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A REVIEW ON MUCOADHESIVE STRENGTH ANALYSIS BY USING DIFFERENT TECHNIQUES

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ABSTRACT

Drug effect can be improved by developing new drug delivery systems, such as the mucoadhesive system. Mucoadhesion is a beneficial strategy for drug delivery systems, this system has several benefits like improved residence time at application sites, drug protection, enhanced drug permeation, and enhanced drug availability. The use of mucoadhesive polymeric materials to improve the efficacy of therapeutic treatments, have great interest in the field of pharmaceutical sciences. The pharmaceutical dosage with mucoadhesive properties is important in order to understand and characterize the in-vivo interaction between the formulation and the biological substrate, which help in development of new mucoadhesive systems with effectiveness, quality, and safety. There are several in vivo, in vitro, and ex vivo methods for the evaluation of the mucoadhesive properties of drug delivery systems. But, there also is a lack of standardization of these methods, which makes comparison between the results difficult. The aim of this study was to review the mucoadhesive polymer, mechanisms, theories and several methods for evaluating mucoadhesion.

KEYWORDS

Mucoadhesive system, Mucoadhesion, polymer, drug delivery, in vivo, in vitro, ex vivo

INTRODUCTION

For the drug delivery the most common route or most convenient route is oral route. Since past 20 year, advances and progress are done by pharmaceutical industry and the drug development industries have overcome the drawbacks of oral route and giving interest on alternate routes for administration of drug (4). Mucoadhesion has provided great interest in pharmaceutical field and they have great application in enhancing the residence time as well as controlled release of drug through various bioadhesive dosage forms. Mucoadhesion may be define as the attractive forces between the biological membrane and mucous membrane or mucus. The mucous membranes adhere to the epithelial surfaces like GIT, lungs, vagina, eye, nasal. Mucoadhesive drug delivery system is defined as the drug delivery systems that utilize the property of bioadhesion of certain water-soluble polymers which become adhesive on hydration and hence used for targeting the drug in particular region of body for extended period of time.(1) The drug delivery across the mucus membrane provides several advantages over other routes that is to overcome hepatic first pass

metabolism and also the degradation of drugs by hazardous environment and by various gastrointestinal enzymes as well as intestinal flora.(2) Here the drug directly access to the systemic circulation through internal jugular vein which avoids acid hydrolysis in the GIT tract and bypasses the drugs from first pass metabolism which lead to high bioavailability ,through which there is rapid cellular recovery of the buccal mucosa which is the advantage of this route(3). But the Disadvantages of this drug delivery system is they have low permeability of the buccal membrane as compared to sublingual membrane and a smaller surface area (3). Selecting of a suitable route for drug delivery within the oral cavity is mainly depend upon anatomical and permeability differences which is exist across the various oral mucosal routes (1).

The various mucoadhesive polymers has been achieved the significant interest in formulating the sustained release, extended release and also prolonged the release of dosage forms. These polymers are either natural or synthetic macromolecules, they are capable to adhere in mucosal surfaces. From last three decades, the employment of varied mucoadhesive polymers has achieved a good interest within the field of pharmaceutical technology. Nowadays, the employment of mucoadhesive polymers has been accepted as a vital strategy to prolong the continuance and to improve the localized effect of drug delivery systems on the various mucus membranes of a biological system (2). Moreover, the oral cavity is easily accessible for self-medication and therefore the administration drug is to be promptly terminated just in case of toxicity by removing the dosage form from cavity (5).

In the early 1980's, Professor Joseph R. Robinson at the University of Wisconsin pioneered the concept of mucoadhesion as a brand-new strategy to prolong the residence time of varied drugs on the ocular surface. Over the years, mucoadhesive polymers were shown to be able to adhere to numerous other mucosal membranes. the potential to stick to the mucus gel layer which covers epithelial tissues makes such polymers are very useful excipients for the drug delivery (4).

The design of a mucoadhesives depend on two factors i.e., they have the ability to prolong the release of drug from the matrix and strong attachment to the mucus. The adhesion with mucus membrame is done by different types of polymers like chitosan, lectins, acrylates, poloxamers, hydroxypropyl methylcellulose, xanthan gum, polyvinyl alcohol, methyl cellulose, carrageenan, polyvinyl pyrrolidone, guar gum, Hyaluronic acid, Sulfated polysaccharide, PEG, cashew gum, pectin's, gellan gum, gellan gum, starch, guar gum etc (6). This review focuses on aspect and describes many experimental methods that has been projected over the years for the evaluation of adhesion ability (7).

MUCOADHESIVE POLYMER

Mucoadhesive polymers are water soluble and also water insoluble polymers, that are swellable networks. These polymers possess best polarity to create certain that they enable comfortable wetting by mucus secretion and optimal fluidity that allow the mutual adsorption and interpenetration of polymer and mucus secretion to the required place (8). The use of bio adhesive compound determines the varied parameters such as mucoadhesive strength, in-vitro unleash, thickness and therefore the residence time of drug delivery device. However, the polymers with high mass square measure most well-liked because; they show effective release rate of the controlling properties (2).

The development of the mucoadhesion theory and enhancements in sensible strategies were accompanied investigation of many polymers employed in prescription drugs and the new materials and their mixtures for the presence of mucoadhesive properties. The classification of mucoadhesive polymer with example are given below; **Table-1**(9,10)

Sr.no	criteria	categories	example
1	Source	Natural polymer / Semi polymer	chitosan, gelatin, hyaluronic acid, carrageenan, pectin, sodium alginate, agarose
		Synthetic	Cellulose derivatives CMC, Thiolated CMC, Na CMC, hydroxyethylcellulose, HPC, HPMC, methylcellulose, methylhydroxyethylcellulose Polymers based on poly(meth)acrylic acid Carbopol, polycarbophil, polyacrylic acid, polyacrylates, copolymer of acrylic acid and PEG, and methacrylic acid, poly-2-hydroxyethylmethacrylate, copolymer of acrylic acid and ethylhexylacrylate, polymethacrylate, polyalkylcyanoacrylates: polyisobutylcyanoacrylate, Others Poly-N-2-hydroxypropylmethacrylamide, PVA, PVP
2	Charge	cationic	chitosan, dimethylaminoethyl (DEAE)-dextran, trimethylatedchitosan, aminodextran
		Anionic	Carbopol, pectin, PAA, sodium CMC, xanthan gum, chitosan-EDTA, carboxymethylcellulose, pectin, Na alginate
		Non-ionic	HPC, poly (ethylene oxide), scleroglucan, Hydroxyethylated starch, HPC, PEG, PVA, PVP
3	solubility	Water-soluble	Carbopol, HEC, HPC (waterb38 8C), HPMC (cold water), sodium alginate, PAA, Na CMC
			Others Poly-N-2-hydroxypropylmethacrylamide, polyhydroxyethylene, thiolated polymer, PVP, PVA
4	Possible bioadhesive bonds	Hydrogen bonds	Acrylates [hydroxylated methacrylate, poly(methacrylicacid)], PVA, Carbopol, Pectin
		Covalent	Cyanoacrylate
		Electrostatic interactions	Chitosan

ADVANTAGE OF MUCOADHESIVE (11,12,13,14,15)

- ✓ Reduction in fluctuation in steady state levels and for better control of disease condition and also reduced intensity of local or systemic side effects.
- ✓ Drugs which show poor bioavailability via the oral route is administered conveniently.
- ✓ Buccal mucosa perfused with blood vessels and give a greater permeability than the skin.
- ✓ Drugs which are unstable within the acidic environment are destroyed by enzymatic or alkaline environment of intestine may be administered by this route. Eg. Buccal sublingual, vagina.
- ✓ Here the drugs bypass first pass metabolism for which there is increases in bioavailability.
- ✓ Here the drug can easily administer and extinction of therapy in the emergency can be facilitated.
- ✓ In unconscious patient and trauma patient, the drug can be administered.
- ✓ It offers a passive diffusion for drug absorption and doesn't require any activation.
- ✓ Excellent accessibility, relatively immobile mucosa, presence of smooth muscle, makes it suitable for administration of the retentive dosage forms.
- ✓ The ocular, vaginal and rectal mucosae have some specific advantages, but they have poor patient acceptability limits for local drug delivery, rather than systemic administration of drugs.
- ✓ A large contact surface of oral cavity gives rapid and extensive drug absorption.
- ✓ Comparison of TDDS the mucosal surfaces do not have stratum corneum. so, the major barrier layer to transdermal drug delivery is not a factor in transmucosal routes of administration.
- ✓ A substantial reduction in dose can be achieved there by reducing dose related side effects.
- ✓ This route gives an alternative for the administration of various narcotic analgesic, enzymes, hormones, steroids, cardiovascular agents etc.
- ✓ Enhanced in safety margin of high potency drugs for better control of plasma levels.
- ✓ Drug administration is easy.

DISADVANTAGE OF MUCOADHESIVE (16)

- ✓ Over hydration may cause disrupt structurally the formulation or form slippery surface for which hydration and swelling of the bioadhesive polymers.
- ✓ Drugs can irritate the oral mucosa, having an unpleasant taste, odour and drug which are unstable at buccal pH can't be administered by this route. Eating and drinking may be restricted.
- ✓ In vaginal drug delivery, the drug has to be stable in acidic pH. The vaginal formulation may leak and cause disorderliness. The vaginal formulation might cause contraindicated in pregnancy.
- ✓ less dose of medicament is required for administration.
- ✓ In ocular formulations, the formulation might cause uneasiness and blur vision.
- ✓ Drugs with passive diffusion absorption can be administered by this route.
- ✓ Drugs can be swallowed along with the saliva and lose the advantages of buccal route.

FACTOR AFFECTING MUCOADHESIVE DRUG DELIVERY SYSTEM

The factor affecting mucoadhesive drug delivery system are shown in **figure.1** (16)

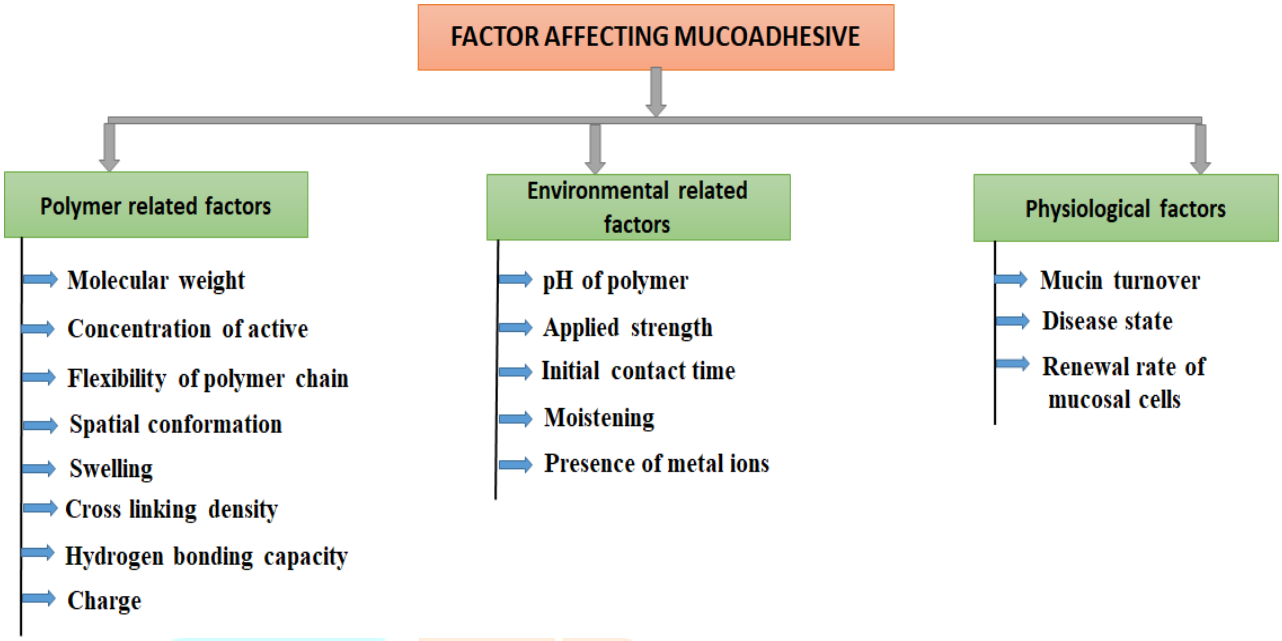


Fig.1- factor affecting mucoadhesion

MECHANISM OF MUCOADHESION

The mucoadhesion is a phenomenon in which two material within which one is artificial such as mucoadhesive polymer and alternative is also the mucin layer of the mucosal tissue, are unit control along by means that of interfacial forces of attraction. "Mucoadhesive" is outlined as a synthetic substance i.e., capable of interacting with the mucus membrane and being retained on them or holding them together for extended or prolonged amount of time. Throughout the method of adhesion, usually the two stages have been identified i.e., contact stage, Consolidation stage (2). The mucoadhesive must spread in the substrate to initiate close contact and also enhance surface contact, which may promote the diffusion of its chains within the mucus. Repulsion and attraction forces arise and, for successful mucoadhesive, the attraction forces must be dominate. Each step can be simplified by the nature of the dosage form and by its administration. As an example, a partially hydrated polymer can be adsorbed by the substrate because of the attraction by the surface water (Lee, Park, Robinson et al., 2000) (17,18).

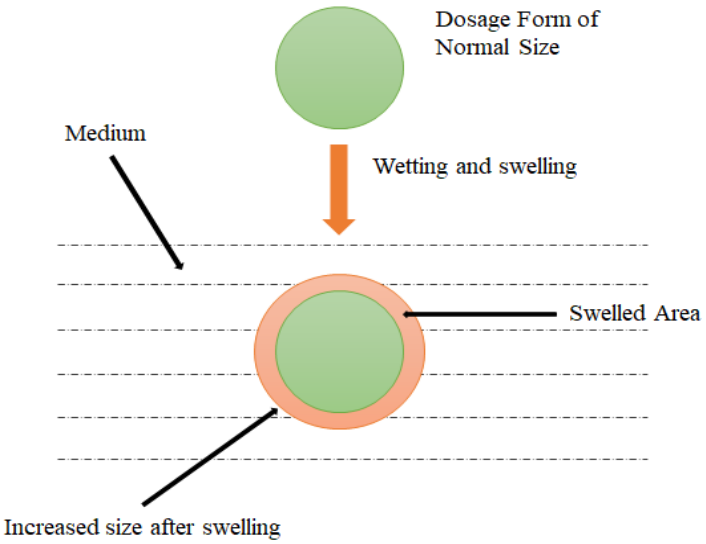


Fig.2- wetting and swelling of polymer

There are two stage i.e., contact stage and consolidation stage:

Contact stage: it is the first stage, in which there contact between the mucoadhesive and the mucous membrane, by spreading and swelling of formulation, which initiate its deep contact with the mucus layer (17). Here an intimate wetting occurs between the mucoadhesive and mucous membrane. In presence of mucosal membrane this wetting of mucoadhesive is done by mucus (2).

Consolidation stage: In this stage, there is activation of mucoadhesive materials in presence of moisture where the mucoadhesive molecules break away and link up again by weak Van der Waals and hydrogen bonds. Basically, two theories explain the consolidation step: the theory of diffusion and the theory of dehydration (16). After they get joint to the mucus membrane, they give prolong lasting mucoadhesion. This is called consolidation stage. After both the stages, the process of mucoadhesion completes (2).

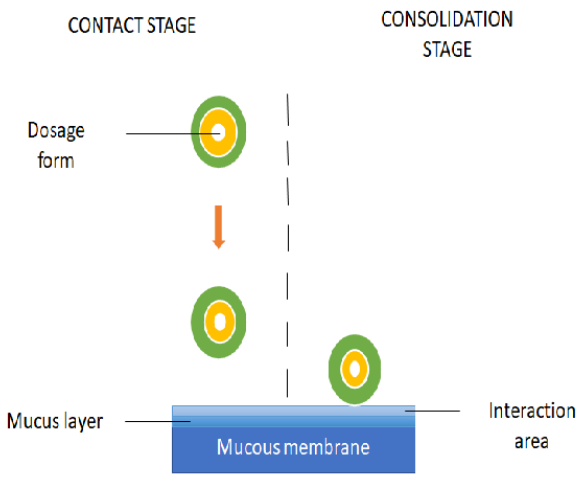


Fig.3 - Two stage of mucoadhesion process

THEORIES OF MUCOADHESION:

The theoretical outline for polymer- polymer adhesion can be simply extended to describe the bioadhesion of polymeric materials with biological surfaces (19). Mucoadhesion is a complex process and several theories have been projected to explain the mechanisms involved. These theories include mechanical interlocking, electronic, diffusion interpenetration, adsorption, fracture, wetting and cohesive theory (20).

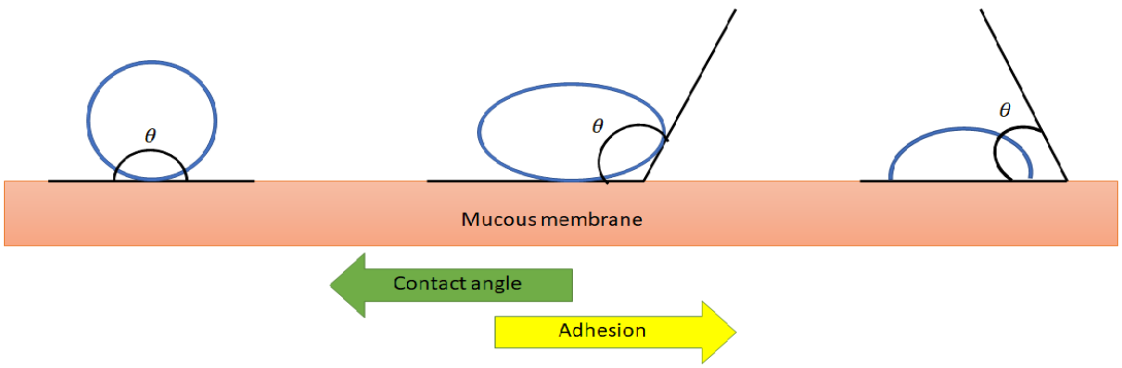


Fig.4 - Influence of Contact angle between device and mucous membrane on bioadhesion. (with permission)

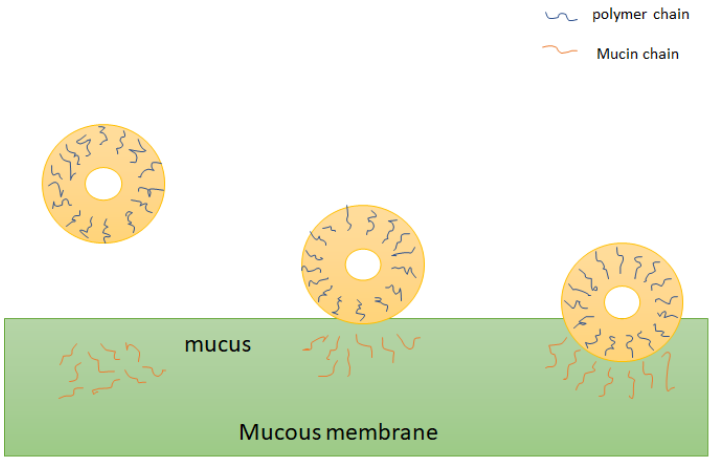


Fig.5 - The secondary interactions resulting from inter-diffusion of polymer chains of bioadhesive device and of mucus. (with permission)

a- Electronic Theories - It defines that the adhesion taking place by means the of electron transfer between the mucus and also the mucoadhesive system, arising through variances in their electronic structures. The electron transfer between the mucus secretion and also the mucoadhesive end up in the formation of double layer of electrical charges at the mucus and mucoadhesive interface. The net result of such a method is that the formation of attractive forces within this double layer (20).

b- Diffusion Theories - According to this theory is based on formation of semi-permanent adhesive bond by the interpenetration of the both polymer and mucin chain. Adhesion forces increases with increase in penetration. The diffusion coefficient, nature of mucoadhesive chain, mobility, flexibility and contact time are the most important parameter because penetration rate depends on this parameter. The solubility of one compartment in other is required for happening the diffusion. (21)

c- Wetting Theories – wetting theory relies on the mechanism of spreadability, in which the dosage form which will cross the biological layer. this theory is very much applicable to low viscous or liquid mucoadhesive system. Here the active components penetrate into the surface irregularities and it will gets harden and then finally results mucoadhesion. (2)

d- Adsorption Theories – According to this theory the mucoadhesive bonds are formed between mucoadhesive device and mucus by secondary chemical interaction such as Van Der Waals and hydrogen bonds, electrostatic attraction or hydrophobic interaction. (21)

e- Fracture Theories - This theory defines the bioadhesion strength through tensile experiment. It examines the force required to separate both surfaces after adhesion is established. The "fracture theory" narrates the force for polymer detachment from the mucus to the strength of their adhesive bond. When the polymer network strands are longer the work of fracture has been found to be grater. (21)

f- Mechanical Theories –In this theory, the diffusion of the liquid adhesives into the micro-cracks and irregularities present on the substrate surface there by forming an interlocked structure which can rise to adhesion. (22)

g- Cohesive Theories - The phenomena of bioadhesion are mainly because of the intermolecular interactions amongst like molecules. (22)

METHODS FOR EVALUATING OF MUCOADHESIVE STRENGTH

The most important step in developing a new mucoadhesive system is confirmation of its ability to adhere to the mucus. The literature defines several description methods that has been developed in different laboratories. The detail data about several in vitro, ex vivo and in vivo methods used to measure mucoadhesivity are given in following heads. (7)

***In-vitro* methods**

In vitro tests are the very common for the determination of mucoadhesivity of polymers as compare to ex vivo and in vivo. The in-vitro methods play an important role in any study due to its various advantages over other methods, these are including cost-effectiveness and easy to perform. However, these methods also suffer from some limitations such as poor in reliability. These tests have evolved from simple measurements of the force of detachment to a complicated and very expensive setups.

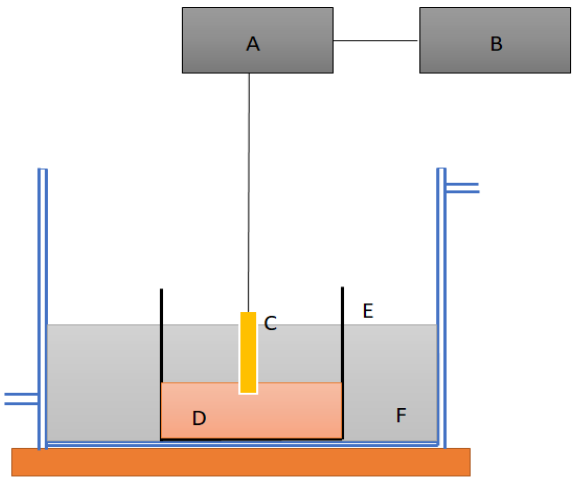
Shear stress method –

For the determination of mucoadhesivity, shear stress method is most reliable method. To illustrate the mucoadhesive strength, the shear stress method can be used. Depending on viscosity several concentrations (w/v) of solution have to prepare for mucoadhesive agent. Spread quantified amount of prepared solution on 3 glass plate. Put another clean slide over the first plate and spread over polymer solution uniformly between two glass plates by placing 100 g weight on the glass plates. Then, allow undisturbed for 15, 30 and 60 min respectively. Then, one side of the glass plate were fixed in a hook and then other connected to a twin passing over a pulley and at the end pan attached. After the said times 15, 30 and 60min weight should be place in an increasing manner till the plates attached with polymer get detach off, weight which is required to detach plate, represent the adhesive strength.

In several ways, this technique can be measured to be a good choice because of simple, ease in handling and cost-effective. However, at the same time, this technique suffers from some drawbacks; i.e., there is an insignificant correlation with in vivo measurement because of no relationship between glass plates and mucosal tissue. (23,8)

Wilhelm plate technique –

To describe the mucoadhesive strength, Wilhelmy's method can be used. A glass plate of small size was taken and coated by dipping into a 1 % w/v solution of mucoadhesive agent. The mucin was taken from the goat intestine and then kept in an appropriate container and then, temperature was maintained at 30 °C. Attach the nylon thread at the one end of glass plate and allow it to undisturbed for 5, 10, 15 and 30 minutes. Provision should be given to raise the weight from the other end. At definite intervals, weight added to separate the coated glass plate from gel and the force required to pull the plate out of the gel can be determine under experimental condition. However, now-a-days this is the only technique which is used frequently to measure the mucoadhesivity. The software needed to compare the results which will make the method easy but the correlation of test data, sometimes not fitted with accurate results. (8)



A- Microforce balance, B- Recorder, C- Glass plate, D- Homogeneized mucus, E- Glass recipient, F- Water bath

Fig.6- Apparatus to determine mucoadhesion in vitro, using wilhemy's technique

Adhesion weight method-

This method describes, the adherent particle weight was determined by poured a suspension of an exchange resin particles over the inner mucosal surface of a section of animal intestine (guinea pig). This method has partial value due to poor data reproducibility because it goes into rapid degradation and biological variation of the tissue. but it is probable to determine the effect of the particle size and charge on the adhesion with adverted intestine after 5 minutes contact. (5)

Falling liquid film method -

This method describes that the mucous membrane is placed in longitudinally cut stainless steel cylindrical tube where the support has been placed inclined in a cylindrical cell which have temperature controlled at 37°C in thermostatic bath. The isosmotic solution is pumped over the mucous membrane by peristaltic pump and collected in a container. Later, in the particulate systems, the quantity remaining on the mucous membrane has been counted with the aid of a coulter counter. For semi-solid systems, the non-adhered mucoadhesive can be measured by HPLC. This procedure allows the visualization of development of liquid-crystalline mesophase on the mucous membrane after the flowing of the fluids and through analysis by means of polarized light microscopy. (16)

Fluorescent probe method -

In this method, pyrene and fluorescein isothiocyanate has been use to label the membrane lipid bilayer and membrane proteins separately. Mix the mucoadhesive agents with cells and changes in fluorescence spectra are observed. This method will give suggestion of polymer binding and its role in the polymer adhesion. (16)

Detachment Force Measurement -

To describe the mucoadhesive strength, the detachment force method can be used. First-of-all collect goat intestine from the slaughter house and then, transfer to tyrode solution. During this experiment we have to place the intestine on one glass slide and tied from the both side of the assembly. The intestine on the affix glass slides one side floor below the modified physical balance. Past the mucoadhesive agent tablet (125 mg) on another glass slide and balanced on the assemble physical balance with a beaker on other side which can be used to hold the water, quantity of water in gram which will require to detach the tablet which were recorded. (8)

Flow channel method -

This procedure is known as a flow chamber, and considered the first in vitro method for microparticles. It is based on the fluid-mechanic principles associated with the flow of fluid around a sphere that is in contact with a substrate. By measuring the fluid viscosity and flow rate, it is possible to determine the bioadhesive force. Mucin or animal mucosa can be placed at the centre of the apparatus with temperature control. The test formulation is attached to this substrate surface and then the flow channel is connected with a gas cylinder containing air for the simulated physiologic flow to be introduced. The channel is placed between a light source and a microscope with a contact angle goniometer. Furthermore, a video camera can be attached to the goniometer to observe the detachment and movement of particles. This procedure was used to evaluate the adhesiveness of 2-hydroxyethyl methacrylate microparticles on the mucin gel and used a video cassette recorder to tape a movement particle and determine the mode of incipient motion. This technique has the advantage of determining whether only the molecular interactions, such as van der Waals, contribute to the adhesion force between the polymer particle and surface, or if a chain interpenetration phenomenon in the contact area is important to the adhesiveness. However, as for specific techniques to determine the adhesive

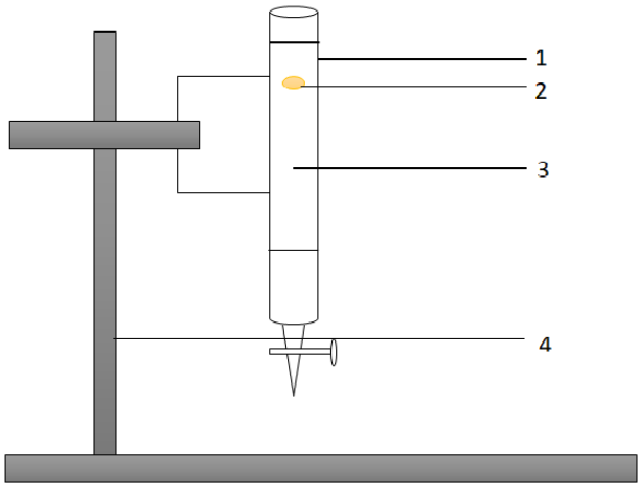
potential of microspheres, the specificity of the dosage form is the greatest limitation to this method. On the other hand, the surface mucosal tissue is also an important source of variability in the results because using an irregular mucosa leads to both low adhesive and high adhesive particles remaining on the tissue for a long period of time. (24)

Mechanical Spectroscopic method –

For investigating the effect of polymer chain length, pH and interaction between polyacrylic acid and glycoprotein gel mechanical spectroscopy was used. The method to investigate the effect of carbopol 934 on the rheological behaviour of mucus gel. They also investigated the role of mucus glycoprotein and the effect of various factors such as polymer molecular weight and ionic concentration and the introduction of anionic, cationic and neutral polymers on the mucoadhesive mucus interface. (5)

Falling Sphere method –

To characterize the mucoadhesive strength, the falling sphere method is used. Here, in this method a clean burette was taken and filled with 10% mucus solution and fixed in a stainless-steel tube. Then, the polymer solution is prepared of various concentrations and the mustard grain which retained on sieve size # 12 were taken and dipped in this polymer solutions. After sometime, each mustard grain slowly placed on mucus layer. The time taken by the grain to fall 50 divisions in the burette, the result was noted and values were calculated. (5)



1. Glass burette, 2. Mustard grains of uniform size, 3. Homogenized mixture of 10% mucus solution, 4. Burette stand.

Fig.7 – falling sphere methods

Rotating Cylinder Method -

Rotating cylinder method is used to characterize the mucoadhesive strength. Compress the tablet of mucoadhesive agent 125 mg. In rotary compression machine, use 6.0mm punch by keeping constant compression pressure for polymer. The tablet prepared in such a way, that it can adhere on freshly excised intestine of goat by just hydrating it with little amount of water. The complete system than adhered on the stainless-steel basket of USP XXVI apparatus with the aid of thread and the basket was immersed in the

dissolution jar which is filled with phosphate buffer pH 7.2 at 37 °C temperature and was rotated at 125 rpm. Then, the time required for the detachment was recorded, disintegration or erosion of the test disc. (8)

Atomic Force Microscopy (AFM) -

Generally, AFM for the use in bioadhesion studies bases on the change in surface roughness by polymer binding to a biological tissue. Forming bonds between polymer and tissue lead to higher surface roughness. Atomic Force Microscopy can be used to study the surface properties as well as the force which is needed to remove the adhesive formulation or polymer from a tissue. The technique to illustrate the surface structure bases on visualisation of atoms depicted as tridimensionality images. Determining the adhesive force between a polymer and a suitable tissue AFM is used in the force–distance model. It determined the bioadhesive force between Pluronic–PAA copolymer and mucin-coated surfaces. A colloidal-sized spherical particle was attached to an AFM cantilever which was brought in contact with mucin, which was in turn attached to an epoxy adhesive layer positioned on a glass microscope slide. It was noticeable that the surface structure was quite heterogeneous, so different locations of measurement led to differences in the results. Furthermore, changes in measurement time, pertest speed as well as withdrawal speed had a significant influence on the results.

The advantage of this method is the possibility to study the surface properties as well as defining the bioadhesive forces. Disadvantages are the time dependency and the missing adaptability for various dosage forms. (25)

Ellipsometry –

This optical technique is based on changes in the state of polarization of polarized light upon reflection at an interface. The presence of a thin film on the surface determines these changes. In this sense, there are numerous diverse methods available that yield different information with varying fields of application, in special mucoadhesive systems. Thus, ellipsometry assesses the mucoadhesive properties at physiologically relevant conditions, since the interaction between samples and mucin-coated silica surfaces can be investigated. Consequently, this method enables the characterization of adsorption layers, in terms of adsorbed amount, layer thickness, and refractive index. This method has demonstrated a high degree of simple experimental setup with intuitive direct measurements of polymer or particle adsorption to a surface, most frequently silica. Moreover, this is performed in a liquid and non-invasive environment. This technique has assessed the mucoadhesion measurements of polymers, such as cellulose derivatives, chitosan, carbopol, polyvinyl pyrrolidone, and particles, like surface-modified particles with chitosan and cubosome particles. (24)

BIACORE® method -

The BIACORE® method, also called the surface plasmon resonance (SPR) method, is based on an optical phenomenon measured by refractive index and range according to the solute concentration in a solution in contact with a sensor chip, which provides resonance units (RU). The SPR response is acquired when the solute concentration in the sensor chip increases, since the detected molecule is linked to the surface of the sensor chip and the analyte is bound to the detected molecule. The sensor chip is a glass surface coated with a thin layer of gold. The ability to monitor the changes in response in real time and its label free detection are reported to be the major advantages of the BIACORE® instrument. During the measurements, the response on the activated sensor surface increases as the sample passes over it, suggesting that binding occurs between polymers and mucin. Conversely, a constant signal will be seen when the equilibrium is reached. In this sense, the slope and value of RU response determines the rate of reaction and the strength of binding, respectively. Consequently, the increase in RU response occurs because of variation in the refractive index at the sensor chip surface, when immobilized molecules interact with binding molecules. Over the years, this method and its experimental conditions were quietly modified, according to the analyte. For example, the concentration of polymer solution used in the second injection was increased. Other modifications include the

immobilization of mucin solution over the sensor chip instead of the polymer solution. In this sense, the sensor chip was first pre-coated with the mucin solution and, afterwards, the polymer solution was injected into the flow cell. Subsequently, if the polymer solution binds to the mucin surface, the refractive index is modified, leading to a quantitative RU signal. (24)

Tensiometer method -

Bernkop-Schnurch and Steininger developed this method in 2000 by using lyophilized polymer conjugates, controls and unmodified polymer followed by compressing it in flat-faced discs. Tensiometry studies is carried out with these discs on native porcine intestinal mucosa with a force of 2.5 mN. The test disc and the mucosa have contact time for 30 min in 100 mM Tris-HCl buffered saline of pH 6.8 in room temperature, the mucosa pulls at a rate of 0.1 mm/s from the disc. Total work of adhesion signifies the area under the force/distance curve and the maximum detachment force which can be resolute by Win-Wedge software in combination with Excel. This technique is best way for quantitative measurement of mucoadhesivity because it is based on Win-Wedge and Excel software. But, the use of mucosal tissue, balance, jacketed water bath and software make the process quite complicated and hence, have limited application. (23)

Colloidal Gold Staining –

In this technique, mucin gold conjugate, are formed by stabilizing the red colloidal gold particle over mucin molecules, a red colour is developed on the bioadhesive hydrogel surface. By measuring the intensity of red colour on the hydrogel surface the interaction between them is easily quantified or by the measurement of the decrease in the concentration of the conjugates from the absorbance at wavelength 525nm. (5)

Thumb test -

This is a qualitative method used to determine the peel strength of adhesive formulations and is a useful tool in the development of buccal adhesive delivery systems as films, because it provides preliminary knowledge on the adhesive strength. In this method, the thumb presses the film for a predefined time, and the adhesiveness is measured by the difficulty in removing the thumb from the adhesive. (24)

Everted sac technique –

A section of intestinal tissue of rat is removed, everted, and one of its ends sutured and filled with saline. The sacs are presented into tubes which contain the system under analysis at known concentrations, stirred, incubated and then removed. The % adhesion rate of the release system into the sac is determined by subtracting the residual mass from the primary mass.

Other techniques use non-everted gut sac. filled rats' intestines with liposome suspensions. The sacs have been sealed and incubated in the saline. After a specified time, the number of liposomes adhered before and after incubation was measured with a coulter counter and the percent mucoadhesive was expressed by equation 8.

The mucoadhesive effect of a system can also be estimated by increases in GIT. fused fluorescent tracers into a system and computed them by fluorescence spectroscopy in the intestinal and stomach mucus as a function of time. (17)

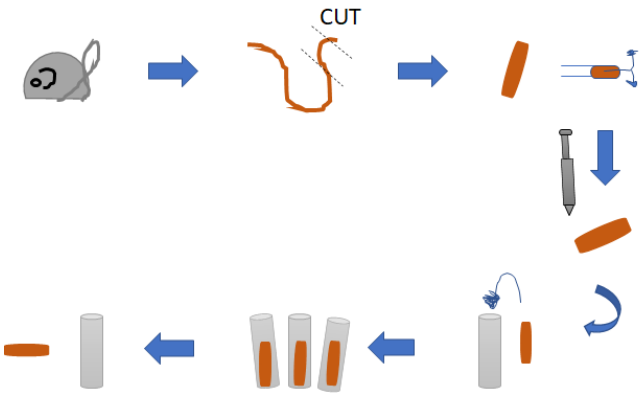


***Fig.*8 – Everted gut sac procedure**

Quick stick method -

In this test method, the tape is dragged away from the substrate at 90 °C at a speed of 12 inches/min. To break the bond between the adhesive and the substrate the peel force is required, and then measured and recorded as tack value, which is expressed in ounces or grams per inch width. To indicate the higher degree of tack, the higher values of force is required. (23)

Probe tack method -

In this method, probe tack tester is used in which a tip of the clean probe with a definite surface roughness is taken into contact with the adhesive, and when the bond is formed between probe and adhesive, the succeeding removal of the probe mechanically breaks it. To pull the probe, the force required away from the adhesive at a fixed rate is recorded as tack which is expressed in grams. (23)

Rolling ball tack method -

The method is based on the principal of the falling sphere viscometer which will measures the softness of a polymer that relates to tack. In this test method, a stainless-steel ball of 7/16 inches in diameter is roll over the inclined track, which will roll down and comes in contact with horizontal, upward facing adhesive. The distance travel by ball along the adhesive track delivers the measurement of tack, which is usually expressed in inch. The less tacky adhesive, farther the ball will travel. This method will give detail about the nature of mucoadhesion and again it fails to measure the adhesion strength. (23)

Ex-vivo method –

The ex-vivo are very popular these days because the results are very similar to in-vivo methods. Here, the single mucosal tissue can be used in different setups to measure mucoadhesivity. The limitation of ex-vivo methods include the sacrifice of an animal is required but the reliability made these methods promising.

Visualization with dyes -

Dyes are used to defining the retention time of drug delivery systems. The retention of mucoadhesive delivery systems on the vaginal tissues to test this, we have to formulate 2% blue lake dye which was intravaginally administered into mice using a micropipette tip. The homogeneity of mixture was sufficient and it follow the remaining vehicle on the mucosal tissue. After 1 hour of administration, mice were sacrificed and the retention of the mucoadhesive delivery systems at the administered sites was visualized by the blue colour of the dye. (23)

Sacs technique –

The sacs technique is to quantity mucoadhesion of poly (methacrylate) and N-trimethylated chitosan polymers using rat intestinal tissue models. The rats kept starved for an overnight before euthanasia by cervical dislocation. The intestine was removed after a midline incision, and the jejunum rapidly removed and flushed with the oxygenated medium. 6 sacs which was 5 cm long, were cut from the isolated jejunum. Then, the Sacs were placed in the oxygenated TC199 medium at 37 °C.

Then, the sacs were tied tightly at one end with silk suture and a small animal vascular catheter was tied into the other end. 1ml syringe with a sterile 26-gauge micro lance was fixed to the catheter. In-some cases, intestinal sacs were pre-treated with 10 mM NAC for 15 min, which was flushed out with 20 ml of medium. Then the sacs were filled with 0.5 mL polymer solution 1 mg/mL via the catheter. Each sac was placed in a separate sealed 50ml flask containing 15 mL of the oxygenated TC-199 medium on a shaking water bath for 30 minutes at 37 °C. Duplicate 50µL samples of incubation medium were removed from the bath after 30 min to assess leakage. Then, the sacs were removes out from the bath and the internal contents improved using a fresh 1mL volume syringe.

Following objective of the polymer-loaded donor compartment, sacs were then washed serially 4 times with a total of 5mL of medium and the washes collected for assay. Then, Samples were adjusted to pH 7.4 by addition of sodium citrate (10 mM) and assayed by fluorescence technique. The adhesion to sacs was calculated by subtraction and expressed as µg polymer/cm2. (23)

Modified Setnikar-Fanteli technique -

This can be a way for ex-vivo mucoadhesion/retention studies as outlined within which the distilled water is spread through the 2 small side arms into the glass cell with a pump evaluated the adherence of a brand-new dosage form for clotrimazole comprising a mucoadhesive polymer (polycarbophil, HPMC and hyaluronic sodium salt) in pessaries product of semisynthetic solid triglycerides using the test technique of Satnikar-Fantelli in a very modified way. This test simulates physiological vaginal conditions and verifies the efficiency of the polymers in prolonging the permanence of the dosage form with in the location where it's applied.

On the opposite hand, there was an improvement with in the adhesiveness of the pessaries with in the in-vitro adhesion test and a prolongation of the liquefaction time, test with in the presence of mucoadhesive polymers, which can increase with increasing in polymer concentration. The presence of the mucoadhesive had a big impact on the adherence of the drug on the simulated application site. Among the employed mucoadhesive polymers (polycarbophil, HPMC and hyaluronic sodium salt), polycarbophil with in the highest tested concentration turned out most promising. (23)

In-vivo methods –

in-vivo mucoadhesive studies are less commonly seen in the literature than in-vitro testing because of high cost, time limitation and ethical considerations. Despite these concerns in-vivo testing is important, if the true mucoadhesive potential of a system is to be determined. As such in-vivo techniques have found there most extensive use in the analysis of potential oral mucoadhesive dosage forms. (23)

Spectrophotometry -

Spectrophotometric methods described the in vitro methods section and a few findings are reported in literature using the methods for in vivo tests. However, it is also possible to evaluate the in vivo mucoadhesion. For example, RITC-labeled microspheres were administrated in rats to determine the gastric mucoadhesiveness in vivo. After administration, the stomach is removed to recover the remaining microspheres by fluorescence spectrophotometry, and the % of retention in the stomach is determined with the fluorescence signal. (24)

Gamma Scintigraphy Technique -

It is a tool; used in the development of the pharmaceutical dosage forms. The use of this method, it is possible to obtain information non-invasively. This technique is useful in oral dosage form and delivers information across the different regions of GI tract, the time and site of disintegration of dosage forms, the site of drug absorption, the effect of disease, food, and also the size of the dosage form in the in-vivo presentation of the dosage forms. (5)

Microscopy -

The fluorescein-resin complex can be used to label microparticles containing amoxicillin, and the system is administered directly to the stomachs of rats. After 3 different times, the rats were sacrificed and their stomachs and intestine were opened and observed through a fluorescence microscope, to inspect the distribution of formulations in several sections of the gastrointestinal tract (GIT). In the same way, in-vivo gastrointestinal transit can be used to evaluate chitosan microspheres. Cao and collaborators orally administered coated and uncoated pellets in rats. (24)

Mucoadhesion time (direct examination) -

This mucoadhesion technique is performed by the direct examination of mucoadhesion performance of formulations by the residence time. Generally, it is not necessary to use equipment for human models. On the other side, for animal models, the use of cameras or fiberscopes is required. Mucoadhesion time has been broadly employed for local application, mainly for buccal administration, but also for ocular and nasal routes. As well, this technique has been also explored for nasal and ocular routes with the support of blue dyes. The mucoadhesive properties of polymeric solutions and gel for nasal and ocular administration, respectively, was assessed. In the same manner as formulations for buccal mucosa, these systems were placed in their respective mucosa, and the adhesion time was determined. For rabbit nose, the adhesion time was observed using a fiberscope, while for rabbit eye, pictures were taken at determined times. (24)

GI transit using radio-opaque technique -

This technique is very simple and includes the use of radio-opaque markers, for example- barium sulfate, encapsulated in mucoadhesive polymers to determine the effects of mucoadhesive polymers on GI transit time. Faeces collection and X-ray inspection provide a non-invasive method of monitoring total GI residence time without affecting normal GI motility. Mucoadhesives labelled with Cr-51, Tc-99m, In-113m, or I-123 has used to study the transit of the microspheres in the GI tract. (23)

Radioisotopes and fluorescent labelling techniques -

The time measurements of the residence time of mucoadhesive at the application site provide quantitative information on their mucoadhesive properties. The GI transit times of various mucoadhesive preparations have been examined using radioisotopes and fluorescent labelling techniques. (23)

Gamma Scintigraphy Technique

This tool used in the development of pharmaceutical dosage forms. This method, help to obtain information non-invasively. This technique is useful in oral dosage form and provides evidence across the different regions of GI tract, the time and site of disintegration of dosage forms, the site of drug absorptions, and the effect of disease, food and size of the dosage form on the in-vivo presentation of the dosage forms. (5)

CONCLUSION

The use bioadhesive materials in contact with mucosal surfaces, as approach to improve the effect of therapeutic treatments, has a great interest in the pharmaceutical field, since the early developments in mucoadhesion. Mucoadhesive system has a progress area, whose aim is to develop new devices and more "intelligent" polymers, and also the creation of new methods that can better explain the mucoadhesion phenomenon. Mucoadhesive dosage forms are ease to administer, enhanced retention time at the site of action. Mucoadhesive polymers play important role to improve the bioavailability of the drug by extending the retention time at the site of action and bypass the first pass metabolism in the GIT and hepatic first-pass elimination. In this review article, several methods are explained to evaluate the mucoadhesive performance of the systems have been described, which is based on in vivo, ex vivo or in vitro analyses. Despite the various number of techniques are there for researchers, but it is impossible to find appropriate one because few studies are compared with different methods demonstrate irregularity between the conclusions drawn from them.

REFERENCES

1. Manohar S.D., *et al*. Drug Delivery from the Oral Cavity: A Focus on Mucoadhesive Buccal Drug Delivery Systems. PDA J Pharm Sci and Tech 2012, (66): 466-500.
2. Singh R., *et al.* Review on Mucoadhesive Drug Delivery System with Special Emphasis on Buccal Route: An Important Tool in Designing of Novel Controlled Drug Delivery System for the Effective Delivery of Pharmaceuticals. J Dev Drugs 2017, 6(1):1-12.
3. Reddy C., *et al.* A review on bioadhesive buccal drug delivery systems: current status of formulation and evaluation methods. DARU J of Pharmaceutical Sciences 2011, 19(6): 385-403.
4. Kaul M., *et al*. An Overview on Buccal Drug Delivery System. Int J Pharmaceutical Science Research. 2011; Vol. 2(6): 1303-1321.
5. Singh J., *et al*. A Review Article on Mucoadhesive Buccal Drug Delivery System. Int J Pharmaceutical Sci Research. 2013; 4(3): 916-927.
6. Singh T.G., *et al*. Mucoadhesion Drug Delivery System: A Propitious Approach. Int. J. Pharm. Sci. Rev. Res. 2018, 50(2): 72-88.
7. Bianco-Peled H., *et al*. Mucoadhesion: a review of characterization techniques. Expert Opin. Drug Deliv. (2010), 7(2): 259-271.
8. Singh S., *et al*. A Review on in vitro - in vivo Mucoadhesive Strength Assessment. Ph Tech Med 2013, 1(2): 221-229.
9. Kharenko E.A., *et al*. Mucoadhesive Drug Delivery System (Review). Pharmaceutical Chemistry Journal 2009, 43(4) 200-208.
10. Salamat-Miller N., *et al*. The use of mucoadhesive polymers in buccal drug delivery. Advanced Drug Delivery Reviews. 2005, 1666–1691.
11. Alexander A., *et al*. Mechanism Responsible for Mucoadhesion of Mucoadhesive Drug Delivery System: A Review. Int Journal of Applied Biology and Pharmaceutical Technology. 2011, 2(1):434-445.
12. Sheoran R., *et al*. Buccal Drug Delivery System: A Review. Int. J. Pharm. Sci. Rev. Res., 2018, 50(1): 40-46.
13. Syed I.A., *et al*. Buccal Mucoadhesive Based Drug Delivery Devices. World Journal of Pharmaceutical research. 2012, 1(3):548-575.
14. Vidyasagar N., *et al*. A Review on Buccal Drug Delivery System. Journal of Pharmaceutical Research and Development. 2012,1(2):29 – 35.
15. Patel P.S., *et al*. Buccal Drug Delivery System: A Review. Int. J. Drug Dev. & Res., 2013, 5 (3): 35-48.
16. Tandel H.T., *et al*. A Systematic Review on Mucoadhesive Drug Delivery System. World Journal of Pharmaceutical Research. 2017, 6(9): 337-366.
17. Carvalho F.C., *et al*. Mucoadhesive drug delivery systems. Brazilian Journal of Pharmaceutical Sciences. 2010, 46(1): 1-17.

18. Lee J.W., et al. Bioadhesive-Based Dosage Forms: The Next Generation. Journal of Pharmaceutical Science, 2000, 89(7):850-866.
19. Hussain M.A., *et al*. A Comprehensive Review on Buccal Drug Delivery System. American Journal of Advanced Drug Delivery. 2013, 1(3):300-312.
20. Boddupalli B.M., *et al*. Mucoadhesive drug delivery system: An overview. Journal of Advanced Pharmaceutical Technology & Research. 2010 1(4): 381-387.
21. Gaikwad S.S., et al. Buccal Tablet as a Promising Mucoadhesive Drug Delivery. Inventi Rapid: Pharm Tech. 2012, (3): 1-8.
22. Anil A., *et al*. Mucoadhesive Polymer: A Review. Journal of Pharmaceutical Research, 2018, 17(1): 47-55.
23. Semwal R., *et al*. Mucoadhesive assessment – An encyclopedic review. Curr Med Drug Res, 2018, 2 (2).
24. Bruschi L., *et al*. A critical review about methodologies for the analysis of mucoadhesive properties of drug delivery systems. Drug Development and Industrial Pharmacy. 2017.
25. Kleinebudde P., et al. Assessment of test methods evaluating mucoadhesive polymers and dosage forms: An overview. European Journal of Pharmaceutics and Biopharmaceutics. 2013.