talukder and Fassihi, 2004b; Jain et al., 2005, & Hwang et al., 1998). On the other hand, the multiple unit dosage forms may be an attractive alternative since they have been shown to reduce inter and intra subject variabilities in drug absorption as well as to lower the possibility of dose dumping (Bechgaard and Ladefoged, 1978a; Bechgaard and Nielson, 1978b, & Vervaet et al., 1995). Various formulations like floating microparticles, pellets, tablets, capsules, etc., were evaluated as a GRDS. Among these formulations, the multiparticulate systems (El-Kamel et al., 2001, & Efentakis et al., 2000) like microparticles and pellets are more advantageous than single unit systems like tablets and capsules. They distribute uniformly within the gastric content and gradually empty from the stomach, possibly resulting in longer lasting effects and reduced inter subject variability.

A floating system made of multiple unit forms has relative merits compared to a single unit preparation (Iannuccelli et al., 1998). Indeed, the gastric emptying of a multiparticulate floating system would occur in a consistent manner with small individual variations. On each subsequent gastric emptying, sunksen particles will spread out more uniformly over a large area of absorption sites, increasing the opportunity for drug release profile and absorption in a more or less predictable way and reduce the risk of local irritation (Acikgoz et al., 1995; Kawashima et al., 1992, & Stithit et al., 1998). Moreover, since each dose consists of many subunits, the risk of dose dumping is reduced (Iannuccelli et al., 1998).
2.2.3. Intragastric drug delivery systems - the research perspective

Various multiple-unit floating systems have been developed in different forms and principles such as air compartment multiple-unit system (Iannuccelli et al., 1998), hollow microspheres (microballoons) prepared by the emulsion solvent diffusion method (Sato et al., 2003, 2004a, b, & Jain et al., 2005), microparticles based on low density foam powder (Streubel et al., 2002, & 2003a), beads prepared by emulsion gelation method (Talukder and Fassihi, 2004b, & Srimornsak et al., 2005). Use of swellable polymers and effervescent compounds is another approach for preparing multiple unit FDDS. An FDDS developed by coating the sustained release pills or granules with tartaric acid layer, sodium bicarbonate layer and polymeric film consisting of polyvinyl acetate and shellac (Ichigawa et al., 1991).

The floating system using ion exchange resin loaded with bicarbonate and then coated by a semipermeable membrane was also proposed (Atyabi et al., 1996). Recently, a floating alginate beads were prepared using gas forming agents like calcium carbonate and sodium bicarbonate (Choi et al., 2002).

Low density porous carriers have been used by researchers for formulation of FDDS (Yuasa et al., 1996a, & Streubel et al., 2003b).

An alginate floating dosage form was introduced as early as in the 1980s (Stochwell and Davis, 1986). Its benefits are cheap and abundant sources, excellent biocompatibility, and total degradation without hazardous byproducts.

Recent development of alginate floating dosage forms by three different groups (Whitehead et al., 1998; Iannuccelli et al., 1998, & Murata et al., 2000) Calcium alginate is the result of the complexation of the polyguluronic sequences by calcium ion, known to be insoluble and resistant to acidic media (Grant et al., 1973).

Iannuccelli et al. (1998) developed a different bubble type floating alginate dosage form with an alginate core was designed. However, excellent buoyancy was only achieved in water. In acidic media, the units did not float, and even after modification of membrane permeability with PVA addition, buoyancy was still not as good as in water. No drug release characteristics were reported in this case.
Whitehead et al. (1998) reported that freeze drying of calcium alginate gel beads produced approximately 2.5 mm in diameter and floated on agitated acidic media for over 12 h. These floating beads were radiolabelled with pertechnetate and in vivo test revealed gastric retention of these beads ranged from 5.5 to 9 h. There was little sustained release of the drug. The majority of drug was released in a burst during the first 60 min. and higher drug loadings could be achieved at the expense of faster release and lower buoyancy. A modification to this method, vegetable oil for extra buoyancy or chitosan for extra bioadhesion (Gaserod et al., 1998) was added into the alginate gel beads. In contrast to the findings of Whitehead et al.’s, non oily gel beads failed the buoyancy test and they were found to have a lower loading of the drug metronidazole than the oily beads. The floating gel beads were found to be able to maintain a stable serum concentration of metronidazole for 4 h rather than the 2 h of the conventional dosage in guinea pigs (Murata et al., 2000).

In a later study, a multi unit calcium alginate dosage form with enhanced buoyancy using sunflower oil as a dispersed phase to generate a uniform emulsion to create multiple tiny chambers in the alginate matrix for better buoyancy (Murata et al., 2003).

Sustaining the release of both hydrophilic and hydrophobic drugs over a reasonable duration, since prolonged retention without sustained release is of little practical value (Tang et al., 2007). Previous efforts of establishing a relationship between the drug release profile and drug solubility (Whitehead et al., 1998; Iannuccelli et al., 1998, & Murata et al., 2000) appeared not to produce sustained release of more than 2–3 h. Three drugs with different hydrophilicities, ibuprofen (less hydrophilic), niacinamide (hydrophilic) and metoclopramide HCl (highly hydrophilic) were studied as the model drugs.

A completely erodible multi-unit floating GRDS has been developed with an intention of achieving a controlled release profile that is consistent with prolonged retention time. Gel beads made solely of calcium alginate was found to lack sufficient and consistent buoyancy over long hours of administration. In contrast, the oil incorporated alginate gel beads floated constantly under the condition of body temperature and continuous agitation for more than 24 h. Drug release profiles were
influenced by the relative hydrophilicities of drugs. A slow first order ibuprofen release was achieved with drug encapsulation in oil. Sustained release for more than 12 h was also achieved for hydrophilic drugs such as niacinamide and metoclopramide HCl, with the rate-control being achieved by means of an acid resistant Eudragit coating. Such an alginate based GRDS is hence thought to be able to sustain the release of both hydrophobic and hydrophilic drugs over 12 h, while remaining afloat in the gastric fluid (Tang et al., 2007).

In another study, a new multiple-unit FDDS based on gas formation technique was developed. The system consists of drug-containing cores coated with effervescent layer and Eudragit RL 30D as a polymeric membrane. The floating ability and drug release of the system were dependent on amount of the effervescent agent layered on the core pellets, and type and coating level of the polymeric membrane. Only the system using polymeric membrane could float as Eudragit RL 30D had high water and low CO$_2$ permeability with high flexibility (Sungthongjeen et al., 2006).

Low density porous carriers have been used by researchers for formulation of FDDS (Yuasa et al., 1996a, & Streubel et al., 2003b). Porous carriers are low density solids with open or closed pore structure and provide large exposed surface area for drug loading. Their hydrophobicity varies from hydrophilic carriers, which immediately disperse or dissolve in water, to completely hydrophobic ones, which float on water for hours. Due to wide range of useful properties, porous carriers have been used in pharmaceuticals for many purposes; some of these includes development of novel drug delivery systems like floating drug delivery systems, sustained drug delivery systems; improvement of solubility of poorly soluble drugs; enzyme immobilization etc. (Hanawa et al., 1996; Byrne and Deasy, 2002; Streubel et al., 2003b, & Ito et al., 2005). Examples of pharmaceutically exploited porous carriers include porous silicon dioxide (Sylsia®), polypropylene foam powder (Accurel®), porous calcium silicate (Florite®), magnesium aluminometa silicate (Neusilin®), porous ceramic, etc. Florite RE® (FLR) is a porous calcium silicate and possesses a lot of pores particularly of size 0.15µm on its surface (Yuasa et al., 1996a). FLR has been used to adsorb oily and other drugs, as a compressive agent in pharmaceuticals and to improve solubility (Yuasa et al., 1994, 1996b, and Kinoshita et al., 2003).
A blend of floating and pulsatile principles of drug delivery system would have the advantage that a drug can be released in the upper GI tract after a definite time period of no drug release. A multiparticulate floating-pulsatile drug delivery system was developed using porous calcium silicate (Florite RE®) and sodium alginate, for time and site specific drug release of meloxicam. Floating time was controlled by density of beads and hydrophobic character of drug. A pulsatile release of meloxicam was demonstrated by a simple drug delivery system which could be useful in chronopharmacotherapy of rheumatoid arthritis. Developed formulations showed instantaneous floating with very less drug release in acidic medium followed by a pulse drug release in simulated gastric fluid (Sharma et al., 2006).

As far as floating devices are concerned, air included within a multiple unit compartment system resulted in excellent buoyancy in vitro and prolonged the GRT relative to controls in vivo in the fed state (Iannuccelli et al., 1998a, b, & 2000). However, in the fasted state, the intragastric buoyancy of the devices did not affect the GRT. Hollow microspheres (microballoons) were developed in order to prolong the GRT of the dosage form (Kawashima et al., 1991). This gastrointestinal transit-controlled preparation is designed to float on the surface of gastric juice with a specific density of less than 1. When in vivo evaluation of microballoons (MB) was performed, extreme difficulty was encountered with respect to examination of the flotation behavior of MB in the stomach of animals such as rats and dogs. For many drugs like Riboflavin that are absorbed mainly from the proximal small intestine, controlled release in the stomach would result in improved bioavailability. Prolongation of the urinary excretion of riboflavin could be obtained by ingestion of water as well as “fed” conditions. This phenomenon was attributable to the buoyancy properties of MB in the stomach and an increase in the gastric residence time (Sato et al., 2004).

Streubel et al., (2002) reported floating microparticles consisting of (i) polypropylene foam powder; (ii) verapamil HCl as model drug; and (iii) Eudragit RS, ethylcellulose or polymethyl methacrylate as polymers were prepared with an o/w solvent evaporation method showed good floating properties, high encapsulation efficiencies, high pay loads of the drugs and sustained drug release over several hours.
A multiple unit, oral, floating system, which generates carbon dioxide, was developed using ion exchange resin particles which were loaded with bicarbonate and coated with a semipermeable membrane. Upon exposure to gastric media, exchange of bicarbonate and chloride ions took place and led to the formation of carbon dioxide. The gas was trapped within the membrane causing the particles to float (Atyabi et al., 1996a, & b).

An interesting multi particulate FDDS was developed known as hollow microspheres (microballoons) consisting of Eudragit S, an enteric polymer, loaded with drug in the outer polymer shells (Kawashima et al., 1991, 1992). They prepared a solution of polymer and drug in ethanol/methylene chloride was poured into an agitated aqueous solution of polyvinyl alcohol. The ethanol rapidly partitioned into the external aqueous phase and the polymer precipitated around methylene chloride droplets. The subsequent evaporation of the entrapped methylene chloride led to the formation of internal cavities within the microparticles. However, many drugs are not released in significant amounts from this type of microparticles at the pH of gastric fluids (Lee et al., 1997). A floating drug delivery system being less dense than gastric juice due to the incorporation of at least one porous structural element, such as foam or a hollow body has been patented (Muller and Anders, 1989).

A floating microparticulate sustained release system for ketoprofen designed using Eudragit S100 (ES) with Eudragit RL (ERL) to increase its residence time in the stomach without contact with the mucosa was prepared by the emulsion-solvent diffusion technique. The formulation containing ES to ERL at 1:1 ratio exhibited high percentage of floating particles in 0.1 N HCl containing 0.02 % Tween 20 and simulated gastric fluid without pepsin (El-Kamel et al., 2001).

Casein gelatin beads have been prepared by emulsification extraction method and cross-linked with D, L-glyceraldehyde in an acetone–water mixture 3:1 (v:v). Casein emulsifying properties cause air bubble incorporation and the formation of large holes in the beads. The high porosity of the matrix influences the bead properties such as drug loading, drug release and floatation (Bulgarelli et al., 2000).

A gastrointestinal drug delivery system can be made to float in the stomach by a gelling process of hydrocolloid materials or by incorporating a flotation chamber,
vacuum or gas filled (Chien, 1992). In this way a bulk density less than that of the
gastric fluid is produced. However, most of the devices generating gas or gelling need
time to be floated and this parameter must be checked carefully in order to prevent the
dosage form from transiting into the small intestine together with food before floating
in the stomach.

2.2.4. Classification of microbeads/microparticles

Microbeads/ microparticles can be classified as effervescent floating micro
beads and non effervescent floating micro particles.

2.2.4.1. Effervescent floating microparticles

Gas forming agents such as carbonates and bicarbonates are incorporated in
the microbeads which when comes in contact with acidic gastric medium causes the
release of CO$_2$ and due to this effervescence the system floats.

Floating alginate beads using gas forming agents (calcium carbonate and
sodium bicarbonate) were prepared and studied the effect of CO$_2$ generation on the
physical properties, morphology, and release rates. The study revealed that the kind
and amount of gas-forming agent had a profound effect on the size, floating ability,
pore structure, morphology, release rate, and mechanical strength of the floating
beads. It was concluded that calcium carbonate formed smaller but stronger beads
than sodium bicarbonate. Calcium carbonate was shown to be a less effective gas
forming agent than sodium bicarbonate but it produced superior floating beads with
enhanced control of drug release rates. In vitro floating studies revealed that the beads
free of gas-forming agents sank uniformly in the media while the beads containing
gas-forming agents in proportions ranging from 5:1 to 1:1 demonstrated 100 %
floating (Choi et al., 2002).

A floating system using ion exchange resin that was loaded with bicarbonate
by mixing the beads with sodium bicarbonate solution was reported. The loaded beads
were then surrounded by a semipermeable membrane to avoid sudden loss of CO$_2$.
Upon coming in contact with gastric contents an exchange of chloride and bicarbonate
ions took place that resulted in CO$_2$ generation thereby carrying beads toward the top
of gastric contents and producing a floating layer of resin beads (Atyabi et al., 1996b).
A multiple unit floating drug delivery system based on gas formation technique was developed in order to prolong the gastric residence time and to increase the overall bioavailability of the dosage form. The system consists of the drug containing core pellets prepared by extrusion spheronization processes, which are coated with double layers of an inner effervescent layer (sodium bicarbonate) and an outer gas entrapped polymeric membrane of an aqueous colloidal polymer dispersion (Eudragit® RL 30D, RS 30D, NE 30D). Only the system using Eudragit® RL 30D as a gas entrapped polymeric membrane could float. The time to float decreased as amount of the effervescent agent increased and coating level of gas entrapped polymeric membrane decreased. The optimum system could float completely within 3 min and maintained the buoyancy over a period of 24 h (Sungthongjeen et al., 2006).

2.2.4.2. Non effervescent floating micro particles

Polycarbonate microspheres were developed by solvent evaporation technique. Polycarbonate in dichloromethane was found to give hollow microspheres that floated on water and simulated biofluids as evidenced by scanning electron microscopy. High drug loading was achieved and drug loaded microspheres were able to float on gastric and intestinal fluids. It was found that increasing the drug to polymer ratio increased both their mean particle size and release rate of drug (Thanoo et al., 1998).

Bulgarelli et al., (2000) studied the effect of matrix composition and process conditions on casein gelatin beads prepared by emulsification extraction method. Casein by virtue of its emulsifying properties causes incorporation of air bubbles and formation of large holes in the beads that act as air reservoirs in floating systems and serve as a simple and inexpensive material used in controlled oral drug delivery systems. It was observed that the percentage of casein in matrix increases the drug loading of both low and high porous matrices, although the loading efficiency of high porous matrices is lower than that of low porous matrices. Casein gelatin beads have been prepared by emulsification extraction method and cross linked with D, L-glyceraldehyde in an acetone water mixture 3:1 (v:v). Casein emulsifying properties cause air bubble incorporation and the formation of large holes in the beads. The high porosity of the matrix influences the bead properties such as drug loading, drug release and floatation. These effects have been stressed by comparison with low
porous beads, artificially prepared without cavities. The percentage of casein in the matrix increases the drug loading of both low and high porous matrices, although the loading of high porous matrices is lower than that of low porous matrices. As a matter of fact, the drug should be more easily removed during washing and recovery because of the higher superficial pore area of the beads. This can explain the drug release rate increase, observed in high porous matrix, in comparison with beads without cavities. This is due to the rapid diffusion of the drug through water filled pores. The study shows that cavities act as an air reservoir and enable beads to float. Therefore, casein seems to be a material suitable to the inexpensive formation of an air reservoir for floating systems (Bulgarelli et al., 2000).

Floating alginate beads incorporating amoxicillin were reported recently. The beads were produced by dropwise addition of alginate into calcium chloride solution, followed by removal of gel beads and freeze drying. The beads containing the dissolved drug remained buoyant for 20 h and high drug loading levels were achieved (Whitehead et al., 2000).

Floating microparticles of ketoprofen were prepared by emulsion solvent diffusion technique. Four different ratios of Eudragit S 100 with Eudragit RL were used. The formulation containing 1:1 ratio of the 2 abovementioned polymers exhibited high percentage of floating particles in all the examined media as evidenced by the percentage of particles floated at different time intervals. This can be attributed to the low bulk density, high packing velocity, and high packing factor. A sustained release system for ketoprofen designed to increase its residence time in the stomach without contact with the mucosa was achieved through the preparation of floating microparticles by the emulsion-solvent diffusion technique. Four different ratios of Eudragit S100 (ES) with Eudragit RL (ERL) were used to form the floating microparticles. Floating ability in 0.1 N HCl, 0.1 N HCl containing 0.02 % Tween 20 and simulated gastric fluid without pepsin was also tested. The formulation containing ES:ERL 1:1 exhibited high percentage of floating particles in all examined media (El-Kamel et al., 2001).

Illum and Ping, (2000) developed microspheres that released the active agent in the stomach environment over a prolonged period of time. The active agent was
encased in the inner core of microspheres along with the rate-controlling membrane of a water-insoluble polymer. The outer layer was composed of bioadhesive (chitosan). The microspheres were prepared by spray drying an oil/water or water/oil emulsion of the active agent, the water insoluble polymer, and the cationic polymer.

Streubel et al., (2002) prepared floating microparticles composed of polypropylene foam, Eudragit S, ethyl cellulose, and polymethylmethacrylate by solvent evaporation technique. Good floating behaviour was observed as more than 83 % of microparticles were floating for at least 8 h.

Multiple-unit hollow microspheres capable of floating invivo in aqueous media for 12 h were reported. Drug and acrylic polymer were dissolved in an ethanol-dichloromethane mixture, and poured into an aqueous solution of PVA with stirring to form emulsion droplets (emulsion solvent diffusion technique). Radiographical studies proved that microballoons orally administered to humans were dispersed in the upper part of stomach and retained there for 3 h against peristaltic movements (Kawashima et al., 1991)

A floating dosage form of piroxicam based on hollow polycarbonate microspheres was reported. The microspheres were prepared by the solvent evaporation technique. Encapsulation efficiency of 95 % was achieved. In vivo studies were performed in healthy male albino rabbits. Pharmacokinetic analysis was derived from plasma concentration vs time plot and revealed that the bioavailability from the piroxicam microspheres alone was 1.4 times that of the free drug and 4.8 times that of a dosage form consisting of microspheres plus the loading dose and was capable of sustained delivery of the drug over a prolonged period (Joseph et al., 2002).

Recently an emulsion gelation method to prepare oil entrapped calcium pectinate gel beads capable of floating in the gastric condition was designed and tested. The type and percentage of oil play an important role in controlling the floating of oil entrapped beads. The results suggested that oil entrapped beads were promising as a carrier for intragastric floating drug delivery (Sriamornsak et al., 2004).
It was reported that floating microspheres consisting of calcium silicate as porous carrier; orlistat, an oral anti-obesity agent; and Eudragit S as polymer, were prepared by solvent evaporation method and evaluated their gastro retentive and controlled release properties. The gamma scintigraphy of the optimized formulation was performed in albino rabbits to monitor the transit of floating microspheres in the gastrointestinal tract. Microsphere formulation, containing 200 mg calcium silicate, showed the best floating ability (88% buoyancy) in simulated gastric fluid as compared with other formulations. Prolonged gastric residence time of over 6 h was achieved in all rabbits for calcium silicate based floating microspheres of orlistat. The enhanced elimination half-life observed after pharmacokinetic investigations in the study is due to the floating nature of the formulations (Jain et al., 2006).

Badve et al., (2007) reported hollow calcium pectinate beads for floating-pulsatile release of diclofenac sodium intended for chronopharmacotherapy. Floating pulsatile concept was applied to increase the gastric residence of the dosage form having lag phase followed by a burst release. To overcome limitations of various approaches for imparting buoyancy, hollow/porous beads were prepared by simple process of acid base reaction during ionotropic crosslinking. The floating beads obtained were porous (34% porosity), hollow with bulk density <1 and had 50% of the particles were floated for 14–24 h. In vivo studies by gamma scintigraphy determined on rabbits showed gastro retention of beads up to 5 h.

Hollow microspheres (microballoons) floatable in JPXIII No.1 solution were reported as a dosage form characterized by excellent buoyant properties in the stomach. Microballoons were prepared by the emulsion solvent diffusion method utilizing enteric acrylic polymers dissolved with drug in a mixture of dichloromethane and ethanol. The release properties of five different drugs exhibiting distinct water solubilities (aspirin, salicylic acid, ethoxybenzamide, indomethacin and riboflavin) entrapped within microballoons were investigated. Buoyancy of the microballoons decreased with increasing drug release rate. In addition, by incorporating a polymer such as hydroxypropylmethylcellulose within the shell of microballoons, the release rate of riboflavin from the microballoons could be controlled while maintaining high buoyancy (Satoa et al., 2004b).
Hollow microspheres (microballoons) floatable on JPXIII No.1 solution were developed as a dosage form capable of floating in the stomach. Hollow microspheres were prepared by the emulsion solvent diffusion method using enteric acrylic polymers with drug in a mixture of dichloromethane and ethanol. It was found that preparation temperature determined the formation of cavity inside the microsphere and the surface smoothness, determining the floatability and the drug release rate of the microballoon. The correlation between the buoyancy of microballoons and their physical properties, e.g. apparent density and roundness of microballoons were elucidated. The optimum loading amount of riboflavin in the microballoon was found to impart ideal floatable properties to the microballoons (Sato et al., 2003).

Vegetable oil held in the alginate gel matrix improves the buoyancy of alginate gel beads. Murata et al. (2000) prepared two types of alginate gel beads capable of floating in the gastric cavity. The first, alginate gel bead containing vegetable oil, its buoyancy is attributable to vegetable oil held in the alginate gel matrix. The second, alginate gel bead containing chitosan (ALCS), is a dried gel bead with dispersed chitosan in the matrix. When ALCS containing metronidazole was administered orally to guinea pigs, it floated on the gastric juice and released the drug into the stomach. Furthermore, the concentration of metronidazole at the gastric mucosa after administration of ALCS was higher than that in the solution, though the metronidazole serum concentration was the same regardless of which type of gel was administered.

A floating dosage form that is able to sustain release both hydrophobic and hydrophilic drugs within its extended gastric retention time was reported. Floating dosage forms enable the sustained delivery of drugs in the gastrointestinal tract. In the study, a type of multi unit floating gel bead was synthesized with calcium alginate, sunflower oil, and a drug of interest through an emulsification/gelation process. The alginate beads with oil addition were able to continuously float over the medium for 24 h under constant agitation while the non oily beads could not (Tang et al., 2007).

The potential of Accurel MP 1000®, a low density porous as gastroretentive drug delivery system was reported. Low density porous carriers are widely used in the pharmaceutical applications. Ibuprofen was adsorbed on Accurel MP 1000® by
solvent evaporation using two organic solvents methanol and dichloromethane. SEM showed the penetration and adsorption of the drug in and on the microporous polymer. All batches showed excellent in vitro floating property (Sher et al., 2007).

Floating microcapsules containing melatonin by the ionic interaction of chitosan and a negatively charged surfactant, sodium dioctyl sulfosuccinate (DOS) were reported. The use of DOS solution in coagulation of chitosan produced well-formed microcapsules with round hollow core and 31.2 % incorporation efficiencies. Most of the hollow microcapsules developed tended to float over simulated biofluids for more than 12 h. and the data obtained suggest that the floating hollow microcapsules produced would be an interesting gastroretentive controlled-release delivery system for drugs (El-Gibaly, 2002).

An air compartment multiple unit system containing PVA coated alginate beads was developed by Iannuccelli et al. and pre-formulation study was carried out to optimize the in vitro floating ability. Each unit was formed by a coated bead composed of a calcium alginate core separated by an air compartment from a calcium alginate or calcium alginate: polyvinylalcohol membrane. The floating ability depended on the presence of the air compartment and on membrane porosity. The porous structure generated by the leaching of PVA, employed as a water-soluble additive in the coating composition, increased the membrane permeability preventing air compartment shrinkage. In this way, units were produced which were able to float immediately upon contact with artificial gastric juice and for a long period of time. The floating ability increased with the increase in PVA concentration and molecular weight and it was found to be excellent when using PVA 100000 at a concentration of at least 5 % (Iannuccelli et al., 1998).

Srivastava et al., (2005) prepared and evaluated floating microspheres with cimetidine as model drug for prolongation of gastric residence time. The microspheres were prepared by the solvent evaporation method using polymers hydroxypropylmethyl cellulose and ethyl cellulose. The prepared microspheres exhibited prolonged drug release (8 h) and remained buoyant for more than 10 h.
2.3. EVALUATION OF MICRO PARTICULATE DELIVERY SYSTEMS

Various parameters that need to be evaluated in gastroretentive formulations include floating duration, dissolution profiles, specific gravity, content uniformity, hardness, and friability in case of solid dosage forms. In the case of multiparticulate drug delivery systems, differential scanning calorimetry, particle size analysis, flow properties, surface morphology, and mechanical properties are also performed. The tests for floating ability and drug release are generally performed in simulated gastric fluids at 37ºC (Desai et al., 1993).

2.3.1. Mechanical strength

Many scientists have reported mercury load cell method for the determination of mechanical strength or crushing strength of the microbeads. Usually large beads prepared using 26G needles were used for this study (Choi et al., 2002).

2.3.2. Total moisture content

The total moisture content in the floating beads can be measured using Karl Fisher titration method (Badve et al., 2007).

2.3.3. Micromeritic properties

2.3.3.1. Apparent particle density

Apparent particle density of the microbeds/particles can be determined by the projective image count method as follows. Microparticles were placed on a glass plate. Heywood diameter and no of microparticles were measured by an image processing and analysis System. Subsequently, the apparent particle density was calculated according to Eq.

\[
\frac{W}{V} = \frac{W}{\sum(\pi d^3 n/6)}
\]

Where W, weight microparticles, V, its volume, d, Heywood diameter, and n, number of microparticles (Sato et al., 2004). The density of beads can also be measured using an air comparison pycnometer (Bulgarelli et al., 2000).
2.3.3.2. Roundness

Roundness of microballoons is measured by an image processing and analysis system. In this system, roundness can be calculated according to the equation

\[
\text{Roundness of microparticles} = \frac{L^2}{4\pi S}
\]

Where \( L \) is circumference of a projective image, and \( S \) area of a projective image. If the roundness of microparticles is close to 1, the microparticles closely resemble spherical particles.

2.3.3.4. Particle size analysis

- Using a calliper: The particle sizes of microparticles can be determined by using a caliper (Murata et al., 2000)
- Heywood diameters: The Heywood diameter was introduced as a single variable to evaluate the particle sizes of the beads as the beads are not perfect spheres. Heywood diameters of the microparticles are obtained with a stereo zoom optical microscope with Image Analysis software, if beads are not perfectly spherical and no uniform diameter, the Heywood diameter was employed as an approximation so that every bead could be evaluated with a single variable to quantify its size. The size of microspheres can be determined using a microscope fitted with an ocular micrometer and stage micrometer. (Wang and Liao, 2002, & Tang et al., 2007)
- Size distribution: Determined by sieving the floating microparticles in standard test sieves.
- Dynamic light scattering or lasser scattering techniques using particle analysers

2.3.3.5. Measurement of flow properties

- Angle of repose: Angle of repose of different formulations can be determined by a fixed funnel method.
- Bulk density: Bulk density was measured by tapping method.
- Packing properties: Packing properties of floating microparticles were measured also by tapping method.
• The packing rate: The packing rate \((b, k)\) was calculated from the following equations

\[
\frac{n}{c} = \frac{1}{ab} + \frac{n}{a}
\]

Where \(c = \frac{V_0}{V_n}\), \(n\) is the tap number and \(n\) is the tap number, and \(V_o\) and \(V_n\) are the bed volumes of microbeads at initial and tapped state, respectively

\[
pf - pn = (pf - po)e^{-kn}
\]

Where \(pf\), \(pn\) and \(po\) are the apparent densities at equilibrium, \(n^{th}\) tapped state and initial state, respectively.

• The packing factor: The packing factor can also calculated as the ratio of bulk density after tapping to bulk density before tapping.

• Compressibility: Compressibility was computed according to the following equation

\[
Compressibility(\%) = \frac{(p_t - p_b)100}{p_t}
\]

Where, where \(p_t\) is the tapped bulk density and \(p_b\) is the initial bulk density (Lin and Kao, 1989).

• Porosity: The porosity was determined by mercury intrusion technique. This technique is based on the fact that the \(P\) pressure required to drive mercury through a pore increases as the pore diameter decreases, as described by the Washburn equation.

\[
P = \frac{-4\sigma \cos \theta}{d}
\]

Where \(d\) is the pore diameter, \(\sigma\) is the mercury air interfacial tension and \(\theta\) is the contact angle at the mercury air pore wall interface. A plot of the volume of mercury versus pressure is a common way to display the raw data. The shape of the porosimetry curve provides information about the pore morphology (Bulgarelli et al., 2000).
2.3.4. *In vitro* buoyancy test

2.3.4.1. Agitating glass flask/bottle method

The floating behaviour of microbeads and microparticles are carried out in glass flasks containing buffer solutions which are agitated by means of mechanical or magnetic shakers and the number of floating particles/beads is counted at the end of a specific time period. There are some reported works by researchers.

Floating behavior studies can be performed by placing a specific number of particles into 30 mL glass flasks and subsequent addition of 30 mL preheated 0.1 N HCl pH 1.2, containing 0.02 % w/v Tween 20 (37 °C) to exclude floating due to non-wetted surfaces, followed by horizontal shaking (37 °C, 75 rpm). At predetermined time intervals, the flasks were allowed to stand for 5 min without agitation and the numbers of settled particles are counted (Streubel *et al.*, 2002).

In another study the buoyancy of the gel beads can be tested by visual inspection. For each sample of gel beads, 20 individual beads were placed in the test bottles (10 mL volume capped bottle) filled with 10 mL SGF (about pH 1.0). The test bottles were kept in a water bath at 37±1 °C under constant agitation of 250 rpm with a magnetic stirrer for 24 h. The samples were considered buoyant only if 20 individual beads remained afloat after the prescribed test time (Murata *et al.*, 2003).

2.3.4.2. Agitating beaker method

In the agitating beaker method beakers are used in place of bottles compared to the flask method. The following are some of the reported methods.

Previously weighed floating microparticles were placed in 50 mL beakers to this twenty milliliters of 0.1 N HCl or 0.1 N HCl containing 0.02 % Tween 20 or simulated gastric fluid without pepsin (USP, 1995) was added. The beakers were shaken horizontally in a water bath at 37±0.1 °C. Floated particles were collected at 1, 2, 4 and 6 h and dried in a desiccator till constant weight. The percentage of floating microparticles was calculated as the ratio of weight of floating microparticles to the initial weight of floating microparticles multiplied by 100. (El- Kamel *et al.*, 2001).
Murata et al., (2000) reported that sample beads (ten granules) were steeped in 50 mL of each test solution (distilled water, 0.9 % NaCl or JPXIII 1st fluid) and their buoyancy was observed visually. The preparation was considered to have buoyancy in the test solution only when all of the granules floated in it.

Jain et al., (2005) used fifty milligrams of the floating microparticles, which are place in simulated gastric fluid (pH 2.0, 100 mL) containing 0.02 w/v % Tween 20. The mixture was stirred at 100 rpm in a magnetic stirrer. After 8 h, the layer of buoyant microparticles was pipetted and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in a desiccator until constant weight. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

\[
Buoyancy(\%) = \left( \frac{W_f}{W_f + W_s} \right) \times 100
\]

Where \( W_f \) and \( W_s \) are the weights of the floating and settled microparticles, respectively. All the determinations were made in triplicate.

The floating ability of the beads can be evaluated by placing fifty milligrams of the floating microparticles in 50 mL beakers. Twenty milliliters of 0.1 N HCl or 0.1 N HCl containing 0.02 % Tween 20 or simulated gastric fluid without pepsin (USP, 1995) were added. The beakers were shaken horizontally in a water bath at 37±0.1 °C. Floated particles were collected and dried in a desiccator till constant weight.

\[
\% Floating microparticles = \frac{weight \ of \ floating \ microparticles}{initial \ weight \ of \ microparticles}
\]

The percentage of floating microparticles was calculated by the above equation (El-Kamel et al., 2001).

2.3.4.3. USP Paddle method

USP dissolution apparatus II (paddle type) was made use of determination of floating microparticles. There are few reported methods for different type of microparticles.
The floating ability of the multiple unit floating beads was determined by using the USP paddle method at 50 rpm and 37±0.2 °C in 900 mL of water or simulated gastric fluid (pH 1.2; USP XXIII, without pepsin) or HCl solutions at two different pH values (3.0 and 5.0). Then, 20 units or their separate components were placed in the medium and the percentage of floating samples and the floating times were measured by visual observation (Ivanucelli et al., 1998).

2.3.5. Drug content and entrapment efficiency

The entrapment efficiency and drug content in microparticles can be determined by extracting the drug in suitable buffer or solvent and then analysing the drug content by suitable analytical techniques (Srivastava et al. 2005; Ibrahim El-Gibaly et al., 2002, & Streubel et al., 2002). The following equation can be used for the determination of the drug content in the formulations:

\[
\text{Drug content(%) or entrapment efficiency} = \frac{\text{Weight of drug in the microparticles}}{\text{Weight of microparticles recovered}} \times 100
\]

2.3.6. Bead water uptake and equilibrium swelling studies

The water uptake of beads were determined explain the possible influence on the polymeric materials used for the preparation of the beads on drug release behaviour (Tang et al., 2007). Bead water uptake was presented as normalized weight gain ratio defined in the following equation.

\[
Y = \frac{m_w}{m_d}
\]

Where \(Y\) is the normalized weight gain ratio, \(m_w\) the bead weight after swelling (including water uptake), and \(m_d\) is the initial dry bead weight.

It can also be represented as swelling ratio (SR) with following equation (El-Gibaly, 2002).

\[
SR = \frac{W_e - W_o}{W_o}
\]

Where \(W_o\) is the initial weight of the dry microparticles and \(W_e\) is the weight of the swollen microparticles at equilibrium swelling in the media.
The rate of entry of the acidic media (pH 1.2, 3.0 and 5.0) into the floating units was determined by means of the Enslin apparatus. One unit of each sample was placed into contact with the medium and the amount absorbed was measured at determined intervals of time at 25±0.2 °C.

2.3.7. Drug release studies and kinetics of dissolution

Drug release is an important parameter in the dosage form development. The factors which determine the drug release profile are the dissolution medium used and the various conditions at which the dissolution studies are carried out and various linear regression models have been used for predicting the drug release from the polymeric microparticles. The data obtained for in vitro release were fitted into equations for the zero-order, first-order, Higuchi and Peppas release models.

Drug dissolution from solid dosage forms has been described by kinetic models in which the dissolved amount of drug \( Q \) is a function of the test time, \( t \) or \( Q=f(t) \). Some analytical definitions of the \( Q \) (\( t \)) function are commonly used, such as zero order, first order, Hixson–Crowell, Weibull, Higuchi, Baker–Lonsdale, Korsmeyer–Peppas and Hopfenberg models.

2.3.7.1. Zero order kinetics model

The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. The following relation can, in a simple way, express this model

\[
Q_t = Q_o + K_o t
\]

Where \( Q_t \) is the amount of drug dissolved in time \( t \), \( Q_o \) is the initial amount of drug in the solution. Most times, \( Q_o = 0 \) and \( K_o \) is the zero order release constant.

2.3.7.2. First order kinetics model

The following relation can express first order kinetic models and also in a way a graphic of the decimal logarithm of the released amount of drug versus time will be linear
\[ Q_t = Q_0 e^{-Kt} \text{ or } \log Q_t = \log Q_0 + Kt/2.303 \]

Where \( Q_t \) is the amount of drug released in time \( t \), \( Q_0 \) is the initial amount of drug in the solution and \( K \) is the first order release constant.

**2.3.7.3. Higuchi model**

In a general way it is possible to resume the Higuchi model to the following expression (generally known as the simplified Higuchi model)

\[ f_t = K_H t^{1/2} \]

Where \( K_H \) is the Higuchi dissolution constant treated sometimes in a different manner by different authors and theories. Higuchi describes drug release as a diffusion process based in the Fick’s law, square root time dependent.

**2.3.7.4. Korsmeyer Peppas model**

Korsmeyer et al. (1983) developed a simple, semiempirical model, relating exponentially the drug release to the elapsed time (t).

\[ f_t = a t^n \]

where \( a \) is a constant incorporating structural and geometric characteristics of the drug dosage form, \( n \) is the release exponent, indicative of the drug release mechanism, and the function of \( t \) is \( M_t/Ma \) (fractional release of drug). If the diffusion is the main drug release mechanism, a graphic representing the drug amount released, in the referred conditions, versus the square root of time originate a straight line. Under some experimental situations the release mechanism deviates from the Fick equation, following an anomalous behaviour (non-Fickian). In these cases a more generic equation can be used:

\[ M_t/Ma = a t^n \]

Peppas (1985) used this \( n \) value in order to characterise different release mechanisms, concluding for values for a slab, of \( n=0.5 \) for Fick diffusion and higher values of \( n \), a non-Fickian model. In the case of a cylinder, \( n=0.45 \) instead of 0.5, and 0.89 instead of 1.0.
2.3.8. In vivo gastro retention Studies on the microbeads

In vivo gastric residence time of a floating dosage form is determined by X-ray diffraction studies and gamma scintigraphy (Timmermans et al., 1994) or roentgenography (Babu et al., 1990).

Atyabi et al., (1996) studied the in vivo behavior of coated and uncoated beads were monitored using a single channel analyzing study in 12 healthy human volunteers of mean age 34 yrs.

Whitehead, et al., (1998) studied the gastroretention of Calcium alginate multiple units floating beads in seven healthy males (21–55 years). After fasting from midnight the night before the subjects consumed cereal (30 g) with milk (150 mL) to which was added 20 Ci . 99 m Tc-DTPA. An anterior image of stomach was obtained with √ camera. Anterior images were acquired at suitable intervals and subjects remained standing/sitting for the duration of the study.

Sato et al., (2004) administered riboflavin containing microballoons (MB) orally to each of three healthy volunteers and the pharmacokinetics of riboflavin was investigated by analysis of the urinary excretion. Prolongation of the urinary excretion of riboflavin could be obtained by ingestion of water as well as “fed” conditions. This phenomenon was attributable to the buoyancy properties of MB in the stomach and an increase in the gastric residence time (GRT). The excretion half-life time following administration of MB (particle size: 500–1000 µm) exhibiting high buoyancy was longer than that of MB (particle size: <500 µm) displaying low buoyancy. Therefore, the intragastric floating properties of MB are potentially beneficial as far as a sustained pharmacological action is concerned.

The intragastric behaviour of a floating multiple unit system can also be investigated in humans. The floating units used in this study, composed of a calcium alginate core separated by an air compartment from a calcium alginate and polyvinylalcohol membrane, and the in vivo study was conducted in three different sessions (fasted state, fed state after a meal and fed state after a succession of meals) by administering to each subject at the same time both floating and control systems, loaded with barium sulfate, and monitoring them in the gastric region at determined
time intervals using X-ray apparatus. Unlike the control, the floating system remained buoyant on the gastric content under both fasted and fed states. In the fasted state, the intragastric buoyancy of the system did not influence its gastric residence time (GRT). In the fed state after a meal, all the floating units showed a floating time (FT) of about 5 h and a GRT prolonged by about 2 h over the control. In the fed state after a succession of meals, most of the floating units showed a FT of about 6 h and a GRT prolonged by about 9 h over the control, though a certain variability of the data owing to mixing with heavy solid food ingested after the dosing was observed (Iannuccelli et al., 1998).

Table 2.1. List of drugs reported as floating microparticles / beads

<table>
<thead>
<tr>
<th>Drug</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Niacinamide</td>
<td>Ibrahim El-Gibaly, 2002</td>
</tr>
<tr>
<td>Verapamil</td>
<td>A.Streubelet et al., 2002</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>Jain et al., 2005</td>
</tr>
<tr>
<td>Riboflavin microballoons</td>
<td>Sato et al., 2004</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>Badve et al., 2007</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Ishak et al., 2007</td>
</tr>
<tr>
<td>Aspirin, griseofulvin and p-nitroaniline</td>
<td>Thanoo et al., 1993</td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>Sheth et al., 1979</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>Jayanthi et al., 1995</td>
</tr>
<tr>
<td>Tranilast</td>
<td>Kawashima et al., 1991</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>El-Kamel et al., 2001</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Streubel et al., 2002</td>
</tr>
</tbody>
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