Review Article

Stomach-Specific Mucoadhesive Microsphere as a Controlled Drug Delivery System

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ABSTRACT

Stomach-specific mucoadhesive microspheres as a controlled drug delivery system have been developed to increase gastric retention time of the dosage forms. It is known that differences in gastric physiology, such as gastric pH and motility, exhibit both intra- as well as inter-subject variability demonstrating significant impact on gastric retention time and drug delivery behavior. This article presents the polymers use for mucoadhesive microsphere, factor affecting the mucoadhesion, and preparation techniques of mucoadhesive microsphere. Developments in the techniques for *in vitro* and *in vivo* evaluation of mucoadhesive microspheres have also been discussed.

Introduction

Oral controlled release (CR) dosage forms (DFs) have been developed for the past three decades due to their considerable therapeutic advantages.[1] However, this approach has not been suitable for a variety of important drugs, characterized by a narrow absorption window in the upper part of the gastrointestinal tract, i.e. stomach and small intestine. This is due to the relatively short transit time of the DF in these anatomical segments. Thus, after only a short period of less than 6 h, the CR-DF has already left the upper gastrointestinal tract and the drug is released in nonabsorbing distal segments of the gastrointestinal tract. This results in a short absorption phase that is often accompanied by lesser bioavailability.

The medications that are included in the category of narrow absorption window drugs are mostly associated with improved absorption at the jejunum and ileum due to their enhanced absorption properties, e.g. large surface area, in comparison to the colon or because of the enhanced solubility of the drug in the stomach as opposed to more distal parts of the gastrointestinal tract.[2]

It was suggested that compounding narrow absorption window drugs in a unique pharmaceutical DF with gastro retentive properties would enable an extended absorption phase of these drugs. After oral administration, such a stomach-specific mucoadhesive microsphere would be retained in the stomach and release the drug there in a controlled and prolonged manner, so that the drug could be supplied continuously to its absorption sites in the upper gastrointestinal tract. This mode of administration would best achieve the known pharmacokinetic and pharmacodynamic advantages of stomach- specific mucoadhesive microsphere for these drugs.[3]

Under certain circumstances prolonging the gastric retention of a delivery system is desirable for achieving greater therapeutic benefit of the drug substance. For example, drugs that are absorbed in the proximal part of the gastrointestinal tract and drugs that are less soluble in or are degraded by the alkaline pH may benefit from prolonged gastric retention. In addition, for local and sustained drug delivery to the stomach and proximal small intestine to treat certain conditions, prolonged gastric retention of the therapeutic moiety may offer numerous advantages including improved bioavailability and therapeutic efficacy, and possible reduction of dose size. It has been suggested that prolonged local availability of antibacterial agents may augment their effectiveness in treating *H. pylori* related peptic ulcers.[4-6]

Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1-1000 μm in diameter and consist either entirely of a mucoadhesive polymer or having an outer coating of it, respectively. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery, but coupling of mucoadhesive properties to microspheres has additional advantages, e.g. efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of drugs to the absorption site achieved by anchoring plant lectins, bacterial adhesions, antibodies, etc. on the surface of the

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microspheres. Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in stomach, thus offering the possibilities of localized as well as systemic controlled release of drugs. The application of mucoadhesive microspheres to the mucosal tissues of gastric epithelium is used for administration of drugs for localized action. Mucoadhesive microspheres are widely used because they release the drug for prolong period, reduce frequency of drug administration and improve the patient compliance.[7]

Biological aspects of GRDFs

Physiological considerations

The intrinsic properties of the drug molecule and the target environment for delivery are the major determining factors in bioavailability of the drug. Factors such as pH, enzymes, nature and volume of secretions, residence time, and effective absorbing surface area of the site of delivery play an important role in drug liberation and absorption. In stomach there are several types of cells that secrete up to 2-3 l of gastric juice daily. For example, goblet cells secrete mucus, parietal cells secrete hydrochlororic acid, and chief cells secrete pepsinogen.[8,9] The contraction forces of the stomach churn the chyme and mix it thoroughly with the gastric juice. The average length of the stomach is about 0.2 m, and the apparent absorbing surface area is about 0.1 m^2 [Table 1].

A brief survey of relevant physiological features that pose challenge to the development of an effective gastro-retentive delivery system is presented below.

Gastric pH: The gastric pH is not constant rather it is influenced by various factors such as diet, disease, presence of gases, fatty acids, and other fermentation products.[10] In addition, the gastric pH exhibits intra- as well as inter-subject variation. This variation in pH may significantly influence the performance of orally administered drugs. Radio telemetry, a noninvasive device, has successfully been used to measure the gastrointestinal pH in human. It has been reported that the mean value of gastric pH in fasted healthy subjects is 1.1 $\pm$ 0.15.[11-13] In contrast, the mean gastric pH in fed state in healthy males has been reported to be 3.6 $\pm$ 0.4,[14] and the pH returns to basal level in about 2-4 h. However, in fasted state, basal gastric secretion in women is slightly lower than that of in men.[15,16]

Gastric pH may be influenced by age, pathological conditions, and drugs. About 20% of the elderly people exhibit either diminished (hypochlorohydria) or no gastric acid secretion (achlorohydria) leading to a basal pH value over 5.0.[17] Pathological conditions such as pernicious anemia and AIDS may significantly reduce gastric acid secretion leading to elevated gastric pH.[18,19] In addition, drugs like H2 receptor antagonists and proton pump inhibitors significantly reduce gastric acid secretion. The pH in the proximal duodenum may rise as high as 4 pH units from the stomach.[20] This increase in pH is caused by the bicarbonate secreted by the pancreas and the duodenal mucosa that neutralize the acidic chyme peristalsed from the stomach. The mean pH value in fasted duodenum has been reported to be 5.8 $\pm$ 0.3 in healthy subjects[21] while the fasted small intestine has been observed to have a mean pH of 6.0 $\pm$ 0.14.[13] Passing from jejunum through the mid small intestine and ileum, pH rises from about 6.6-7.5.[22]

Gastric pH is an important consideration in selecting a drug substance, excipients, and drug carrier(s) for designing intragastric delivery systems.

Gastric motility and emptying of food from the stomach

The motility of the stomach is mostly contractile, which causes food grinding into smaller particles, mixing with gastric juices, forward and backward movements of gastric contents and emptying, with all of the actions occurring together[23,24] There is a marked difference between motility in the fasting state and the fed state: The motoric activity in the fasting state, termed interdigestive myoelectric motor complex (IMMC), is a 2-h cycle of peristaltic activity that is generated in the stomach and progresses aborally to the ileocecal junction. Its aim is to clear the stomach and the small intestine of indigested debris, swallowed saliva, and sloughed epithelial cells.[12] It is composed of four phases: Phase 1 lasts 45-60 min, is quiescent, with rare low amplitude contractions; phase 2 with a length of 30-45 min, has intermediate amplitude contractions,[25] and involves bile secretion;[26] and phase 3 is also termed "housekeeper wave" and extends for 5-15 min. It is initiated in the stomach in most cases (71%) or in the duodenum.[12] Very high amplitude contractions, with a frequency of 4-5 per min[10] and maximal pyloric opening,[27] characterize this phase. This enables efficient evacuation of the stomach contents; phase 4 has a length of less than 5 min and connects between the maximal amplitude contractions to the basal phase.[25]

The motor activity in the fed state is induced 5-10 min after ingestion of a meal and persists as long as food remains in the stomach. The larger the amount of food ingested, the longer the period of fed activity, with usual time spans of 2-6 h, and more typically 3-4 h. Its phasic contractions are similar to those seen during phase 2 of the IMMC. The stomach churns food while suspended fine particles, typically in a size of less than 1 mm,[12] are emptied every 20 s to the duodenum.[11] This controlled rate enables proper digestion and absorption of the food in the small intestine.[28]

Generally, the residence time of the food in the stomach depends upon its nutritive and physical properties; emptying of liquid nutrients has a rate of 200 kcal/h, regardless of whether those calories are in the form of fats, proteins, or carbohydrates. Non-nutrient liquids empty rapidly, with a time to 50% emptying of 8-18 min. Solids empty much more slowly than liquids. Digestible nonfat solids are first ground for up to 1 h, and then emptied in zero order kinetics. Solid or semisolid fats, after being consumed and warmed to body temperature in the stomach, are converted into a liquid. Due to a nervous mechanism inhibiting gastric peristalsis and floating over gastric liquids, liquid fats empty much more slowly than aqueous liquids.[12]

In the cases where the stomach contractions during food digestion

Table 1: Salient features of upper gastrointestinal tract[11-14,17,18,22-33]

Section	Length (m)	Transit time (h)	pH	Microbial count	Absorbing surface area (m^2)	Absorption pathway
Stomach	0.2	Variable	1-4	< 103	0.1	P, C, A
Small Intestine	6-10	3 $\pm$ 1	5-7.5	103-1010	120-200	P, C, A, F, I, E, CM

P - Passive diffusion; C - Aqueous channel transport; A - Active transport; F - Facilitated transport; I - Ion-pair transport; E - Entero- or pinocytosis; CM - Carrier-mediated transport

and in the second phase of IMMC activity are unable to empty undigested matter, including no disintegrating DFs, through the relaxed pyloric sphincter, a retropulsion reflex is activated to deliver the material from the pylorus and distal antrum to the proximal antrum and stomach body.[29-31] Gastric emptying also depends upon posture[32] gender, age,[33] osmolarity and pH of food,[10] mental stress, and disease state.[20]

Emptying of DFs from the stomach

When no disintegrating DFs, like other indigestible solids, are administered in the fasting state, they typically are not retained in the stomach for over 2 h due to the IMMC. On the other hand, in the fed stomach the gastric retention time (GRT) of no disintegrating DFs depends mostly on the DF size as well as the composition and the caloric value of food;[34] indigestible spheres smaller than 1 mm in diameter freely pass into the intestine, often at rates faster than solid nutritive food. Spheres with diameters of 1-2.4 mm pass with the calorie-containing components of a solid meal.[12]

In general, the GRT of DFs and in particular large DFs is longer in the fed state in comparison to the fasting state. Large DFs are retropelled from the pyloric-antrum for further digestion and evacuation in the end of the fed state or are retained until the arrival of the subsequent "housekeeper wave." In such cases, the GRT is a function of the length of the digestive process. Thus theoretically, continuous feeding can prolong GRT of the DF for more than 24 h.[20]

Efforts were made to identify a cut-off size above which the DF will be retained in the stomach for prolonged periods of times. Large DFs, such as 13 mm diameter no disintegrating tablets, were retained in the stomach for 171 $\pm$ 29 min, almost an hour more than 7 mm tablets, after a light breakfast of 360 kcal.[35] It was suggested that 7 mm tablets empty during the fed state while 13 mm tablets are retained until arrival of the subsequent sweeping "housekeeper wave." This emphasizes the need for substantial size enlargement of the DF at the stomach in order to prolong GRT.

In addition to some prolongation in GRT due to the retropulsion reflex, gastroretentivity may simply be achieved by large dimensions that are physically unable to pass through the pyloric sphincter. The dimensions that are desirable in order to prevent rapid evacuation of DFs from the human stomach can be determined from reports on foreign bodies retained in the stomach where medical intervention was required to draw them out using gastro-copy. It has been suggested that the size is a length of more than 5 cm or a diameter larger than 3 cm.[36] As opposed to foreign bodies, DFs should be tailored to degrade, disintegrate, be minimized in size or 'collapse' in the stomach at a plausible time interval, i.e. before the subsequent dosing time.

The "housekeeper wave" does not always completely clear the stomach from non-disintegrating DFs.[28] For instance, a radio telemetric capsule for pH measurements ('Heidelberg capsule', 25 $\times$ 8 mm, length $\times$ diameter) was randomly retained in the stomach of one healthy subject from a group of eight for over 12 h. During that time three "housekeeper waves" were recorded.[37] Other studies supported that a radio telemetric capsule is unable to induce fed state motility.[38]

Drugs incorporated into GRDFs[39-41]

Acyclovir, Alendronate, Atenolol, Captopril, Cinnarizine, Ciprofloxacin, Cisapride, Furosemide, Ganciclovir, Glipizide, Ketoprofen, Levodopa, Melatonin, Metformin, Minocyclin, Misoprostol, Nicardipine, Riboflavin, Sotalol, Tetracycline, Verapamil.

Polymers used for mucoadhesive microspheres[42]

The properties of the mucoadhesive microspheres, e.g. their surface characteristics, force of bioadhesion, release pattern of the drug, and clearance, are influenced by the type of polymers used to prepare them. Suitable polymers that can be used to form mucoadhesive microspheres include soluble and insoluble, nonbiodegradable and biodegradable polymers. These can be hydrogels or thermoplastics, homopolymers, copolymers or blends, natural or synthetic polymers.

Characteristics of an ideal mucoadhesive polymer[43]

1. The polymer and its degradation products should be nontoxic and should be no absorbable from the GI tract.
2. It should be nonirritant to the mucus membrane.
3. It should preferably form a strong no covalent bond with the mucin-epithelial cell surfaces.
4. It should adhere quickly to most tissue and should possess some site specificity.
5. It should allow easy incorporation of the drug and should offer no hindrance to its release.
6. The polymers must not decompose on storage or during the shelf life of the dosage form.
7. The cost of polymer should not be high so that the prepared dosage form remains competitive.

Robinson and his group using the fluorescence technique concluded that:

1. Cationic and anionic polymers bind more effectively than neutral polymers.
2. Polyanions are better than polycations in terms of binding/potential toxicity, and further, that water-insoluble polymers give greater flexibility in dosage form design compared with rapidly or slowly dissolving water-soluble polymers.
3. Anionic polymers with sulfate groups bind more effectively than those with carboxylic groups.
4. Degree of binding is proportional to the charge density on the polymer.
5. Highly binding polymers include carboxy methyl cellulose, gelatine, hyaluronic acid, carbopol, and polycarbophyl.

Molecular characteristics

Investigations into polymers with various molecular characteristics have led to a number of conclusions regarding the molecular characteristics required for mucoadhesion. The properties exhibited by a good mucoadhesive may be summarized as follows:

1. Strong hydrogen-bonding groups [-OH, -COOH]
2. Strong anionic charges
3. Sufficient flexibility to penetrate the mucus network or tissue crevices
4. Surface tension characteristics suitable for wetting mucus/mucosal tissue surface
5. High molecular weight

Table 2: Some mucoadhesive polymers[44]

Natural	Synthetic	Biocompatible	Biodegradable
Na alginate	Polyvinyl alcohol, Polyamides, polycarbonates, Polyalkylene glycols, polyvinyl ethers,	Esters of haluronic acid,	Poly (lactides),
Pectin	Esters and halides, polymethacrylic acid, polymethyl methacrylic acid,	Polyvinyl acetate,	Poly(glycolides),
Tragacanth	methylcellulose, ethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose,	ethylene glycol	Poly(lactide-co-glycolides), Polycaprolactones,
Gelatin	Sod. carboxymethylcellulose		Polyalkyl cyanoacrylates, Polyorthoesters, Polyphosphoesters, Polyanhydrides,
Carrageenan			Polyphosphazenes,
			Chitosan,
			Poly ethylene oxide

The examples of some mucoadhesive polymers are given in Table 2

Factors affecting mucoadhesion[43]

Polymer-related factors

Molecular weight

The optimum molecular weight for maximum bioadhesion depends upon type of mucoadhesive polymer at issue. It is generally understood that the threshold required for successful bioadhesion is at least 100 000 molecular weight. For example, polyethylene glycol (PEG), with a molecular weight of 20 000, has little adhesive character, whereas PEG with 200 000 molecular weight has improved, and PEG with 400 000 has superior adhesive properties. The fact that mucoadhesiveness improves with increasing molecular weight for linear polymers implies two things: (1) interpenetration is more critical for a low-molecular-weight polymer to be a good mucoadhesive, and (2) entanglement is important for high-molecular-weight polymers. Adhesiveness of a nonlinear structure, by comparison, follows a quite different trend. The adhesive strength of dextran, with a high molecular weight of 19 500 000 is similar to that of PEG, with a molecular weight of 200 000. The reason for this similarity may be that the helical conformation of dextran may shield many of the adhesive groups, which are primarily responsible for adhesion, unlike the conformation of PEG.

Concentration of active polymer

There is an optimum concentration for a mucoadhesive polymer to produce maximum bioadhesion. In highly concentrated system, beyond the optimum level, however, the adhesive strength drops significantly because the coiled molecules become separated from the medium so that the chain available for interpenetration becomes limited.

Flexibility of polymer chains

Chain flexibility is critical for interpenetration and entanglement. As water soluble polymers become cross-linked, the mobility of an individual polymer chain decreases and thus the effective length of the chain that can penetrate into the mucus layer decreases, which reduces mucoadhesive strength.

Spatial conformation

Besides molecular weight or chain length, spatial conformation of a molecule is also important. Despite a high molecular weight of 19 500 000 for dextrans, they have adhesive strength similar to that of PEG, with a molecular weight of 200 000. The helical conformation of dextran may shield many adhesively active groups, primarily responsible for adhesion, unlike PEG polymers, which have a linear conformation.

Swelling

Swelling characteristics are related to the mucoadhesive itself and its environment. Swelling depends on the polymer concentration, the ionic strength, and the presence of water. During the dynamic process of bioadhesion, maximum bioadhesion *in vitro* occurs with optimum water content. Overhydration results in the formation of a wet slippery mucilage without adhesion.

Environment-related factors

pH of polymer-substrate interface

pH can influence the formal charge on the surface of the mucus as well as certain ionizable mucoadhesive polymers. Mucus will have a different charge density depending on pH due to the difference in dissociation of functional groups on the carbohydrate moiety and the amino acids of the polypeptide backbone. Some studies had shown that the pH of the medium is important for the degree of hydration of cross-linked polycyclic acid, showing consistently increased hydration from pH 4 through pH 7, and then a decrease as alkalinity or ionic strength increases, for example polycarbophil does not show a strong mucoadhesive property above pH 5 because uncharged, rather than ionized, carboxyl group reacts with mucin molecule, presumably through numerous hydrogen bonds. However, at higher pH, the chain is fully extended due to electrostatic repulsion of the carboxyl ate anions.

Applied strength

To place a solid mucoadhesive system, it is necessary to apply a defined strength. Whatever the polymer, poly (acrylic acid/divinyl benzene) or carbopol 934, the adhesion strength increases with the applied strength or with the duration of its application, up to an optimum. The pressure initially applied to the mucoadhesive

tissue contact site can affect the depth of interpenetration. If high pressure is applied for a sufficiently long period of time, polymers become mucoadhesive even though they do not have attractive interactions with mucin.

Initial contact time

Contact time between the mucoadhesive and mucus layer determines the extent.of swelling and interpenetration of the mucoadhesive polymer chains. More mucoadhesive strength increases as the initial contact time increases.

Physiological factors

Mucin turnover

The natural turnover of mucin molecules from the mucus layer is important for at least two reasons. Firstly, the mucin turnover is expected to limit the residence time of the mucoadhesives on the mucus layer. No matter how high the mucoadhesive strength, they are detached from the surface due to mucin turnover. The turnover rate may be different in the presence of mucoadhesives, but no information is available on this aspect. Secondly, mucin turnover results in substantial amounts of soluble mucin molecules. These molecules interact with mucoadhesives before they have chance to interact with the mucus layer. Surface fouling is unfavorable for mucoadhesion to the tissue surface. Mucin turnover may depend on the other factors such as the presence of food. The gastric mucosa accumulates secreted mucin on the luminal surface of the tissue during the early stages of fasting. The accumulated mucin is subsequently released by freshly secreted acid or simply by the passage of ingested food; the exact turnover rate of the mucus layer remains to be determined. Lehr *et al*. calculated a mucin turnover time of 47- 270 min. The ciliated cells in the nasal cavity are known to transport the mucus to the throat at the rate of 5 mm/min. The mucociliary clearance in the tracheal region has been found to be at the rate of 4-10 mm/min.

Disease state

The physiochemical properties of the mucus are known to change during disease conditions such as the common cold, gastric ulcers, ulcerative colitis, cystic fibrosis, bacterial, and fungal infections of female reproductive tract, and inflammatory conditions of the eye. The exact structural changes taking place in mucus under these conditions are not clearly understood. If mucoadhesives are to be used in the disease states, the mucoadhesive property needs to be evaluated under the same conditions.

Preparation of Mucoadhesive Microspheres

Mucoadhesive microspheres can be prepared using any of the following techniques [Table 3].

Table 3: Comparison of the various processes used for the preparation of mucoadhesive microspheres[7,43]

Process used	Particle size (μm)	Polymers
Solvent evaporation	1-100	Relatively stable polymers, e.g. polyesters, polystyrene
Hot melt microencapsulation	1-1000	Water labile polymers, e.g. polyanhydrides, polyesters; with a molecular weight of 1000-50000
Solvent removal	1-300	High melting point polymers especially polyanhydrides
Spray drying	1-10	—
Ionic gelation and size extrusion	1-300	Chitosan, CMC, alginate
Phase inversion	0.5-5.0	Polyanhydrides

Evaluation of the mucoadhesive microspheres

The best approach to evaluate mucoadhesive microspheres is to evaluate the effectiveness of the mucoadhesive polymer to prolong the residence time of drug at the site of absorption, there by increasing absorption and bioavailability of the drug. The methods used to evaluate mucoadhesive microspheres include the following.

Measurement of adhesive strength/in vitro tests[44]

The quantification of the mucoadhesive forces between polymeric microspheres and the mucosal tissue is a useful indicator for evaluating the mucoadhesive strength of microspheres. *In vitro* techniques have been used to test the polymeric microspheres against a variety of synthetic and biological tissue samples, such as synthetic and natural mucus, frozen and freshly excised tissue, etc. The different *in vitro* methods include the following.

Tensile stress measurement[45]

Wilhelmy plate technique

The Wilhelmy plate technique is traditionally used for the measurement of dynamic contact angles and involves the use of a microtensiometer or a microbalance. The CAHN dynamic contact angle analyzer (model DCA 322, CAHN instruments, Cerritos) has been modified to perform adhesive microforce measurements. The DCA 322 system consists of an IBM compatible computer and a microbalance assembly. The microbalance unit consists of stationary sample and tare loops and a motor powered translation stage. The instrument measures the mucoadhesive force between mucosal tissue and a single microsphere mounted on a small diameter metal wire suspended from the sample loop in microtensiometer. The tissue, usually rat jejunum, is mounted within the tissue chamber containing Dulbecco's phosphate buffered saline containing 100 mg/dl glucose and maintained at the physiologic temperature. The chamber rests on a mobile platform, which is raised until the tissue comes in contact with the suspended microsphere. The contact is held for 7 min, at which time the mobile stage is lowered and the resulting force of adhesion between the polymer and mucosal tissue is recorded as a plot of the load on microsphere versus mobile stage distance or deformation. The plot of output of the instrument is unique in that it displays both the compressive and the tensile portions of the experiment. By using the CAHN software system, three essential mucoadhesive parameters can be analyzed. These include the fracture strength, deformation to failure, and work of adhesion.

- *Fracture strength*: It is the maximum force per unit surface area required to break the adhesive bond.
- *Deformation to failure*: It is the distance required to move the stage before complete separation occurs. This parameter is dependent on the material stiffness and the intensity of strength of adhesion.
- *Work of adhesion*: It is a function of both the fracture strength and the deformation to failure. It tends to be the strongest indicator of the mucoadhesive potential.

This technique allows the measurement of mucoadhesive properties of a candidate material in the exact geometry of the proposed microsphere delivery device and the use of a physiological tissue chamber mimics the *in vivo* conditions. From a single tensile experiment, 11 mucoadhesive parameters can be analyzed out of which three are direct predictors of the mucoadhesive potential.

The CAHN instrument, although a powerful tool has inherent limitations in its measurement technique, makes it better suited for large microspheres (with a diameter of more than 300 μm) adhered to tissue *in vitro*. Therefore, many new techniques have been developed to provide quantitative information of mucoadhesive interactions of the smaller microspheres.

Novel electromagnetic force transducer

The electromagnetic force transducer (EMFT) is a remote sensing instrument that uses a calibrated electromagnet to detach a magnetic loaded polymer microsphere from a tissue sample. It has the unique ability to record remotely and simultaneously the tensile force information as well as high magnification video images of mucoadhesive interactions at near physiological conditions. The EMFT measures tissue adhesive forces by monitoring the magnetic force required to exactly oppose the mucoadhesive force. To test a microsphere, it must first be attached to the sample of tissue; magnetic force is then generated by an electromagnet mounted on the microscope vertically above the tissue chamber. After the computer has calculated the position of microsphere, the tissue chamber is slowly moved down, away from the magnet tip. As the tissue slowly descends away from the magnet, the video analysis continuously calculates the position of microsphere until the latter is completely pulled free of the tissue. The computer can display the results either as raw data or convert it to a force versus displacement graph. The primary advantage of the EMFT is that no physical attachment is required between the force transducer and the microsphere. This makes it possible to perform accurate mucoadhesive measurements on the small microspheres, which have been implanted *in vivo* and then excised (along with the host tissue) for measurement. This technique can also be used to evaluate the bioadhesion of polymers to specific cell types and hence can be used to develop BDDS to target-specific tissues.

Shear stress measurement[46]

The shear stress measures the force that causes a mucoadhesive to slide with respect to the mucus layer in a direction parallel to their plane of contact. Adhesion tests based on the shear stress measurement involve two glass slides coated with a polymer and a film of mucus. Mucus forms a thin film between the two polymer-coated slides, and the test measures the force required to separate the two surfaces.

Mikos and Peppas designed the *in vitro* method of the flow chamber. The flow chamber made of plexiglass is surrounded by a water jacket to maintain a constant temperature. A polymeric microsphere placed on the surface of a layer of natural mucus is placed in a chamber. A simulated physiologic flow of fluid is introduced in the chamber and movement of microsphere is monitored using video equipment attached to a goniometer, which also monitors the static and dynamic behavior of the microparticle.

Other tests to measure the adhesive strength[47]

Adhesion number

Adhesion number for mucoadhesive microspheres is determined as the ratio of the number of particles attached to the substrate to the total number of applied particles, expressed as a percentage. The adhesion strength increases with an increase in the adhesion number.

Particle size of microspheres

The particle size of the microspheres was determined by using the optical microscopy method. Microspheres were counted for particle size using a calibrated optical microscope.

Swelling index of microspheres

For estimating the swelling index, the microspheres were suspended in simulated gastric fluid (pH 1.2). The particle size was monitored by the microscopy technique using an optical microscope. The increase in particle size of the microspheres was noted for every time interval and the swelling index was calculated using the following formula

$$\%\,\text{Swelling} = \frac{\text{Final diameter} - \text{Initial diameter}}{\text{Initial diameter}} \times 10 \quad (1)$$

In vitro wash-off test for microspheres

The mucoadhesive properties of the microspheres were evaluated by the *in vitro* wash-off test as reported by Lehret *et al*. A 1 cm × 1 cm piece of rat stomach mucosa was tied onto a glass slide (3 inch × 1 inch) using a thread. Microspheres were spread onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid (pH 1.2). At the end of every time interval, the number of microspheres still adhering on to the tissue were counted and there adhesive strength was determine using the following formula

$$\%\,\text{Adhesive strength} = \frac{N_o - N_s}{N_s} \times 100 \quad (2)$$

where N_o = Initial number of the microsphere spread from the mucosal surface

N_s = Number of the microsphere detaching from the mucosal surface

Falling liquid film method

It is a simple, quantitative *in situ* method; wherein an excised intestinal segment cut lengthwise is spread on a plastic flute and positioned at an incline. The suspension of microspheres is allowed to flow down the intestinal strip. Particle concentrations entering

the segment from the dilute suspension reservoir and leaving the intestinal segment can be determined with the help of Coulter counter to quantify the steady state fraction of particles adhered to the intestinal mucosa [Figure 1]. The percent of particles retained on the tissue is calculated as an index of mucoadhesion using Eq. (2).

Everted sac technique[40]

The everted intestinal sac technique is a passive test for mucoadhesive and involves polymeric microspheres and a section of the everted intestinal tissue. It is performed using a segment of intestinal tissue excised from the rat, everted, ligated at the ends, and filled with saline. It is then introduced into a tube containing a known amount of the microspheres and saline, and agitated while incubating for 30 min. Sac is then removed, microspheres are washed and lyophilized, and the percentage of binding to the sac is calculated from difference in the weight of the residual spheres from the original weight of the microspheres.

The advantage of the technique is that no external force is applied to the microspheres being tested; microspheres are freely suspended in buffer solution and made to come in contact with the everted intestinal tissue randomly. The CAHN technique and the everted intestinal sac technique, both predict the strength of mucoadhesive in a very similar manner. Correlation between the two *in vitro* mucoadhesive assay methods, thereby allows one to confidentially utilize a single mucoadhesive assay to scan a variety of mucoadhesive polymers.

Novel rheological approach[48]

The rheological properties of the mucoadhesive interface (i.e. of the hydrated gel) are influenced by the occurrence of interpenetration step in the process of mucoadhesive. Chain interlocking, conformational changes, and the chemical interaction, which occur between mucoadhesive polymer and mucin chains, produce changes in the rheological behavior of the two macromolecular species. The rheological studies provide an acceptable *in vitro* model representative of the *in vivo* behavior of mucoadhesive polymers. Due to intermolecular interactions between the two polymers (mucin and the mucoadhesive polymer), experimentally measured viscosity of the mixture is generally higher than the viscosity calculated as a weighted average of the viscosities of the individual components. Thus, the magnitude of the intermolecular interactions can be quantitated by the relative change of the solution viscosity. A synergistic increase in the viscosity of the gastric mucus glycoprotein has been observed with polyacrylates, which thereby re-inforce the gastroduodenal mucus. It has been reported that an optimum polymer concentration is required for rheological synergy to be evident, above which any synergy is masked by the rheological properties of the polymer alone. The effect of pH on the mucus/polymer rheological synergism of polyacrylates has been examined using dynamic oscillatory rheology. It has been shown that an optimum mucus polymer interaction occurs not only at the pK_a value but also at the pH regimes unique to each polymer type, being influenced by the hydrogen-bonded interactions.

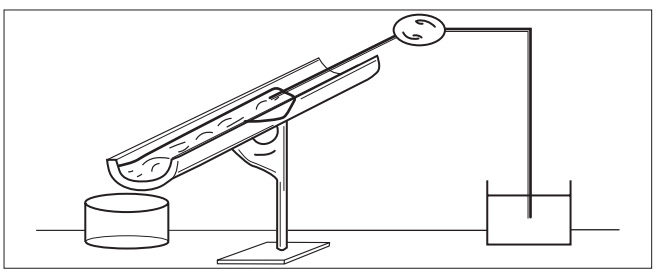

Figure 1: Falling liquid film technique to measure percentage adhesive strength

Measurement of the residence time/in vivo techniques

Measurements of the residence time of mucoadhesives at the application site provide quantitative information on their mucoadhesive properties. The GI transit times of many mucoadhesive preparations have been examined using radioisotopes and the fluorescent labeling techniques.

GI Transit using radio-opaque microspheres[49]

It is a simple procedure involving the use of radio-opaque markers, e.g. barium sulfate, encapsulated in mucoadhesive microspheres to determine the effects of mucoadhesive polymers on GI transit time. Feces collection (using an automated feces collection machine) and X-ray inspection provide a non-invasive method of monitoring total GI residence time without affecting normal GI motility. Mucoadhesives labeled with Cr-51, Tc-99m, In-113m, or I-123 have been used to study the transit of the microspheres in the GI tract.

Gamma scintigraphy technique[49]

Distribution and retention time of the mucoadhesive microspheres can be studied using the gamma scintigraphy technique. A study has reported the intensity and distribution of radioactivity in the genital tract after administration of technetium-labeled HYAFF microspheres. Dimensions of the stomach part of the sheep can be outlined and imaged using labeled gellan gum, and the data collected are subsequently used to compare the distribution of radiolabeled HYAFF formulations. The retention of mucoadhesive-radiolabeled microspheres based on HYAFF polymer was found to be more for the dry powder formulation than for the pessary formulation after 12 h of administration to stomach epithelium. The combination of the sheep model and the gamma scintigraphy method has been proved to be an extremely useful tool for evaluating the distribution, spreading, and clearance of administered stomach mucoadhesive microspheres.

Surface characterization of the mucoadhesive icrospheres[49]

The surface morphology of microspheres and the morphological changes produced through polymer degradation can be investigated and documented using scanning electron microscopy (SEM), electron microscopy, and scanning tunneling microscopy (STM). To assess the effect of surface morphology on the mucoadhesive properties, the microsphere samples are lyophilized and analyzed under SEM at 150 × and 1000 ×. The smooth texture of the microsphere surface leads to weak mucoadhesive properties, while the coarser surface texture improves the adhesion through stronger mechanical interactions. The morphological surface changes occurring due to the hydrolytic degradation of the polymers, e.g. polyanhydrides can be studied after incubating the microspheres in the PBS buffer for different intervals of time.

Conclusion

Mucoadhesive microspheres offer a unique carrier system for many pharmaceuticals and can be tailored to adhere to any mucosal tissue, including those found in eyes, oral cavity, and throughout the respiratory, urinary, and gastrointestinal tracts. The mucoadhesive microspheres can be used not only for controlled release but also for targeted delivery of the drugs to specific sites in body. Recent advances in medicine have envisaged the development of polymeric drug delivery systems for protein/peptide drugs and gene therapy. These challenges put forward by the medicinal advances can be successfully met by using increasingly accepted polymers, e.g. HYAFF, polyacrylates, chitosan, and its derivatives, polyphosphazenes, etc. Many studies have already been undertaken for exploring the prospects of mucoadhesive microspheres in gene therapy, delivery of peptides (insulin, calcitonin, and desmopressin), localized and targeted release of antitumor agents and mucosal vaccination (influenza vaccine).

Although significant advancements have been made in the field of mucoadhesives, there are still many challenges ahead in this field. Of particular importance is the development of universally acceptable standard evaluation methods and development of new site directed polymers. Efforts have been initiated on these lines in the form of novel EMFT techniques for evaluation of mucoadhesive strength of microspheres to specific cell types. Polymeric science needs to be explored to find new mucoadhesive polymers with the added attributes of being biodegradable, biocompatible, mucoadhesive for specific cells or mucosa and which could also function as enzyme inhibitors for the successful delivery of proteins and peptides. A multidisciplinary approach will therefore be required to overcome these challenges and to employ mucoadhesive microspheres as a cutting edge technology for site-targeted controlled release drug delivery of new as well as existing drugs.

Future Prospects[50]

The effort will be carried out to transfer the research work to a large industrial scale for commercialization in order to provide cost-effective, patient compliance, product life extension. Also for publications and patents.While the control of drug release profiles has been a major aim of pharmaceutical research and development in the past two decades, the control of GI transit profiles could be the focus of the next two decades and might result in the availability of new products with new therapeutic possibilities and substantial benefits for patients. Soon, the so-called once-a-day formulations may be replaced by novel gastroretentive products with release and absorption phases of approximately 24 h.

References

1. Hoffman A. Pharmacodynamic aspects of sustained release preparations. Adv Drug Del Rev 1998;33:185-99.
2. Hwang SJ, Park H, Park K. Gastric retentive drug-delivery systems. Crit Rev Ther Drug Carrier Sys 1998;15:243-84.
3. Hoffman A, Stepensky D. Pharmacodynamic aspects of modes of drug administration for optimization of drug therapy. Crit Rev Ther Drug Carrier Sys 1999;16:571-639.
4. Deshpande AA, Rhodes CT, Shah NH, Malick AW. Controlled-release drug delivery systems for prolonged gastric residence: An overview. Drug Dev Ind Pharm 1996;22:531-9.
5. Singh BN, Kim KH. Floating drug delivery systems: An approach to oral controlled drug delivery via gastric retention. J Contol Release 2000;63:235-59.
6. Moes AJ. Gastroretentive dosage forms. Crit Rev Ther Drug Carrier Syst 1993;10:143-95.
7. Vasir JK, Tambwekar K, Garg S. Mucoadhesive microspheres as a controlled drug delivery system. Int J Pharmaceut 2003;255:13-32.
8. Washington N, Washington C, Wilson CG. Physiological Pharmaceutics. Vol. 2. New York: Taylor and Francis; 2001.
9. Bannister LH. Alimentary system. In: Williams, PL, editor. Gray's Anatomy. XXXVIII, New York: Churchill Livingstone; 1995. p. 1683-812.
10. Rubinstein A, Li VH, Gruber P, Robinson JR. In: Yacobi A, Halperin-Walega E, editors. Oral sustained release formulations: Design and evaluation. New York: Pergamon Press; 1988. p. 125-56.
11. Thibodeau GA, Patton KT. Anatomy and physiology. III, Mosby, St. Louis 1996.
12. Hasler WL. In: Yamada T, editor. Textbook of Gastroenterology II. Philadelphia: J.B. Lippincott; 1995. p. 181-206.
13. Dressman JB, Berardi RR, Dermentzoglou LC, Russel TL, Schmaltz SP, Barnett JL, et al. Upper gastrointestinal (GI) pH in young, healthy men and women. Pharm Res 1990;7:756-61.
14. Russell TL, Berardi RR, Barnett JL, Dermentzoglou LC, Jarvenpaa KM, Schmaltz SP, *et al*. Upper gastrointestinal pH in seventy-nine healthy, elderly, North American men and women. Pharm Res 1993;10:187-96.
15. Munk J, Gannaway R, Hoare M, Johnson A. In: Duthie HL, editor. Gastrointestinal motility in health and disease. Lancaster: MTP Press; 1978. p. 349-59.
16. Salessiotis N. Measurement of the diameter of the pylorus in man. I Experimental project for clinical application. Am J Surg 1972;124:331-3.
17. Ogihara H, Suzuki T, Nagamachi Y, Inui K, Takata K. Peptide transporter in the rat small intestine: Ultrastructural localization and the effect of starvation and administration of amino acids. Histochem J 1999;31:169-74.
18. Barley NF, Howard A, O'Callaghan D, Legon S, Walters JR. Epithelial calcium transporter expression in human duodenum. Am J Physiol Gastrointest Liver Physiol 2001;280:285-90.
19. Cousins RJ, McMahon RJ. Integrative aspects of zinc transporters. J Nutr 2000;130:1384-7.
20. Read NW, Sugden K.Gastrointestinal dynamics and pharmacology for the optimum design of controlled-release oral dosage forms. CRC Crit Rev Ther Drug Carrier Syst 1987;4:221-63.
21. Kararli TT. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm Drug Dispos 1995;16:351-80.
22. Davis SS, Wilding EA, Wilding IR. Gastrointestinal transit of a matrix tablet formulation: Comparison of canine and human data. Int J Pharm 1993;94:235-8.
23. Rubinstein A. Approaches and opportunities in colon-specific drug delivery. Crit Rev Ther Drug Carrier Syst 1995;12:101-49.
24. Rubinstein A, Tirosh B, Baluom M, Nassar T, David A, Radai R, *et al*. The rationale for peptide drug delivery to the colon and the potential of polymeric carries as effective tools. J Control Release 1997;46:59-73.
25. Minami H, McCallum RW. The physiology and pathophysiology of gastric emptying in humans. Gastroenterology 1984;86:1592-610.
26. Gruber P, Rubinstein A, Li VH, Bass P, Robinson JR. Gastric emptying of nondigestible solids in the fasted dog. J Pharm Sci 1987;76:117-22.
27. Ehrlein HJ. Motility of the pyloric sphincter studied by the inductograph method in conscious dogs. Am J Physiol 1988;254:650-7.
28. Wilding I. Site-specific drug delivery in the gastrointestinal tract. Crit Rev Ther Drug Carrier Syst 2000;17:557-620.
29. Ehrlein HJ. A new technique for simultaneous radiography and recording of gastrointestinal motility in unanesthetized dogs. Lab Anim Sci 1980;30:879-84.
30. Kelly KA, Morley KD, Wilbur BG. Effect of corporal and antral gastrojejunostomy on canine gastric emptying of solid spheres and liquids. Br J Surg 1973;60:880-4.
31. Shalaby WS, Blevins WE, Park K. Use of ultrasound imaging and fluoroscopic imaging to study gastric retention of enzyme-digestible hydrogels. Biomaterials 1992;135:289-96.
32. Backon J, Hoffman A. The lateral decubitus position may affect gastric

emptying through an autonomic mechanism: The skin pressure-vegetative reflex. Br J Clin Pharmacol 1991;32:138-9.
33. Mojaverian P, Vlasses PH, Kellner PE, Rocci ML. Effects of gender, posture, and age on gastric residence time of an indigestible solid: Pharmaceutical considerations. Pharm Res 1988;5:639-44.
34. Dressman JB, Amidon GL, Reppas C, Shah VP. Dissolution testing as a prognostic tool for oral drug absorption: Immediate release dosage forms. Pharm Res 1998;15:11-22.
35. Khosla R, Davis SS. The effect of tablet size on the gastric emptying of nondisintegrating tablets. Int J Pharm 1990;62:9-11.
36. Hamilton JK, Polter DE. In: Sleisenger MH, Fordtran JS, editors. Gastrointestinal Disease. Vol. 1. Philadelphia: W.B. Saunders; 1993. p. 286-92.
37. Coupe AJ, Davis SS, Evans DF, Wilding IR. Correlation of the gastric emptying of nondisintegrating tablets with gastrointestinal motility. Pharm Res 1991;8:1281-5.
38. Mojaverian P, Reynolds JC, Ouyang A, Wirth F, Kellner PE, Vlasses PH. Mechanism of gastric emptying of a nondisintegrating radiotelemetry capsule in man. Pharm Res 1991;8:97-100.
39. Klausner EA, Lavy E. Michael Friedman Expandable gastroretentive dosage forms. J Control Release 2003;90:24-6.
40. Santos CA, Jacob JS, Hertzog BA, Freedman BD, Press DL, Harnpicharnchai P, et al. Correlation of two mucoadhesive assays: The everted sac technique and the CAHN microbalance. J Control Release 1999;61:113-22.
41. Available from: http://www.patentstorm.us/patents/6123965.html.
42. Asane GS, Nirmal SA, Rasal KB, Naik AA, Mahadik MS, Rao YM. Polymers for mucoadhesive drug delivery system: A current status. Drug Dev Indus Pharm 2008;34:1246-66.
43. Ahuja A, Khar RK, Ali J. Mucoadhesive drug delivery systems. Drug Develop Indus Pharm 1997;23:489-515.
44. Chickering D, Jacob J, Mathiowitz E. Poly (fumaric-cosebacic) microspheres as oral drug delivery systems. Biotechnol Bioeng 1996;52:96-101.
45. Kamath KR, Park K. Mucosal adhesive preparations. In: Swarbrick J, Boylan JC, editors. Encyclopedia of Pharmaceutical Technology. Vol. 10. New York: Marcel Dekker; 1994. p. 133-63.
46. Teng CL, Ho NF. Mechanistic studies in the simultaneous flow and adsorption of poly coated latex particles on intestinal mucus. I. Methods and physical model development. J Control Release 1987;6:133-49.
47. Riley RG, Smart JD, Tsibouklis J, Dettmar PW, Hampson F, Davis JA, et al. An investigation of mucus/polymer rheological synergism using synthesised and characterised poly (acrylic acid)s. Int J Pharm 2001;217:87-100.
48. Mathiowitz E, Jacob JS, Jong YS, Carino GP, Chickering DE, Chaturvedi P, *et al*. Biologically erodable microspheres as potential oral delivery systems. Nature 1997;386:410-4.
49. Richardson JL, Armstrong, TI. Vaginal delivery of calcitonin by hyaluronic acid formulations. In: Mathiowitz E, Chickering DE, Lehr CM, editors. Mucoadhesive Drug Delivery Systems-Fundamentals, Novel Approaches and Development '98. New York: Marcel Dekker; 1999. p. 563-99.
50. Cremer K. Drug delivery: Gastro- remaining dosage forms. Pharm J 1997;259:108.

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