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Controlled Release Drug Delivery System with Stomach Specific Mucoadhesive Nanoparticles

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Abstract

In recent years scientific development has been effected, by overcoming physiological difficulties, in the rate controlled oral drug delivery system such as short gastric residence time and unpredictable gastric emptying time. This review article mostly focuses on the dosage forms which are retained in the stomach for a prolonged and predictable period of time; which are most logical, economical and safest among all approaches to retain the dosage form in the stomach, aimed at enhancing the oral bioavailability of a drug. This study discusses concept of gastric emptying, absorption window, potential drug candidates, technological development evaluation and applications for stomach-specific mucoadhesive nanoparticles. Marketed products for oral nanoparticulate drug delivery systems are also discussed in this review.

Keywords: Gastroretentive, Mucoadhesive, Nanoparticles, Controlled release, Gastric residence time.

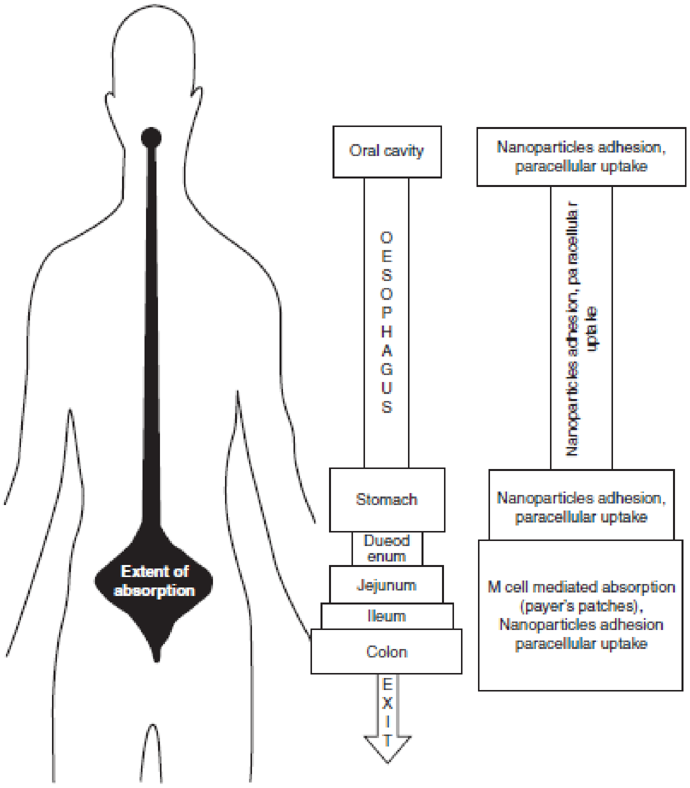
1. Introduction

The GIT is the preferred route for the delivery of drugs. The physiological properties which favor absorption are the large volume of fluid available, the peristaltic movements, the extensive mucosal area and the extensive mesenteric circulation blood flow [1]. Due to the preference for this oral route, research is towards the development of effective oral dosage forms. Bioadhesives may delay the gastric emptying and intestinal transit of drugs by interaction with mucosa of the GIT [2]. This approach to improving bioavailability of drugs is desirable, since localization of modification permeability and protease inhibition may also be achieved [3]. These are important implications for the oral delivery of proteins and polypeptide drug molecules. A substantial improvement in the concept by means of specific receptor mediated interactions between the mucosal cell surface and bioadhesive may be achieved. Although, such interactions can be observed in vitro, this concept appears to ignore the fact that these adhesives must penetrate prior to attachment to the mucosal cell surface. The success, therefore, depends on the rapid and successful diffusion of adhesive into the mucus layer. Removal of the bioadhesive drug delivery system by the movements of the luminal contents may be delayed by the presence of the mucus layer; as the bonds formed upon contact with the mucosal surface, must withstand the forces of mucus turnover and transit along the GIT, to offer advantages over non-specific bioadhesive [4].

The absorption of drugs through the mucus/mucosal lining of the gastrointestinal tract (GIT) may be achieved by means of bioadhesives. A bioadhesive is defined as a synthetic or biological material, capable of adhering to a biological substrate or tissue. When the biological substrate is mucus, the term "mucoadhesive" is employed. If the stomach is biological tissue involved termed as "gastroadhesive. Other definitions suggest that bioadhesives should remain attached to the biological substrate "for an extended period of time", although this period of time is never quantified. The period of time a bioadhesive is required to remain attached to a biological substrate will vary according to the target site and the condition being treated. For *drug targeting in GIT, the phrase "for an extended period of time" should be replaced* with the phrase "for a period of time which allows a reduction in dosage frequency in comparison to conventional, non-adhesive dosage forms" [4].

Nanoparticulate dosage forms are retained in the stomach by adhering to the mucosal layer of the stomach called as stomach specific mucoadhesive nanoparticles (SSMN) as shown in figure 1. SSMN can improve controlled delivery of drugs, by continuously releasing the drug for a prolonged period ensuring optimal bioavailability. Drugs with poor absorption show improved absorption at the jejunum and ileum due to the enhanced absorption properties of these sites (e.g. large surface area), or because of enhanced solubility in the stomach and continuously releasing drug at a controlled rate to the site of absorption i.e. stomach and upper part of small intestine.

Fig.1 *Absorption mechanism and extent of nanoparticles absorption from different regions of GIT [5].*



The types of drugs benefiting from using stomach specific mucoadhesive nanoparticles includes-:

- Drugs that act locally in the stomach (e.g. tetracycline and antacids),
- Drugs with low solubility at high pH values (e.g. verapamil, diazepam, propranolol, metoprolol and chlordiazepoxide),
- Drugs that are primarily absorbed in the stomach (e.g. salbutamol, albuterol, sotalol and levodopa),
- Drugs with a narrow window of absorption, i.e. drugs that are absorbed mainly from the proximal part of the small intestine (e.g. riboflavin, acyclovir, nitrofurantoin and allopurinol),
- Drugs that absorb rapidly from the gastro intestinal tract (e.g. amoxicillin),
- Drugs that degrade in the colon (e.g. ranitidine and metoprolol), and
- Drugs that is unstable in intestinal fluids (e.g. captopril and famotidine).

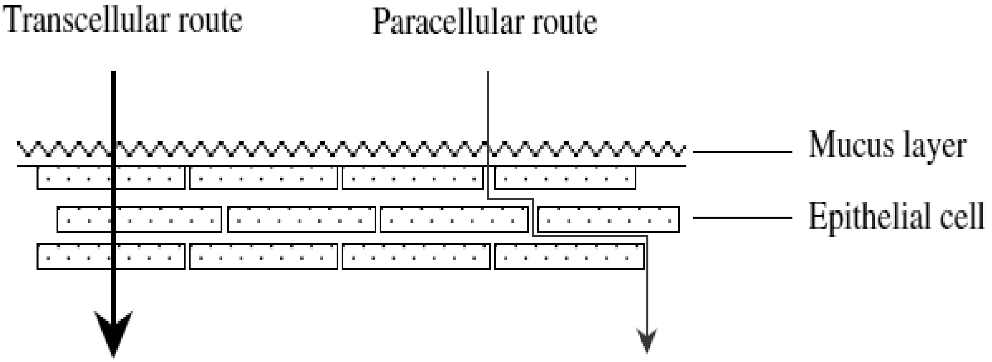
Longer residence time in the stomach could be advantageous for local action especially in the upper part of the small intestine, namely for the treatment of peptic ulcer disease [6,7]. The list of potential drugs for stomach specific mucoadhesive drug delivery system is given in table 1.

Table.1 *Potential Drug Candidates for SSMN [7]*

Acyclovir	Riboflavin
Alendronate	Riserdonate
Atenolol	Fluorouracil
Captopril	Diazepam
Ciprofloxacin	Verapamil HCl
Furosemide	Chlordiazepoxide HCl
Ganciclovir	Ranitidine HCl
G-CSF	Diltiazem
Ketoprofen	Chlorpheniramine maleate
Levodopa	Sotalol
Melatonin	p-nitroaniline
Metformin	Tranilast
Minocyclin	Amoxycillin trihydrate
Misoprostol	Ibuprofen
Verapamil	Tetracycline

Reddy et al. reviewed that mucoadhesive system can be applied for both mucosal (local effect) and trans-mucosal (systemic effect) drug delivery. In the first case, the aim is to achieve a site-specific release of the drug on the mucosa, whereas the second case involves drug absorption through the mucosal barrier to reach the systemic circulation. The drug transport mechanism through the mucosa involves two major routes: transcellular (intracellular) and paracellular (intercellular) pathways as shown in figure 2.

Fig.2 *Schematic representation of penetration routes in mucosal drug delivery [8].*



1.1 Advantages of SSMN

- SSMN greatly improve stomach pharmacotherapy through local drug release, which leads to high drug concentrations at the gastric mucosa (eradicating Helicobacter pylori from submucosal tissue of the stomach), in the treatment of duodenal ulcers, gastritis and oesophagitis, and to reduce the risk of gastric carcinoma.
- SSMN can be used as carriers for drugs with so-called absorption windows. These substances are antiviral, antifungal and antibacterial agents (e.g. sulfonamides, quinolones, penicillins, cephalosporins and tetracycline) are taken up only from very specific site of gastrointestinal tract (GIT).
- SSMN have been recommended to achieve sustained drug delivery. Improved patient compliance and convenience have been reported due to less frequent drug administration and the nature of the drug's release kinetics. Reduction of fluctuation in drug blood concentration and maximum utilization of the drug with a decrease in total adverse effects have been reported, with improved absolute bioavailability of the drug in SSMN (e.g. famotidine). SSMN provide maintenance of systemic drug concentration within the therapeutic window, and provide site specific drug delivery. Drugs with absorption sites in the upper small intestine, such as furosemide and riboflavin can be typically formulated using this system [7].

1.2 Limitations Of SSSMN

- Adsorption prevention to any ingested food, SSMN would necessitate administration on an empty stomach [9].
- SSMN are not suitable for the drugs that have solubility or stability problems in the gastric fluid and may cause irritation to gastric mucosa [10].
- Drugs like nifedipine, which is well absorbed along the entire GIT and which undergoes significant metabolism, may not be desirable candidates for SSMN since the slow gastric emptying may lead to reduced systemic bioavailability [10].

2. Gastro-Intestinal Mucus

The composition of gastro-intestinal mucus:

Mucus is composed predominately of water (95%), the remainder being glycoprotein, sloughed epithelial cells, proteins, electrolytes, bacteria and DNA, in certain disease states. However, even though the glycoprotein molecules only constitute 2-3 % of native mucus, are responsible for the bulk of mucus gel properties. The gastro-intestinal tract (GIT) is lined with a layer of mucus which performs a number of physiological functions. One such function is that of providing a barrier to acid in the stomach by presenting insipid layer into which bicarbonate ions are secreted by the surface epithelium. The bicarbonate ions secreted actively, neutralize hydrogen ions (secreted by parietal or oxyntic cells) as the latter diffuse towards the epithelium from the lumen. A pH gradient across the mucus gel layer, from low pH on the luminal side to high pH on the epithelial side, has been demonstrated in rabbit, in rat and in human gastric mucosa as shown in figure 3. The gel also resists auto digestion of the GIT by presenting a diffusional barrier to the progress of enzymatic molecules, such as pepsin. There is continual secretion of mucus in order to maintain the mucus layer intact, as constant loss from enzymatic degradation and physical erosion occurs. It is this delicate balance of mucus secretion, loss and its buffering capacity, which endows the mucus layer with its ability to protect the underlying epithelium. A further role of mucus is to facilitate the passage of food along the GIT. As mucus is a visco-elastic gel, it allows the food to "slip over" the underlying epithelia without causing damage.

The thickness of the mucus layer varies with the region, the species and the methodology employed to measure it. The mucus layer in the human stomach was reported to be 576 ± 8l μm whereas another researcher reported mean thickness of 192 μm. The blanket of mucus covering the entire length of the GIT is essential to the physiological function of the alimentary tract. However, with respect to drug absorption, the mucus layer, once regarded simply as insipid water layer, is now regarded as an important potential barrier affecting drug diffusion.

Mucus is a viscoelastic material. It exhibits both the flow properties of liquids and the elastic properties of a solid, although only one of these properties will be obvious under certain conditions. When subjected to low intensity stress or when a stress is applied for a short period of time, the gel may appear completely elastic and recover from any deformation. However, at stress of higher intensity or for longer periods of time, the mucus gel will flow. Both these features are essential for mucus to exert its protective effect since the solid or elastic behavior enables it to provide support to the underlying epithelium from abrasion by food, and the ability to flow, facilitates the passage of solid contents [11].

3. Theories of Mucoadhesion

The four main theories that describe the possible mechanisms of mucoadhesion are following:

- The electronic theory assumes that transfer of electrons occurs between the mucus and the mucoadhesive due to differences in their electronic structures. The electron transfer between the mucus and the mucoadhesive leads to the formation of a double layer of electrical charges at the interface of the mucus and the mucoadhesive. This result, in attraction forces, inside the double layer.
- The adsorption theory concerns the attraction between the mucus and the mucoadhesive achieved via molecular bonding caused by secondary forces such as hydrogen and van der Waals bonds. The resulting attractive forces are considerably larger than the forces described by the electronic theory.
- The wetting theory correlates the surface tension of the mucus and the mucoadhesive with the ability of the mucoadhesive to swell and spread on the mucus layer and indicates that interfacial energy plays an important role in mucoadhesion. By calculating the interfacial energy from the individual spreading coefficients of the mucus and the mucoadhesive or by calculating a combined spreading coefficient, predictions about the mucoadhesive performance can be obtained. The wetting theory is significant, since spreading of the mucoadhesive over the mucus is a prerequisite for the validity of all the other theories.
- The diffusion theory concerns the interpenetration to a sufficient depth and physical entanglement of the protein and polymer chains of the mucus and the mucoadhesive, depending on their molecular weight, degree of cross-linking, chain length, flexibility and spatial conformation. None one of these theories gives a complete description of the mechanism of mucoadhesion. The total phenomenon of mucoadhesion is a combined result of all these theories. First, the polymer gets wet and swells (wetting theory). Then, non covalent (physical) bonds are created within the mucus– polymer interface (electronic and adsorption theory). Then, the polymer and protein chains interpenetrate (diffusion theory) and entangle together, to form further non-covalent (physical) and covalent (chemical) bonds (electronic and adsorption theory) [12].

4. Polymers Used for Mucoadhesive Nanoparticles

The concept that bioadhesion enhances the efficiency of drug delivery through an intimate and prolonged contact between the delivery device and the absorption site, has resulted in considerable efforts to develop and evaluate bioadhesive polymers. The use of bioadhesive polymers in controlled release drug delivery systems provides potential advantages, including

1. Prolonged residence time at the site of absorption
2. Increased time of contact with the absorbing mucosa
3. Localization in specific regions to enhance drug bioavailability

Several important physicochemical properties contribute to the adhesive potential of candidate polymers. These properties include the following

- High molecular weight (i.e., >100,000 Da) needed to produce interpenetration and chain entanglement.
- Hydrophilic molecules containing a large number of functional groups capable of forming hydrogen bonds with mucin.
- Anionic poly electrolytes with a high charge density of hydroxyl and carboxyl groups.
- Highly flexible polymers with high chain segment mobility to facilitate polymer chain interpenetration and inter-diffusion.

- Surface properties similar to those of the biological substrate to provide a low interfacial free energy between the adhesive and the substrate

Although these properties are not all required for bioadhesion, they have been found to enhance the bioadhesive characteristics of the polymers [13].

Table.2 *List of some mucoadhesive polymers [8].*

Criteria	Category	Examples
Source	Semi-natural/natural	Agarose, chitosan, gelatin Hyaluronic acid Various gums (guar, hakea, xanthan, gellan, carragenan, pectin, and sodium alginate)
	Synthetic	Cellulose derivatives: [MC, CMC, thiolated CMC, HPMC, HEC, HPC] Poly(acrylic acid)-based polymers: [CP, PC, PAA, copolymer of acrylic acid and PEG] Others: PVA, PVP, thiolated polymers
Aqueous solubility	Water-soluble	CP, HEC, HPMC (cold water), PAA, sodium CMC, sodium alginate
	Water-insoluble	Chitosan (soluble in dilute aqueous acids), EC, PC
Charge	Cationic	Aminodextran, chitosan, dimethylaminoethyl (DEAE)-dextran, trimethylated chitosan
	Anionic	Chitosan-EDTA, CP, CMC, pectin, PAA, PC, sodium alginate, sodium CMC, xanthan gum
	Non-ionic	Hydroxyethyl starch, HPC, poly(ethylene oxide), PVA, PVP, scleroglucan
Potential bioadhesive forces	Covalent	Cyanoacrylate
	Hydrogen bond	Acrylates [hydroxylated methacrylate, poly(methacrylic acid)], CP, PC, PVA
	Electrostatic interaction	Chitosan

5. Characteristics of an ideal Mucoadhesive Polymer

- Rapid adherence to mucosa.
- Exhibit strong interaction with the mucin epithelial tissue.
- Minimum impact on drug release.
- Good spreadability, wetting, swelling and solubility and bio degradability properties.
- Unaffected by the hydrodynamic conditions, food and pH changes.
- Easy to incorporate in various dosage forms.
- Possess peel, tensile and shear strengths at the bioadhesive range.
- Show bioadhesive properties in both dry and liquid state.
- Demonstrate local enzyme inhibition and penetration enhancement properties.
- Demonstrate acceptable shelf life.
- Optimum molecular weight.
- Possess adhesively active groups.
- Possess required spatial conformation.
- Sufficiently cross-linked but not to the degree of suppression of bond forming groups.
- Possess good viscoelastic properties and no breakdown at the mucosa [14].

6. Targeting of Nanoparticles using Ligands

6.1 Targeting ligands to epithelial cells in the GI tract

Targeting strategies to improve the interaction of nanoparticles with adsorptive enterocytes and M-cells of Peyer's patches in the GI tract can be classified into those utilizing specific binding to ligands or receptors and those based on nonspecific adsorptive mechanism. The surface of enterocytes and M cells display cell-specific carbohydrates, which may serve as binding sites to colloidal drug carriers containing appropriate ligands. Certain glycoprotein's and lectins bind selectively to this type of surface structure by specific receptor-mediated mechanism. Different lectins, such as bean lectin and tomato lectin, have been studied to enhance oral peptide adsorption. Vitamin B-12 absorption from the gut under physiological conditions occurs via receptor-mediated endocytosis. The ability to increase oral bioavailability of various peptides (e.g., granulocyte colony stimulating factor, erythropoietin) and particles by covalent coupling to vitamin B-12 has been studied. For this intrinsic process, mucoprotein is required, which is prepared by the mucus membrane in the stomach and binds specifically to cobalamin. The mucoprotein completely reaches the ileum where resorption is mediated by specific receptors [15].

6.2 Targeting ligands to mucus

A variety of ligands have been attached to the surface of nanoparticles which are specific to lymphoid tissue in order to improve bioavailability. These are diverse and have included invasins, lectins and vitamin B12. Invasin-C192 coated 500 nm polystyrene nanoparticles have achieved modest uptake following single gavage in rat. In the same study significantly lower mucin coated control systemic uptake. The difficulty of resolution of results such as these reflects the highly complex experimental environment involved. In this case the result has been questioned, as to why the porcine mucin coating interfered with systemic uptake while initial particles would have gained a mucin coating. As an explanation it has been suggested that the relatively high density of mucin coupled with low mucin secretion in rat model may have been contributing factors to these results. Lectin conjugated nanoparticles are reported to have improved uptake through interaction with mucus and epithelial cells. A study utilizing tomato-lectin functionalized polystyrene nanoparticles administered to rat by oral gavage with water as the liquid phase over a 5 day period remarkably resulted in a quarter of the particles reaching the systemic circulation. This is in spite of the presence of formidable biological barriers. The route of nanoparticle uptake in this study was attributed primarily to the non-lymphoid intestinal surface [16].

7. Technological development in gastroretentive mucoadhesive dosage forms

Katayama et al. prepared a sustained release liquid preparation using sodium alginate. For evaluating the gastric retention time of the preparation, the remaining percent of ampicillin when an aqueous ampicillin solution vs. the sodium alginate preparation were administrated in isolated perfused rat stomachs was compared, 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 [17].

Liu et al. prepared mucoadhesive microspheres of amoxicillin by an emulsification/evaporation method, using ethyl cellulose as matrix and carbopol 934P as a mucoadhesive polymer. They found 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. Finally, studies on the in vivo clearance of H. pylori revealed that, in a single-dosage administration (4 mg/kg to 14.8 mg/kg), the mucoadhesive microspheres had a better effectiveness (expressed by the ratio of colony counts between amoxicillin powder and microspheres) compared to amoxicillin powder (3.2 to 9.7, respectively). In parallel, a multi dosage administration regimen (3.5 mg/kg, twice a day for 3 consecutive days) showed a complete eradication of H. pylori with microspheres in five of six rat stomachs, whereas amoxicillin powder showed four times less effectiveness [18].

Jaccob et al. developed a composite formulation for selective, high efficacy delivery to specific regions of the GIT. The formulation is typically in the form of a tablet or capsule which may include microparticles or beads. The formulation uses bioadhesive and controlled release elements to direct release to specific regions where bioadhesive elements are exposed at the time the formulation reaches the region of desired release. This can result in enhanced amounts relative to the formulation in the absence of the bioadhesive and/or controlled release elements. This is demonstrated by several examples showing delivery of different drugs greater area under the curve (AUC) relative to the reference immediate release dosage form i.e., the AUC of the composite bioadhesive formulation is greater than 100% of the AUC of the immediate release drug and/or the drug in a formulation of only the controlled release or bioadhesive elements [19].

Mathiowitz et al. encapsulated insulin in the form of mucoadhesive particles by using poly (fumaric anhydride) and poly (lactide-co-glycolide) 50:50 (P (FA: PLGA)). Particles were administered to groups of fasted rats that were injected with an initial glucose load. It was found that upon administration of insulin containing particles by rats, blood glucose was controlled successfully at the fasting levels [20].

Umamaheshwari et al. formulated mucoadhesive gliadin nanoparticles (GNP) containing amoxicillin by desolvation method and evaluated their effectiveness in eradicating *H. pylori*. For evaluation in vivo gastric mucoadhesive property in albino rats Rhodamine isothiocyanate-entrapped GNP formulations were prepared. It was reported that on increasing gliadin concentration, the mucoadhesive property of GNP increased. In vitro antimicrobial activity of GNP containing amoxicillin on an isolated *H. pylori* strain shown that the time required for complete eradication was higher in GNP containing amoxicillin than in amoxicillin because of the controlled drug delivery of amoxicillin from GNP containing amoxicillin. They concluded that GNP containing amoxicillin eradicated *H. pylori* from the gastrointestinal tract more effectively than amoxicillin because of the prolonged gastrointestinal residence time attributed to mucoadhesion [21].

Shishu et al. developed multiple-unit-type oral floating dosage form (FDF) of 5- fluorouracil (5-FU) to prolong gastric residence time, target stomach cancer, and increase drug bioavailability. The floating bead formulations were prepared by ionotropic gelation using calcium carbonate and a mixture of sodium alginate and hydroxypropyl methylcellulose solution. The multiple-bead FDF was found to reduce the tumor incidence in mice by 74%, while the conventional tablet dosage form reduced this incidence by only 25%. Results indicate that FDF performed significantly better than the simple tablet dosage form [22].

Mitragotri et al. invented a novel intestinal mucoadhesive patch system for oral drug delivery. The patch system comprises an impermeable backing layer, a drug reservoir and a mucoadhesive layer. The drug reservoir and the mucoadhesive layer may be combined into a single layer. When the patches are introduced into the gastrointestinal tract, the mucoadhesive layer sticks to the lumenal wall due to its mucoadhesive properties, then the drug releases from the reservoir in a unidirectional way through the mucoadhesive layer into the intestine mucosa. This improved method is advantageous in enhancing bioavailability of poorly absorbed drugs such as polar molecules or bioactive peptides and proteins [23].

Makhlof et al. developed mucoadhesive particulate system for the oral delivery of peptide drugs by combining safe permeation enhancers by ionic interaction of spermine (SPM) with polyacrylic acid (PAA) polymer. Cytotoxicity studies in Caco-2 monolayers revealed the safety of the delivery system in the concentration range used for permeation enhancement. The cellular transport of fluorescein isothiocyanate dextran (FD4) showed higher permeation enhancing profiles of SPM–PAA NPs, as compared to SPM solution or PAA NPs prepared by ionic gelation with MgCl2 (Mg-PAA NPs). The permeation enhancing properties of SPM–PAA NPs were further evaluated in vivo after oral administration to rats, using FD4 and calcitonin as models of poorly permeating drugs. Confocal microscopy images of rats' small intestine confirmed previous findings in Caco-2 cells and revealed a strong and prolonged penetration of FD4 from the mucosal to the basolateral side of the intestinal wall [24].

Suwannateep et al. developed mucoadhesive drug carriers for the gastro-intestinal tract (GIT). Here, a monopolymeric carrier made from ethyl cellulose (EC) and a dipolymeric carrier made from a blend of methylcellulose (MC) and EC (ECMC) were prepared through a self-assembling process and yielded the highest reported curcumin loading of 48 to 49%. The in vivo evaluation of their adherence to stomach mucosa and their ability to release curcumin into the circulation were carried out through quantification of curcumin levels in the stomach tissue and in blood of mice orally administered with the two spheres. Direct evidence of the adherence of the C-EC and C-ECMC particles along the mucosal epithelia of the stomach is also presented for the first time through SEM images [25].

Irache et al. developed bioadhesive nanoparticles for the oral delivery of poorly available drugs. The bioadhesive potential of Gantrez nanoparticles fluorescently labeled with rhodamine B isothiocyanate was determined. The adhesive potential of Gantrez was found to be stronger when formulated as nanoparticles than in the solubilized form. Conventional nanoparticles displayed a tropism for the upper areas of the gastrointestinal tract, with a maximum of adhesion 30 min post-administration and a decrease in the adhered fraction along the time depending on the given dose. Finally, nanoparticles were coated with either gelatin or albumin. In the first case, the presence of gelatin dramatically decreased the initial capacity of these carriers to interact with the gut mucosa and the intensity of these phenomenons. In the latter, bovine serum albumin coated nanoparticles (BSA-NP) showed an important tropism for the stomach mucosa without further significant distribution to other parts of the gut mucosa [26].

Arora et al. prepared chitosan-alginate polyelectrolyte complex (CS-ALG PEC) nanoparticles of amoxicillin and optimized for various variables such as pH and mixing ratio of polymers, concentrations of polymers, drug and surfactant, using 3x3 Box-Behnken design. Various studies like particle size, surface charge, percent drug entrapment, *in-vitro* mucoadhesion and *in-vivo* mucopenetration of nanoparticles on rat models were conducted. The optimized FITC labeled CS-ALG PEC nanoparticles have shown comparative low *in-vitro* mucoadhesion with respect to plain chitosan nanoparticles, but excellent mucopenetration and localization as observed with increased fluorescence in gastric mucosa continuously over 6 hours, which clinically can help in eradication of *H. pylori* [27].

Park et al. stated that highly charged carboxylated polyanions are good potential bioadhesives for drug delivery. They described a new, simple experimental technique that can quantitatively measure bioadhesive properties of various polymers. The technique consists of labeling the lipid bilayer of cultured human conjunctival epithelial cells with the fluorescent probe pyrene. Addition of polymers to this substrate surface compresses the lipid bilayer causing a change in fluorescence as compared to control cells. The fluorescent probe, pyrene, provides information on membrane viscosity, which is proportional to polymer binding. In addition to the use of pyrene, membrane proteins were labeled with fluorescein isothiocyanate, and depolarization of probe labeled proteins was measured before and after polymer treatment. By using these fluorescent probes, it was possible to compare charge sign. Charge type and density, and backbone structure as to their influence on polymer adhesion [28].

Bhat et al. evaluated the extent of drug binding to mucin; a purified model mucus system containing primarily the large glycoprotein fraction (400 kDa) of gastric mucus was developed for use in drug binding studies. The extent of binding of six selected compounds (albuterol, rifampicin, p-amino-salicylic acid, isoniazid, pyrazinamide, and pentamidine) to mucus glycoproteins was studied. The binding of each drug to a model plasma protein, bovine serum albumin (BSA), was also investigated. Binding studies were performed by diafiltration, which combines characteristics of equilibrium dialysis and ultra filtration in a continuous system. All the compounds selected showed affinities of the same order of magnitude to mucin despite being chemically dissimilar and exhibiting differing ionization states. This suggests that binding to gastric mucus glycoproteins is non-specific in nature with similar types of binding forces involved in the binding of all the compounds tested. Based on these results, it can be concluded that the binding behavior of drugs to gastric mucin is non-specific in nature with binding constants of a low magnitude [29].

Tur et al. carried out study to demonstrate that the addition of a bioadhesive polymer can greatly increase the bioavailability of griseofulvin with normal particle size form. Four formulations: A, 30 mg drug (mean particle size of 14/tm); B, 30 mg drug and 300 mg poly(acrylic acid) crosslinked with 2,5-dimethyl-l,5-hexadiene (PADH); C, 30 mg per 10 ml aqueous suspension; and D, 30 mg per 10 ml oil-in-water emulsion were employed in this experiment. New Zealand white rabbits were orally administered with the above dosage forms and the blood samples were collected from the marginal vein at different time intervals for 24 h. The plasma concentrations were determined with a high performance liquid chromatography (HPLC). The result indicates that the addition of PADH to griseofulvin can increase the total absorption by 2.9-, 4-, and 2.9-folds when compared with drug powder, aqueous suspension and emulsion, respectively. The mechanism of improvement is probably due to the increase in gastro-intestinal transit time and the intimacy of the drug with the absorbing membrane brought about by the bioadhesive polymer [30].

In order to circumvent the problem of poor bioavailability with some drugs Ponchel et al. proposed, association of drug to polymeric nanoparticulate systems (or small particles in the range of the micrometer in size) because of their tendency to interact with the mucosal surface. Bioadhesion can be obtained by the building of either non-specific interactions with the mucosal surface, which are driven by the physicochemical properties of the particles and the surfaces, or specific interactions when a ligand attached to the particle is used for the recognition and attachment to a specific site at the mucosal surface. The relative merits of those systems are discussed. Their fate in the gastrointestinal tract, including at least three different pathways: (i) bioadhesion, (ii) translocation through the mucosa and (iii) transit and direct fecal elimination [31].

Hillery concluded that microparticulate carriers offer considerable potential for drug and vaccine delivery via mucosal routes. Perhaps greater therapeutic success can be achieved by using microparticles as carriers for vaccines, rather than for therapeutic drugs, because of the lower relative amount of antigen that is required to induce an immune response in comparison with the amount of drug required to produce a pharmacological response. Also, mucosal vaccines offer the potential to be highly efficient because of their ability to induce local protection at mucosal surfaces [32].

Sakuma et al. investigated the mucoadhesion of polystyrene nanoparticles having surface hydrophilic polymeric chains in the gastrointestinal (GI) tract in rats. Radio labeled nanoparticles were synthesized by adding hydrophobic 3-(trifluoromethyl) - 3-(*m*-[125I]iodophenyl)diazirine in the final process of nanoparticle preparation. The radioiodonated diazirine seemed to be incorporated in the hydrophobic polystyrene core of nanoparticles. The change in blood ionized calcium concentration after oral administration of salmon calcitonin (sCT) with nanoparticles showed that the in vivo enhancement of sCT absorption by radio labeled nanoparticles was the same as that by non-labeled nanoparticles. The GI transit rates of nanoparticles having surface poly (*N*-isopropylacrylamide), poly (vinylamine) and poly (methacrylic acid) chains, which can improve sCT absorption, were slower than that of nanoparticles covered by poly (*N*-vinylacetamide), which does not enhance sCT absorption at all. These slow transit rates were probably the result of mucoadhesion of nanoparticles. The strength of mucoadhesion depended on the structure of the hydrophilic polymeric chains on the nanoparticle surface. The mucoadhesion of poly (*N*-isopropylacrylamide) nanoparticles, which most strongly enhanced sCT absorption, was stronger than that of ionic nanoparticles, and poly (*N*-vinylacetamide) nanoparticles probably did not adhere to the GI mucosa. These findings demonstrated that there is a good correlation between mucoadhesion and enhancement of sCT absorption [33].

Lehr reviewed recent developments in the area of bioadhesive drug delivery systems. The area of bioadhesion in drug delivery had started some 20 years ago by using so-called mucoadhesive polymers. Many of these polymers were already used as excipients in pharmaceutical formulations. This has facilitated the development of the first bioadhesive drug products, which are now commercially available. A major disadvantage of the hitherto known mucoadhesives, however, is their non-specificity with respect to the substrate. In particular for gastro-intestinal applications, this may cause some premature inactivation and moreover limits the duration of mucoadhesive bonds to the relatively fast mucus turnover. In contrast to the mucoadhesive polymers, lectins and some other adhesion molecules specifically recognize receptor-like structures of the cell membrane and therefore indirectly to the epithelial cells themselves (cytoadhesion) rather than to the mucus gel layer. Furthermore, when bioadhesion is receptor-mediated, it is not only restricted to mere binding, but may subsequently trigger the active transport of large molecules or nanoscalic drug carrier systems by vesicular transport processes (endo-/transcytosis). Rather than only acting as a platform for controlled release systems, the concept of lectin-mediated bioadhesion therefore bears the potential for the controlled delivery of macromolecular biopharmaceuticals at relevant biological barriers, such as the epithelia of the intestinal or respiratory tract [34].

Pan et al. prepared Insulin-loaded CS-NPs by ionotropic gelation of CS with tripolyphosphate anions. The ability of CS-NPs to enhance intestinal absorption of insulin and increase the relative pharmacological bioavailability of insulin was investigated by monitoring the plasma glucose level of alloxan-induced diabetic rats after oral administration of various doses of insulin-loaded CS-NPs. Insulin association was found up to 80% and its in vitro release showed a great initial burst with a pH-sensitivity property. CS-NPs enhanced the intestinal absorption of insulin to a greater extent than the aqueous solution of CS in vivo. Above all, after administration of 21 I.U./kg insulin in the CS-NPs, the hypoglycemia was prolonged over 15 h and the average pharmacological bioavailability relative to SC injection of insulin solution was up to 14.9% [35].

Muller et al. prepared nanosuspension of buparvaquone for use in experimental clinics against the gastrointestinal persisting parasite *Cryptosporidium parvum* by high pressure homogenization. Main advantages of nanosuspensions (amongst others) are their increase of saturation solubility and dissolution velocity, improving the bioavailability of drugs. The buparvaquone nanosuspension had a bulk population of about 600 nm (analyzed by photon correlation spectroscopy (PCS)). The additional analysis performed with laser diffraction showed that only a very small content of microparticles occurred, which is, for the special features of nanosuspensions, negligible because they were still below 3 μm. Another feature of nanosuspensions is the adhesion properties to surfaces, e.g. mucosa. To further increase the adhesion time of the buparvaquone nanosuspension to *C. parvum*, the nanosuspension was formulated with hydrogels made from mucoadhesive polymers, e.g. different types of carbopol and chitosan. Only a small increase of the particle size of the bulk population occurred directly after the incorporation of buparvaquone nanosuspension into the hydrogels. The nanosuspension/hydrogel systems were physically long-term stable over a period of 6 months as indicated by the unchanged particle sizes [36].

Vasir et al. reviewed the spectrum of potential applications of bioadhesive microspheres in controlled drug delivery ranging from the small molecules, to peptides, and to the macromolecular drugs such as proteins, oligonucleotides and even DNA. They studied the development of mucus or cell-specific bioadhesive polymers and the concepts of cytoadhesion and bioinvasion provide unprecedented opportunities for targeting drugs to specific cells or intracellular compartments. They also discussed developments in the techniques for in vitro and in vivo evaluation of bioadhesive microspheres [37].

Arbosa et al. evaluated the potential of specific bioadhesive nanoparticles to increase the oral bioavailability of presystemic degraded drugs, using 5-fluorouridine (FURD) as model. For this purpose, poly (methylvinylether-co-maleic anhydride) nanoparticles (NP), NP coated with albumin (BSA-NP) and NP treated with albumin and 1, 3-diaminopropane (BD-NP) were used. All the formulations displayed a similar size and drug loading. However, BSA-NP showed a tropism for the stomach, NP developed adhesive interactions with both the stomach and middle portions of the small intestine and BDNP with the distal regions of the small intestine. These formulations were orally administered to laboratory animals and the FURD levels in plasma, tissues and urine were quantified at different times. From the urine data, the FURD bioavailability when loaded in either BSA-NP or NP was about 79% and 21%, respectively. For the control oral solution and BD-NP this parameter was 11% and 2%, respectively. In summary, the use of bioadhesive nanoparticles with tropism for the stomach mucosa may be considered as an adequate alternative to increase the bioavailability of some pre-systemic metabolized drugs [38].

Salman et al. evaluated the bioadhesive potential of a polymeric vector obtained by the association between Gantrez AN nanoparticles and flagella-enriched Salmonella enteritidis extract. Fluorescently labeled nanoparticles (SE-NP) were prepared, after incubation between the polymer and the extract, by a solvent displacement method and cross-linkage with 1, 3-diaminopropane. SE-NP displayed a size close to 280 nm and the amount of associated bacterial extract was 18 Ag/mg nanoparticles. Flagellin represents more than 80% of the total proteins associated with SE-NP, which was identified by SDSPAGE and confirmed by Western blotting. Concerning the bioadhesive properties, SE-NP shows an important tropism for the ileum. In fact, about 50% of the given dose of SE-NP was found in this gut region for at least 3 h. Interestingly, the bioadhesive ability of SE-NP correlated well with the described colonization profile for Salmonella enteritidis. This fact was corroborated by competitive tissue distribution studies. Thus, when SE-NP and Salmonella cells were administered together by the oral route, both the bacteria and the nanoparticles displayed a similar distribution within the intestinal mucosa. However, the ability of SENP to be taken up by Peyer's patches appeared to be negatively affected by the presence of the bacteria. Similarly, when SE-NP was administered 30 min before cells, SE-NP were found broadly distributed in Peyer's patches, whereas the bacteria were neither able to adhere to nor penetrate this lymphoid tissue. In summary, SE-NP demonstrated their Salmonella-like gut colonization, which can be a useful vector for oral targeting strategies [39].

Yao et al developed a novel gastro-mucoadhesive delivery system for Riboflavin-5'- phosphate sodium salt (RF5P), which is site-specifically absorbed from the upper gastrointestinal tract, based on ion-exchange fiber. Gastrointestinal transit studies of the RF5P fiber complexes in rats and gamma imaging study in volunteer was carried out to evaluate the gastro-retentive behavior of the fiber. The pharmacokinetic profile and parameters of riboflavin via analysis of urinary excretion of riboflavin on man were measured. Study on rat and man provide evidence for the validity of the hypothesis that the drug fiber provided good mucoadhesive properties *in vivo* and should therefore be of considerable interest for the development of future mucoadhesive oral drug delivery dosage forms [40].

Madhav et al. overviewed a wide range of orotransmucosal routes being potentially useful for transmucosal drug delivery. Oral mucosal drug delivery is an alternative method of systemic drug delivery that offers several advantages over both injectable and enteral methods and also enhances drug bioavailability because the mucosal surfaces are usually rich in blood supply, providing the means for rapid drug transport to the systemic circulation and avoiding, in most cases, degradation by first-pass hepatic metabolism. The systems contact with the absorption surface resulting in a better absorption, and also prolong residence time at the site of application to permit once or twice daily dosing. For some drugs, this results in rapid onset of action via a more comfortable and convenient delivery route than the intravenous route. Transmucosal drug delivery promises four times the absorption rate of skin. Drugs considered for oral transmucosal delivery are limited to existing products, and until there is a change in the selection and development process for new drugs, candidates for oral transmucosal delivery will be limited [41].

Moghaddam et al. evaluated the in vitro mucoadhesion and permeation enhancement properties of thiolated chitosan (chitosan-glutathione) coated poly (hydroxyl ethyl methacrylate) nanoparticles. Core-shell nanoparticles were prepared by radical emulsion polymerization method initiated by cerium (IV) ammonium nitrate. Different molecular weights of chitosan were utilized for nanoparticles preparation. Incorporation of fluorescein isothiocyanate dextran (FD4, MW 4400 Da), which was used as the model macromolecule, was achieved by incubation method. The intestinal mucoadhesion and penetration enhancement properties of nanoparticles were investigated using excised rat jejunum. All nanoparticle systems showed mucoadhesion and improved apparent permeation coefficient (Papp) of FD4. Nanoparticles prepared by thiolated chitosan with medium molecular weight revealed the most mucoadhesion and penetration enhancement properties [42].

Tao et al. prepared acyclovir-loaded mucoadhesive microspheres (ACV-ad-ms) using ethyl cellulose as matrix and Carbopol 974P NF as mucoadhesive polymer for the purpose of improving the oral bioavailability of acyclovir. In vitro and in vivo mucoadhesion of the microspheres was evaluated. Eggshell membrane was found to have a potential use for in vitro mucoadhesion measurement in place of stomach mucosa. In vitro drug release profiles and oral bioavailability of acyclovir in rats were also investigated. The release of the drug was influenced markedly by the medium pH and the proportion of Carbopol incorporated in the microspheres. The result of mucoadhesion study showed prolonged residence time of ACV-ad-ms in rats' gastrointestinal tract. In pharmacokinetics study, relatively steady plasma drug concentrations were observed within 8 h after oral administration of ACV-ad-ms to rats. The $AUC0\!-\!t$ and mean residence time (MRT) of ACV-ad-ms (6055.9 ng h/mL and 7.2 h) were significantly higher than that of ACV suspension (2335.6 ng h/mL and 3.7 h) ($P < 0.05$), which indicated that the bioavailability of acyclovir was greatly improved due to the prolonged retention of ACV-ad-ms in gastrointestinal tract [43].

Dudhani et al formulated bioadhesive chitosan nanoparticles (CS NPs) for encapsulation of catechin and evaluation of their mucoadhesive potential that leads to enhanced oral bioavailability of catechin. CS NPs and catechin loaded CS NPs were obtained by ionic gelation between the CS and sodium tripolyphosphate (TPP). Particle size distribution analysis confirmed the size ranges, 110 ±5 nm and 130 ±5 nm for CS NPs and catechin loaded CS NPs, respectively. TEM indicated smooth and spherical nanoparticles. FTIR and DSC showed no significant interactions between catechin and CS after encapsulation and cross-linking. Entrapment efficiency of 90% was achieved with a weight ratio of 2:1 (CS: TPP) at pH 5.5. In vitro release of catechin from CS NPs was 32% within 24 h and exhibited 40% and 32% mucoadhesivity for catechin loaded CS NPs and CS NPs, respectively, demonstrating potential for controlled release of catechin in GIT [44].

Plapied et al. developed a new nano carrier made of fungal Chitosan promising for oral gene delivery and oral DNA vaccination due to its mucoadhesive properties. Chitosan (CS) produced under GMP conditions from fungal source was used to encapsulate a plasmid DNA coding for a reporter gene. Nanoparticles made by complex coacervation of CS and DNA had a size around 200 nm, a positive zeta potential, a high association of DNA and protected the plasmid against nuclease degradation. Confocal microscopy studies showed that CS/DNA and PEI/DNA nanoparticles were found at the apical surface of cell monolayers and DNA was co-localized within the nucleus. Quantification seemed to show that more DNA was associated with the cells when incubated with CS nanoparticles and that the presence of M cells slightly influenced DNA uptake when complexed with CS [45].

Meng et al. engineered a tenofovir loaded chitosan based nanoparticles (NPs) by Box-Behnken design to assess the influence of formulation variables on the size of NPs and drug encapsulation efficiency. The effect of the NPs on vaginal epithelial cells and Lactobacillus crispatus viability and their mucoadhesion to porcine vaginal tissue were assessed by cytotoxicity assays and fluorimetry, respectively. In the optimal aqueous conditions, the EE% and NPs size were 5.83% and 207.97 nm, respectively. With 50% (v/v) ethanol/water as alternative solvent, these two responses increased to 20% and 602 nm, respectively. Unlike small size (182 nm) exhibiting burst release, drug release from medium (281 nm) and large (602 nm)-sized NPs fitted the Higuchi (r2 = 0.991) and first order release (r2 = 0.999) models, respectively. These NPs were not cytotoxic to both the vaginal epithelial cell line and L. crispatus for 48 h. When the diameter of the NPs decreased from 900 to 188 nm, the mucoadhesion increased from 6% to 12%. However, the combinatorial effect of EE% and percent mucoadhesion for larger size NPs was the highest. Overall, large-size, microbicide loaded chitosan NPs appeared to be promising nanomedicines for the prevention of HIV transmission [46].

Yadav et al. prepared mucoadhesive microspheres by the emulsion solvent evaporation technique consisting of (I) chitosan mucoadhesive (ii) repaglinide, an oral hypoglycemic agent; and (iii) Eudragit RS-100 as polymer to increase its residence time in the stomach. The microspheres were evaluated for surface morphology, particle shape, microencapsulation efficiency, in vitro wash-off mucoadhesion test, in vitro drug release and in vivo study. The microspheres were found to be spherical and free flowing. The microencapsulation efficiency was in the range of 61.44±1.16 to 79.90±1.17 and microspheres exhibited good mucoadhesive property in the in vitro wash off test. The drug release was also found to be slow and extended for 24 h. The hypoglycemic effect obtained by mucoadhesive microspheres was for more than 16 h whereas repaglinide produced an antidiabetic effect for only 10 h suggesting that mucoadhesive microspheres are a valuable system for the long term delivery of repaglinide [47].

Gaba et al. prepared mucoadhesive microspheres of glipizide as the site of absorption of glipizide is from stomach, to improve drug efficiency and decrease dose requirements. Microsphere carrier systems made by using polymer galactomannan having strong mucoadhesive properties and easily biodegradable could be an attractive strategy to formulate. Prepared formulation was evaluated for its in vitro characteristics and in vivo performance for sustained glucose lowering effect and improvement in diabetic condition as compared to immediate release of glipizide [48].

8. Characterization of Stomach Specific Mucoadhesive Nanoparticles

8.1 Particle Size

It has been shown that particle size and size distribution are the most important characteristics of nanoparticles systems. Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system. Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and available to a wider range of biological targets due to their small size and relative mobility. For example, body distribution studies have shown that nanoparticles larger than 230 nm accumulate in the spleen due to the capillary size in this organ. Different in vitro studies indicate that the particle size also influences the cellular uptake of nanoparticles. In some cell lines, only submicron nanoparticles can be taken up efficiently but not the larger size microparticles [36].

Drug release is affected by particle size. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. While, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out. Smaller particles also have greater risk of aggregation of particles during storage and transportation of nanoparticle dispersion. It is always a challenge to formulate nanoparticles with the smallest size possible but maximum stability. Currently, the fastest and most routine method of determining particle size is by photon-correlation spectroscopy (PCS) or dynamic light scattering (DLS). PCS is industrially preferred method of sub-micron particle size analysis. The sample analyzed in the PCS device should consist of well dispersed particles in liquid medium. In such conditions the particles are in constant random motion, referred to as Brownian motion and PCS measures the speed of this motion by passing a laser. PCS determines the average particle size and polydispersity index (PI) which is a range of measurement of the particle sizes within measured samples. The accurate measurement of particle size must be below 0.7 (70%). Dynamic light scattering (DLS) theory is a well established technique for measuring particle size over the size range from a few nanometers to a few microns. The concept uses the idea that small particles in a suspension move in a random pattern. Observation of larger particles compared to smaller particles will show that the larger particles move more slowly than the smaller ones if the temperature is the same [36,49].

8.2 Particle Morphology

Manipulation of the physicochemical properties of materials at the nanoscale has the potential to revolutionize electronic, diagnostic, and therapeutic applications. Because of the potential large-scale use of nanomaterials, it is important to determine if there is any unique toxicity of the nanoscale materials as compared to the bulk. It is essential for the purposes of interpreting results from cell culture and animal models that the nanomaterials are thoroughly characterized and that correlations are made between observed toxicological responses and the physicochemical characteristics of the materials. The morphology of nanoparticles was examined by two techniques.

The atomic force microscope (AFM) or scanning force microscope (SFM) is a very high-resolution type of scanning probe microscope, with demonstrated resolution of fractions of a nanometer, more than 1000 times better than the optical diffraction limit. The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. SEM has the required nanometer resolution for sizing in the submicron range and is invaluable to determine the particle morphology. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity [2].

8.3 Surface Charge

Many techniques have been developed and used to study the surface modification of nanoparticles (NPs). The efficiency of surface modification can be measured either by estimating the surface charge, density of the functional groups or an increase in surface hydrophilicity. One method used to measure the surface modification is to determine zeta potential of the aqueous suspension containing NPs. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. The main reason to measure zeta potential is to predict colloidal stability. The interactions between particles play an important role in colloidal stability. The use of zeta potential measurements to predict stability is an attempt to quantify these interactions. The zeta potential is a measure of the repulsive forces between particles. And since most aqueous colloidal systems are stabilized by electrostatic repulsion, the larger the repulsive forces between particles, the less likely they will be to come close together and form an aggregate. Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles [36,37].

8.4 Loading and Release

8.4.1 Drug loading

Drug may be bound to nanoparticles either (i) by polymerization in the presence of the drug- in most cases in the form of a solution (incorporation method) or (ii) by adsorbing the drug after the formation of nanoparticles by incubating them in the drug solution. Depending on the affinity of the drug to the polymer, the drug will be surface adsorbed, dispersed in the particle polymer matrix in the form of a solid solution, or solid dispersion, or in some case, the drug may be covalently bound to the polymer. Therefore it is apparent that a large amount of drug can be entrapped by the incorporation method when compared to the adsorption. The macromolecule or protein shows greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption. The drug loading of the nanoparticles is generally defined as the amount of drug bounded per mass of polymer (usually moles of drug per mg polymer or mg drug per mg polymer) it could also be given on a percentage basis based on the polymer [50].

8.4.2 Determination of drug entrapment

Binding of drug to the protein nanoparticles was measured by centrifuging part of the particle suspension. For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (w) by UV-spectrophotometry, fluorescence spectrophotometer or by a validated HPLC method. A standard calibration curve of concentration versus absorbance was plotted for this purpose. The amount of drug in supernatant (w) was then subtracted from the total amount of drug added during the formulation (W). Effectively, (W-w) will give the amount of drug entrapped in the pellet.

Then percentage entrapment of a drug is obtained by using following equation

$$\% \text{ Drug Entrapment} = (W-w) \times 100 / W$$

Finally, the encapsulation efficiency refer to the ratio of the amount of drug encapsulated/absorbed to the total (theoretical) amount of drug used, with regard to the final drug delivery system of the dispersion of nanoparticles [51].

8.5 Drug release

Release profiles of the drugs from nanoparticles depend upon the nature of the delivery system. In the case of nanospheres, drug is uniformly distributed/ dissolved in the matrix and the release occurs by diffusion or erosion of the matrix. If the diffusion of the drug is faster than matrix degradation, then the mechanism of drug release occurs mainly by diffusion, otherwise it depends upon degradation. Many theoretically possible mechanisms may be considered for the release drug from protein nanoparticles: (a) Liberation due to polymer erosion or degradation, (b) self diffusion through pores, (c) release from the surface of the polymer, (d) pulsed delivery initiated by the application of an oscillating magnetic or sonic field. In many case, some of these processes may coexist, so that the distinction between the mechanisms is not always trivial. When drug release occurs by a self diffusional process, a minimum drug loading is necessary before drug release is observed. This is easy to understand since the process involves diffusion through aqueous channels created by the phase separation and dissolution of the drug itself. This mechanism rarely occurs with drug loaded nanoparticles since, as explained before, the encapsulation efficiency of most drugs is generally too low. In fact, release from the surface and erosion or bulk polymer degradation is usually the most important processes affecting the liberation of drug from nanoparticles. Method for quantifying drug release in vitro are: (i) side by- side diffusion cells with artificial or biological membranes; (ii) equilibrium dialysis technique; (iii) reverse dialysis sac technique; (iv) ultracentrifugation; (v) ultra filtration; or (vi) centrifugal ultra filtration technique [52,53].

8.6 Test methods used to study bioadhesion

In vivo techniques represent the ultimate test for bioadhesives which appear promising from initial screening techniques in vitro. However, it is questionable whether current in vitro techniques are able to identify potential bioadhesives which would be of value clinically. Attempting to extrapolate results obtained in vitro to what may happen in vivo should be treated with extreme caution, since in vitro tests are performed in a controlled environment and may bear no relationship to the ultimate performance of the bioadhesive. Biological variables such as GI motility, mucus turnover, presence of endogenous materials (e.g. enzymes, electrolytes, bile) and exogenous materials (e.g. food, drink, drugs) are difficult, if not impossible, to mimic in an in-vitro model. In addition, the presence of both drug and, more importantly, excipients, are likely to influence greatly the overall durability of the BDDS, which may not be accounted for in in-vitro testing.

8.6.1 In vivo test methods

The three main techniques which have been used to monitor bioadhesion in vivo include gamma Scintigraphy, perfused intestinal loops, and transit studies with radiolabelled dosage forms.

8.6.1.1 Gamma Scintigraphy

The formulation to be investigated is labeled with a radionuclide, technetium-99m being the most commonly employed, and the dosage form is ingested by human volunteers. Accurate positioning of the volunteer in front of the gamma camera enables images or scintigraphs to be produced at selected time intervals and the course of the dosage form throughout the GIT (except in the small intestine) can be easily followed. The results from this technique are invaluable since they give a clear picture of the durability of the BDDS (i.e. how long it remains bioadhesive) as a whole [54].

8.6.1.2 Perfused intestinal loop

By isolating a section of intestine and anastomosis of the remaining intestine, one has the opportunity of investigating bioadhesion over a known area in a relatively controlled manner [4].

8.6.1.3 Radiolabelled transit studies

The radiolabelled BDDS under test was placed into a surgically incised stomach of the rat which was then resealed and the animal allowed regaining consciousness. After set time intervals, the animals were sacrificed and the stomach and intestines removed. These were further cut into segments and the radioactivity remaining in each segment measured by scintillation counting [1].

8.6.2 In vitro test methods

In vitro test methods were initially designed to screen potential bioadhesive with a view to in vivo testing if successful.

8.6.2.1 Adhesion strength tests

The method is based on the measurement of shear stress required to break the adhesive bond between a mucosal membrane and the formulation. The formulation is sandwiched between two mucosal membranes fixed on flexible supports in the assemblies for a sufficient period of time. After the adhesive bond has formed, the force (weight) required to separate the bond was recorded as mucoadhesive strength [55].

8.6.2.2 Perfusion techniques

Assessment of the duration of adhesion is a more realistic measurement of adhesive performance and this parameter can be evaluated by perfusion techniques (in addition to adhesion strength tests), of which three types exist. The first one of these is the flow channel method which examines, with the aid of a video camera, the movement of a bioadhesive particle placed on a bed of mucus whilst humid air is passed over the surface. The second perfusion technique has been termed the “falling liquid film” method and involves dripping a suspension of the material under test onto a section of excised tissue, cut lengthwise and mounted in tubing positioned on an inclined platform. The eluted particles are sampled in a Coulter Counter so that an estimation of numbers of particles adhering as a function of time can be determined. The third type of perfusion technique is similar to the falling liquid film method except that an entire segment of intestine is used rather than one that has been cut lengthwise. The radiolabelled bioadhesive formulation, which must be syringeable, is allowed to interact with the tissue for a period of time, after which perfusion is commenced and the eluted fractions collected and sampled for radioactivity [4].

8.6.2.3 Rheological tests

Rheological evaluation of mucin/polymer mixtures gives some information on the extent and magnitude of interaction between the two, since the increase in viscosity which results from mixing the two has been claimed to correlate with mucoadhesive function [2].

9. Applications of SSMN

- **Sustained Drug Delivery SSMN:** can remain in the stomach for long periods and hence can release the drug over a prolonged period of time. The problem of short gastric residence time encountered with an oral controlled release formulation, hence, can be overcome with these systems.
- **Site Specific Drug Delivery:** These systems are particularly advantageous for drugs that are specifically absorbed from stomach or proximal part of the small intestine e.g., riboflavin, furosemide and misoprostal.
- **Absorption Enhancement:** Drugs that have poor bioavailability because of site specific absorption from the upper part of the GIT are potential candidate to be formulated as floating drug delivery systems, thereby maximizing their absorption.
- **Maintenance of Constant Blood Level:** These systems provide an easy way of maintaining constant blood level by once a day administration and constant release of drug.
- **Patient Compliance:** Once a day administration of dosage form provide better patient compliance.
- **Improved Therapeutic Efficacy:** Once a day administration and continuous release of drug at specified place for prolonged period, improve therapeutic efficiency of drug.

Table.3 *List of nanotechnology based oral formulations in pharmaceutical market and in clinical trials[16].*

Product	Drug	Nanotechnology	Dosage form	Indication	Company/alliance	Commercial/therapeutic benefits
Rapamune	Sirolimus	Nanosuspensions	Tablet	Immuno-suppressant	Wyeth Pharmaceuticals. Elan Drug Delivery	Enabled development of tablet dosage form over previous oral solution. Enhanced patient compliance Greater bioavailability as compared to solution
Megace ES	Megestrol acetate	Nanosuspensions	Nano-sus-pen-sion	Treatment of anorexia, cachexia, or an unexplained significant weight loss in AIDS patients	Par Pharmaceuticals- Elan Drug Delivery	1/4th Reduction in dose volume as compared to previous oral suspension (from 20 mL to 5 mL). Elimination of variability because of food effect
Emend	Aprepitant	Nanosuspensions	Capsule	Antiemetic	Merck-Elan Drug Delivery	Higher oral bioavailability
Tricor	Fenofibrate	Nanosuspensions	Tablet	Antihyperlipidemic agent	Abbott Labs	Dose reduction Elimination of variability because of food effect
Panzem NCD	2-Methoxy estradiol	Nanosuspensions	Nano-suspension	Estrogen metabolite with anti-proliferative and anti-angiogenic effect	EntreMed Inc.	Being evaluated in Phase II clinical trial Douse reduction and higher oral bioavailability

Sandummine Neoral	Cyclosporine	Spontaneously emulsifying systems	Soft gelatine Capsule (SGC)	Immunosuppressant	Novartis	Increased bioavailability of cyclosporine as compared to earlier oily formulation Sandimmun1 and reduction in inter and intra-individual pharmacokinetic variability
Gengraf	Cyclosporine	Spontaneously emulsifying systems (SES)	Hard gelatine capsule	Immunosuppressant	Abbott Labs	Less expensive than Neoral
Norvir	Ritonavir	SES	SGC	Anti-retroviral (anti-HIV)	Abbott Labs	

10. Conclusion

Among the currently available drugs in clinical use having narrow absorption window may be benefited by compounding into a SSMN. It can be concluded that the therapeutic potential of colloidal drug carriers after oral administration is probably not to deliver the drug+ directly in the blood flow, but to increase bioavailability by protecting the drug from denaturation in the gastro-intestinal lumen or by increasing the drug concentration for a prolonged period of time directly at the surface of the mucous membrane. Improvements in all aspects of this delivery system are required, so that efficient systems will emerge.

11. References

1. Wade, A. (Ed.) (1980) *Drug absorption. In: Pharmaceutical Handbook*, The Pharmaceutical Press, London, 19, pp.294-334.
2. Veillard, M. (1990) Buccal and gastrointestinal drug delivery systems, In: Gurny R. and Junginger HE. (Eds.), *Bioadhesion-Possibilities and Future Trends*, Wissenschaftliche Verlagsgesellschaft, Stuttgart, pp.124-139.
3. Robinson, J. R. (1990) Rationale of bioadhesion/ mucoadhesion, In: Gurny R. and Junginger HE. (Eds.), Bioadhesion Possibilities and Future Trends, Wissenschaftliche Verlagsgesellschaft, Stuttgart, pp.13-15.
4. Helliwell, M. (1987) The use of bioadhesives in targeted delivery within the gastrointestinal tract, *Advanced Drug Delivery Reviews*, 11, pp.221-251.
5. Harde, H., & Das, S. (2011) Solid lipid nanoparticles: an oral bioavailability enhancer vehicle, *Expert Opinion in Drug Delivery*, 8(11), pp.1407-1424.
6. Kumar, R., & Philip, A. (2007) Gastroretentive dosage forms for prolonging gastric residence time*, International Journal of Pharmaceutical Medicine*, 21(2), pp.157-171.
7. Kharia. A. A., & Hiremath, S. et al., (2011) Gastro retentive drug delivery system: an overview, *Indian Drugs*, 48(05), pp.7-15.
8. Reddy, C. P., & Chaitanya, K. S. C. et al., (2011) A review on bioadhesive buccal drug delivery systems: current status of formulation and evaluation methods, *Daru*, 19(6), pp.385-403.
9. Deshpande, A. A., & Rhodes C. T. et al., (1996) Development of a novel controlled release system for gastric retention, *Drug Development and Industrial Pharmacy*, 22(6), pp.105-113.
10. Singh, B. N., & Kim, K. H. (2006) Floating drug delivery system- an approach to oral controlled drug delivery via gastric retention, *Jourrnal of Controlled Release,* 63, pp.235-254.
11. Angela, Mac. A. (1993) The effect of gastro-intestinal mucus on drug absorption, *Advanced Drug Delivery Reviews*, 11, pp.201-220.
12. Dodou, D., & Breedveld, P. et al., (2005) Mucoadhesives in the gastrointestinal tract: revisiting the literature for novel applications, *European Journal of Pharmaceutics and Biopharmaceutics,* 60, pp.1-16.
13. Shojaei, A. H., & Berner, B. (2006) Gastric retentive dosage forms, In: Li X, Jasti BS.(Eds.), Design of controlled release drug delivery systems, McGraw-Hill Companies, USA, pp.173-201.
14. Roy, S. K., & Prabhakar, B. (2010) Bioadhesive Polymeric Platforms for Transmucosal Drug Delivery Systems - a Review.

Tropical Journal of Pharmaceutical Research, 9(1), pp.91-104.

15. Hunter, A. C., Elsom, J. et al., (2012) Polymeric particulate technologies for oral drug delivery and targeting: a pathophysiological perspective, *Nanotechnology, Biology and Medicine*, 8, S5-S20.
16. Desai, P. P., & Date, A. A, et al., (2012) Overcoming poor oral bioavailability using nanoparticle formulations - opportunities and limitations, *Drug Discovery Today*: *Technologies*, 9(2), E87-95.
17. Katayama, H., & Nishimura, T. et al., (1999) Sustained release liquid preparation using sodium alginate for eradication of Helicobacter Pylori, *Biological & Pharmaceutical Bulletin*, 22(1), pp.55-60.
18. Liu, Z., & Lu, W. et al., (2005) In vitro and in vivo studies on mucoadhesive microspheres of amoxicillin, *Jourrnal of Controlled Release,* 102(1), pp.135-144.
19. Jacob, J. S., & Mathiowitz, E. et al., (2006) Controlled regional oral delivery, US patent 20060045865 A1.
20. Mathiowitz, E., & Jacob, J. S. et al., (1997) Biologically erodible microspheres as potential oral drug delivery system, *Nature*, 386, pp.410-414.
21. Umamaheshwari, R. B., & Ramteke, S. et al., (2004) Anti-*Helicobacter Pylori* effect of mucoadhesive nanoparticles bearing amoxicillin in experimental gerbil's model, *AAPS PharmSciTech*, 5(2), pp.1-9.
22. Shishu, & Gupta, N., et al., (2007) Stomach-specific drug delivery of 5-fluorouracil using floating alginate beads, *AAPS PharmSciTech*, 8(2), E1-E7.
23. Mitragotri, S., & Shen, Z. (2003) Methods for oral drug delivery. US patent 20030017195.
24. Makhlof, A., & Werle, M. et al., (2011) A mucoadhesive nanoparticulate system for the simultaneous delivery of macromolecules and permeation enhancers to the intestinal mucosa, *Journal of Controlled Releas,* 149, pp.81-88.
25. Suwannateep, N., & Banlunara, W. et al., (2011) Mucoadhesive curcumin nanospheres: Biological activity, adhesion to stomach mucosa and release of curcumin into the circulation, *Journal of Controlled Release,* 151, pp.176-182.
26. Irache, J. M., & Huici, M. et al., (2005) Bioadhesive properties of Gantrez nanoparticles, *Molecules*, 10, pp.126-145.
27. Arora, S., & Gupta, S. et al., (2011) Amoxicillin Loaded Chitosan - Alginate Polyelectrolyte Complex Nanoparticles as Mucopenetrating Delivery System for H. Pylori*. Scientia Pharmaceutica*, 79, pp.673-694.
28. Park, K., & Robinson, J. R (1984) Bioadhesive polymers as platforms for oral-controlled drug delivery: method to study bioadhesion*, International Journal of Pharmaceutics*, 19, pp.107-127.
29. Bhat, P. G., & Flanagan, D. R. et al., (1996) Drug binding to gastric mucus glycoproteins, *International Journal of Pharmaceutics*, 134, pp.15-25.
30. Tur, K. M., & Cheng, H. S. et al., (1997) Use of bioadhesive polymer to improve the bioavailability of griseofulvin, *International Journal of Pharmaceutics*, 148, pp.63-71.
31. Ponchel, G., & Irache JM (1998) Specific and non-specific bioadhesive particulate systems for oral delivery to the gastrointestinal tract, *Advanced Drug Delivery Reviews*, 34, pp.191-219.
32. Hillery, A. M. (1998) Microparticulate delivery systems: potential drug/vaccine carriers via mucosal routes, *Pharmaceutical Science & Technology Today*, 1(2), pp.69-75.
33. Sakuma, S., & Sudo, R. et al., (1999) Mucoadhesion of polystyrene nanoparticles having surface hydrophilic polymeric chains in the gastrointestinal tract, *International Journal of Pharmaceutics*, 177, pp.161-172.
34. Lehr, C. M. (2000) Lectin-mediated drug delivery: The second generation of bioadhesives, *Journal of Controlled Release,* 65, 19-29.
35. Pan, Y., & Li, Y. J. et al., (2002) Bioadhesive polysaccharide in protein delivery system: chitosan nanoparticles improve the intestinal absorption of insulin in vivo, *International Journal of Pharmaceutics*, 249, pp.139-147.
36. Muller, R. H., & Jacobs, C. (2002) Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long-term stability, *International Journal of Pharmaceutics*, 237, pp.151-161.
37. Vasir, J. K., & Tambwekar, K. et al., (2003) Bioadhesive microspheres as a controlled drug delivery system, *International Journal of Pharmaceutics,* 255, pp.13-32.
38. Arbosa, P., & Campanero, M. A. et al., (2004). Nanoparticles with specific bioadhesive properties to circumvent the pre-system-

ic degradation of fluorinated pyrimidines, *Journal of Controlled Release,* 96, pp.55-65.

39. Salman, H. H., & Gamazo, C. et al., (2005) Salmonella like bioadhesive nanoparticles, *Journal of Controlled Release,* 106, pp.1-13.
40. Yao, H., & Xu, L. et al., (2008) A novel riboflavin gastro-mucoadhesive delivery system based on ion-exchange fiber, *International Journal of Pharmaceutics,* 364, pp.21-26.
41. Madhav, N. V. S., & Shakya, A. K. et al., (2009) Orotransmucosal drug delivery systems: A review, *Journal of Controlled Release,* 140, pp.2-11.
42. Moghaddam, F. A., & Atyabi, F. et al., (2009) Preparation and in vitro evaluation of mucoadhesion and permeation enhancement of thiolated chitosan-pHEMA core-shell nanoparticles, *Nanotechnology, Biology and Medicine*, Vol. 5, pp.208-215.
43. Tao, Y., & Lu, Y. et al., (2009) Development of mucoadhesive microspheres of acyclovir with enhanced bioavailability, *International Journal of Pharmaceutics,* 378, pp.30-36.
44. Dudhani, A. R., & Kosaraju, S. L. (2010) Bioadhesive chitosan nanoparticles: Preparation and characterization, *Carbohydrate Polymers,* 81, pp.243-251.
45. Plapied, L., & Vandermeulen, G. et al., (2010) Bioadhesive nanoparticles of fungal chitosan for oral DNA delivery, *International Journal of Pharmaceutics*, Vol. 398, pp.210-218.
46. Meng, J., & Sturgis, T. F. et al., (2011) Engineering tenofovir loaded chitosan nanoparticles to maximize microbicide mucoadhesion, *European Journal of Pharmaceutical Sciences,* Vol. 44, pp.57-67.
47. Yadav, V. K., & Kumar, B. et al., (2011) Design and evaluation of mucoadhesive microspheres of repaglinide for oral controlled release, *International Journal of Drug Delivery*, Vol. 3, pp.357-370.
48. Gaba, P., & Singh, S. et al., (2011) Galactomannan gum coated mucoadhesive microspheres of glipizide for treatment of type 2 diabetes mellitus: In vitro and in vivo evaluation, *Saudi Pharmaceutical Journal*, Vol. 19, 143-152.
49. Ahmed, H. E., & Kamel, O. A. et al., Ammonium methacrylate unit's polymer content and their effect on acyclovir colloidal nanoparticles properties and bioavailability in human volunteers, *Colloids and Surfaces B: Biointerfaces*, 75, 398-404.
50. Bellare, J., & Banerjee, R. et al., (2005) Aspirin loaded albumin nanoparticles by coacervation: implications in drug delivery *Trends* in Biomaterials & *Artificial Organs*, 18(2), 203-211.
51. Tripath,i A., & Gupta, R. et al., (2010) PLGA nanoparticles of anti tubercular drug: drug loading and release studies of a water in-soluble drug, *International Journal* of *PharmTech Research*, 2, 2116-2123.
52. Jahanshahi, M., & Babaei, Z. (2008) Protein nanoparticle: A unique system as drug delivery Vehicles, *African Journal* of *Biotechnology*, 7(25), 4926-4934.
53. Amany, O. K., & Gehanne, A. S. (2009) Preparation of intravenous stealthy acyclovir nanoparticles with increased mean residence time*, AAPS PharmSciTech*, 10(4), 1427-1436.
54. Khoshla, R., & Davis, S. S. (1987) The effect of polycarbophil on the gastric emptying of pellets, *Journal of Pharmacy and Pharmacology,* 39, 47-49.
55. Wirth, M. (1991) Instrumental color measurement: method for judging the appearance of tablet, *Journal of Pharmaceutical Sciences*, 80, 1177-1179.