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Gastroretentive microparticles for drug delivery applications

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Abstract

Many strategies have been proposed to explore the possibility of exploiting gastroretention for drug delivery. Such systems would be useful for local delivery, for drugs that are poorly soluble at higher pH or primarily absorbed from the proximal small intestine. Generally, the requirements of such strategies are that the vehicle maintains controlled drug release and exhibits prolonged residence time in the stomach. Despite widespread reporting of technologies, many have an inherent drawback of variability in transit times. Microparticulate systems, capable of distributing widely through the GI tract, can potentially minimize this variation. While being retained in the stomach, the drug content is released slowly at a desired rate, resulting in reduced fluctuations in drug levels. This review summarises the promising role of microencapsulation in this field, exploring both floating and mucoadhesive microparticles and their application in treatment of *H. pylori*, highlighting the clinical potential of eradication of this widespread infection.

1. Introduction

The oral delivery route is the most common of all drug administration routes and accounts for about half of all drug administration. This is partly due to the fact that the gastrointestinal tract offers a wide range of flexibility in dosage form design than other routes. Also, it is a convenient route of administration for easy access to the systemic circulation. However, drug absorption via this route can be unsatisfactory and variable even following promising *in vitro* release profiles (Davis, 2005, Streubel *et al.*, 2006). This makes it difficult to predict the *in vivo* performance of a drug delivery system (DDS), even though the *in vitro* data are reproducible. There are several physiological factors that could work against achieving successful delivery of drugs via the oral route and such factors include unpredictable gastric emptying times, shorter gastrointestinal transit time of the dosage form, partial drug release from the dosage form and the absorption site of the particular drug.

The average residence time of formulations in the stomach depends on the type of dosage form. Tablets, pellets, capsules and solutions have an average residence time of 2.7 ± 1.5 hours, 1.2 ± 1.3 hours, 0.8 ± 1.2 hours and 0.3 ± 0.07 hours respectively in the fed state (Chawla *et al.*, 2003). The effective duration of release from non-retentive controlled release delivery systems such as oral matrix or osmotic systems cannot not extend beyond normal gastrointestinal (GI) transit time, and so is unpredictable and limited to around 12 hours maximum.

2. Gastroretentive systems

Gastroretentive dosage forms (GRDFs) are designed to be retained in the stomach for an extended duration in order to improve the residence time of dosage forms in the stomach, thereby leading to enhanced bioavailability of the drug.

Not all drugs, however, are good candidates for gastroretention, but those that are and have been formulated in a range of gastroretentive systems include:

- drugs that act locally in the stomach, e.g. misoprostol (Oth *et al.*, 1992), antacids (Fabregas *et al.*, 1994) and antibiotics (Yang *et al.*, 1999, Whitehead *et al.*, 2000)
- drugs that are absorbed primarily in the stomach, e.g. metronidazole (Adebisi and Conway, 2010)
- drugs that are poorly soluble at alkaline pH, e.g. diazepam (Sheth and Tossounian, 1984) and verapamil hydrochloride (Streubel *et al.*, 2002); thereby preventing solubility from being the rate-limiting step to the absorption of the drug by restricting the drug to the stomach
- drugs that have a narrow absorption window in the stomach or in the upper small intestine, e.g. levodopa (Erni and Held, 1987), para-amino benzoic acids (Ichikawa *et al.*, 1991a, Ichikawa *et al.*, 1991b) and furosemide (Menon *et al.*, 1994)
- drugs that are absorbed rapidly from the GI tract, e.g. amoxicillin
- drugs that degrade or are unstable in the colonic / intestinal environment, e.g. captopril (Matharu and Sanghavi, 1992, Nur and Zhang, 2000) and metronidazole (Nayak *et al.*, 2010).

2.1. Requirements for gastroretentive devices

In order for a drug dosage form to achieve gastroretention, it must satisfy certain conditions. One major requirement is that the dosage form must be strong enough to withstand the peristaltic waves of the stomach, the contractions and forces within the stomach. Another important requirement is that it must be easily removed from the stomach, once the drug content is released from the delivery device (Anilkumar, 2008).

2.2. Physiology of the stomach

The stomach is the organ involved in the liquefaction of food and it also releases the churned food in a controlled manner into the intestines. It is divided into two major parts based on their functions. The fundus and the body of the stomach produce contractions in the muscle walls and cause compaction of the stomach contents, while the antrum causes peristaltic phase movement leading to the comminution of the food into small particles of about 2 mm. For the stomach contents to be able to pass through the pyloric valve into the small intestine, the particle size should be within the range of 1 to 2 mm (Deshpande *et al.*, 1996).

In the fasted state, the stomach has a residual volume of 25-50 ml (Waugh *et al.*, 2001) with a small amount of fluid and a pH ranging from 1 to 3 (Bowman *et al.*, 1968). The GI tract is in a state of continuous motility. The motility is in two modes – the inter-digestive motility pattern (also called the migrating motor complex) and digestive motility pattern.

The inter-digestive motility pattern presides in the fasted state, with the main function being the clearing of the stomach of the residual contents of the upper GI tract.

Under fasted conditions, the inter-digestive myoelectric motor complex (IMMC) is a 2-hour cycle of peristaltic activity that regulates motility patterns (Washington *et al.*, 2001). It is organised in cycles of activity and quiescence (Deshpande *et al.*, 1996). Each of the cycles lasts for a period of 90 to 120 minutes consisting of four phases and the duration of the phases depends on the concentration of the hormone, motilin. The ingestion of food interferes with the inter-digestive motility cycle and the digestive cycle takes over. The digestive cycle is induced 5-10 minutes after the ingestion of food and remains active for as long as there is food in the stomach. Therefore, the larger the meal, the longer the period of fed activity, with usual times being 2-6 hours and more usually 3-4 hours with contractions similar to Phase II of IMMC (Pawar *et al.*, 2011).

For a formulation to be gastroretentive, it must be able to resist the pressures and forces of the IMMC, especially the strong intense contractions of phase III, (Soppimath *et al.*, 2001a) for a considerable period of time. The gastric residence time (GRT) of a particular formulation will depend on the stage of the IMMC is active at the time of drug administration.

In the fed state, after the churning of food to smaller particle sizes, the residence time of the food, depends on what type of food is consumed. Liquids and small food particles will be easily transferred into the duodenum, while solids and larger food particles are removed much more slowly (Conway, 2005).

2.3. Factors affecting gastric residence time

- There are several factors affecting the GRT of dosage forms (Gruber *et al.*, 1987) and they include: 2.3.1. Fed or fasted conditions – GRT is longer in the

fed state than in the fasted state. In the fasted state, the gastric residence of dosage forms is usually not longer than an hour and it is common for dosage forms to move rapidly through the small intestine, for not more than 3 hours (Khandai *et al.*, 2010, Naisbett and John, 1995). This phenomenon is due to the fact that the IMMC moves the undigested food material from the stomach and if the time of drug administration occurs around the time of IMMC, the formulation will be expelled out of the stomach, leading to a short gastric residence. In the fed state however, the IMMC (related to fasted state) is delayed, thereby increasing the gastric residence time. Lin *et al.* observed that stomach emptying of 1.6 mm diameter spheres were affected by a 300 g steak meal (Lin *et al.*, 1992). The presence of food caused a delay in the housekeeper wave. Most of the drug particles were uniformly distributed among the food particles in the stomach, thereby delaying the stomach emptying process. Also, when 3-5 mm diameter tablets were taken with a meal, the emptying process was delayed in humans (Khosla *et al.*, 1989). In addition, a high fat meal may delay gastric emptying from 3 to 5 hours (Gad, 2008).

2.3.2. Density of formulation - The density of a dosage form has an impact on its ability to stay in the stomach for a prolonged period of time (Figure 1). A high density formulation e.g. coated pellets, which have a density greater than that of gastric contents (1.004 g/cm^3), will sink to the lower part of the antrum. This type of coating is achieved by the use of heavy inert materials such as barium sulphate, zinc oxide, and titanium dioxide (Patel, 2007). In addition, a low density formulation, with a density less than the density of the gastric contents, is expected to remain buoyant in the gastric fluid (Singh and Kim, 2000). For example, the hypotensive action of diltiazem

was heightened when administered to humans in a floating controlled release tablet compared to an equivalent non-floating tablet (Gu et al., 1992). For particulate systems, it has been reported that particles of different densities ranging from 0.5 to 2.9 g/cm³, emptied from the stomach of fasted dogs in a similar manner (Gruber *et al.*, 1987). The results by Gruber and co-workers are similar to those observed in fasted humans as there was no difference observed between the rate of gastric emptying of floating (density=0.96 g/cm³) and non-floating (1.96 g/cm³) single unit dosage forms (Davis *et al.*, 1986). Inconsistencies and variability in results are likely to be a consequence of fed versus fasted conditions in the stomach, thereby reducing the impact of density on gastroretention.

- 2.3.3 Size of formulation - If the size of a dosage form is larger than the diameter of the pylorus, the dosage form will be retained in the stomach, even during the housekeeper wave. Therefore, such a dosage form will be initially of a smaller size to facilitate swallowing, then it increases in size when it gets to the stomach (Streubel *et al.*, 2006). The size of the dosage form required, may be greater than 5 cm in length and a diameter greater than 3 cm (Klausner *et al.*, 2003a). A diameter of more than 7.5 mm is also stated to favour gastroretention more than dosage forms with diameter, greater than 9.9 mm (Timmermans and Moes, 1994).
- 2.3.4 Shape of formulation -The shape of the dosage form has an effect on its gastroretentive ability. Ring-shaped and tetrahedral devices have been shown to demonstrate a longer GRT , in comparison with dosage forms of other

shapes (Garg and Sharma, 2003). In addition, ring-shaped GRDFs with a flexural modulus of 22.5 kilo pounds per square inch and tetrahedral GRDFs (modulus of 48 kilo pounds per square inch) were reported to exhibit 90-100% retention at 24 hours, compared to dosage forms with shapes defined as continuous stick, planar disc, planar multi-lobe and string (Pawar *et al.*, 2011).

- 2.3.5. Single or multiple unit formulation - When compared to single unit formulations, multiple unit formulations show more predictable and more reliable gastric emptying. Single unit formulations exhibit the “*all or nothing concept*” and failure of the unit, while in the case of multiple unit systems, the particles are distributed freely throughout the GI system and their distribution or movement is less affected by the transit time than with single unit dosage form (Bechgaard and Ladefoged, 1978, Whitehead *et al.*, 1998). Also, as the drug release kinetics of multiple unit systems are more predictable, there is a reduced likelihood of localised mucosal damage or dose dumping (Rouge *et al.*, 1997), and they allow the co-administration of units with different release profiles or those containing substances that are incompatible (Ishak *et al.*, 2007).

- 2.3.6. Nature of meal and food intake - Factors such as the nature of food, frequency of feeding and caloric content have important effects on gastro-retention of dosage forms. Fatty acid salts and indigestible polymers such as cellulose, poly-dextrose and reffinose tend to extend GRT. . The gastric retention of water was reported to follow an exponential pattern with a half life ($t_{1/2}$) of 10 minutes (Hunt, 1968). An increase in the volume of water increases the gastric emptying; however, gastric emptying of a liquid meal can be

affected by the chemical and osmotic properties of the meal. Foods high in proteins and fats can increase GRT by 4-10 hours. The gastric retention of dosage forms is improved by the presence of food in the stomach; this increased residence of drug in the stomach therefore, helps in improving the bioavailability of the drug. Enteric-coated or enteric matrix tablets may be retained longer, if administered with heavy meals or breakfast (Gad, 2008).

- 2.3.7. Gender - Females have been found to show a comparatively lower mean ambulatory GRT, than their male counterparts. The gastric emptying **time of the Heidelberg capsule is** slower in women than in men (Mojaverian *et al.*, 1988). Also, the mean GRT in females (4.6 ± 1.2 h) is higher than in males (3.4 ± 0.6 h) of the same age and race.
- 2.3.8. Posture - It has been reported that posture does not have a significant effect on GRT (Mojaverian *et al.*, 1988); however, another study showed that for both floating and non-floating systems, the GRTs of the dosage forms vary based on the subject's posture (Van Gansbeke *et al.*, 1991). Floating systems taken by subjects in an upright position were found to float for a longer period of time, thereby extending gastroretention. However, non-floating systems settled to the bottom of the stomach and were easily evacuated by peristaltic contractions. In a supine position however, the reverse occurred as the floating units were more easily emptied from the stomach, than the non-floating units (Timmermans and Moes, 1994).
- 2.3.9. Concomitant drug administration- Examples of drugs that have an effect on GI transit times include anti-cholinergic drugs such as atropine, opiates,

e.g. codeine and pro-kinetic drugs, e.g. metoclopramide. All these prolong GRT, while drugs like octreotide and erythromycin enhance gastric emptying.

- 2.3.10. Biological factors- disease states such as Crohn's disease have been associated with delayed gastric emptying (Grill et al., 1985, Annese et al., 1995). Diabetes has also been linked to delayed gastric emptying with this delay occurring in 30-50% of patients with long standing diabetes mellitus (Horowitz et al., 1996). Duodenal ulcers lead to an increase in gastric emptying, while gastric ulceration reduces antral motility causing a normal emptying of liquids but resulted in delayed emptying of solids (Miller et al., 1980).
- 2.3.11. Age – The effect of age on the gastric residence of the Heidelberg capsule was assessed in 12 healthy elderly males over 65 years. It was observed that the mean GRT after a 500-kcal breakfast. was significantly longer, compared to that observed in young male volunteers (Mojaverian *et al.*, 1988).

3. Gastroretentive dosage forms

Various approaches have been developed to achieve gastroretention. Passage delaying agents such as triethanolamine myristate (Gröning and Heun, 1984), have been used to have an influence on gastric transit of DDS based on the fact that the lipid vehicles tend to reduce the motility of the stomach. However, this deliberate slowing down of gastric motility may have an effect on the emptying of the entire contents of the stomach.

DDS have been developed to achieve gastroretention and they include bioadhesive systems (Ponchel and Irache, 1998); swelling and expanding systems (Urguhart and Theeuwes, 1994); high density systems (Rednick and Tucker, 1970); floating systems (Deshpande *et al.*, 1996), and modified shape systems (Fix *et al.*, 1993).

3.1. Swelling and Expandable Systems

Swelling systems exploit the restrictions on the removal of large particles from the stomach, if they are larger than the pyloric sphincter opening (Nayak *et al.*, 2010). . Important features of these formulations are that the dosage form must be small enough for it to be easily swallowed; the onset of swelling must be fast, so as to prevent its evacuation from the stomach, before getting a chance to swell (Conway, 2005); it must not cause any form of gastric obstruction, either singly or by accumulation (Nayak *et al.*, 2010) and must regain a small size to be evacuated after complete drug release (Groning *et al.*, 2007).

The increase in size of the formulation is normally achieved by swelling (through the process of osmosis) or by unfolding on contact with the contents of the stomach (Klausner *et al.*, 2002). There are several drawbacks to the use of this kind of system, as large single unit dosage forms may cause obstruction, intestinal adhesion, and gastropathy (Klausner *et al.*, 2003b). Such dosage forms should not possess sharp edges or cause local damage to the stomach on extension and the system must be made from biodegradable polymers.

3.2. High density systems

High density systems are dosage forms that have a density greater than the density of normal stomach contents which is 1.004 g/cm³ (Figure 1). The density of the

formulation should be close to 2.5 g/cm^3 , for it to be retained in the stomach for any considerable length of time (Clarke *et al.*, 1993). Rouge *et al.*, also reported that above densities of $2.4 - 2.8 \text{ g/cm}^3$, formulations can be retained in the lower part of the stomach (Rouge *et al.*, 1998). Inert materials used to increase the density of formulations are used either by coating the drug with it or by mixing the material with the drug (Vyas and Khar, 2006). On addition of such inert materials, the formulation density can increase by up to $1.5\text{--}2.4 \text{ g/cm}^3$ (Clarke *et al.*, 1993). One major drawback, however is that they are difficult to manufacture, requiring relatively large quantities of active drug as the dry material constituent of the formulation reacts with the gastric fluid to release its contents. There is no formulation utilising this strategy currently on the market (Nayak *et al.*, 2010, Garg and Sharma, 2003) and *in vivo* data in animal) or clinical studies are also rather scarce.

3.3. Bioadhesive systems/ Mucoadhesive systems

Bioadhesive DDS , as introduced in the 1980s, adhere to epithelial surfaces, thus maintaining a more intimate contact with the mucosal barrier (Park and Robinson, 1984) and thereby prolonging GRT due to this feature. A subset of bioadhesive systems is mucoadhesive systems, which adhere to the thick mucus gel layer that covers the mucosal surfaces in the stomach (Conway, 2005). Chitosan for example, has been used to achieve mucoadhesion (Lehr *et al.*, 1992) and adhesion to porcine stomach (Gåserød *et al.*, 1998) in some formulations. The concept of mucoadhesive systems is based on the self protecting mechanism of the GI tract. Mucus plays a cytoprotective role by protecting the surface mucosal cells from acid and peptidases. It is a viscoelastic, gel-like, stringy slime consisting mainly of glycoproteins and

serves as a lubricant for the passage of solids and as a barrier to antigens, bacteria and viruses (Chawla *et al.*, 2003). The process of mucoadhesion is complex and its mechanism, has been explained through various theories such as electrical,, adsorption, wetting and diffusion theories (Peppas and Buri, 1985, Park and Robinson, 1987, Rillosi and Buckton, 1995). Mucoadhesion has been said to occur in two stages ; the contact (wetting) stage and then the consolidation stage, which is the stage where adhesive interactions are established (Smart, 2005). The mucosal surface is negatively charged; therefore a polymer that has a positive charge might assist the mucoadhesion process. An initial step of mucoadhesion could be electrostatic attraction, followed by mechanical interlocking of the polymer chains, van der Waals force, hydrogen bonding and other forces (Lehr *et al.*, 1993). The different mechanisms of bioadhesion are summarized in Table 1.

One drawback associated with such systems is that the mucus on the stomach walls is constantly being renewed, thereby making adherence of a formulation to this mucus unpredictable (Chun *et al.*, 2005b). In addition, the contents of the stomach are highly hydrated, thereby reducing the level of adhesiveness of the polymers. Other factors that can affect effective *in vivo* mucoadhesion include the composition of mucus, different behaviour of mucoadhesive devices over the pH range, and disease conditions (Vasir *et al.*, 2003). Also, the prospect of oesophageal binding might be daunting, regarding the safety aspects of such formulations (Wang *et al.*, 2000). The specificity of the formulation could also be a major drawback, as it is difficult to specifically target bioadhesive polymers to the gastric mucosa, for example, Carbopol® will adhere to various surfaces (Khosla and Davis, 1987).

However, the advantages of such systems in the treatment of *H. pylori* infections may outweigh these concerns, because they have the potential to maintain contact with the mucus layer and provide controlled release of drugs e.g. antibiotics, in a localised environment. An additional consideration with this application is the avoidance of any local drug overdose, which could lead to irritation of the gastric mucosa (Ch'ng *et al.*, 1985).

3.4. Floating systems

Early studies based on floating systems date as far back as 1968 (Davis, 1968). Floating delivery systems (Figure 1) generally have a bulk density, that is less than the density of the gastric contents and thus remain buoyant in the stomach, without affecting the gastric emptying rate for a prolonged period of time. A floating system could lead to high drug levels in the fundal area of the gastric mucosa and this may be a useful strategy for the delivery of narrow spectrum antibiotics for peptic ulcer disease (Umamaheswari *et al.*, 2002) and for drugs that are primarily absorbed in the stomach or the upper small intestine (Sungthongjeen *et al.*, 2006), e.g. metronidazole.

The drug content should be released slowly as the dosage form remains floating on the gastric contents. At the end of the release period, the DDS should exit from the stomach. This type of DDS has been demonstrated to increase gastroretention and reduce fluctuations in drug plasma concentrations (Singh and Kim, 2000).

3.4.1. Specific criteria for a floating DDS:-

- It must have a structure to form a cohesive gel barrier.

- It must maintain a density lower than that of gastric contents (1.004-1.010 g/cm³).
- It should dissolve slowly enough to serve as a drug depot (Desai, 2007).

Floating systems include designs such as hydro-dynamically balanced systems (HBS), gas generating systems, raft forming systems and hollow microspheres. These systems can be achieved by entrapping air into the formulation, e.g. hollow microspheres (Krogel and Bodmeier, 1999) or through the inclusion of oils (Sriamornsak *et al.*, 2004) or foam powder (Streubel *et al.*, 2002). Floating dosage forms include granules (Yuasa *et al.*, 1996), powders (Dennis and Timmins, 1992), capsules (Franz and Oth, 1992), tablets (Sheth and Tossounian, 1979), laminated films (Machida *et al.*, 1989) and hollow microspheres (Kawashima *et al.*, 1991).

There are several advantages attributed to the use of floating DDS including improvement in patient compliance; achievement of better therapeutic effect of drugs with a short half life; enhancement of absorption of drugs, which are soluble primarily in the stomach and achievement of site-specific delivery of drug to the stomach (Pawar *et al.*, 2011).

The limitations to the use of these formulations, include the requirement for the presence of fluids in the stomach (dosage form is administered typically with fluid of about 200-250 ml (Soppimath *et al.*, 2001a)), in order to maintain buoyancy effect of the formulation in the stomach. Also, drugs that cause gastric mucosa irritation and those with solubility and/or stability issues in gastric fluids and those that cause gastric mucosa irritation like biomolecules, such as proteins and peptides (which are liable to proteolysis in gastric fluid) are not suitable for incorporation into this type of

delivery system. In addition, drugs which are well absorbed along the entire GI tract and undergoes significant first pass metabolism are not suitable candidates, since the slow gastric emptying could lead to a reduction in systemic bioavailability (Pawar *et al.*, 2011).

3.4.2. Formulations for floating systems

Floating systems can be divided into two categories: - gas generating systems and non-effervescent systems (Garg and Gupta, 2008).

3.4.2.1. Gas generating / Effervescent systems

These are systems designed so that when they contact the gastric contents, gas bubbles are released causing the dosage form to float on gastric contents. This is achieved by the incorporation of vacuum, air or inert gas into a floatation chamber (Iannuccelli *et al.*, 1998). The gas can be added to the formulation by the volatilization of an organic solvent such as ether or cyclopentane, causing inflation on contact with gastric fluid or through carbon dioxide produced due to the chemical reaction between organic acids and carbonate-bicarbonate salts (Sakr, 1999). These formulations make use of swellable polymers such as methylcellulose and hydroxypropylmethylcellulose (HPMC); polysaccharides (e.g. chitosan) and effervescent materials, such as sodium bicarbonate, citric acid (Rubinstein and Friend, 1994), tartaric acid or floating chambers that contain liquids that turn into a gaseous state at body temperature (Pawar *et al.*, 2011). The required stoichiometric ratio of citric acid and sodium bicarbonate for the generation of gas has been reported to be 0.76:1 (Garg and Sharma, 2003).

In a study in 1978, Umezawa developed floating pepstatin minicapsules containing sodium bicarbonate, coated with an inner HPMC layer and an outer pepstatin layer. On contact with gastric fluid, there is a release of carbon dioxide leading a GRT of about 3- 5 hours and prolonged the release of drug from the formulation (Umezawa, 1978) .

Other floating formulations include those using a combination of sodium alginate and sodium bicarbonate (Stockwell *et al.*, 1986); floating mini-capsules consisting a core of sodium bicarbonate, lactose and polyvinyl-pyrrolidone, coated with HPMC and systems produced using ion exchange resin technology (Garg and Gupta, 2008).

The main drawback of these systems is that they do not float immediately after swallowing, as there is a lag time between swallowing and the release of gas. In order for the formulation to be effective, the lag time has to be minimised to avoid premature evacuation from the stomach (Streubel *et al.*, 2003). However, there are many commercially available pharmaceutical products utilising this approach, such as Gaviscon[®] and Madopar[®] HBS capsule (Singh and Kim, 2000).

3.4.2.2. Non-effervescent Systems

In non-effervescent systems, air entrapped in the swollen polymer confers buoyancy on the dosage forms. The systems absorb gastric fluid, on contact, swell and form a colloidal gel barrier (Sheth and Tossounian, 1979), that limits the rate of fluid absorption into the device and subsequently drug release (Sheth and Tossounian, 1984). The air trapped by the swollen polymer, lowers the density and confers buoyancy to the dosage form. A common way of incorporating drug into this type of formulation is by mixing the drug with a gel (typically with a high concentration of

about 25-75 %w/w) that swells on contact with gastric fluid, while still maintaining its integrity of shape and a bulk density less than 1.004 g/cm³. Commonly used polymers for this type of formulation include, cellulosic hydrocolloids such as HPMC and matrix-forming polymers such as poly-acrylate, polycarbonate, polystyrene and poly-methacrylate. Other excipients include polyvinyl acetate, Carbopol[®], agar, sodium alginate, polyethylene oxide and polycarbonates (Garg and Gupta, 2008).

Non-effervescent systems are sub-divided into hydrodynamically balanced systems, alginate beads, microporous compartment systems and hollow microspheres:-

3.4.2.2.1. Hydrodynamically balanced systems (HBS) introduced in 1975 by Sheth and Tossounian are single unit dosage forms, containing the active ingredient with one or more gel forming hydrocolloids, which remain floating on stomach contents. These systems are suitable for drugs that have a better solubility in the acidic pH of the stomach and those that have a specific site of absorption in the upper part of the small intestine (Rocca *et al.*, 2003). Excipients commonly used in this type of formulations include HPMC, hydroxyethylcellulose (HEC), sodium carboxymethylcellulose (NaCMC), polycarbophil, polyacrylate, polystyrene, agar, carrageenans or alginic acid (Hwang *et al.*, 1998, Reddy and Murthy, 2002, Nayak *et al.*, 2010).

3.4.2.2.2. Alginate beads

Floating alginate dosage forms were introduced in the 1980s (Stockwell *et al.*, 1986). Alginates are linear anionic block copolymer hetero-polysaccharides made up of monomers of (β -d-mannuronic acid) (M) and its C-5 epimer (α -1-guluronic acid) (G) residues, linked to one another by 1, 4-glycosidic linkages. They are extracted from

the cell walls of various species of brown algae (Sanford and Baird, 1983). Alginates from different seaweeds can have different ratios of the component monomers. The ratio and the distribution of the monomers in the alginate chain do have an effect on gel formation and strength. Hydrogel formation occurs by ionotropic gelation on reaction with bivalent alkaline earth metals such as Ca^{2+} , Sr^{2+} and Ba^{2+} or trivalent Fe^{3+} and Al^{3+} ions, due to an ionic interaction and intra-molecular bonding between the carboxylic acid groups present on the polymer backbone and the cations (Patel *et al.*, 2006). The complexation of the polyguluronic sequences of alginate by Ca^{2+} results in the formation of a three-dimensional network usually described by the 'egg box' model (Grant *et al.*, 1973), giving rise to formation of calcium alginate hydrogels. The buckled chain of the guluronic acid units is a two-dimensional analogue of a corrugated egg-box with interstices in which the calcium ions may pack and be coordinated (McHugh, 1987). "The analogy is that the strength and selectivity of cooperative binding is determined by the comfort with which 'eggs' of the particular size may pack in the 'box', and with which the layers of the box pack with each other around the eggs". The calcium alginate formed is porous and is known to be insoluble and resistant in acidic media (Grant *et al.*, 1973).

Generally sodium alginate solution is dropped into an aqueous solution of calcium chloride through the use of hypodermic needles leading to the precipitation of calcium alginate beads (Figure 2). These beads are then filtered and dried by air convection and freeze drying, leading to the formulation of porous systems which can be buoyant for over 12 hours. Such beads have been demonstrated to extend gastric retention time to beyond 5.5 hours (Garg and Gupta, 2008, Whitehead *et al.*, 1998).

3.4.2.2.3. Microporous compartment systems

In these systems, the drug reservoir is inside a compartment containing pores in the surrounding membrane (Harrigan, 1977). The peripheral walls are sealed completely in order to avoid any direct contact of the undissolved drug with the gastric contents. The entrapped air in the floatation chamber causes buoyancy in the gastric fluid (Vyas and Khar, 2006). The gastric fluid passes through the apertures, dissolving the drug, thereby providing a reservoir of dissolved drug for continuous drug transport.

3.4.2.2.4. Hollow microspheres

Microspheres have been widely exploited in this area of gastroretention and floating microspheres or hollow microspheres combine the advantages of floating systems outlined above along with those of multiple unit systems.

4. Microparticles as drug delivery devices

Microencapsulation is the mechanism used by approximately 65% of all sustained release systems (Gad, 2008). It is a process of application of a thin coating to individual core materials with an arbitrary particle size range from 5-5000 nm (Lachman *et al.*, 1986). Microencapsulation may cause an improvement in absorption of drug and reduction of side effects such as gastrointestinal mucosa irritation (Obeidat and Price, 2006). The process is also used to achieve controlled release of drugs (Vandegaer, 1974). Single unit dosage forms are known to have the disadvantage of an all or nothing emptying process from the stomach, whereas multiple unit systems such as microspheres can avoid the unpredictable gastric emptying. There is also a reduced risk of dose dumping, with the use of multiple drug units rather than single unit dosage forms. The uniform distribution of these multiple

unit systems could lead to a more reproducible and predictable drug absorption and lower risk of local drug irritation; thus the combination of floatation (Hirtz, 1985) and mucoadhesive capability with microencapsulation is a promising strategy for success in this application.

The use of microspheres in sustained drug delivery has attracted much attention in recent years, especially with the use of naturally occurring biodegradable polymers, controlling the rate of drug release and targeting drugs to specific sites in the body (Patel and Patel, 2007, Gohel and Amin, 1998, Woo *et al.*, 2001, Capan *et al.*, 2003). Examples of polymers used in the preparation of microspheres of various sizes include chitosan and albumin to deliver drugs at a controlled rate to the target sites (Singla and Chawla, 2001, Gupta and Hung, 1989). Other examples include gelatin, waxes, sodium alginate, and ethyl cellulose (Ahmed *et al.*, 2010). Microspheres have been reported to have a high loading capacity (Daharwal *et al.*, 2007) and can be formulated with sufficient buoyancy to float in the gastric contents and remain in stomach for prolonged periods (Pawar *et al.*, 2011). Such microspheres are prepared by a process of solvent evaporation or solvent diffusion/evaporation methods (Kawashima *et al.*, 1992). In addition to improving patient compliance by decreasing dosing frequency; improved therapeutic effects with drugs with short half-life drugs can be achieved (Gaba 2008). Microspheres can also help mask the taste of bitter drugs, e.g. roxithromycin (Gao *et al.*, 2006).

Several methods have been used by researchers in production of microspheres (Benita *et al.*, 1984, Bodmeier and McGinity, 1987a, Bodmeier and McGinity, 1987b, Jeffery *et al.*, 1993). The methods include solvent evaporation; hot melt microencapsulation; solvent removal; spray drying and phase inversion

microencapsulation. The most common method is the emulsion solvent –evaporation technique (Kawashima *et al.*, 1992) because of its simplicity and the fact that small batches of samples can be produced (Quintanar-Guerrero *et al.*, 1998b, Quintanar-Guerrero *et al.*, 1998a, Swarbrick and Baylan, 1994, Scholes, 1998, Mathiowitz *et al.*, 1999).

4.1. Characterisation of microspheres

Microspheres are characterized by their micromeritic properties such as particle size, tapped density, compressibility index, true density, flow properties including angle of repose, scanning electron microscopy. For floating microspheres, scanning electron microscopy (SEM) can be used to evaluate the internal structure of the microspheres and confirm the hollow nature of microspheres. The particle sizes of microspheres can be measured using laser diffraction particle size analyzers. Larger microspheres can be measured using an optical microscope and the mean particle size can be calculated by determining the mean of 200-300 particles with the use of a calibrated ocular micrometer or through the sieving method. The sieving method involves separating the microspheres into different size fractions by sieving for a required time using a mechanical sieve shaker.

4.1.1. Buoyancy studies

An *in vitro* floatability study is usually performed in a USP II dissolution apparatus. The test is carried out by spreading the microspheres over simulated gastric fluid (for example HCl/NaCl with 0.02% Tween 80, pH1.2) and this fluid simulates the surface

tension of human gastric juice (35-50 mN/m²) (El-Gibaly, 2002). The gastric medium is maintained at 37°C and stirred at 100rpm.. At specific time intervals, the relative proportions of the floating microspheres and the settled microspheres are noted and the buoyancy of the microspheres can be calculated using this formula (Kawashima *et al.*, 1991).

$$\text{Buoyancy (\%)} = \frac{Q_F}{Q_F + Q_S} \times 100 \quad \text{Equation 1}$$

Where Q_f and Q_s are the masses of the floating and settled microspheres respectively.

Some microspheres have been reported to float for more than 12 hours *in vitro* following such studies (Kawashima *et al.*, 1991, Kawashima *et al.*, 1992, Soppimath *et al.*, 2001a).

In vivo floating studies have been assessed by X-ray photographs of the floating microspheres loaded with radio-opaque materials, such as barium sulphate in the stomach of animals, e.g. healthy beagle dogs (Tanwar *et al.*, 2007). In this study, the animals were fasted for 12 hours and the first X-ray was photographed to ensure absence of radio-opaque materials in the stomach. Barium sulphate-loaded cellulose acetate microspheres were administered during the course of the study and the animals were allowed free access to water. The quantity of barium sulphate added was sufficient to ensure visibility by X-ray, but low enough to enable the microspheres to float. The radiographs of the abdomen were taken at predetermined time intervals and the microspheres floated for 3.2 hours *in vivo*, compared with 12 hours in the *in vitro* studies.

Gamma scintigraphy has been used successfully to study gastric residence of drug dosage forms. In a study by Jain *et al.*, optimized floating microspheres consisting of calcium silicate as the porous carrier replaginide and Eudragit[®] were evaluated.

Gamma scintigraphy was used to monitor the transit of floating and non-floating microspheres labelled with Technetium-99 (^{99m}Tc) in twelve one-year old healthy male albino rabbits. The animals were fasted for 12 hours, prior to the start of the experiment. Upon administration of the microspheres, the animals were allowed access to sufficient volume of drinking water. The location of the formulation in the stomach was monitored by keeping the subjects in front of the gamma camera. The sequential camera images indicated that the floating microspheres remained buoyant for over 6 hours and were uniformly distributed in the gastric contents. The non-floating microspheres were buoyant for less than 2 hours (Jain *et al.*, 2006b). *In vitro* studies of similar floating microspheres, showed that more than 80% of the microspheres were buoyant for at least 10 hours (Jain *et al.*, 2005).

Gamma scintigraphy has also been used to assess the buoyancy of microspheres in healthy human volunteers (Ma *et al.*, 2008) and GI transit of floating and non-floating alginate microspheres. *In vitro* studies showed that coating of these microspheres with chitosan had no effect on buoyancy, as both coated and uncoated microspheres floated over simulated gastric fluid (pH 1.2). The human volunteers were not on any regular medication and did not have any history of GI disease as with the animal models. After an overnight fast, the volunteers were allowed to consume a breakfast after taking 100 ml of water containing 20mCi ($^{99m}\text{TcO}_4^-$). *In vivo*, prolonged gastroretention was observed for over 5 hours for coated floating microspheres labeled with Technetium-99 (^{99m}Tc), while non-floating units, sank rapidly to the base of the stomach and were emptied from the stomach within 2.5 hours.

4.1.2. Evaluation of mucoadhesiveness

In vitro evaluation of mucoadhesiveness of amoxicillin microspheres can be studied by assessing the percentage of remaining microspheres on the stomach mucosa of rats (Liu *et al.*, 2005) after an *in vitro* wash off test. The rats were fasted overnight and dissected immediately after being sacrificed. The stomachs were removed and cut into small pieces and rinsed with physiological saline. One hundred microspheres were scattered uniformly on the stomach mucosa with the mucosa mounted in a chamber maintained at 93% relative humidity and at room temperature. After 20 min, the tissues were taken out and fixed on a polyethylene support at an angle of 45°. The stomach was rinsed with pH 1.3, HCl - physiological saline solutions for 5 min at a rate of 22 ml/min. The microspheres remaining on the surface of gastric mucosa were then counted, and the percentage of the remaining microspheres was determined to be 93% for the mucoadhesive microspheres and 89% for non-mucoadhesive microspheres. In this study, *in vivo* studies were carried out using rats that were kept fasted until they were sacrificed 2, 4 and 7 hours after administration of the microspheres. The microspheres remaining in the gastrointestinal tract were counted, and the percentage of remaining microspheres was determined to be 65%, 63% and 4% over the various time intervals for the mucoadhesive microspheres and 55%, 16%, 4% for the non-mucoadhesive microspheres.

GI transit study using radio-opaque markers or radiation emitting doses including X-ray and gamma scintigraphy has also been used to assess mucoadhesion (Säkkinen *et al.*, 2006, Chary *et al.*, 1999)

4.2. Polymers used in microparticulate drug delivery

The type of polymer used in microsphere production determines the properties of the microspheres such as the surface characteristics, force of adhesion, buoyancy,

release pattern and clearance. Examples of polymers used in development of mucoadhesive microspheres include chitosan and Carbopol[®] 934P, which has been used by many researchers (Chickering et al., 1995, Chun et al., 2005a) because of its good mucoadhesive and biodegradable properties (Patel and Chavda, 2008). Other suitable polymers include hydrogels or thermoplastics, homopolymers, copolymers or blends, natural or synthetic polymers.

4.2.1. Carbopol[®]

The mucoadhesive capability of Carbopol[®] can be easily affected by factors such as pH and ionic strength (Singla *et al.*, 2000). At low pH, (i.e. less than 5.0), there is limited ionisation of carboxyl groups resulting in reduced polymer swelling and thus stronger interaction with polysaccharides in mucus. There have been contradictory reports regarding the mucoadhesive properties of Carbopol[®]. Nagahara *et al.* prepared mucoadhesive microspheres by dissolving amoxicillin, curdlan (matrix) and carboxyvinyl polymer (adhesive polymer) in melted hydrogenated castor oil. *In vivo* mucoadhesive tests showed that the microspheres demonstrated longer GRT in the stomach of rat models (Nagahara *et al.*, 1998) . However, in a similar study, it was observed that Carbopol[®] did not increase the gastric residence time of the microspheres Carbopol[®] coated amoxicillin-resin microspheres (Cuna *et al.*, 2001). The strength of the adhesive forces between Carbopol[®] and the mucous layer depends on the distribution state of Carbopol[®] in the microspheres, that is whether the polymer is used as a coat layer or is dispersed within the microspheres (Akiyama *et al.*, 1995). *In vitro* and *in vivo* tests showed those with dispersed Carbopol[®] exhibited stronger mucoadhesion than those with Carbopol[®] coating.

4.2.2. Chitosan

Chitosan has positive charges in its D-glucosamine residue, which can lead to a strong electrostatic interaction with mucus or negatively charged sialic residues of mucin (He *et al.*, 1998, Fiebrig *et al.*, 1995). Also, chitosan has -OH and -NH₂ groups which can lead to hydrogen bonding and the linear molecule possesses some degree of chain flexibility, the conformation of which depends on the ionic strength (He *et al.*, 1998). These properties are considered important for mucoadhesion to occur (Peppas and Buri, 1985, Smart *et al.*, 1984); Robinson *et al.*, 1987 (Robinson and Mlynek, 1995). Its use as a matrix for sustained release granules (Hou *et al.*, 1985) and sustained release tablets (Akbuga, 1993, Kawashima *et al.*, 1985) was widely studied as early as the 1980s.

The importance of the mucoadhesive properties of chitosan has been reported in works by (Lehr *et al.*, 1992, Fiebrig *et al.*, 1995, Illum *et al.*, 1994, Lueben *et al.*, 1994, Aspden *et al.*, 1996). Chitosan microspheres with small particle sizes less than 5 µm, containing anticancer agents such as 5-fluorouracil (5-FU) (Ohya *et al.*, 1993), and magnetic microspheres (Hassan *et al.*, 1992), have been described for site-specific delivery. Its use in the preparation of microspheres are somewhat limited by its comparatively low strength and non-floating properties in simulated gastric fluid (pH 1.2) (Kas, 1997).

4.3. Gastroretentive microparticles

The use of microspheres in oral drug delivery is limited by the short GRT, similar to other conventional dosage forms. Therefore, the use of gastroretentive microspheres tends to permit the localisation of the drug component in the GI mucosal membrane for an extended period of time. This improves the bioavailability, leading to a reduction in the dose and frequency of administration. The control of the location of a

delivery system at a particular site in the GI tract especially the upper GI tract , often improves the absorption of the drug and the therapeutic effect of the drug (Singh and Robinson, 1988).

4.3.1. Mucoadhesive microparticles

Bioadhesive/mucoadhesive microspheres are a useful and promising delivery system, adhering to the mucosal layer, meanwhile releasing their drug contents in a sustained manner (Illum, 1998). Such microspheres can either consist entirely of a bioadhesive polymer or an outer coating by attaching bioadhesive / mucoadhesive materials to the microspheres (Figure 3). Bioadhesive microspheres can be modified to adhere to any form of mucosal tissue. They have the added advantage of efficient absorption and improved bioavailability of drug content due to the high surface to volume ratio, an intimate contact with the mucosal layer and they could help target specific absorption sites (Lehr *et al.*, 1992, Henriksen *et al.*, 1996, Bhaskara and Sharma, 1997, Chowdary and Rao, 2003).. Studies have shown that there was improved bioavailability from mucoadhesive systems containing drugs such as testosterone and its esters, vasopressin (Morimoto *et al.*, 1991), dopamine (Ikeda *et al.*, 1992) , insulin (Nagai *et al.*, 1984) and gentamycin (Illum *et al.*, 1989). There are however, challenges in the development of particles with adequate drug loading for their intended application. Loadings of 26%, as reported for amoxicillin mucoadhesive microspheres raises concerns both about the efficiency of the process and the amount of material that would need to be delivered to achieve therapeutic levels (Liu *et al.*, 2005).

Mucoadhesive acyclovir microspheres were prepared by a emulsion-chemical crosslinking technique using chitosan, thiolated chitosan, Carbopol 71G and Methocel K15M (Dhaliwal *et al.*, 2008). The microspheres showed release of $78.8 \pm 3.9\%$ in 12 hours compared with a release of $90.5 \pm 3.6\%$ in 1 hour from the drug powder. The thiolated chitosan microspheres showed better retention at 8.0 ± 0.8 h. Studies also showed that the administration of these thiolated chitosan microspheres could maintain plasma concentration of the drug for about 24 hours as compared to 5 hours after its administration in solution and showed a nearly four- fold higher AUC_{0-24} value .Chitosan, Carbopol and Methocel showed mucoadhesion times of 3.1 ± 0.4 h, 1.1 ± 0.2 h and 0.2 ± 0.1 h. (Dhaliwal *et al.*, 2008)

Chitosan microspheres containing lacidipine have also been evaluated *in vitro* for the treatment of pyloroplasm. The entrapment efficiency, using glutaraldehyde as the cross-linking agent, was between 14-40.82% and the microspheres exhibited a mucoadhesive property of over 70% in the *in vitro* wash off test using rat stomach mucosa. The entrapment efficiency and the mucoadhesion depended on the polymer concentration, volume of cross-linker and the stirrer speed. The optimal formulation showed controlled release for more than six hours and release followed Higuchi kinetics (Sultana *et al.*, 2009).

4.3.1.1. Application of mucoadhesive microparticles to eradication of *H. pylori*

Helicobacter pylori (Figure 4) is a spiral gram-negative micro-aerophilic bacterium, with unipolar-sheathed flagella. The flagella provide motility and the bacterium has

the ability to penetrate the gastric mucosa, resist gastric emptying and remain in the gastric mucosa, due to its spiral shape and high motility (Conway, 2005).

The major problem relating to the antibiotic treatment of this infection is that after infection, the bacterium resides below the gastric mucus adherent to the gastric epithelium; therefore, the access of drugs to this particular site is rather limited. In addition, the bacteria could have acquired resistance to the commonly used antimicrobial drugs (Iijima *et al.*, 2004). The first line therapy for the treatment of this infection is the use of a triple therapy consisting of one proton pump inhibitor (PPI) and two antibiotics. The antibiotics have to be used in combination, as only one antibiotic cannot achieve adequate eradication when used alone and there is a requirement for adjuvant therapy (Chang *et al.*, 2003). The adjuvant therapy consists of drugs that increase alkalinity in the stomach, in order to allow the local action of antibiotics that are not active in acidic pH of the stomach, for example, proton pump inhibitors used at a dose equivalent to 20mg omeprazole twice daily.

A combination of two antibiotics including clarithromycin, amoxicillin and metronidazole with a gastric acid inhibitor has been classified as the most effective therapy for the eradication of *H. pylori* (Lahej *et al.*, 1999). However, the persistent rise in resistance of this bacterium to these antibiotics; the hostile environment of the stomach; reducing antibiotic bioavailability at the site of action has led to failures in treatment (Batchelor *et al.*, 2007) and has encouraged research into producing alternatives to the commonly used formulations and gastroretentive formulations hold particular promise.

Patel and Chavda (2008) developed amoxicillin microspheres with Carbopol[®] 934P as the mucoadhesive polymer and ethylcellulose as the carrier polymer. The

microspheres produced were spherical, free flowing and with entrapment efficiencies ranging from 20% to 56%. The microspheres adhered to gastric mucous layer over an extended period of time and the release of drug from these microspheres was sustained for more than 12 hours. *In vivo* tests also showed that the microspheres exhibited better *H. pylori* clearance than amoxicillin administered as a dry powder (Patel and Chavda, 2008). In a similar study by Yellanki *et al.*, amoxicillin-trihydrate microspheres were prepared using Carbopol® 934P and ethylcellulose, the entrapment efficiency was between 78-86%. The particle size ranged from 500-560 µm for all the batches produced. *In vitro* tests were carried out using sheep gastric mucosa rinsed with hydrochloric acid buffer for 5 minutes at a rate of 22 ml/minute. More than 84% of the microspheres were retained on the mucosa after 5 minutes and drug release was biphasic, with an initial burst release and then followed by a slow release with more than 80% drug released after 6 hours (Yellanki *et al.*, 2010).

Liu *et al.* prepared ethylcellulose microspheres with Carbopol® 934P as the mucoadhesive polymer and amoxicillin as the active drug. The sizes of microspheres ranged from 400-1000 µm. The microspheres had a dense but porous inner core and release was pH-dependent. In acidic medium (HCl- pH 1.0), 90% of the drug was released in 4 hours, while in phosphate buffer (pH 7.8), the release was about 50%. *In vitro* studies showed that 93.5% of the microspheres containing Carbopol® were retained in the gastric mucosa of rats, compared with 85.8±5.3% of those without Carbopol®. *In vivo* studies in rats showed that the mucoadhesive microspheres were retained for longer, gastric amoxicillin concentrations were higher and there was enhanced clearance of *H. pylori* (Liu *et al.*, 2005).

It was reported in a study that the gastric retention in rats of amoxicillin microspheres prepared by dispersing Carbopol® in waxy hydrogenated castor oil, was about three times higher than that obtained using amoxicillin suspension containing 0.5% w/v methylcellulose, after 2 and 4 hours with about 47% and 20% retained respectively for the microspheres and 17% and 6% for the amoxicillin suspension. In addition, the mucoadhesive microspheres achieved a 10 times higher bactericidal activity, than the amoxicillin suspension in rats (Nagahara *et al.*, 1998).

In a study by Patel and Patel, the *in vitro* and *in vivo* characteristics of mucoadhesive microspheres were evaluated. Chitosan was used as the mucoadhesive polymer and glutaraldehyde as the cross-linking agent. *In vitro* tests showed that the amoxicillin mucoadhesive microspheres were retained more strongly on the gastric mucous layer and could be retained in the GI tract for an extended period of time. The best formulation produced in this research, exhibited a high drug entrapment efficiency of 70% and swelling index of 1.39. The percentage mucoadhesion was 79% after 1 hour and drug release was sustained for more than 12 hours. The *in vivo* clearance studies showed that mucoadhesive microspheres had a better clearance effect on *H. pylori* than amoxicillin powder. Following administration of a dose of 4 mg/kg amoxicillin mucoadhesive microspheres, the colony counts (a measurement of the growth of *H. pylori*) were 23 ± 7.07 , and as the doses were increased to 7.5 and 15 mg/kg the colony counts were reduced to 5.5 ± 0.70 and 2 ± 0 , respectively. However, following administration of amoxicillin powder (4 mg/kg) the colony counts were 78 ± 8.48 , and as the doses were increased to 7.5 and 15 mg/kg, the colony counts were 29 ± 5.65 and 17.5 ± 17.67 , respectively (Patel and Patel, 2007).

Wang *et al.* produced modified gelatin microspheres using aminated gelatine by surfactant-free emulsification in olive oil followed by a cross-linking reaction with glutaraldehyde. These modified microspheres exhibited a greater gastric mucoadhesiveness than the unmodified gelatine microspheres; thereby, presenting a likely new candidate DDS for the eradication of H-pylori. There are however safety concerns in using glutaraldehyde as a cross-linking agent and residual levels need to be controlled (Wang *et al.*, 2000) .

Ramteke *et al.*, (2006), prepared oral mucoadhesive sustained release nanoparticles of clarithromycin, with the aim of simplifying administration, thereby improving compliance, therapeutic effect and reducing dose related side effects of the therapy. The maximum drug entrapment was 73%, while the percentage nanoparticle recovery was reported to be 88%. The drug formulation was proved to reside in the stomach of rats for a longer period of time as some nanoparticles still remained in the stomach of rats 6 hours after administration. They were retained in the stomach for a longer period than clarithromycin suspensions or conventional drug formulations (Ramteke *et al.*, 2006).

Cuna *et al.*, prepared amoxicillin loaded ion-exchange resins encapsulated in mucoadhesive polymers such as polycarbophil and Carbopol[®] 934. An oil-in-oil solvent evaporation technique was modified to produce these microparticles containing multiple amoxicillin-resin cores. Polycarbophil microparticles were spherical, while those containing Carbopol[®] were irregularly shaped. *In vitro* release of amoxicillin was rapid despite the polymer coating. GI transit in rats was investigated by fluorescence microscopy using particles loaded with fluorescein instead of amoxicillin; GRT was longer, and the particles were more evenly

distributed over the stomach when uncoated. It was also observed that Carbopol® did not help increase the GRT of the microspheres. Such discrepancies may be due to the method of administration, the amount of polymer used and the swelling ability of the formulation as outlined previously.

4.3.1.2. Improving targeting of mucoadhesive microspheres

Targeted drug delivery is a selective and effective localization of pharmacologically active compound at specific targets in therapeutic concentrations, while restricting its access to other sites, thus minimising the toxic effects and maximising the therapeutic index (Gregoriadis and Florence, 1993). Mucoadhesive polymers exhibit the ability to stick to wet mucosal surfaces by non-specific physicochemical mechanisms, such as hydrogen bonding. With this non-specific binding, the polymer is unable to differentiate between adherent or shed-off mucus, limiting their ability to target a specific tissue. The development of microspheres coupled with cell specific ligands has increased therapeutic benefit and enhanced the possibility of effective site-specific drug delivery (Chowdary and Rao, 2004). Any ligand that has a high binding affinity for mucin can be linked covalently to microspheres. Examples of such ligands include lectins, adhesins, antibodies and certain amino acid sequences.

4.2.1.2.1. Lectins: There has been interest in lectins due to their ability to bind specifically to membrane bound sugar moieties located at the cell surface of epithelial cells, enhancing the adherence of microparticles to the intestinal epithelium and improving the absorption of drugs (Lee *et al.*, 2000). Lectins are found in plants, vertebrates (Ashwell and Harford, 1982, Stockert and Morell, 1983), bacteria and invertebrates (Lis and Sharon, 1986) but the plant lectins are the largest

group of known lectins. Based on their molecular structure, lectins can be divided into three categories:

- Monolectins - those having only one carbohydrate recognising domain
- Hololectins - those with two or more carbohydrate recognising domains
- Chimerolectins – those with additional unrelated domains

They have the potential to target drugs to different parts of the GI tract or even to different cells (e.g. complex-specific lectins for parietal cells or fuco-specific lectins for M cells). Coating of polystyrene microparticles with tomato lectins has been demonstrated to facilitate specific binding with enterocytes (Gabor *et al.*, 1997). Following cellular uptake, they subsequently exhibit strong binding to nuclear pore membranes (Haas and Lehr, 2002). Another important advantage of lectins in mucoadhesive drug delivery to the GI tract is their resistance to digestion within that environment.

Montisci *et al.* investigated the behaviour of two plant lectin-particle conjugates after oral administration. The two lectins, *Lycopersicon esculentum L.* and *Lotus tetragonolobus* are specific for oligomers of *N*-acetyl-D-glucosamine and L-fucose, respectively and they were conjugated to small poly(lactide) microspheres. The overall GI transit of the particles was strongly delayed, when the microspheres were conjugated to the lectins, mainly due to the gastric retention of the particles. A significant fraction of the conjugates adhered to the gastric and intestinal mucosa. No significant differences were observed after a preliminary incubation of lectin-microsphere conjugates with specific sugars showing that the activity could be maintained (Montisci *et al.*, 2001).

Jain and Jangdey prepared and characterized lectin-conjugated clarithromycin microspheres for the effective colonization of *H. pylori*. Ethylcellulose (EC) microspheres were prepared using the emulsification/evaporation method. Drug entrapment efficiency was about 70% and conjugation with Concanavalin-A (Con-A) was confirmed by IR spectroscopy and differential scanning calorimetry. Con-A is a lectin isolated from the jack bean, *Canavalia ensiformis* and binds specifically to mono, oligo- and polysaccharides with terminal non-reducing α -D-mannopyranosyl-, α -D-glucopyranosyl- or β -D-fructofuranosyl residues. Maximum mucoadhesion of 85% was observed in Con-A conjugated EC microspheres on stomach mucosa of rats, compared with 12% observed in non-conjugated microspheres. A GRT of over 6 hours was reported in rabbits for Con-A conjugated microspheres of clarithromycin, while it was 3 hours for an optimised clarithromycin tablet formulation (Jain and Jangdey, 2009).

In another study by Umamaheshwari and Jain, lectin-conjugated nanoparticles were prepared as a means of attaching acetohydroxamic acid delivery system on the carbohydrate receptors of *H-pylori*. *Ulex europaeus* Agglutinin I (UEA I) and Con-A lectins were bound to gliadin nanoparticles (GNP) by a two-stage carbodiimide coupling technique. The binding efficacy of the lectin to the carbohydrate receptors was evaluated and this showed strong agglutination patterns with mannose-specific Con A-GNP and (L)-fucose specific UEA-GNP formulations. The lectin formulations completely inhibited *H. pylori* binding with human stomach cells. The antimicrobial activity of the formulations was evaluated by percent growth inhibition studies by using isolated *H. pylori* strain. The inhibitory efficacy of UEA-GNP and Con A-GNP was approximately two-fold higher compared to unconjugated nanoparticles (Umamaheshwari and Jain, 2003).

4.2.1.2.2. Bacterial adhesins: Bacterial fimbriae are long lectin-like proteins found on the surface of many bacterial strains, through which they attach to the epithelial surfaces of enterocytes. Their presence has been associated with pathogenicity. Therefore, DDS based on this technique could be an efficient mechanism to enhance adhesion of bioadhesive microspheres to epithelial surfaces (Lee *et al.*, 2000).

Bernkop-Schnurch *et al.* covalently attached a fimbrial protein –K99 to poly (acrylic acid) polymer in order to improve the adhesion of the DDS to the GI epithelium. K99 was isolated from an *E-coli* strain harbouring the fimbriae-encoding plasmid pR19906. In this study, the function of the fimbrial protein was tested using a haemagglutination assay, along with equine erythrocytes expressing the same K99-receptor structures as those of GI-epithelial cells. A 10-fold slower migration of the equine erythrocytes through the K99-poly (acrylic acid) gel, compared to the control gel without the fimbriae was demonstrated, indicating the strong affinity of the K99-fimbriae to their receptor on the erythrocytes (Bernkop-Schnürch *et al.*, 1995).

4.2.1.2.3. Amino acid sequences

Some amino acid sequences have complementary parts on the cell and on the mucosal surfaces and when they are attached to microparticles, this can enhance binding to specific cell surface glycoproteins (Vasir *et al.*, 2003). In the disease state the cell surface glycoproteins are altered and these altered protein sequences can be a target by complementary amino acid sequences attached to drug delivery devices. Dihydroxyphenylalanine (DOPA) an amino acid is found in mussel adhesive protein (MAP) and is believed to lend to the adhesive process. DOPA has been combined with Pluronic to enhance their adhesion (Huang *et al.*, 2002). With its

favourable safety profile, MAP seems to be a suitable compound for the development of mucoadhesive DDS, preferably if these can be manufactured and stored under non-oxidative conditions (Schnurrer and Lehr, 1996).

Antibodies can be produced against some selected molecules present on mucosal surfaces. Antibodies could be a rational choice for designing site-specific mucoadhesives, due to the high level of specificity of the antibodies and this is especially useful in targeting drugs to tumour tissues.

4.3.2. Floating microparticles

Several studies have investigated the effect of formulation and process variables such as polymer type, drug and polymer ratio, type of solvent, organic solvent ratios, concentration of plasticiser in aqueous phase, time of stirring on the yield, particle size, loading, release and floating behaviour of microspheres (Lee *et al.*, 1999, Streubel *et al.*, 2002, Sato *et al.*, 2004, Soppimath *et al.*, 2006). Some studies have shown that the microspheres remained buoyant in the gastric cavity over a long period of time *in vivo* (Jain *et al.*, 2006b) and the encapsulated drug showed a high bioavailability (Joseph *et al.*, 2002, Sato *et al.*, 2003). The encapsulated drug in the polymer matrix exhibited varied release patterns such as zero order release (Gibley, 2002), Higuchi matrix model and Peppas Korsmeyer model (Jain *et al.*, 2006a).

Kawashima *et al.* prepared microspheres containing tranilast – an oral anti-allergic agent (Kawashima *et al.*, 1991). The drug and an acrylic polymer were dissolved in a combination of solvents containing ethanol and dichloromethane. Increasing the polymer ratio led to an increase in the volume of the internal cavity and changing the polymer ratio also led to control of the drug release rate. *In vitro* studies showed the

formulation was buoyant for 12 hours, while *in vivo* radiographical studies showed that the formulations were dispersed in the upper part of the human stomach for over 3 hours.

The effect of co-excipients on the rate of drug release and buoyancy of microspheres were reported in another study (Soppimath *et al.*, 2006). Nifedipine was incorporated into cellulose acetate hollow microspheres prepared by solvent diffusion/evaporation technique in the presence of co-excipients like polyethylene glycol (PEG), dibutyl phthalate (DBP), and poly (ϵ -caprolactone) (PCL) using ethyl acetate as a dispersing solvent. Increasing the concentration of polymer led to an increase in size of the microspheres. The microspheres were buoyant in simulated gastric fluid for over 12 hours, with the blank microspheres being the most buoyant compared with other microspheres produced in the series. The presence of co-excipients affected buoyancy of the microspheres, with cellulose acetate-polyethylene glycol (CA-PEG) microspheres buoyancy was lower and this increased with increasing concentration of PEG. However, by increasing the concentration of PEG from 10 to 40%, the buoyancy decreased from 51.3 to 11.8%. A better buoyancy of 62–82% was observed for microspheres with water-insoluble plasticiser like DBP after 15 h. This might be due to the fact that DBP is a hydrophobic plasticiser and it prevents wetting as well as water uptake. However, the percentage buoyancy of CA–PCL formulations decreases from 26% to 6% with increasing concentration of PCL. The nifedipine content of the microspheres was released in a controlled manner and was easily modified by changing the process parameters of the formulation.

Jain *et al.*, prepared repaglinide floating microspheres through the emulsion- solvent diffusion technique with calcium silicate as a porous carrier, Eudragit[®] S as the

polymer and ethanol with dichloromethane as solvents and polyvinyl alcohol as the surfactant. The microspheres exhibited a high entrapment efficiency of $75 \pm 3.0\%$ due to the poor aqueous solubility of the drug. They were predominantly spherical in nature with an average particle size of 142-825 μm across various batches. True densities ranged from 1.62-1.92 g/cm^3 with angle of repose less than 40° . The compressibility index ranged from 25.0% to 34.6%. *In vitro* buoyancy tests were carried out by spreading the microspheres over the surface of simulated gastric fluid containing Tween 20. The microspheres also exhibited satisfactory buoyancy profile with more than 80% of the microspheres still floating for at least 10 hours. It was observed however, that microspheres of larger sizes exhibited a longer floating time (Jain *et al.*, 2005).

Srivastava *et al.*, prepared and evaluated cimetidine-loaded floating gastroretentive microspheres. The polymers used were hydroxypropylmethylcellulose and ethylcellulose. Effects of the stirring rate during preparation, polymer concentration, solvent composition and dissolution medium on the size of microspheres and drug release were also observed. The drug was released over approximately 8 hours and the microspheres exhibited buoyancy for over 10 hours. Buoyancy percentage of the microspheres was in the range of 69% to 87%. At high polymer concentration, the mean particle increased and the drug release rate decreased (Srivastava *et al.*, 2005).

In a study by Garg and Gupta, silymarin microspheres were prepared using cellulose polymers (hydroxypropyl methyl cellulose and ethyl cellulose) and Eudragit[®] polymers (Eudragit[®] S 100 and Eudragit[®] RL), through the emulsion-solvent evaporation method. *In vitro* floatability studies were carried out using the USP XXIV

dissolution apparatus with 0.1M HCl containing 0.02%v/v Tween 80 as the medium and the paddles rotating at 100rpm over a period of 12 hours. A large proportion of the microspheres remained buoyant with 61% and 75% of the Eudragit[®] and cellulose microspheres respectively floating after 12 hours (Garg and Gupta, 2010).

Floating chitosan beads containing verapamil were prepared by Yassin *et al.* The physical properties, floating characteristics and release profile of the beads were studied. The beads were spherical with a size range of 1.3 to 2.0 mm and the drug loading efficiency was around 42% in all the batches. Beads produced using medium molecular weight chitosan, exhibited the slowest drug release rate and the shortest floating lag time of around 5 minutes and long duration of buoyancy of more than 6 hours (Yassin *et al.*, 2006).

Singh *et al.*, developed floating and non-floating beads containing an anti-ulcer drug pantoprazole through the simultaneous ionotropic gelation of alginate and sterculia gum using calcium chloride as the crosslinker. Calcium carbonate (CaCO₃) was used as the floating aid. The beads produced showed a drug loading of over 65% and were further characterized using SEM and FTIR. Swelling studies showed that the swelling of the beads was dependent on the amount of polymer used, crosslinking agent and the pH of the media. Release of drug from the beads was by Fickian diffusion mechanism and the release of drug from floating beads was less than that observed from non-floating beads (Singh *et al.*, 2010).

In a study by Atyabi *et al.*, microparticles loaded with theophylline and bicarbonate were prepared (Atyabi *et al.*, 1996a). The ion-exchange resin beads (Amberlite IRA-400[®] and Dowex 2X10[®]) were coated with a semi-permeable membrane (Eudragit RS). Carbon dioxide was released on contact of the beads with the acidic gastric

juice (Atyabi *et al.*, 1996b). *In vitro* studies showed that the microparticles exhibited floating times of over 24 hours. The coating of the beads trapped carbon dioxide generated in the beads and thereby prolonged the floating time. *In vivo* studies with human volunteers using gamma-scintigraphy showed a prolonged residence time for coated beads (40% to 65% of the dose remained in the beads in the upper stomach 3 hours after a light breakfast) compared to the uncoated beads for which no beads remained in the stomach after 3 hours.

4.3.2.1 Application of floating microparticles for eradication of *H. pylori*

Floating acetohydroxamic acid microspheres were developed using polycarbonate as the polymer and were prepared by the emulsion solvent evaporation technique. *In vitro* analysis showed that the microspheres exhibited buoyancy with over 70% of the microspheres floating over simulated gastric fluid (pH 1.2) containing Tween 20 after 12 hours (Umamaheshwari *et al.*, 2003). In this study, floating of up to 10 hours was considered to be satisfactory. An increase in the concentration of polymers led to a reduction in buoyancy of the microspheres, due to an increase in density. Also, the microspheres required a less amount of drug dose in achieving its anti- *H. pylori* activity in rat models, when compared with the drug alone. The microspheres cleared the microorganism more effectively than the drug, due to the prolonged GRT of the microspheres resulting from the buoyancy of the microspheres.

There is little research reported on the use of floating microspheres in the eradication of *H. pylori* in the treatment of peptic ulcer and this is an area of research that looks promising. However, floating beads have been extensively researched for

eradication of *H. pylori* and results may be used to inform subsequent studies using microparticles.

Metronidazole (MZ) was incorporated into chitosan-treated alginate beads by the ionotropic gelation method (Ishak *et al.*, 2007). A (3 × 2 × 2) factorially designed experiment in which three viscosity-imparting polymers- methylcellulose, Carbopol® 934P and κ-carrageenan; two concentrations (0.2 and 0.4% w/v) of chitosan as encapsulating polymer and two concentrations (2.5 and 5% w/w) of the low density magnesium stearate as a floating aid were tested. The bead formula containing 0.5% κ-carrageenan, 0.4% chitosan and 5% magnesium stearate showed immediate buoyancy, optimum drug entrapment efficiency and extended drug release. The histopathological examination of mice stomachs and *in vivo H. pylori* clearance tests were carried out by orally administering MZ floating alginate beads or MZ suspension, to *H. pylori* infected mice under fed conditions as a single daily dose for three successive days in different doses- 5, 10, 15 and 20 mg/kg. Groups receiving MZ in the form of floating alginate beads at doses 10, 15 and 20 mg/kg showed better *H. pylori* eradication than the corresponding suspension form. The *in vivo H. pylori* clearance tests showed that MZ floating beads with a dose of 15 mg/kg provided 100% clearance rate, whereas the MZ suspension with a dose of 20 mg/kg gave only 33.33%.

Rajnikanth and Mishra prepared matrix, gellan gum-based, clarithromycin floating beads. Formulation variables such as concentrations of gellan, calcium carbonate and drug loading, influenced the *in vitro* drug release. Differential scanning calorimetry confirmed the absence of interactions between drug and polymer. There was good anti-microbial activity against isolated *H. pylori* strain with complete growth

inhibition after 12 hours. 80% of the beads remained floating after 1 hour; after 4 and 6 hours about 60% and 50% of the beads remained floating in rabbit stomach. The stability studies of beads did not show any significant changes after storage of beads, at 40° C/75% relative humidity for 6 months. The preliminary results from this study suggest that floating beads of gellan can be used to incorporate antibiotics like clarithromycin and may be effective when administered locally in the stomach against *H. pylori*. (Rajinikanth and Mishra, 2009).

Metronidazole-loaded alginate beads consisting of calcium silicate as a porous carrier or NaHCO₃ as a gas-forming agent were prepared for local eradication of *H. pylori*. The silicate based beads showed slower release pattern, compared to the gas-forming beads due to network structure strengthening effect of the calcium silicate. In addition, the gas-forming-based beads had a shorter buoyancy lag time because the NaHCO₃ produced larger pores than those of silicate treated ones. Drug entrapment efficiency was over 60% for the formulations (Javadzadeh *et al.*, 2010)

Murata *et al.* prepared two types of floating alginate beads. The first alginate gel bead contained vegetable oil (ALGO) and its buoyancy was due to the oil in the alginate gel matrix. The second, (ALCS), was 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. In addition, the concentration of drug at the gastric mucosa after administration of ALCS was higher than that obtained from metronidazole solution, though the metronidazole serum concentration was the same, regardless of which type of gel was administered. These release properties of alginate gels are

applicable not only for sustained release of drugs, but also for targeting the gastric mucosa (Murata et al., 2000).

4.3.3. Floating bioadhesive microparticles

Dual functioning systems are currently being explored in gastroretentive drug delivery. This is achieved through a combination of both the floating and bioadhesive systems, which can be exploited to achieve synergy and also help to overcome the drawbacks associated with each system. This theory was explored by Chitnis *et al* (1991) and it was proposed that these systems target *H. pylori*-induced infected sites more effectively and could serve to optimize antibiotic monotherapy of *H. pylori*-based infections (Umamaheswari *et al.*, 2002). In the research by Umamaheswari *et al*, floating microspheres containing acetohydroxamic acid were prepared. Also, these microspheres were further coated with polycarbophil. The microspheres floated for longer than 12 hours, due to the low densities of the formulation 0.61-0.85 g/cm³. The coating of the microspheres reduced the release rate of the drug and exhibited a better *in vitro* and *in vivo* percentage *H. pylori* growth inhibition.

Zheng *et al.* also explored this strategy with chitosan alginate-ethylcellulose microparticles. The formulation was prepared through a combination of emulsification/evaporation and internal/ion gelation methods. *In vitro* tests showed that 74% of the microspheres remained buoyant on an acetate buffer solution for 8 hours and 90% of the loaded drug was released in a sustained manner over this period. *In vivo* mucoadhesive studies showed that 61% of the microparticles were retained in the stomach of male Sprague-Dawley rats for 4 hours. Pre-treatment

with omeprazole led to an increase in clarithromycin concentration with the microparticles in the gastric mucosa compared to clarithromycin solution (Zheng *et al.*, 2006).

A floating bioadhesive system was developed for the eradication of *H. pylori* with ethylcellulose as matrix polymer and Carbopol® 934P as the mucoadhesive polymer. The microspheres exhibited both strong mucoadhesive and good buoyancy profiles. They also demonstrated significant anti *H. pylori* effect *in vivo* in Mongolian gerbil models. In addition, on comparison with conventional clarithromycin suspension, the new formulation required a lower dose of drug for eradication of the microorganism. The microspheres also improved the gastric stability of clarithromycin and eradication of *H. pylori* from the GI tract more than conventional formulations due to the prolonged gastric retention time of the formulation (Rajinikanth *et al.*, 2008).

In a randomised clinical trial floating bioadhesive microspheres were compared with conventional clarithromycin suspension. In 876 patients, it was observed that at low doses of 60 and 90mg/kg of clarithromycin, *H. pylori* was mostly cleared with a 98-100% clearance rate and 83% inhibition at clarithromycin dose of 30mg/kg. This formulation exhibited a better eradication profile than the suspension and the microbial clearance was further confirmed by polymerase chain reaction analysis (Vaicunas *et al.*, 2010).

Gattani *et al.* developed alginate/hydroxypropyl methylcellulose-based floating/mucoadhesive beads containing clarithromycin to extend the contact time of the antibiotic with *H. pylori*. The beads were prepared by the ionic-gelation technique with calcium chloride as the gelating agent and liquid paraffin (LP) was incorporated to aid the floating of the beads. Drug loading efficiency was more than 80%, in all

batches of the formulation with particle size within the range of 0.7 mm-1.1 mm. SEM images of the beads presented a rough surface with characteristic large wrinkles and micropores. Floating ability of beads depended upon the percentage of LP used in the preparation of beads. The formulations without LP were not buoyant and those containing more than 10% LP showed 100% buoyancy. As the concentration of LP increased, the release rate decreased. *In vitro* mucoadhesion studies showed that alginate beads exhibited up to 80% mucoadhesion and there was no significant effect of the LP on the mucoadhesive property of the beads, while Alg-HPMC beads showed 100% mucoadhesion. *Ex-vivo* mucoadhesion study shows floating-mucoadhesive beads of Alg-HPMC have better adhesive effect in the stomach and might stay longer in the stomach for more effective *H. pylori* clearance. *In vivo* X-ray imaging study showed that the Alg-HPMC beads of CL remained buoyant for at least 6 h in rabbit stomach and that they had good floatability *in vivo* (Gattani *et al.*, 2010).

Sahasathian et al prepared amoxicillin- loaded alginate beads, coated with 0.5% w/v chitosan and the beads exhibited drug encapsulation efficiency and mucoadhesiveness which was over 90%, 100% buoyancy was achieved and the beads achieved sustained release of amoxicillin for over 6 hours in simulated gastric fluid (Sahasathian *et al.*, 2010).

5. Conclusion

There is no doubt that the oral route is the most common and probably most complex route of drug delivery. The major barriers against achieving successful delivery of drugs via the oral route include unpredictable gastric emptying times, shorter gastrointestinal transit time of the dosage form, partial drug release from the

dosage form and the absorption site of the particular drug. The application of controlled release gastroretentive microparticles has the potential to resolve most of these problems. However, in the eradication of *H. pylori*, the ideal dosage form should in addition to overcoming all these barriers, also be able to target the bacterium. Most studies show excellent *in vitro* sustained release and eradication profiles, which do not often correlate to *in vivo* results. Therefore, more research is required into gastroretentive microparticulate formulations that will exhibit excellent *in vitro* and *in vivo* eradication results against *H. pylori* and a reliable *in vitro* method to assess their potential.

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Theory	Mechanism of Bioadhesion	Comments
Electronic theory	There are attractive electrostatic forces between the glycoprotein mucin network and the bioadhesive material	Electron transfer occurs between the mucin and the bioadhesive material forming a double layer of electric charge at the interface
Adsorption theory	There are surface forces resulting in chemical bonding	The surface forces include strong primary forces which are covalent bonds and weak secondary forces which include ionic bonds, hydrogen bonds and van der Waal's forces
Wetting theory	The ability of bioadhesive polymers to spread and develop intimate contact with the mucus membranes	Spreading coefficients of polymers must be positive. Contact angle between polymer and cells must be near to zero
Diffusion theory	Physical entanglement of mucin strands and the flexible polymer chains	For maximum diffusion and best bioadhesive strength: solubility parameters (δ) of the bioadhesive polymer and the mucus glycoproteins must be similar interpenetration of mucin strands into the porous structure of the polymer substrate
Fracture theory	Analyses the maximum tensile stress developed during detachment of the bioadhesive drug delivery system from the mucosal surfaces	Does not require physical entanglement of bioadhesive polymer chains and mucin strands, hence appropriate to study the bioadhesion of hard polymers, which lack flexible chains

Table 1: Mechanism of bioadhesion-Adapted from Vasir *et al.*, (2003). Used with permissions.