Introduction

Over the past three decades oral controlled release dosage forms have been developed and patented due to their considerable therapeutic advantages such as ease of administration, patient compliance and suppleness in formulation. Though, this approach is problematic with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract (GIT) due to variable gastric emptying and motility. Furthermore, the relatively short residence time of the drug in humans which normally averages 2-3 h through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose.\(^{[1,2]}\) Therefore, control of placement of a drug delivery system in a specific region of the GI tract offers advantages for a variety of important drugs characterized by a narrow absorption window in the GIT or drugs with a stability problem.\(^{[3]}\) These considerations have led to the development of a unique oral controlled release dosage form with gastroretentive properties. There are numerous approaches which have been adopted to develop gastroretentive dosage form to prolong the gastric residence time. Gastroretentive dosage form may be broadly classified into mucoadhesive systems, floating systems, high density systems, expendable systems, super porous hydrogel systems and magnetic systems.\(^{[4-7]}\) They enable oral therapy of drugs with narrow absorption window in upper part of GIT, having short half life (\(t_{1/2}\) 2-8 h) or drugs with poor stability. Furthermore the gastroretentive system can act locally within the stomach and prolong the intimate contact with the absorbing membrane thus increasing its efficacy. The detailed literature on classification of gastroretentive systems has been well reviewed elsewhere.\(^{[8-10]}\) The most common approach was gastroretention based on floating system. The disadvantage of floating devices administered in a single-unit form such as hydrodynamically balanced systems (HBS) are unreliable in prolonging the GRT owing...
to their ‘all-or-none’ emptying process thus, they may cause high variability in bioavailability and local irritation due to a large amount of drug delivered at a particular site of GIT. In contrast, multiple-unit particulate dosage forms (e.g. mucoadhesive microspheres) have the advantages that they pass uniformly through the GIT to avoid the vagaries of gastric emptying and provide an adjustable release, thereby, reducing the inter-subject variability in absorption and risk of local irritation. A multi-particulate system, such as one containing microspheres can become mixed with the food and as a consequence, will usually empty with the food over an extended period of time. This review will highlight the detailed study of research done by various scientists in terms of measurement of adhesive strength, formulation of microspheres using mucoadhesive polymers, in vitro and in vivo evaluation techniques and its current status.

**Basic gastrointestinal physiology and transit**

The GIT is composed of several regions differing in anatomy, biochemical environment, microbial flora, expression of transporters, and absorption characteristics. There are several processes that may occur simultaneously following drug release from a dosage form (DF) in the GIT, including; chemical/enzymatic/ bacterial degradation, absorption (passive and/or active), precipitation, efflux by P-glycoprotein pump, and metabolism by CYP450 enzymes. As a consequence the pharmacokinetic profile of a drug may be influenced by its delivery site. Anatomically the stomach is divided into three regions namely fundus, body and antrum (pylorus). The proximal part made of fundus and body acts as a reservoir for undigested material, whereas the antrum is the main site for mixing motions and acts as a pump for gastric emptying by propelling actions. Gastric emptying occurs during fasting as well as fed states. The pattern of motility is however distinct in the two states. During the fasting state, an inter-digestive series of electrical event takes place, which cycle both through stomach and intestine every 2 to 3 h. This is called the inter-digestive myoelectric cycle or migrating myoelectric cycle (MMC), which is further divided into following four phases as described by Wilson and Washington.

The Phase I (basal phase) lasts from 40 to 60 min with rare contractions. Phase II (pre-burst phase) lasts for 40 to 60 min with intermittent action potential and contractions. As the phase progresses the intensity and frequency also increases gradually. Phase III (burst phase) lasts for 4 to 6 min. It includes intense and regular contractions for short period. It is due to this wave that all the undigested material is swept out of the stomach down to the small intestine. It is also known as the housekeeper wave. Phase IV lasts for 0 to 5 min that occurs between phases III and I of 2 consecutive cycles. After the ingestion of a mixed meal, the pattern of contractions changes from fasted to that of fed state. This is also known as digestive motility pattern and comprises continuous contractions as in phase II of fasted state. These contractions result in reducing the size of food particles (to less than 1 mm), which are propelled toward the pylorus in a suspension form. During the fed state onset of MMC is delayed resulting in slowdown of gastric emptying rate. Scintigraphic studies determining gastric emptying rates revealed that orally administered controlled release dosage forms are subjected to basically -two complications that of short gastric residence time and unpredictable gastric emptying rate.

The pH of the stomach has been measured from 1.4 to 2.1. The pH of stomach changes when food is present increasing to nearly 4.0. The small intestine is divided into three regions i.e., duodenum followed by jejunum and ileum. The entire length of small intestine is 5 m. The pH of small intestine ranges between 6.0 to 7.8.

The transit of a drug through the GIT determines how long a compound will be in the contact with its preferred absorptive site. In humans, the small intestine transit time is around 3h for a drug formulation to pass from the ileo-caecal junction. Transit through the colon is much longer and can be 20 h or more.

**Factor affecting gastric retention**

The gastric retention time (GRT) of dosage forms is controlled by several factors. The density and size of the dosage form, Fed and fasted stomach, dietary component such as fat, certain amino acid and peptides can slow gastric emptying and intestinal transit. The patiens position, posture, age, sex, sleep and disease state of the individual (e.g., gastrointestinal diseases and diabetes) can also altered motor activity, thus slowing transit time. Certain Drug combinations that contain gastro-kinetic agents such as metoclopramide, cisapride have been marketed can also effect gastric retention. The detailed study of factor affecting gastroretention has been well reviewed elsewhere.

**Mucoadhesive microspheres**

Mucoadhesive microspheres include microparticles and microcapsules of 1 to 1000 μm in diameter consisting either of drug entirely of mucoadhesive polymer or having an outer coating with adhesive property. Microspheres have the potential to be used for controlled as well as spatial drug delivery. Incorporating mucoadhesiveness to microspheres leads to efficient absorption and enhanced bioavailability of drug. Specific targeting of drug to the absorption site is achieved by using homing devices (ligand) like plant lactin, bacterial adhesion etc. on the surface of the microspheres. Mucoadhesive microspheres can be tailored to adhere to mucosal linings of GIT, thus offering the possibilities of localized as well as systemic absorption of drug in controlled manner.

**Polymers for mucoadhesive microspheres**

The properties of mucoadhesive microspheres, e.g., their surface characteristics, force of mucoadhesion, release pattern of the drug, and clearance, are influenced by the type of polymers used to prepare them. Polymer microspheres can be used to deliver drug in a rate controlled manner and sometimes in targeted manner. The polymers that are commonly employed in the manufacture of mucoadhesive drug delivery platforms that adhere to mucin- epithelial surfaces may be conveniently divided into three broad categories as defined by Park and Robinson.

**First generation mucoadhesive polymer**

First-generation mucoadhesive polymers may be divided into three main sub-categories, namely: Anionic polymers, Cationic polymers and non-ionic polymers. Of these, anionic and cationic polymers have been shown to exhibit the greatest mucoadhesive strength. Consequently, such charged polymeric systems will now be examined in more detail.
Anionic polymers
Anionic polymers are the most widely employed mucoadhesive polymers within pharmaceutical formulation due to their high mucoadhesiveness, functionality and low toxicity. Typical examples include alginites, carrageenan, polyacrylic acid (PAA) and its weakly cross-linked derivatives and sodium carboxymethylcellulose (NaCMC). PAA and NaCMC possess excellent mucoadhesive characteristics due to the formation of strong hydrogen bonding interactions with mucin. Polycarbophil and carbomer (Carbopol, Kelco, Inc), which are cross-linked with allyl alcohol and allyl sucrose or allylpentaerythritol, whereas polycarbophil polymers are cross-linked with divinyl glycol. Both compounds have the same acrylic backbone but vary in their cross-link density that is often tailored to suit pharmaceutical or cosmetic performance.

Cationic polymers
Chitosan is the most extensively investigated within the current scientific literature. Chitosan is a cationic polysaccharide, the most abundant polysaccharide in the world, next to cellulose. The most explored mucoadhesive polymers, chitosan is gaining increasing importance due to its good biocompatibility, biodegradability and due to their favourable toxicological properties. The linearity of chitosan molecules also ensures sufficient chain flexibility for interpenetration. Whilst chitosan may provide improved drug delivery via a mucoadhesive mechanism, it has also been shown to enhance drug absorption via the paracellular route through neutralisation of fixed anionic sites within the tight junctions between mucosal cells. Anionic polymers are the most widely employed mucoadhesive polymers within pharmaceutical formulation due to their high mucoadhesiveness, functionality and low toxicity. Typical examples include alginites, carrageenan, polyacrylic acid (PAA) and its weakly cross-linked derivatives and sodium carboxymethylcellulose (NaCMC). PAA and NaCMC possess excellent mucoadhesive characteristics due to the formation of strong hydrogen bonding interactions with mucin. Polycarbophil and carbomer (Carbopol, Kelco, Inc), which are cross-linked with allyl alcohol and allyl sucrose or allylpentaerythritol, whereas polycarbophil polymers are cross-linked with divinyl glycol. Both compounds have the same acrylic backbone but vary in their cross-link density that is often tailored to suit pharmaceutical or cosmetic performance.

Thiolated polymers
Thiolated polymers (thiomers) are a type of second-generation mucoadhesive derived from hydrophilic polymers such as polyacrylates, chitosan or deacetylated gelan gum. The presence of thiol groups allows the formation of covalent bonds with cysteine-rich sub domains of the mucus gel layer, leading to increased residence time and improved bioavailability. In this respect thiomers mimic the natural mechanism of secreted mucus glycoproteins that are also covalently anchored in the mucus layer by the formation of disulphide bonds. Whilst first-generation mucoadhesive platforms are facilitated via non-covalent secondary interactions, the covalent bonding mechanisms involved in second generation systems lead to interactions that are less susceptible to changes in ionic strength and or the pH. Moreover the presence of disulphide bonds may significantly alter the mechanism of drug release from the delivery system due to increased rigidity and cross-linking. In such platforms a diffusion-controlled drug release mechanism is more typical, whereas in first-generation polymers anomalous transport of drugs into bulk solution is more common.

Methodologies used in preparation of mucoadhesive microspheres
Mucoadhesive microspheres can be prepared using one of the following methods:

Emulsion cross-linking method/chemical denaturation
It was described by Thanoo and associates. This method utilizes the reactive functional group of polymer to crosslink with aldehyde group of cross linking agent. In this method water-in-oil (w/o) emulsion was prepared by emulsifying the polymer aqueous solution in the oily phase. Aqueous droplets were stabilized using a suitable surfactant like span 80 or dioctyl sodium sulphosuccinate. The stable emulsion was cross linked by using an appropriate cross-linker like gluteraldehyde to harden the droplets. Microspheres were filtered and washed repeatedly with hexane or petroleum ether to remove traces of oils. They were finally washed with water to remove cross linkers and then dried at room temperature for 24 h.

Emulsification and ionotropic Gelation
Singla and associates used the dispersed phase consisting of chitosan aqueous acetic acid solution which was added to the continuous phase consisting of hexane and Span 80 (0.5% w/v) to form a w/o emulsion. After 20 minutes of mechanical stirring, sufficient quantity of 1(N) sodium hydroxide solution was added at the rate of 5ml/min at 15-min interval. Stirring speed of 2000 to 2200 rpm was continued for 2.5 h. The microspheres were separated by filtration and subsequently washed with petroleum ether, followed by distilled water and then air dried.
Solvent evaporation

It is the most extensively used method of microencapsulation, first described by Ogawa and co-workers. In this method a buffered or plain aqueous solution of the drug contained a stabilizing or viscosity modifying agent. It was added to an organic phase having polymer solution. This resulting solution was kept for continuous stirring to form water in oil emulsion. This emulsion was then added to a large volume of water containing an emulsifier like poly vinyl alcohol (PVA) or poly vinyl pyrrolidone (PVP) to form the multiple emulsions (w/o/w). The double emulsion, so formed was then subjected to stirring until most of the organic solvent get evaporated, leaving solid microspheres. The microspheres were then washed, centrifuged and lyophilised to obtain the free flowing and dried microspheres.[52]

Hot melt microencapsulation

This method was first used by Mathiowitz and Langerto prepare microspheres of poly(anhydride copolymer of poly [bis(P-carboxy phenoxyl) propane anhydride] with sebacic acid. In this method, the polymer was first melted and then mixed with solid particles of the drug that had been sieved to less than 50 μm. The mixture was suspended in a non-miscible solvent (like silicone oil), continuously stirred, and heated to 5 °C above the melting point of the polymer. When the emulsion was stabilized it was left for cooling until the polymer particles solidified. The resulting microspheres were washed with petroleum ether. The main objective for developing this method was to develop a microencapsulation process suitable for the water labile polymers, e.g., poly(anhydrides). Microspheres with diameter of 1-1000 μm could be obtained and the size distribution could be easily controlled by changing the stirring rate. The major limitation of this method is that it is not suitable for thermolabile substances.[53]

Solvent removal

It is a non-aqueous method of microencapsulation, also suitable for water labile polymers such as the poly(anhydrides). Carino and co-workers used this method for preparing microspheres. In this method, drug was dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride. This mixture was then suspended in silicone oil containing Span 85 and methylene chloride. After pouring the polymer solution into silicone oil, petroleum ether was added and stirred until solvent was extracted into the oil solution. The resulting microspheres were then dried under vacuum.[54]

Ionic gelation (hydrogel microspheres)

Microspheres made of gel-type polymers, such as alginate, were produced by dissolving the polymer in an aqueous solution, suspending the active ingredient in the mixture and extruding through a precision device, producing microdroplets which were made to fall into a hardening bath, which was slowly stirred. The hardening bath usually contains calcium chloride solution, whereby the divalent calcium ions crosslink the polymer forming gelled microspheres. The method involved an “all-aqueous” system and avoided residual solvents in microspheres. Lim and Moss[55] developed this method for encapsulation of live cells, as it does not involve harsh conditions, which could kill the cells. The surface of these microspheres can be further modified by coating them with polycationic polymers, like polylysine after fabrication. The particle size of microspheres could be controlled by using various size extruders or by varying the polymer solution flow rates.

Spray drying

This method is based on drying of atomized droplet in stream of hot air. In this method polymer was first dissolved in aqueous solution, drug was then dissolved or dispersed in the solution and then, a suitable cross-linking agent was added. This solution or dispersion was then atomized in a stream of hot air. Atomization leads to the formation of free flowing particles. The quality of spray-dried microspheres could be improved by the addition of plasticizers, e.g., citric acid, which promote polymer coalescence on the drug particles and hence promote the formation of spherical and smooth surfaced microspheres. The size of microspheres could be controlled by the rate of spraying, the feed rate of polymer drug solution, nozzle size, and the drying temperature. This method of microencapsulation was particularly less dependent on the solubility characteristics of the drug and polymer and was simple, reproducible, and easy to scale up.[56]

Phase inversion microencapsulation

The process involves addition of drug to a dilute solution of the polymer (usually 1-5%, w/v in methylene chloride). The mixture was poured into an unstirred bath of a strong non-solvent (petroleum ether/hexane/acetone) in a solvent to non-solvent ratio of 1:100, resulting in the spontaneous production of microspheres through phase inversion. The microspheres in the size range of 0.5-5.0 μm were then filtered, washed with petroleum ether and dried with air.[57] This simple and fast process of microencapsulation involves relatively little loss of polymer and drug.

Comparison of various processes used for preparation of mucoadhesive microspheres is given in Table 1.

Evaluation of mucoadhesive microspheres

The best approach to evaluate mucoadhesive microspheres is to evaluate the effectiveness of mucoadhesive polymer to prolong the residence time of drug at the absorption site, thereby increasing absorption and bioavailability of the drug. The methods used to evaluate mucoadhesive microspheres include the following:
**Table 1: Comparison of various processes used for preparation of mucoadhesive microspheres**

<table>
<thead>
<tr>
<th>Methods</th>
<th>Size (μm)</th>
<th>Polymers</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase inversion microencapsulation</td>
<td>0.5-5</td>
<td>Polyanhydrides</td>
<td>Low polymer and low drug loss during preparation</td>
</tr>
<tr>
<td>Spray drying</td>
<td>1-10</td>
<td>Poly(lactide-co-glycolide)</td>
<td>Primarily for microspheres used for intestinal imaging</td>
</tr>
<tr>
<td>Solvent evaporation</td>
<td>1-100</td>
<td>Relatively stable polymer like polystyrenes and polyanhydrides</td>
<td>Labile polymers may degrade during the fabrication process due to the presence of water</td>
</tr>
<tr>
<td>Solvent removal</td>
<td>1-300</td>
<td>High melting point polymers like polyanhydrides</td>
<td>Only organic solvents are used</td>
</tr>
<tr>
<td>Ionic gelation and size extrusion</td>
<td>1-300</td>
<td>Chitosan, Alginate</td>
<td>Used for encapsulation of live cells</td>
</tr>
<tr>
<td>Hot melt microencapsulation</td>
<td>1-1000</td>
<td>Water labile polymers like e.g. polyanhydrides and polystyrenes; with a molecular range of 1000-5000</td>
<td>Smooth and dense external surfaces of microspheres</td>
</tr>
</tbody>
</table>

**In vitro techniques**

**Measurement of adhesive strength**

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 used are:

**Method based on measurement of tensile strength**

The Wilhelmy plate technique is an old concept used for the measurement of dynamic contact angles and involves the use of a microtensiometer or a microbalance. The CAHN dynamic contact angle analyser (model DCA 322, CAHN instruments, Cerritos, California, USA) has been modified to perform adhesive microforce measurements. The DCA 322 system consists of an IBM compatible computer and a microbalance assembly.[10] 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.[10] The tissue, usually rat jejunum, is mounted within the tissue chamber containing Dulbecco’s phosphate

**Table 2: Literature review on mucoadhesive microspheres in gastroretentive delivery systems**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Polymer</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyclovir (ACY)</td>
<td>Chitosan, Thioltated chitosan, Carbopol 71</td>
<td>Retention time at its absorption site increases but thiolated chitosan show highest mucoadhesiveness</td>
<td>96</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>Sodium alginate</td>
<td>In vivo studies showed the gastric residence time of more than 4 h which revealed that optimized formulation could be a good choice for gastroretentive system</td>
<td>88</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>Ethylcellulose and Carboxopol74P</td>
<td>The bioavailability of acyclovir was greatly improved due to the prolonged retention of ACV in gastrointestinal tract</td>
<td>74</td>
</tr>
<tr>
<td>Famotidine</td>
<td>Sodium CMC &amp; sodium alginate</td>
<td>With increase in polymer concentration the mucoadhesion increases</td>
<td>100</td>
</tr>
<tr>
<td>Atenolol</td>
<td>HPMC, K15M and carboxopol 971P</td>
<td>In vivo radioimaging studies in rabbits showed the residence of Mucoadhesive microspheres for 6-8 h in upper part of GIT</td>
<td>101</td>
</tr>
<tr>
<td>Delapril hydrochloride</td>
<td>Polyglycerol esters of fatty acids</td>
<td>Mean residence time of drug is increased and plasma concentration of active metabolite are sustained</td>
<td>102</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Gelatin</td>
<td>The period of time drug release from erythromycin loaded microspheres was prolonged compared with that of erythromycin without gelatin microspheres</td>
<td>103</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>Chitosan</td>
<td>Showed good mucoadhesion up to 8 hrs</td>
<td>104</td>
</tr>
<tr>
<td>Dextran</td>
<td>Thioltated chitosan</td>
<td>Effective mucoadhesive potential</td>
<td>105</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>Chitosan</td>
<td>Enhanced bioavailability with sustained release</td>
<td>106</td>
</tr>
<tr>
<td>Amoxicillin/clarithromycin</td>
<td>PAA⁺ with PVP</td>
<td>Dissolution rate of complex microspheres were significantly slower with that of PVP alone microspheres</td>
<td>107</td>
</tr>
<tr>
<td>Enorflaxin</td>
<td>Chitosan-PAA</td>
<td>Enhanced mucoadhesive potential than chitosan alone</td>
<td>108</td>
</tr>
<tr>
<td>Theophylline, Thymine disulphide</td>
<td>Dextran derivative, CAB⁻</td>
<td>Improved bioavailability of drug</td>
<td>109</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Ethylcellulose/Carboxopol 94P</td>
<td>Sustained effect and have sound mucoadhesive potential when EC⁺-CP⁻ is 17:3</td>
<td>110</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Carboxyvinyl polymer</td>
<td>H. pylori eradication rate will be increased</td>
<td>111</td>
</tr>
<tr>
<td>Lacidipine</td>
<td>Chitosan</td>
<td>The value of zeta potential 23.68±0.8mV indicated the affinity of microspheres for mucin in stomach. The release was found to be controlled for more than 6 h</td>
<td>112</td>
</tr>
<tr>
<td>Captopril</td>
<td>Sodium alginate, HPMC, CP93MP, chitosan and cellulose acetate phthalate</td>
<td>The sustained delivery of captopril with mucoadhesive potential in gastric region</td>
<td>113</td>
</tr>
<tr>
<td>Amoxicillin trihydrate</td>
<td>Carbopol 934P and ethylcellulose</td>
<td>The prolonged gastrointestinal residence time might make contribution to the H. Pylori clearance</td>
<td>114</td>
</tr>
</tbody>
</table>

*a*=Poly (acrylic acid), *b*=Poly vinyl pyrrolidone, *c*=Celulose acetate butyrate, *d*=Ethyl cellulose, *e*=Carbopol
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 microspheres. 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 analysed. These include the fracture strength, deformation to failure and work of adhesion.\cite{60}

The CAHN instrument, although a powerful tool has inherent limitations in its measurement technique. It 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.

The novel 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.\cite{61} 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 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 \textit{in vivo} and then excised (along with the host tissue) for measurement. This technique can also be used to evaluate the mucoadhesion of polymers to specific cell types and hence can be used to develop mucoadhesive drug delivery system to target-specific tissues.

Recently, tensile test using texture analyzer has been reported for studying the mechanical characteristics of mucoadhesiveness of polymers and dosage forms.\cite{62} Several surface substrates such as porcine stomach tissue, chicken pouch tissue,\cite{63} bovine sublingual mucosa,\cite{64,65} bovine duodenal mucosa,\cite{66} mucin disc,\cite{66} and mucin gel\cite{67} have been used as a model substrate using texture analyzer. The validation of the test using texture analyzer has been performed under simulated gastric condition using pig gastric mucosa\cite{68} or simulated buccal conditions using chicken pouch tissues, in order to elucidate test conditions and instrumental parameters influencing the mucoadhesive test results.

**Method based on measurement of shear stress**

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.\cite{69} Adhesion tests based on the shear stress measurement involve two glass slides coated with the 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\cite{70} designed the \textit{in vitro} method of flow chamber. The flow chamber made of Plexiglas 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 behaviour of the microsphere.\cite{69}

**Novel mucoadhesion test for polymer**

**Mucin particle method**

This method evaluates the mucoadhesion of polymers with commercially available porcine mucin particles. In this test mucin particles were suspended in a suitable buffer solution having a concentration 1% w/v and then were mixed with an appropriate amount of polymer solution. The change in the surface property of mucin particle was detected by measuring the Zeta potential with the zeta master (Malvern instrument, Worcestershire, UK). In one of the experiments when coarse mucin particle suspension was mixed with the solution of chitosan (CS) and carbopol (CP) the zeta potential of the mucin particle was changed but in another experiment when hydroxyl propyl methyl cellulose (HPMC) solution was added to the mucin suspension the zeta potential was unchanged. This result indicates that carbopol and chitosan have mucoadhesive property.

A modified mucin particle method can be performed using the submicron sized mucin particle (200-300 nm) produced by sonication to the coarse mucin suspension. When the suspension is mixed with a polymer solution, the mucin particle may aggregate if the polymer has the mucoadhesive property and the extent of aggregation is directly proportional to the mucoadhesive property of the polymer.\cite{71}

**Biacore system**

The system is based on principle underlying an optical phenomenon called surface plasmon resonance (SPR). The SPR response is the measurement of refractive index, which varies with the solute content in a solution that contains a sensor chip. When a detected molecule is attached to the surface of sensor chip, or when the analyte binds to the detected molecule, the solute concentration on the sensor chip surface increases, leading to an SPR response. When the analyte (mucin particle) binds to the ligand molecule (polymer) on the sensor chip surface, the solute concentration and the refractive index on that surface changes, increasing the resonance unit (RU) response. When they dissociate, the RU response falls. Later, the analyte can be removed from the ligand by using a regenerating agent. The response will then turn back to the equilibrium state as the beginning step.\cite{72}

**In vitro mucoadhesion test on mice stomach mucosa**

The mucoadhesive properties of microspheres were evaluated by the method designed by Ranga and coworkers using stomach isolated from mice.\cite{73} First, mice were fasted for 24 h and the stomach was dissected immediately after the mice were sacrificed. The stomach mucosa were removed and rinsed with physiological saline. Hundred particles of drug loaded formulation were scattered uniformly on the surface of the stomach mucosa. Then, the stomach mucosa with microspheres was placed in a chamber maintained at 93% relative humidity at room temperature. After 30min, the tissues were taken out and fixed on a plate at an angle of 45°. The stomach mucosa was rinsed with simulated gastric fluid (pH 1.3, without enzymes) for 5 min at a rate of 22 mL/min. The microspheres remaining at the surface of stomach mucosa were counted, and the percentages of the remaining microspheres were calculated and the statistical significance of the differences between two groups was analyzed using the two-tailed t-test. A P\textsubscript{value} < 0.05 was termed significant.
**In vitro mucoadhesion test using eggshell membrane as substitute mucosa**

Eggshell membranes were employed as a substitute model for in vitro mucoadhesion evaluation. The eggshell membranes were obtained from fresh chicken eggs. After emptying the egg of its content, the external shell was removed, and the underlying membrane was isolated. Then similar procedure was carried out as mice mucosa to measure the in vitro mucoadhesion of the microspheres. The number of microspheres remaining on the surface of eggshell membrane was counted, and the adhering percent was calculated and statistically analyzed as above.[74]

**Others in vitro tests**

Other tests to measure the adhesive strength are mucoadhesion studies via rotating cylinder,[75] falling liquid film method,[76] everted sac technique,[77] *In Vitro* Wash-off Test[78] and novel rheological approach.[79]

**In vitro release studies**

No standard in vitro method has yet been developed for dissolution study of mucoadhesive microspheres. The apparatus of varying design, different dissolution media, and different stirring speeds for microspheres of different drugs used by different workers have been summarized in Table 3.

**Morphology analysis and size determination of mucoadhesive microspheres**

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). The volume mean diameter of the microspheres were determined in the ultra pure water (Sation 9000, Barcelona, Spain) by laser diffraction (Fraunhofer model) (Coulter LS 230, Florida, USA) reported by Lemoine and associates.[80] The surface charge was measured in terms of Zeta potential and the measurement was done with Brookhaven Instrument ZetaPALS (Phase Analysis Light Scattering) Ultra-Sensitive Zeta Potential Analyzer (NY, USA).[81] The mucoadhesion mechanism of various mucoadhesive polymer was studied by using atomic force microscopy (AFM).[82]

**In vivo techniques**

**Measurement of the residence time**

In vivo mucoadhesion measurements have consisted of transit time or relative bioavailability assays. The established methods for monitoring gastrointestinal transit time of radio-opaque or radiation emitting doses include X-ray and gamma scintigraphy. Relative bioavailability measurements are made by comparing the plasma level concentrations of drugs administered in mucoadhesive per oral dosage forms compared to standard per oral dosage forms and intravenous infusions.[83,84] Each of these methods provides data that support or reject the mucoadhesiveness of a material, which can be correlated indirectly to parameters measured in vitro.

**GI transit using radio-opaque microspheres**

Radio-opaque marker, e.g., barium sulphate encapsulated in mucoadhesive polymer is used to study the GIT transit time. Mucoadhesive labeled with Cr-51, In- 113m, I-123, Tc-99m have been used to study the transit of the microsphere in the GIT[85] Faeces collection (using an automated faeces collection machines) and X-ray inspection provides a non-invasive method of monitoring GI residence time without effecting normal GI motility.

**Gamma scintigraphy technique**

Several methods currently exist to study the fate of formulations in the rodents and primates gastrointestinal tract, such as gamma scintigraphy and radiological studies.[86,87] The greatest advantage of gamma scintigraphy over radiological studies is that it allows visualization over time of the entire course of transit of a formulation through the digestive tract, with reasonably low exposure of subjects to radiation. Location of microspheres on oral administration, extent of transit through the GIT, distribution and retention time of the mucoadhesive microspheres in GIT can be studied using the gamma scintigraphy technique. Some mucoadhesive microspheres were labeled with Tc-99m and administered to rabbits. The imaging was performed after 0.5, 2, 4, 6 and 24 h of dosing using a, large field view gamma camera (Siemens AG, Munich, Germany). In Gamma scintigraphy analysis, the section of GIT was critically analyzed and much differentiation was present at 0.5 h and 2 h after oral administration as shown in Figures 1a, b. The presence of microspheres was marked in the stomach at 4h Figure 1c but after 2 h the formulation moved towards small intestine which could be seen very clearly at 6 h of gamma scintigraphy study Figure 1d which revealed that the optimized formulation demonstrated gastroretention in vivo for 4h.[88] The percent radioactivity had significantly decreased ([Ig/1g of 99mTc-pertechnetate is 5-6 h], and the presence of microspheres in GIT could not be assessed clearly after 24 h of administration due to negligible radioactivity.[89]

Studies on the behaviour of chitosan formulations in humans are few, and more studies are therefore needed to demonstrate what happens to chitosan formulations in the human gastrointestinal tract. In a recent study, we used neutron activation-based gamma scintigraphy to visualize the gastro-retentive properties of chitosan formulations in the human stomach. Sakkinen and coworkers have...
In vitro/in vivo correlation of mucoadhesive force for gastric retention

To investigate the mucoadhesive properties of the gastric environment, an in vivo quantitative mucoadhesive fracture strength test was developed to correlate the data established with in vitro experimentation. Mucoadhesive and non-mucoadhesive bioerodible polymers with potential for use in oral drug delivery were tested for mucoadhesive fracture strength both in vivo and in vitro. Surprisingly, no statistically significant difference was found between the mucoadhesive fracture strength of fast eroding polyanhydride and slowly eroding hydrophobic polymers in vivo but in vitro results was statistically different. The lack of IVIVC (in vitro/in vivo correlation) among mucoadhesive fracture strengths reflects the clinical finding that polymers that produced strong mucoadhesive forces in vitro may not achieve prolonged gastric retention in vivo due to differences between the in vitro screening conditions and the in vivo bioadhesive environment. Laulitch and coworkers reported a novel means of obtaining in vivo mucoadhesive fracture strength by testing through a surgically implanted, re-closable gastric cannula. Investigating the link between in vitro and in vivo mucoadhesion experiments will lead to improved screening methods for mucoadhesive materials and improved translational research outcomes when transitioning from bench top to preclinical trials. Quantitative in vivo mucoadhesion measurements are useful in establishing if the results obtained in vitro reflect the in vivo environment. The new technique for comparing in vivo to in vitro mucoadhesion measurements quantitatively provides a means for analyzing the correlation between in vitro and in vivo mucoadhesive performance indicator, fracture strength. For more detailed literature on in vivo to in vitro mucoadhesion measurements of gastroretentive systems has been well reviewed elsewhere.

Conclusion

A new approach investigated to over ride normal gastric emptying is the use of mucoadhesive microspheres for gastroretention. Based on this approach mucoadhesive microspheres in gastroretentive delivery system present the promising area for continued research. This delivery system offers the advantages of controlled release with enhanced bioavailability. The degree of success of this approach lies on the thorough understanding of mucoadhesive polymers, methodologies for preparation and evaluation techniques for mucoadhesive microspheres.

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