Global SciTech Ocean

Current Medical and Drug Research

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Review article

Mucoadhesive assessment – An encyclopedic review

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Article history

Received : June 07, 2018
Accepted : August 12, 2018

Keywords

Transmucosal drug delivery
Soft palates
Sublingual cavities
Bio-polymers

ABSTRACT

Primary objectives behind the use of mucoadhesive drug delivery devices are to prolong their residential time at the particular site to make them target specific, and to enhance the drug absorption process. In this way, the measurement of mucoadhesivity is a crucial step to design the mucoadhesive drug delivery systems. The strategy of the use of mucoadhesive polymeric materials to improve the efficacy of therapeutic treatments has been introduced as long ago and the approach is still of a great interest in the field of pharmaceutical sciences. Nowadays, various methods used to measure the mucoadhesivity are included in the present review. These systems are usually *in vitro* or *ex vivo* in nature, and due to their relative ease of implementation and cost-effectiveness, these are limited in scope and still a serious need to improve their trait. The selection of such systems for the *in vivo* studies is a big challenge. Hence, the new discovery in the field of mucoadhesive drug delivery could be a great achievement to the scientific society.

INTRODUCTION

In the past few decades, a number of novel drug delivery systems have been introduced, and still, the researches to investigate more improved drug delivery systems are in progress (Semwal et al., 2014). Their considerable therapeutic advantages with the drugs make them more specific and interesting. Mucoadhesive drug delivery system (MDDS) is one of the most popular drug delivery systems currently in use or under consideration. MDDS showed rapid absorption as well as higher bioavailability both in topical and local systems due to its considerable surface area and high blood flow (Kharenko et al., 2009). Mucoadhesivity is the bonding of a polymer with mucosal linin or any other biological substrate (Henriksen et al., 1996).

Around 40 years ago, the concept of mucoadhesive polymers has been introduced in the area of pharmaceutical sciences which is accepted as a promising strategy to prolong the residence time of drug onto targeted membranes. Over the past 30 years, the market of transmucosal drug delivery systems has significantly increased approximately 3.5% a year and reached to the estimated value of $7.9 billion by 2010 (Kalorama Information Report, 2007). This growth could be related to the ease with which transmucosal products may be designed and administered (Andrews et al., 2009).

The bioadhesion can be obtained with two different interaction techniques which can be either specific or nonspecific (Ponchel and Irache, 1998). Nonspecific interactions are driven by physico-chemical properties of the particles and intestinal surfaces whereas the specific interactions occur when a ligand is attached to the particle used for recognition and attaching to a specific site at the mucous surface. The main functions of the mucoadhesive controlled-release devices are to improve drug efficacy by preventing dilution of the drug in body fluids, retaining its concentration between active and toxic levels, and letting drug to target to a particular site (Huang et al., 2000). The measurement of mucoadhesivity is the most important step in the development of a new drug delivery.

Although many *in vitro* and *ex vivo* methods to measure mucoadhesivity are already in practice, this task is still puzzling due to higher cost and difficulty in installation of available methods.

Moreover, these methods cannot be selected for *in vivo* experiments. Herein, this paper describes comprehensive information of the available tools to measure mucoadhesivity together with their merits and demerits, and also highlight the challenges and possibilities to introduce new tools in near future.

MERITS AND LIMITATIONS OF MDDS

Mucoadhesive drug delivery system (MDDS), delivering drugs to the mucosal site, has numerous advantages including ease of administration or termination. It allows drugs to localise in the mucosal membrane for an extended time which results in the increased bioavailability (Semwal et al., 2015). MDDS could be administered to bed-ridden patients, and a dose can be reduced to achieve minimal side effects of the drug. This route is also suitable for acid labile drugs which are usually affected in an enzymatic or alkaline environment of the intestine. It also gives an alternative to administer hormones, narcotics, steroids, enzymes, etc. (Khairnar and Sayyad, 2010).

Mucoadhesive drug delivery system offers a passive system for drug adsorption which is rapid and systematic. Unlike in the case of transdermal routes, MDDS in presence of ensures a relatively large amount of water for drug dissolution. Moreover, the mucosal membrane is highly perfused with blood vessels which provided an increased permeability than the skin (Satheesh-Madhav et al., 2012).

On the other hand, the administration of a drug via buccal mucosa has lots of limitations. The drugs those are irritating to the mucosal membrane and sensitive to mucosal pH and enzymatic reactions cannot be administered as a mucoadhesive system while only those drugs, which are absorbed by passive diffusion, can be administered (Satheesh-Madhav et al., 2012).

In MDDS, low dose of the drug is required that may swallow with saliva and loses the advantages of the buccal route. During the administration of the drug to the oral mucosa, eating and drinking might be restricted because over hydration can lead to the formation of the slippery surface which resulted to the disruption of the structural integrity of the formulation by swelling and hydrating of the bioadhesive polymers (Miller, 2005).

MUCOUS MEMBRANE

Membranes of the internal tracts of the body such as gastrointestinal tract (GIT), buccal and sublingual cavities, hard and soft palates, eye, ear, nose, vagina and rectum are covered with a thick gel-like structure known as mucin. It is important to note that whatever the mucoadhesive using for drug delivery systems must interact with the mucin layer during the process of attachment. Mucous helps to form a linkage between the adhesive and the membrane. Mucous is a network of mucin glycoproteins that form a continuous layer that intimately covers the internal tracts of the body (Serra et al., 2009).

Mucin is synthesised by goblet cells and special endocrine cells together with mucous acini (Finkbeiner et al., 2011). In total secreted mucous, only 5% of the content is glycoprotein which comprises of about 160-200 oligosaccharide chains in the glycosylated region while each oligosaccharide unit contains 8-10 monosaccharide units. At physiological pH, the mucin network has a negative charge due to the presence of sialic acid (pKa value = 2.6). Moreover, the presence of sulphate residues also contributes to the negative charge, making the glycoprotein as an anionic polyelectrolyte. Thus, bioadhesive mucin consists of highly hydrated, cross-linked, linear, flexible and random coil glycoprotein molecules with a negative charge.

MUCOADHESIVE FORCES

Mucoadhesion, an electromagnetic force, acts either between molecules or separated regions of macromolecules. These are usually electrostatic or electrodynamic interactions, and their nature could be either attractive or repulsive. These forces can be divided into two major classes, i.e. short-range and long-range forces.

The short-range forces are repulsive if molecules do not tend to interact chemically otherwise it would be the attractive forces or valence forces (Sudhakar et al., 2006). On the other hand, long-range forces, also known as van der Waals or attractive forces, account for various physical phenomena including friction, surface tension, adhesion and cohesion of liquids and solids and viscosity. (Glantz et al., 1999).

Various theories, i.e. electronic, adsorption, wetting, diffusion and fracture have been well described to understand mucoadhesive forces (Ahuja et al., 1997). However, for any type of charged surface, such as muco-surfaces, it is common to distinguish between pure electrostatic repulsive forces and attractive forces. This balanced relationship between repulsive and attractive interactions is expressed in the Derjaguin Landau Verwey Overbeek (DLVO) theory (Elwing et al., 1988).

In biological systems, the interactions take place in the presence of macromolecules and in high ionic strength aqueous media which results in a more complex interaction. Therefore, electrostatic contributions may be less important in favour of force components such as stearic forces, hydrophobic interactions and hydration forces. Various forces involve with the mucoadhesive systems are compiled in Table 1.

Table 1. Various forces involve with the mucoadhesive systems

S.No.	Forces	Principal	Attributes	References
1.	London dispersion forces	Van der Waal's attraction	The attraction between temporarily induced dipoles in nonpolar molecules and interactions involve a force of about 0.5 to 1 Kcal/mol.	Whitesides et al., 1991
2.	Dipole-dipole interactions		Weak in nature because only partial charges are involved and have the force of 1 to 7 Kcal/mol.	Rawlins, 1984
3.	Debye type forces		Interactions between permanent and induced dipoles with force of about 1 to 3 Kcal/mol.	Martin et al., 1994
4.	Hydrogen bonding	Electrostatic interaction	The force is short range and highly directional and magnitude of bond energy is between 10 and 20 KJ/mol	Nylander et al., 1994
5.	Disulphide bridging	Strong covalent interaction	Showed the strongest mucoadhesive properties via thioldisulphide exchange reaction and an oxidation process	Leitner et al., 2003
6.	Hydration forces	Short-range repulsive interaction	Originated from the binding of water molecules to polar surface sites and prevents contact even in the absence of charge-charge repulsion	Claesson and Christensson, 1988
7.	Electrostatic double-layer forces	Attraction and repulsion	Increases adhesion to negatively charged surfaces and assigned to less repulsion between the surface and the adhering cells	Larsson and Glantz, 1981
8.	Hydrophobic interactions	Attractive interactions between non-polar molecules	The hydrophobic effect can be nullified to a certain extent by lowering the temperature of the solution to near zero degrees and strength of these interactions is about 0.37 kcal/mol.	Martin et al., 1994
9.	Steric forces	Repulsive interaction	The maximum possible number of molecular contacts between an adhesive and its substrate may be greatly restricted by the steric aspects of molecular geometry	Glantz et al., 1999

MECHANISM OF MUCOADHESION

The mechanisms involve with mucoadhesion are spreading, wetting, swelling and dissolution of mucoadhesive polymer at the interface; inter-diffusion and interpenetration between the chains of the adhesive polymer and the mucus/epithelial surface resulting physical cross-links or mechanical interlocking; adsorption of the polymers at the interface so that adhesive bonding across the interface is possible; and formation of secondary chemical bonds between the polymer chains and mucin molecules (Sudhakar et al., 2006). The diagrammatical representation of mechanisms involved with mucoadhesion is depicted in Fig. 1.

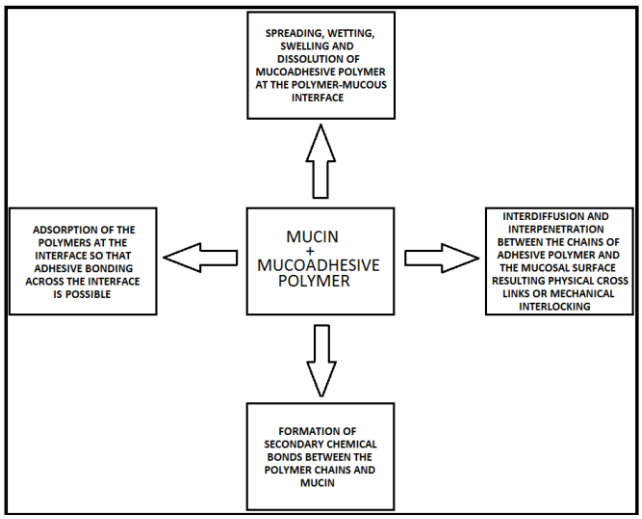

Fig.1. Various mechanisms of mucoadhesion

MEASUREMENT OF MUCOADHESIVITY

Measurement of mucoadhesivity plays an important role in the development of mucoadhesive systems and based on mucoadhesivity, the rank and selection of a polymer are decided (Semwal et al., 2015). The detail information about various *in vitro, ex vivo* and *in vivo* methods used to measure mucoadhesivity are given in following heads.

In vitro methods

In vitro tests are by far the most common for the determination of mucoadhesivity of polymers as compare to *ex vivo* and *in vivo* (Accili et al., 2004). 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 expensive setups.

Shear stress method

The shear stress method is the oldest and most reliable method for the determination of mucoadhesivity (Chen and Cry, 1970). In this method, two smooth, polished plexiglass blocks are used in which one block is fixed with a levelled table with the help of Araldite, an adhesive, and its level is adjusted with the help of a spirit lamp. A

thread is tied with the upper block and passed down through a pulley (Fig. 2A). The thread length is maintained to be 12 cm from the pulley to a pan of 17 g in which the weights can be added. Experimentally, under this method, different polymers of particular strength can be kept at the centre of the first block and covered by the second block. The polymer can be pressed by applying 100 g weight to spread it in a uniform film between two blocks. After a certain interval of 5, 10, 15 and 30 min, the weights on the pan can be added. The weight (or the shear stress) requires pulling upper block or makes it slide down from the lower block represents the adhesion strength.

In various ways, this technique can be considered to be a good choice because of simple, ease in handling and cost-effective. However, at the same time, the technique suffers from some drawbacks; for example, there is an insignificant correlation with *in vivo* measurement because of no relationship between glass plates and mucosal tissue.

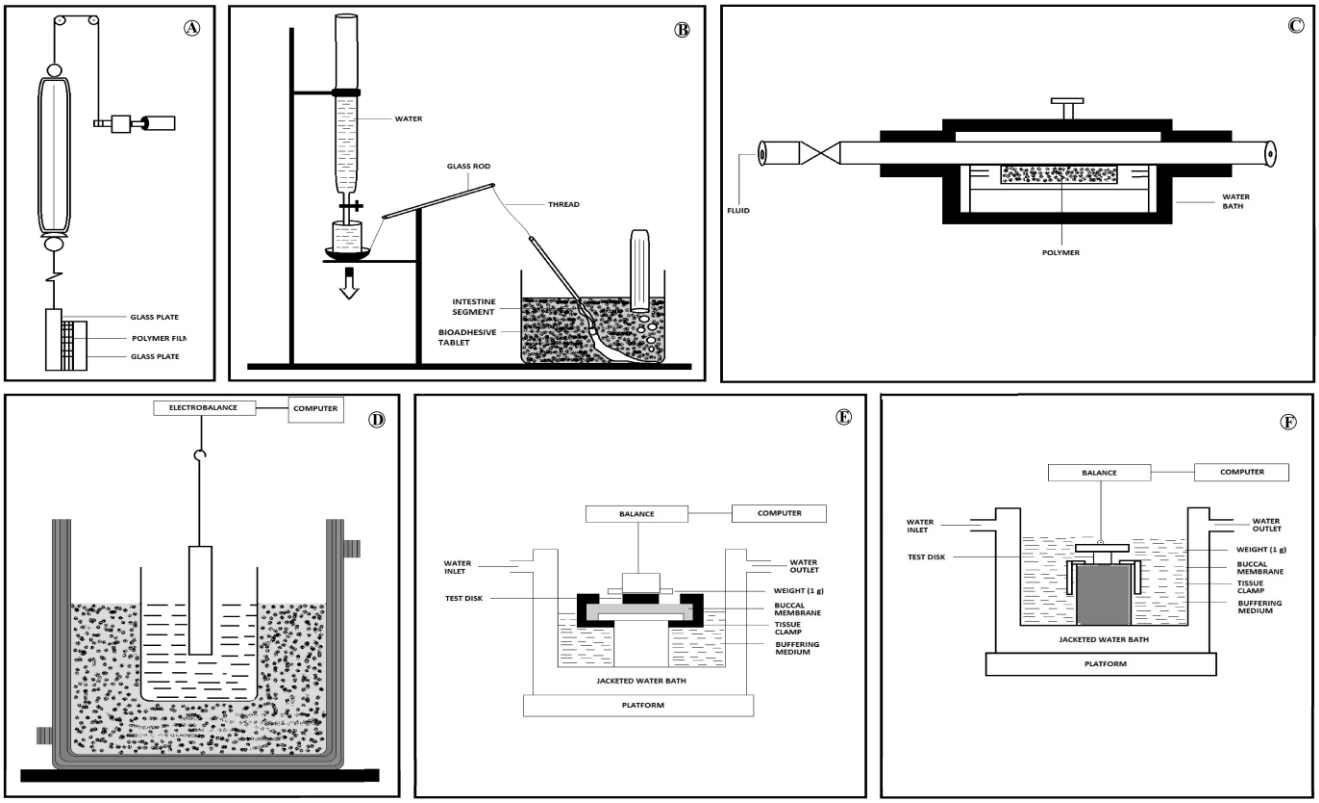

Fig. 2. *In vitro* methods for the determination of mucoadhesivity. A: Shear test apparatus; B: Assembly used in detachment force measurement method; C: Fluid flow chamber for bioadhesive microspheres studies; D: Wilhelmy plate technique; E: Tensiometer assembly for tensile strength measurement; F: Apparatus used for assessing bioadhesion

Detachment force measurement

This method was developed by Marvola (1982) to assess the tendencies of mucoadhesive to adhere to the oesophagus. The assembly (Fig. 2B) consists of a single organ bath, a stand, a glass rod, a pan for keeping beaker and a reservoir for the addition of water into the beaker. Using this method, an experiment was attempted in which an intestine from a freshly killed sheep was removed and kept in the Tyrode solution. A drug tablet of 6 mm was put on one side with mucoadhesive polymer and polymer matrix (2:1). A fine hole was drilled at the centre of the tablet to be tested using a fine needle, and a thread was tied around the tablet. The other end of the thread was tied to the glass rod suspended from the stand. The length of the thread was such that in resting state the tablet should be at the middle of the intestinal piece. To the other end of the glass rod, a pan was tied in which the beaker was placed. After inserting the tablet into the intestinal piece, the assembly was kept undisturbed for a fixed time interval of 30 and 60 min. Then water was added slowly drop by drop into the beaker. The amount of water required to pull out the tablet from the intestinal segment represents the force requires pulling the tablet against the adhesion. [F = 0.00981 W/2], where W is the amount of water. The method is more reliable because the tissue is used in experiments which steer the test near to *in vivo* but at the same time, the animal scarification is needed that makes the test tedious and costly.

Unique flow chamber technique

The unique flow chamber technique was developed by Peppas (1994) for the measurement

of adhesiveness in the polymer microsphere. In this technique, a polymer microsphere was placed on the surface of the natural mucous layer. Fluid, moving with physiological rate, was introduced within the chamber (Fig. 2C), and movement of the microsphere was monitored by video equipment. By measuring the size and speed of the microsphere, it is possible to calculate the bioadhesive force. The technique is based on the principle of electrophoresis. So, it is a reliable qualitative method but the flow of fluid is difficult to maintain as *in vivo* flow of fluid.

Wilhelm plate technique

This technique has traditionally been used for dynamic contact angle measurement and involves a microbalance or Tensiometer and investigated by Smart and co-workers (Smart et al., 1984). In this technique, a glass slide can be coated with the polymer of interest and then dipped into a beaker of synthetic or natural mucous (Fig. 2D). The work required to remove various polymer-coated glass slides could be related to one another by available software and their adhesiveness. This technique has an advantage of allowing the analysis of mucoadhesion under different environmental conditions via a simple modification of instrumental setup. However, afterwards, Mikos and Peppas (1986) pointed out the shortcomings of this technique due to the possible dissolution of the polymer upon testing. They suggested that this effect may be limited if polymer plates of the candidate material can be used instead of polymer-coated glass plates. Further shortcomings were also detailed by Wong et al. (1999), who noted that the lack of biological tissue in such a setup may not represent true mucoadhesion. However, today this is the only technique which is used frequently to measure the mucoadhesivity. The software needed to correlate the results which make the method easy but the correlation of test data, sometimes not fitted with accurate results.

Tensiometer method

This method is developed by Bernkop-Schnürch and Steininger (2000) by using lyophilized polymer conjugates, controls and unmodified polymer followed by compressing it to flat- faced discs (Figure 2E). Tensiometer studies with these discs are carried out on native porcine intestinal mucosa with a force of 2.5 mN. After a contact time between test disc and mucosa of 30 min in 100 mM Tris-HCl buffered saline (pH 6.8) at room temperature, the mucosa pulls at a rate of 0.1 mm/s from the disc. Total work of adhesion represents the area under the force/distance curve and the maximum detachment force which can be determined by WinWedge software (TAL Technologies, Inc., Philadelphia, PA) in combination with Excel (MS software). This technique is best for quantitative measurement of mucoadhesivity because it is based on WinWedge and Excel software. However, the use of mucosal tissue, balance, jacketed water bath and software make the process bit complicated and hence, limited application.

Dual tensiometer method

Dual tensiometer to measure the adhesion force was first used by Leung et al. (1988) in the form of a modified tensile tester named Instron. This apparatus was first time used to measure mucoadhesivity of the adhesive tablet using rabbit stomach tissue. In this procedure, a section of tissue was cut from the fundus of rabbit stomach and secured onto a polyacrylic cylinder (3 cm diameter) using a rubber band to adequately fix the tissue without deforming it. In addition, a rectangular aluminium piece with a hole in the middle was used to support the tablets. This hole has a diameter of 2 mm greater w.r.t. the tablet and allows their swelling due to absorption of the medium. The experiment was carried out in a constant volume of the test medium. After 30 min, the adhesion and shear forces required to separate two parallel surfaces (tablet-tissue) were recorded as a function of time, until the tablet has crossed the tissue surface, and finally, the mechanical parameters can be calculated. This method is based on the dual tensiometer, hence, it has some advantages but the fluctuation appears with the results make its scope limited.

Tensiometer with cyanoacrylate-type adhesive method

The problem behind the mucoadhesivity measurement by tensiometer is that the material does not fix to their places and affects the performance of an instrument. To overcome these problems, Takayama et al. (1990) used cyano-acrylate type adhesives with a tensiometer. They evaluated the adhesion properties of bioadhesive polymer tablets by measuring the force required to separate the tablet from the cyanoacrylate-type adherend (Figure 2F). In this method, the buffer solutions (2 mL; pH 2.0, 3.5 and 5.0) can be gently added on the tablet to hydrate the tablet surface for 5 min at room temperature. The peritoneal membrane of rabbits, sacrificed with pentobarbital injection, can be excised and stored at -10 ˚C and thawed at 4 ˚C in an isotonic saline solution before the experiment. The residual water on the surface can be removed with the help of filter paper. The porcine dermis (round shape pieces; 5 mm diameter) attaches to the tip of an adapter in tensiometer with cyanoacrylate-type adhesive, and the adherent immerses in different buffer solutions (pH 2.0, 3.5 and 5.0) for 5 min at 25 ˚C before the measurement. The holder wearing the sample tablet lifts up in contact with the adherent, which is priorly hydrated within the buffer solution by

applying a loading pressure of 250 g/cm². The tablets and the adherent put in contact with each other for 3 min and thereafter the tablet stretches out from the adherent at an extension rate of 4 mm/s and the force requires to detach the tablet from the adherent can be recorded. Blanco-Fuente and coworkers (Blanco-Fuente et al., 1996) also used cyanoacrylate adhesive for adhesion studies in hydrogel systems. In this study, cyanoacrylate-type adhesives were used to hold the dosage form, which may alter the actual results and these results will be totally different when correlating with in vivo test results. This method is, however, best suited to measure the batch to batch consistency.

Everted sac technique

Santos et al. (1999) developed the everted sac experiment using viable segments of rat jejunum. In this method, the unfasted rats (400 g, male) were sacrificed. The intestinal tissue was excised and flushed with 10 ml of ice-cold phosphate buffered saline, pH 7.2 containing 200 mg/dL glucose (PBSG). Six cm segments of jejunum were everted using a stainless steel rod and lightly washed with PBSG to remove the contents. The tissue was maintained at 4˚C before incubation. The sacs were introduced into a 15 mL tube containing 60 mg of bioadhesive microspheres and PBSG was centrifuged for 30 min. The supernatant fluid was discarded and the sedimented microsphere was then washed three times with 5 mL of distilled water and centrifuged. The supernatant fluid was discarded again and the microspheres were frozen and dried by lyophilisation for 24-48 hrs. The weight of the bound spheres was determined by subtraction of tared weight of the tube and lyophilized spheres from the initial tare weight of tubes and spheres which can be reported as percent binding (Fig. 3). The method is more promising, reliable and quantitative but due to multi-steps, it is complicated and time-consuming. Overall, this method can use to determine muco-adhesivity of all type of dosage forms.

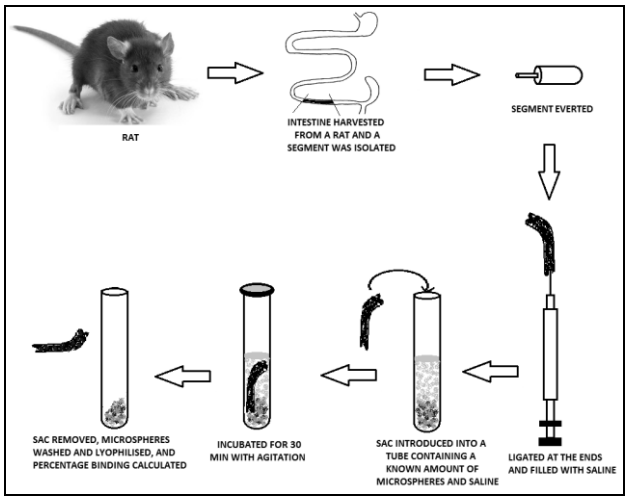

Fig. 3. Everted sac technique procedure

CAHN force measurement technique

Chickering and Mathiowitz (1995) worked with this technique and modified the CAHN Dynamic Contact Angle Analyzer (Model DCA, 322 CAHN Instruments, IN, and Cerritos, CA) to measure the adhesion. The equipment was designed for measuring the contact angles and surface tension using the Wilhelmy plate technique; it also serves essentially an extremely accurate microbalance. The DCA 322 system essentially includes a microbalance stand assembly a CAHN DACS IBM-compatible computer, and an Okidata micro line 320 dot matrix printers (Fig. 4). The microbalance unit consists of stationary sample and tare loop and z-translation stage powered by a motor stopper. The balance can be operated with samples weighing up to 3gms and has sensitivity as low as 1×10^{-5} mN. The stage speed can be varied from 20 to 264 mm/s. This is very sensitive *in vitro* technique but suffer from a major limitation, i.e., very small and very large applied loads are difficult to control.

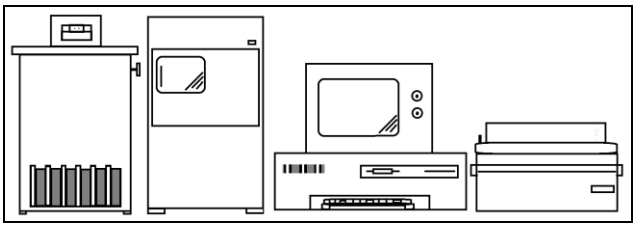

Fig. 4. CAHN DCA 322 dynamic contact angle analyzer

Falling liquid film technique

The technique was first described by Smart et al. (1984), and later on, Belgamwar, et al. (2009) used this technique to characterize the mucoadhesive multi-particulate system containing Metoprolol Tartrate. In this technique, male albino rats (200-250 g) were sacrificed and their intestine rejoin were isolated. Thereafter from the intestine rejoin, jejunum part was separated and cut longitudinally. This separated portion was placed on the semi-cylindrical Plexiglass support and washed with saline for 30 min at the rate of 30 mL/min (Fig. 5). Then 25 number of counted microspheres were hydrated with a little amount of water and were dispersed on the mucosal tissue and left on it for 20 min for interaction with the mucosal surface. During this period, the whole system was placed in a constant humidity chamber which was adjusted to 90% relative humidity. At the end, the system was washed with phosphate buffer pH 7.2 for 20 minutes at the rate of 22 mL/min and the numbers of microspheres remaining on the mucosal surface were counted.

The method is best suited for the qualitative determination of mucoadhesivity of solid particles but this is not well suitable with other formulation

and the same time the counting of solid particles on the the mucosal surface is a tedious job.

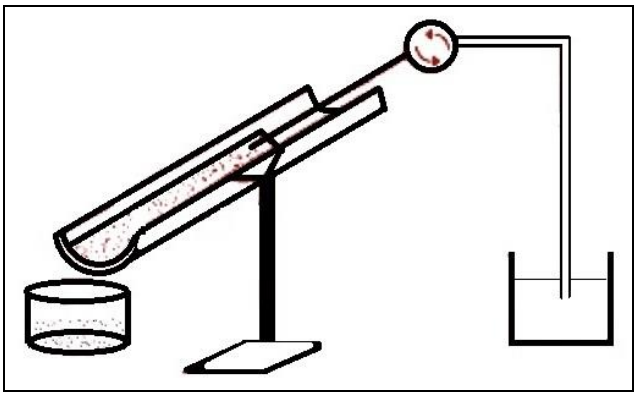

Fig. 5. Falling liquid film technique

Porcine esophageal mucoadhesion test

The porcine oesophagal mucoadhesion test system (Fig. 6) was employed to study the elution behaviour of microparticles placed on a mucosal surface (Smart et al., 2013). The study evaluated low, high and ultra-high molecular weight (MW) polymers (3% solutions) in a dynamic flow model for their ability to bind the tissues from the fundic and pyloric regions of the stomach and the oesophagus of pigs. All the polymers tested were retained on each mucosa for extended periods. It has been found that the high and ultra-high MW polymers showed the greatest retention.

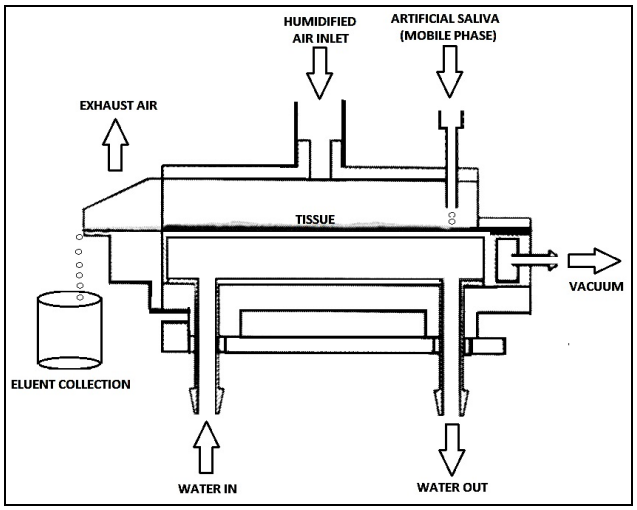

Fig. 6. Porcine oesophagal mucoadhesion test assembly

ASTM D 3359 method using cross-hatch cutter

The ASTM D3359 test method was designed to assess the adhesion of coating films to substrates by applying and removing pressure. A cross-hatch cutter with multiple preset blades was used to make sure that the incisions are properly spaced and parallel (ASTM, 2009). After the tape has been applied and pulled off, the cut area was then inspected and rated according to the percentage of the squared remaining on the test panel. The cross hatch cutter (Fig. 7A) employed in the test was an Elecomer seven blades cross hatch cutter (forming a pattern of 49 squares). The method was recently used to measure mucoadhesion in different drug delivery systems. The method is simple quantitative and suitable for highly adhesive polymer but not sensitive towards moderate and weak adhesive polymers.

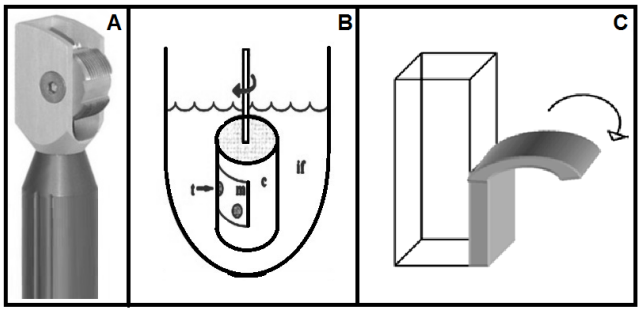

Fig. 7. (A). Cross Hatch Cutter used in STM D 3359 Method; (B). Rotating cylinder for mucoadhesion testing; (C). Peel adhesion tests apparatus

Rotating cylinder method

Bernkop-Schnürch et al. (2003) developed the rotating cylinder method for mucoadhesion testing. In this method, 30 mg of HA-Cys and control tablets were attached to a freshly excised intestinal porcine mucosa, which was fixed on a stainless steel cylinder (diameter: 4.4 cm; height 5.1 cm; apparatus 4-cylinder, USP) (Fig. 7B). Thereafter, the cylinder was placed in the dissolution apparatus according to the USP, entirely immersed with 500 mL of 100 mM phosphate buffer pH 6.8 with 1% NaCl, at 37 °C and agitated with 125 rpm. The detachment of the test tablets was determined visually during an observation time of 48 h. This is a very reliable qualitative method because the adhesion time is observed visually but it requires full-time visual inspection until dosage form does not detach.

Peel adhesion tests

The peel adhesion tests were mainly used for the buccal and transdermal patches or films. The test is based on the calculation of energy required to detach the dosage form from the substrate material (usually excised buccal mucosa) attached through the bioadhesive material in the direction as shown in Fig. 7C. The mucoadhesivity is measured by the following equation-

$$\text{Fracture energy } (G) = \frac{P(1-\cos\theta)}{w} = W°(1+k)$$

Where P is the peel force; w is the peeling width; W° is the intrinsic work of adhesion and k is the proportionality constant that accounts for hysteretic losses.

Peel work is the sum of the surface energy that results from the creation of two free surfaces (energy of dewetting) also referred to as the intrinsic work of adhesion (or cohesion), the bulk energy that dissipates into the stripping member and strain energy in the newly detached strip. Whereas, the intrinsic work of adhesion (or cohesion) is independent of peel rate (speed), peel angle, the thickness of the adhesive and thickness of the stripping member (Bundy et al., 2000). This is a very simple quantitative method but the calculation is based on various parameters which make the results complicated.

The colloidal gold staining method

Park (1989) proposed the colloidal gold staining technique for the study of bioadhesion. The technique employs red colloidal gold particles, which were adsorbed on mucin molecules to form mucin–gold conjugates, which upon interaction with bioadhesive hydrogels develops a red colour on the surface. This can be quantified by measuring at 525 nm either the intensity on the hydrogel surface or the conjugates. This is the reliable qualitative as well as quantitative technique but the use of colloidal gold particles steer this technique towards expensive.

Direct staining method

To overcome the expensiveness and complicacy of gold staining method, a novel technique is reported by Kockisch et al. (2001) to evaluate polymer adhesion to the human buccal mucosa. Adhering polymer was visualized by staining with 0.1% w/v of either Alcian blue or Eosin solution, and the uncomplexed dye was removed by washing with 0.25 M sucrose. The extent of polymer adhesion was quantified by measuring the relative staining intensity of control and polymer treated cells by image analysis. Carbopol 974 P, polycarbophil and chitosan were found to adhere to human buccal cells from 0.10% w/w aqueous dispersions of these polymers. Following *in vivo* administration as a mouthwash, these polymers persisted upon the human buccal mucosa for at least one hour. This method is only suitable for assessing the liquid dosage forms, which are widely employed to enhance oral hygiene and to treat local disease conditions of the mouth such as oral candidacies and dental caries.

Atomic force microscopy method

This method is based on the changes in surface topography when the polymer bound on to buccal cell surfaces and this change was determined by atomic force microscopy. Under the observation of AFM, the unbound cells show relative smooth surface characteristics with many small craters like pits and indentations spread over the cell surfaces; while polymer bound cells will lose crater and indentation characteristics and gained a higher surface roughness (Sudhakar et al., 2006). The Buccastem tablets and Gaviscon oral liquid dosage forms have been evaluated by this method (Deacon et al., 2000). The limitation of this method is that the change in surface characteristics of the polymer can give the qualitative idea but the accurate measurement can't be done by this method.

Electrical conductance method

The method is based on the principal of conductometry, which measure simple electric conductance and useful when the comparison between two polymers is needed, but the method is only suitable for liquid or semisolid dosage forms. Some dosage forms such as Suscard tablets and Orabase oral Base dosage forms have been also evaluated by this method (Ahuja et al., 1997). This method has an advantage that the mucoadhesivity and viscosity both can be evaluated at one time.

Electromagnetic force transducer

This is a quantitative method to measure the mucoadhesivity and based on a calibrated electromagnet to detach a magnetic loaded polymeric drug delivery system from a tissue sample (Hertzog and Mathiowitz, 1999). It has the unique ability to record remotely and simultaneously the tensile force information as well as high magnification video images of bioadhesive interactions at near physiological conditions. EMFT measures tissue adhesive forces by monitoring the magnetic force required to exactly oppose the bioadhesive force (Roy and Prabhakar, 2010). This is only the technique which gives result near to in-vivo methods and the video imaging has made this method more reliable but the preparation of magnetic loaded polymeric system and video imaging made the technique more complicated and expensive.

Fluorescent probe method

Park and Robinson (1984) studied polymer interaction with the conjunctival epithelial cell membrane using the fluorescent probe method to understand structural requirements for bioadhesion in order to design improved bioadhesive polymers for oral use. Sudhakar et al. (2006) labelled the membrane lipid bilayer and membrane proteins with pyrene and fluorescein isothiocyanate, respectively and monitored the fluorescence spectra by mixing the cell with candidate bioadhesive. This gave a direct indication of polymer binding and its influence on polymer adhesion. In this method, the membrane lipid bilayered and membrane proteins were labelled with pyrene and fluorescein

isothiocyanate, respectively. The cells were mixed with the mucoadhesive agents and changes in fluorescence spectra were monitored. This gave a direct indication of polymer binding and its influence on the polymer. The corsodyl gel (oromucosal gel) and corlan pellets (Oromucosal pellet) dosage forms have been evaluated by this method.

Lectin binding inhibition technique

The method was developed by Siegel and Gordon (1985). It involves with avidin-biotin complex and a colorimetric detection system to investigate the binding of bioadhesive polymers to buccal epithelial cells. The marker entities were added to their physicochemical properties. The lectin cancanavalian 'A' has been shown to specifically bind to sugar groups present on the surface of buccal cells. If polymer got bind with the buccal cells, they will mask the surface glycoconjugates, thus reducing or inhibiting the binding of cancanavalian 'A'. The Fentanyl Oralet lozenges and Miconazole tablet, dosage forms have been evaluated by this method (Lehr, 2000). This is an indirect quantitative method to measure mucoadhesivity but the problem arises during experiments, can't be overcome with time because other physiological parameters might reduce the binding of cancanavalian 'A'.

Thumb test method

This test is used for the qualitative determination of peel adhesive strength of the polymer and a useful tool in the development of mucoadhesive drug delivery system. The adhesivity was measured by pulling the thumb from the adhesive as a function of the pressure and the contact time. Although the thumb test may not be conclusive, it provides useful information on the peel strength of the polymer (Ahuja et al., 1997). The Saliveze (artificial saliva) and Tibozole tablet dosage forms have been evaluated by this method. The qualitative measurements of mucoadhesivity by this method is very easy and very less time taking and no need to assemble any instrument but sometimes, polymers can interact with thumb and may cause irritation.

Modified USP disintegration apparatus

The *in vitro* residence time was first determined by Nakane et al. (1996) using a modified USP disintegration apparatus (Fig. 8). The disintegration medium composed of 800 mL isotonic phosphate buffer pH 6.75 was maintained at 37 °C. A segment of rabbit intestinal mucosa, 3 cm long, was glued to the surface of a glass slab, vertically attached to the apparatus. The mucoadhesive tablet was hydrated from one surface using 15 mL IPB and then the hydrated surface was brought into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down so that the tablet was completely immersed in the buffer solution at the lowest point and was out at the highest point. The time necessary for complete erosion or detachment of the tablet from the mucosal surface was recorded. The Enapramil solution and Calcitonin tab dosage forms have been evaluated by this method. The method is complying with in vivo physiological condition so it can consider as more reliable but the hydration of solid particles is required prior to adhesion.

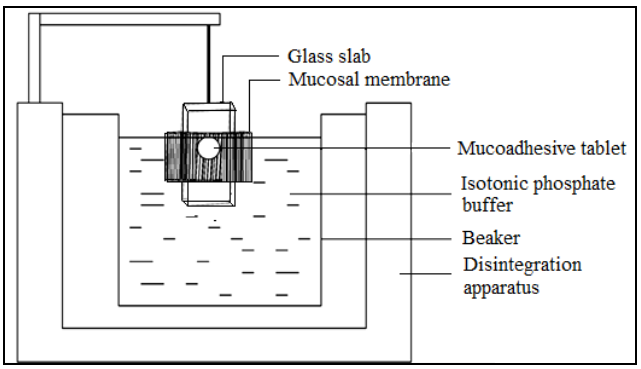

Fig. 8. Schematic diagram of the apparatus used for determining residence time

Scratch test method

The scratch testing method is a comparative test in which critical loads at which failures appear in the samples are used to evaluate the relative cohesive or adhesive properties of a coating or bulk material. In this test, scratches are made on the sample with a spheroconical stylus of 20 to 200 μm size, which is drawn at a constant speed across the sample, under a constant load, or a progressive load with a fixed loading rate. The driving forces for coating damage in the scratch test are a combination of elastic-plastic indentation stresses, frictional stresses and the residual internal stresses (Fig. 9). In the lower load regime, these stresses generally result in conformal or tensile cracking of the coating which still remains fully adherent, whereas in the higher load regime corresponds to the onset of coating detachment from the substrate by spalling, buckling or chipping (Benjamin and Begin 2010).

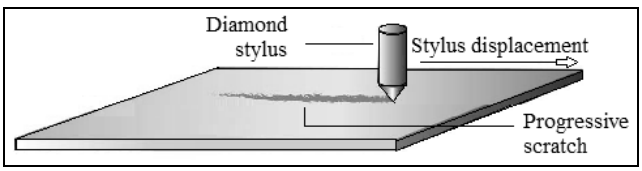

Fig. 9. Scratch test assembly

Adhesion number technique

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 (Kamath and Park, 1994).

Chowdary and Rao (2004) studied the mucoadhesivity of sodium alginate microspheres of nifedipine by adhesion number technique. This is the unique method which tells about the ratio of attached particles to total applied particles and it can be calculated by simple counting of attached and total applied particles. The drawback of this technique is that the counting of the very small particle is a tedious job and at the same time, the method is unable to define the strength of adhesion.

Flow channel method

Mikos and Peppas (1990) invented the flow channel method in which a mucoadhesive polymer particle is placed on a mucous surface in a Plexiglas channel. A laminar flow of air is directed over the microparticles and photographs are taken to analyze the static and dynamic behaviour of the polymer particle. They evaluated the mucoadhesivity of polycarbophil microspheres of Diltiazem with help of this method. The method measures the strength of mucoadhesivity by applying the laminar flow of air to polymer microsphere placed on the mucosal membrane which is totally different with physiological means and this drawback synchronize its use as a reliable measurement. Overall, the method is simple and tells that how efficiently the microspheres would be holding on the mucosal surface *in vivo*.

Novel flow-metrical technique

The flow-metrical technique of mucoadhesion measurements is based on the principal of falling film technique and developed by Ho and Teng (1987). In this method, spherical latex particles are coated with a mucoadhesive material and are suspended in a buffer solution of a known concentration. The rheological properties of the mucoadhesive interface are influenced by the occurrence of interpenetration step in the process of mucoadhesive.

The chain interlocking, conformational changes, and the chemical interaction, which occur between mucoadhesive polymer and mucin chains, produce changes in the rheological behaviour of the two macromolecular species. The rheological studies provide an acceptable *in vitro* model representative of the *in vivo* behaviour of mucoadhesive polymers. With help of this technique, one can just assure the mucoadhesion characteristics of polymers but the accurate quantitative measurement is not possible.

Rolling ball tack method

The method is based on the principal of the falling sphere viscometer which measures the softness of a polymer that relates to tack (ASTM, 2006). In this test, a stainless steel ball of 7/16 inches in diameter is released on the inclined plane or track so that it rolls down and comes in contact with horizontal, upward facing adhesive (Fig. 10). The distance the ball travels along the adhesive track provides the measurement of tack, which is usually expressed in inch. The less tacky the adhesive, the farther the ball will travel. This method only tells about the nature of mucoadhesion and again it fails to measure the adhesion strength.

Fig. 10. Rolling ball tack

Quick stick method

In the quick stick test, the tape is pulled away from the substrate at 90 °C at a speed of 12 inches/min. The peel force required to break the bond between the adhesive and the substrate is measured and recorded as tack value, which is expressed in ounces (or grams) per inch width. The higher values of force required indicate the higher degree of tack (Vyas and Khar, 2002).

Probe tack method

In this method, the probe tack tester is used in which a tip of the clean probe with a defined surface roughness is brought into contact with the adhesive, and when the bond is formed between probe and adhesive, the subsequent removal of the probe mechanically breaks it. The force required to pull the probe away from the adhesive at a fixed rate is recorded as tack which is expressed in grams (Vyas and Khar, 2002).

Wash-off test method

The wash-off test is a very popular method to measures qualitative mucoadhesivity of mucoadhesive substances. Lehr (2000) evaluated the mucoadhesive properties of the microspheres by in vitro wash-off test. In this test, a 1 cm piece of

rat stomach mucosa was tied onto a glass slide (3 by 1 inch) using thread. Microspheres were spread (350) 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 USP (pH 1.2). At the end of 30 minutes, 1 hr, and at hourly intervals up to 10 hrs, the number of microspheres still adhering onto the tissue was counted. The test is also beneficial for all the dosage form like patches, film and tablets and gives reliable results for each. The results obtained from this test can be correlated with in vivo results because the USP disintegrating apparatus offers physiological environment during measurements. The limitation of this test is that the USP disintegration apparatus is used to perform the experiment.

Analytical ultracentrifuge method

The most promising application of this method is to identify the material that is able to form complexes with the mucin. The assay can be done for a change in molecular mass using sedimentation equilibrium. Since complexes can be very large, a more sensible assay procedure is to use sedimentation velocity with change in sedimentation coefficient, s, as their marker for mucoadhesion. Where mucin is available in only miniscule amounts, a special procedure known as Sedimentation Fingerprinting can be used for the assay of the effect on the mucoadhesive. UV absorption optics is used as the optical detection system. However, in this case, the mucoadhesive is invisible, but the pig gastric mucin at the concentrations normally employed is visible. The sedimentation ratio ($S_{complex}/S_{mucin}$), the ratio of the sedimentation coefficient of any complex involving the mucin to that of pure mucin itself, is used as the measure for mucoadhesion. The Emezine™ and Luborant have been evaluated by this method (Sudhakar et al., 2006). This is the indirect test to measure mucoadhesivity but the best method for prediction of the mucorentivity of adhesive material. Sometimes, the gravitational force causes complex which makes the observation more tedious.

Folding endurance method

The folding endurance method was used by Khanna et al. (1997) for the evaluation of mucoadhesion in patches. In this test, they repeatedly folded one patch at the same place till it broke or folded up to 300 times manually, which was considered satisfactory to reveal good patch properties. The number of times of patch could be folded at the same place without breaking gave the value of the folding endurance. This test was done on five patches. The test is simple and reliable but same time it suffers from the limitation like expensiveness because a large number of patches goes to wastage.

In vitro perfusion technique

This is two in one technique to measure the mucoadhesivity as well as perfusion of the drug in same time. In these experiments, a freshly harvested segment of porcine intestine was placed horizontally on a bench top and was connected to the tubing so that the lumen could be perfused with phosphate buffered saline (PBS, pH 7.4, 0.01 M) at a volumetric flow rate of 1 ml/min. A longitudinal incision was made in the intestine to observe the patch's mucoadhesivity (Singh and Rana, 2012). This is the qualitative measurement of mucoadhesivity and based on perfusion of the system.

Texture analyzer method

Alam et al. (2007) worked with XT2 texture analyser method to measure bioadhesive strength of the patches. They used the inverted surface of the chicken pouch as the model tissue to study the bioadhesion. The chicken pouches were kept frozen at –20 °C in a phosphate buffer saline solution (pH 6.75), and only thawed to room temperature before use. The chicken pouch was mounted onto a cylindrical Perspex support of 2 cm diameter and 4 cm length and secured with a string. A foam tape was placed on the Perspex support (underneath the chicken pouch) at the cross-sectional end to provide a cushioning effect. The chicken pouch was further secured and fastened to the foam tape by placing an aluminium cap over the Perspex support. This was to ensure that the tissue adhered firmly to the foam tape and Perspex support so that no movement of the tissue from the foam tape occurred during measurements. A circular hole of 17 mm diameter was made on the top of the cap to expose the chicken pouch for contact with the patches during measurements. The whole perspex support was then positioned at the bottom of the measuring system and held in place by a clamp.

The circular patches of 12 mm diameter were affixed to other Perspex supports of similar dimension using double-sided tape and the support was then screwed onto the upper probe of the instrument. The two Perspex supports were aligned to ensure that the patches come into direct contact with the exposed surface of the chicken pouch when the upper support was lowered. The whole assembly is shown in Fig. 11.

The method gives promising and sensitive quantitative measurement of mucoadhesivity but the assembly is some costly which make study expensive and same time to maintain the experimental conditions are time-consuming.

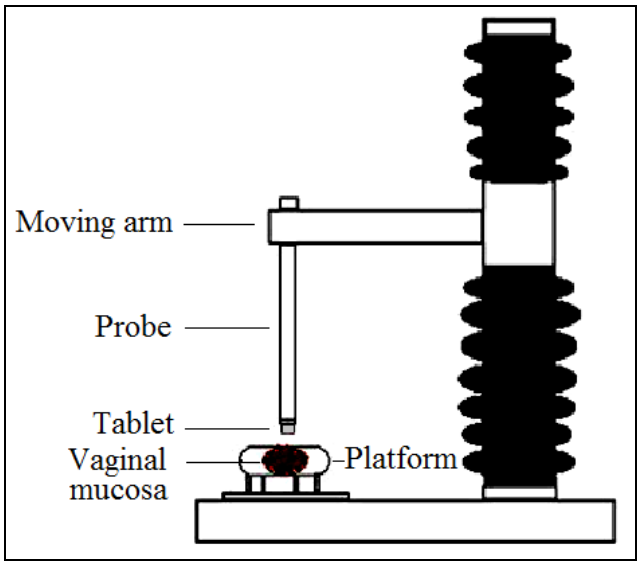

Fig. 11. Texture analyser assembly

Rheological measurement method

The rheological method is a qualitative mean to measure mucoadhesivity and initially suggested by Hassan and Gallo (1990). Within this study, the mucoadhesive potential of polymer candidates was determined by rheologically comparing binary polymer/mucus blends to the rheological sum of similarly concentrated mono-component mucus and polymer systems.

Findings showed that the mucoadhesive polymer/mucus mixtures exhibited synergistic rheological profiles, the causes of which were attributed to bond formation between the polymer and mucus culminating in an increase in total system structure. Since this pioneering work, there have been numerous rheological studies of polymer/mucus interactions. Several authors have suggested that the rheological profiling of polymer–mucus mixtures can provide an acceptable in vitro model representative of the true *in vivo* behaviour of a mucoadhesive polymer (Riley et al., 2001).

Surface force technique

The technique used to study the interaction between mucoadhesive polymers and mucin glycoproteins has done used by Huang et al. (2002) using surface force apparatus (SFA). SFA measures the magnitude and distance dependence of the molecular force acting between two surfaces, with resolutions of the measured force up to 10 nN and distances up to 1 Å.

The method studied the interaction between polymer and mucin so we can also ensure the compatibility between them. Furthermore; the mucoadhesivity can qualitatively ensure by type of interaction takes place between them, for which we need the extra efforts.

Dynamometer apparatus

The dynamometer is a useful instrument for measuring static and dynamic stresses, especially for the measurement of tensile testing of high polymers. This dynamometer measures force by the deformation of a proof ring. Load range and sensitivity can be changed instantaneously by adjusting an attenuator in the electronic circuit; this obviates the necessity for stopping the test and changing the pendulum weight of the conventional test machine, and also gives a much greater range of loads. A fine gain control on the amplifier driving the recorder can be calibrated in terms of specimen thickness, which in conjunction with special scales for use on the recorder chart, makes the instrument record stresses directly in pounds per square inch (Payne and Smith, 1956). Recently, the apparatus is using to measure the mecoadhesivity but the accuracy is questionable with this method because this is not a sensitive apparatus.

Modified balance apparatus

This is the simple and economical method to measure mucoadhesivity because there is no need of a special instrument. In this method, Yong et al. (2001) secured mucosal tissue with a glass vial using a rubber band and an aluminium cap. One vial with a section of tissue is connected to the balance and the other vial is placed on a height-adjustable pan (Fig. 12). The formulation is added onto the vaginal tissue on the other vial. The weights are steadily increased until the two vials are detached. Mucoadhesive force, the detachment stress (dyne/cm^2), is determined from the minimum weight which detaches vials. This is a cheap and best method for the quantitative measurement of mucoadhesivity because the balance exists a sensitive mean at decimal level. The method tells only about adhesivity but it is not the method to measure retentivity.

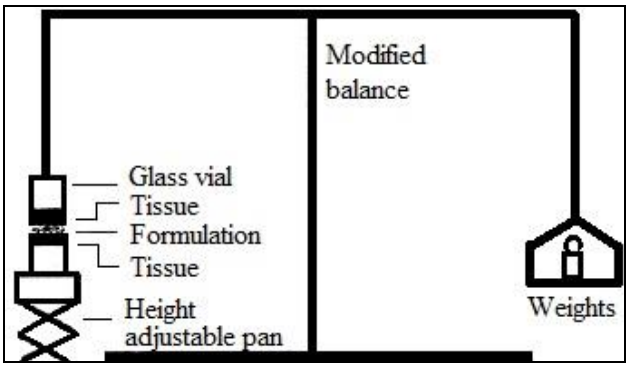

Fig. 12. Mucoadhesive force-measuring device

Agar plate method

Bachav and Patravale (2009) used an agar plate (1%, w/w) to measure the mucoadhesivity of mucoadhesive systems for which they used citrated phosphate buffer of pH 4.5. The test sample is

placed on the centre plate. After 5 min, the agar plate is attached to a USP disintegration test apparatus and moved up and down in pH 4.5 citrate-phosphate buffer at 37±1°C. The sample on the plate is immersed in the solution at the lowest point and is taken out of the solution at the highest point. The residence time of the test samples on the plate is noted visually. The experimental set-up of this method is illustrated in Fig. 13.

This is unique in vitro method to measure mucoadhesivity in qualitative mean, and this method would be a good alternate for *ex vivo* method but the correlation of agar plate with a mucosal tissue does not exist in real experimental protocols.

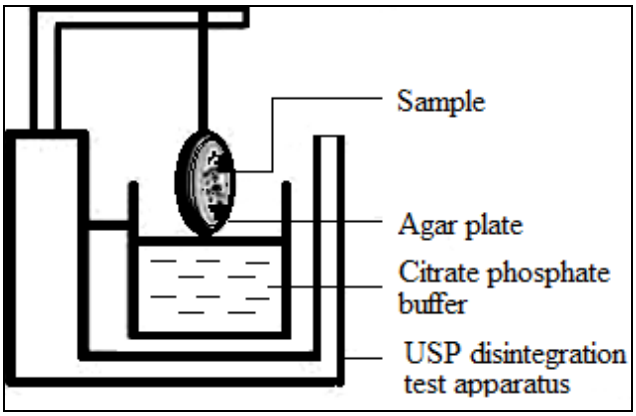

Fig. 13. The schematic configuration of the apparatus used for agar plate method

Glass spheres method

Glass spheres method is a simple, quantitative and realistic in situ method to evaluate the mucoadhesive potential of polymers. In this method, the glass spheres or drug crystals are coated with the polymers and known amounts of these coated particles are placed on mucosal tissue which is kept in a humid environment. The tissue is then washed with a proper buffer solution at a constant rate. The particles percentage retained on the tissue is considered as an index of mucoadhesion (Rao and Buri, 1989).

This is the best method to evaluate mucoadhesivity in solid particles but the liquid or semisolid formulation cannot be evaluated effectively.

Fluorimetric analysis technique

The continual evolution of such *in vitro* techniques has been seen in the work by Batchelor et al. (2002). In this technique, the fluorescently labelled alginate solutions of the known rheological profile were delivered onto the porcine oesophageal tissue. A washing solution was applied at a specified rate to mimic saliva flow, and the eluted material collected with the degree of retention over time measured via fluorimetric analysis. The analytical techniques are well popular for their accuracy and reliability so we can consider such techniques to measure mucoadhesivity.

Imaging technique

An imaging technique that did not use fluorescently labelled polymers was derived by Kockisch et al. (2001). They developed a semi-quantitative image analysis-based technique for the *in vitro* and *in vivo* detections of polymers with an affinity for the mucosal surfaces of the oral cavity. This technique was used to analyse various well-known mucoadhesive polymers, allowing the visualisation of the adhering polymers to buccal cell scrapings. Visualisation of adhesion was aided through staining with 0.1% (w/v) of either Alcian blue (for polyanions) or Eosin (for chitosan) solution with the uncomplexed dye being removed with 0.25 M sucrose washings. The extent of polymer adhesion was then quantified by measuring the relative staining intensity of control and polymer-treated cells by image analysis.

Lowering platform technique

A lowering platform was used to measure the force of detachment of mucoadhesive tablet formulation (Alur et al., 1999). Later on, Yoo et al. (2006) redefined the method and measured the tensile strength of mucoadhesive films. The polymer film was cut into a narrow strip and was placed between the higher and the lower grip of a Chatillon Digital Force Gauge. The two grips were kept at a distance of 10 mm on the same plane, and the hand wheel attached to the lower grip was rotated gradually until the film ruptured. The load at the moment of rupture was recorded and tensile strength was calculated using the following equation. Tensile strength (σ) = Maximum load in Newton (F)/Minimum cross-sectional area of the film specimen in mm2 (MA). The schematic configuration of the platform is shown in Fig. 14. With the help of this method, one can measure the retentivity along with adhesivity but the factors which affect the adhesion in vivo cannot be defined in *ex vivo* study.

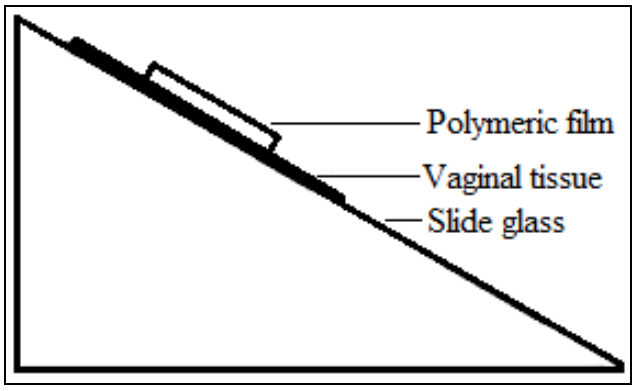

Fig. 14. Lowering platform to measure the detachment force of the mucoadhesive film

BIACORE instrumentation method

A completely different *in vitro* technique was carried out by Takeuchi et al. (2005) who looked at the measurement of mucoadhesion of various adhesive polymers via the BIACORE instrumentation. This system is based on the principle underlying an optical phenomenon called Surface Plasmon Resonance (SPR). Such a system measures the refractive index, which varies with the solute content of a solution that comes in contact with the sensor chip. An SRP response is achieved when a molecule becomes attached to the surface of the sensor chip as the solute concentration on the chip increases. As such, quantitative measurements can be achieved via the binding interaction between the chip surface and one or more functional groups such as NH2, SH, CHO and COOH. The procedure itself involved the immobilization of mucoadhesive polymer on the sensor chip surface with a mucin suspension being passed over the sensor chip for a predefined time. When the mucin particle binds to the polymer on the sensor chip surface, the increased response is measured; when they dissociate, the response will fall. Such an instrument setup allows for the real-time measurement and label-free detection of polymer mucin binding.

Ex vivo methods

The *ex vivo* methods are gaining popularity these days because they provide the results similar to in vivo methods. In these methods, the single mucosal tissue can be utilized in different setups to measure mucoadhesivity. The limitation of *ex vivo* methods includes the sacrifice of an animal is required but the reliability made these methods promising.

Sacs technique

Keely et al. (2005) worked with the sacs technique to measure mucoadhesion of poly (methacrylate) and *N*-trimethylated chitosan polymers using rat intestinal tissue models. They starved rats 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, each 5 cm long, were cut from the isolated jejunum. Sacs were placed in the oxygenated TC199 medium at 37 °C according to the method of Barthe et al. (1998).

The sacs were tied tightly at one end with silk suture and a small animal vascular catheter was tied into the other end. One mL syringe with a sterile 26 gauge micro lance was fixed to the catheter. In some instances, intestinal sacs were pre-treated with 10 mM NAC for 15 minutes, which was flushed out with 20 mL of medium. Sacs were then 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 50 µL samples of incubation medium were removed from the bath after 30 min to assess leakage. Sacs were then removed from the bath and the internal contents recovered using a fresh 1mL volume syringe.

Following aspiration of the polymer-loaded donor compartment, sacs were then washed sequentially four times with a total of 5mL of medium and the washes collected for assay. Samples were adjusted to pH 7.4 by addition of sodium citrate (10 mM) and assayed by fluorescence technique. Adhesion to sacs was calculated by subtraction and expressed as µg polymer/cm^2.

Visualization with dyes

Dyes are used to determining the retention time of drug delivery systems. In research, to test the retention of mucoadhesive delivery systems on vaginal tissues, formulations containing 2% blue lake dye (FD&C blue#1) were intravaginally administered into mice using a micropipette tip. The homogeneity of the mixture was sufficient and it was also easy to follow the remaining vehicle on the mucosal tissue. After 1 hr 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 (Oh et al., 2003).

Modified Setnikar-Fanteli technique

This is a technique for *ex vivo* mucoadhesion/retention studies as outlined in Fig. 15 in which the distilled water is circulated through the two small side arms into the glass cell with a pump (Alam et al., 2007). Ceschel et al. (2001) evaluated the adherence of a new dosage form for clotrimazole comprising a mucoadhesive polymer (polycarbophil, hydroxypropylmethylcellulose and hyaluronic sodium salt) in pessaries made of semisynthetic solid triglycerides using the test technique of Satnikar-Fantelli in a modified way. This test simulates physiological vaginal conditions and verifies the efficiency of the polymers in prolonging the permanence of the dosage form in the location where it is applied. The technological controls demonstrated that the presence of the polymers did not have an influence on the characteristics of the pessaries.

On the other hand, there was an improvement in the adhesiveness of the pessaries in the in vitro adhesion test and a prolongation of the liquefaction time in the liquefaction time test in the presence of mucoadhesive polymers, which increased with increasing polymer concentration. The presence of the mucoadhesive had a significant impact on the

adherence of the drug on the simulated application site. Among the employed mucoadhesive polymers (polycarbophil, hydroxypropylmethylcellulose and hyaluronic sodium salt), polycarbophil in the highest tested concentration turned out most promising.

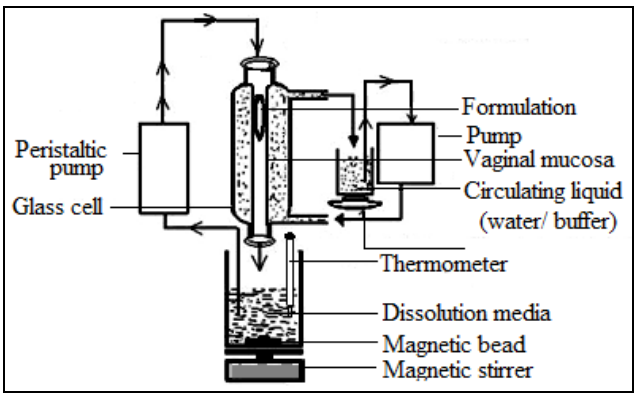

Fig. 15. Apparatus for *ex vivo* experiments for mucoadhesion

In vivo methods

Due to cost, time constraints and ethical considerations, *in vivo* mucoadhesive studies are less commonly seen in the literature than in vitro testing. Despite these concerns in vivo testing is still 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.

Gamma scintigraphy technique

The technique offer the information non-invasively in terms of oral dosage forms across the different regions of GI tract, the time and site of disintegration of dosage forms, the site of drug absorption, and also the effect of food, disease, and size of the dosage form on the *in vivo* performance of the dosage forms (Roy and Prabhakar, 2010). The distribution and retention time of the mucoadhesive intravaginal microspheres have been studied using the gamma scintigraphy technique.

The study has reported the intensity and distribution of radioactivity in the genital tract after administration of technetium labelled hyaluronic acid esters microspheres. Dimensions of the vaginal cavity of the sheep can be outlined and imaged using labelled gellan gum and the data collected is subsequently used to compare the distribution of radiolabelled HYAFF formulations. The retention of mucoadhesive-radio labelled microspheres based on HYAFF polymer was found to be more for the dry powder formulation than for the pessary formulation after 12 hrs of administration to vaginal epithelium.

The combination of sheep model and gamma Scintigraphy method has been proved to be an extremely useful tool for evaluating the distribution, spreading and clearance of vaginally administered mucoadhesive drug delivery system, including microbicides.

GI transit using radio-opaque technique

The technique is very simple and involves the use of radio-opaque markers, *e.g.* barium sulfate, encapsulated in mucoadhesive polymers to determine the effects of mucoadhesive polymers on GI transit time. Faeces collection (using an automated faeces collection machine) 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 been used to study the transit of the microspheres in the GI tract (Mathiowitz et al., 1999).

Radioisotopes and fluorescent labelling techniques

Time measurements of the residence time of mucoadhesive 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 fluorescent labelling techniques (Pandey, 2010).

Surface characterization technique

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 tunnelling microscopy (STM). To assess the effect of surface morphology on the mucoadhesive properties, the microsphere samples are lyophilized and analyzed under SEM at 150 m and 1000 m. 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 surfaces 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 (Pandey, 2010).

Oro-gum adherent technique

Perioli et al. (2007) suggested this method for mucoadhesive tablets. They kept tablet to the oral gum mucosa and evaluated residence times above 12 hrs. The method having some advantages over other in vivo methods, such as the visual inspection can be studied. The oro-gum adherent technique is given in Fig. 16.

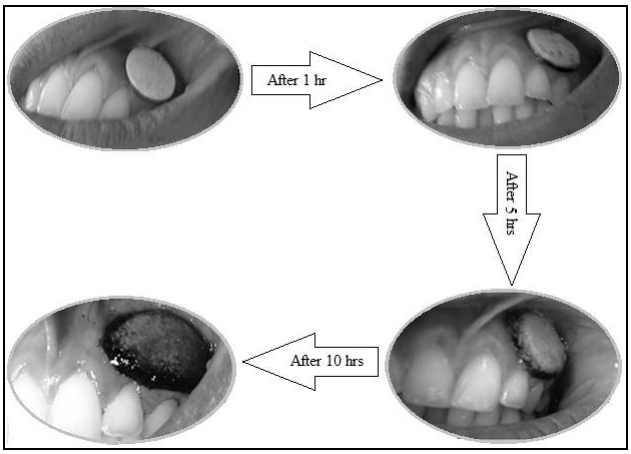

Fig. 16. Oro-gum adherent technique

Magnetic resonance imaging technique

A more advanced non-invasive imaging technique was presented by Albrecht et al. (2006). The investigators used magnetic resonance imaging to localise the point of release of thiolated polymers from dosage forms via the use of gadolinium. In vivo mucoadhesion was determined by ascertaining the residence time of the fluorescently-tagged thiomer on intestinal mucosa of rats after 3 hrs. This technique allowed the comparison of mucoadhesive properties of candidate polymer systems for oral drug delivery *in vivo*.

CONCLUSION

The idea of using bioadhesive materials in contact with mucosal surfaces, as a strategy to improve the efficacy of therapeutic treatments, has been of great interest in the pharmaceutical field since the early developments in mucoadhesion. Despite the lack of a universal test for the measurement of mucoadhesivity, numerous techniques are available that allow for mucoadhesive ranking of polymer systems. Such systems are usually *in vitro* and *ex vivo* in nature due to their relative ease of implementation and cost-effectiveness and as such may present an efficient way of selecting candidate delivery systems for further more intensive *in vivo* testing.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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How to cite this article?

Semwal R, Semwal RB, Semwal DK (2018). Mucoadhesive assessment – An encyclopedic review. Current Medical and Drug Research, 2 (2), Article ID 187.

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