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MUCOADHESIVE VAGINAL DRUG DELIVERY SYSTEM: A REVIEW ON ADVANCE STATUS

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ABSTRACT

Mucoadhesive delivery systems offer several advantages over other oral controlled release systems by virtue of prolongation of residence time of drug, its targeting, and localization of the dosage form at a specific site. These advantages include bypass of first pass metabolism of the drug and hence more concentration of the drug is available for absorption. Mucoadhesion occurs between two surfaces, one of which is a mucous membrane and another is drug delivery system. These mucoadhesive systems are known to provide intimate contact between dosage form and the absorptive mucosa, resulting in a high drug influx through the absorbing tissue. Mucoadhesive formulations use polymers as the adhesive component. Mucoadhesive drug delivery systems are available in the form of tablets, films, patches, and gels for oral, buccal, nasal, ocular, vaginal, rectal and topical routes for both systemic and local effects. To design an effective particulate drug delivery system having mucoadhesive function, several mucoadhesion tests for polymers and for the resultant delivery systems should be developed. This paper lays main emphasis on evaluation parameters. This review article presents the theories of mucoadhesion and factors affecting mucoadhesion and techniques for *invitro* and *in vivo* evaluation of mucoadhesive dosage forms.

Keywords: Mucoadhesion, Theories, Factors affecting mucoadhesion, Vaginal drug administration, Various evaluation parameters.

INTRODUCTION

Mucoadhesion is commonly defined as the adhesion between two materials, at least one of which is a mucosal surface. Over the past few decades, mucosal drug delivery has received a great deal of attention. Mucoadhesive dosage forms may be designed to enable prolonged retention at the site of application, providing a controlled rate of drug release for improved therapeutic outcome. Application of dosage forms to mucosal surfaces may be of benefit to drug molecules not amenable to the oral route, such as those that undergo acid degradation or extensive first-pass metabolism. The mucoadhesive ability of a dosage form is dependent upon a variety of factors, including the nature of the mucosal tissue and the physicochemical properties of the polymeric formulation. This review article aims to provide an overview of the various aspects of mucoadhesion, mucoadhesive materials, factors affecting mucoadhesion, evaluating methods, and finally various mucoadhesive drug delivery systems (buccal, nasal, ocular, gastro, vaginal, and rectal) [1].

The vaginal cavity is an important area of the reproductive tract and acts as a favourable site for drug administration due to avoidance of first pass effect, large permeation area, rich vascularization and relatively low enzymatic activity. In recent years, research has been focused on vaginal drug delivery systems as logical alternatives to oral or parenteral drug administration. Many studies have demonstrated the superiority of vaginal over oral drug administration in terms of minimizing general and gastrointestinal side effects. The search for non-invasive drug delivery systems continues due to poor patient compliance and acceptance, limited market size and drug uses, coupled with the high cost of disease management. The vaginal cavity has a potential for non-invasive, controlled transmucosal delivery of both local and systemic therapeutically active

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compounds. The vagina has a great potential for systemic delivery of a wide range of compounds including proteins and peptides. Formulation and delivery of microbicides is being developed as a new therapeutic approach to prevent HIV and other sexually transmitted diseases (STDs). The vaginal cavity is also an effective site for the uterine targeting of various therapeutic agents such as terbutaline, progesterone and danazol. Recently, the vagina has been studied as a novel route for the delivery of chemotherapeutic agents for treatment of all cancer. Creams, tablets, gels, suppositories, foams, ointments, tampons and inserts are commonly used as vaginal drug delivery systems. The currently available vaginal dosage forms have certain limitations such as messiness, leakage and low residence time, leading to poor patient compliance and loss of therapeutic efficacy. Therefore, novel concepts and dosage forms are needed. Extensive research is ongoing to develop better vaginal drug delivery systems that can full fill the user's requirements. Some of the vaginal products recently introduced into the market and in various stage of development are listed in Table 1. This review highlights several recent advances in vaginal drug delivery [2].

MUCUS MEMBRANES

Mucus membranes (mucosae) are the moist surfaces lining the walls of various body cavities such as the gastrointestinal and respiratory tracts. They consist of a connective tissue layer (the lamina propria) above which is an epithelial layer, the surface of which is made moist usually by the presence of mucus layer. The epithelial may be either single layered (example, the stomach, small and large intestines and bronchi) or multilayered or stratified (example, in the esophagus, vagina and cornea). The former contain goblet cells which secret mucus directly onto the epithelial surfaces; the latter contain, or are adjacent to tissue containing specialized glands such as salivary glands that secrete mucus onto the epithelial surface. Mucus is present either as a gel layer adherent to the mucosal surface or as a luminal soluble or suspended form. The major components of all mucus gels are mucin glycoproteins, lipids, inorganic salts and water, the later accounting for more than 95% of their weight making them a highly hydrated system [3].

MECHANISM OF MUCOADHESION

The mechanism of mucoadhesion is generally divided into two steps; the contact stage and the consolidation stage. The first stage is characterized by the contact between the mucoadhesive and the mucus membrane with spreading and swelling of the formulation, initially its deep contact with the mucus layer.

In the consolidation step the mucoadhesive material are activated by the presence of moisture, Moisture plasticizer the system allowing the mucoadhesive molecules to break free and to link up by weak Vander Waals and hydrogen bonds. Essentially, there are two theories explaining the consolidation step; the diffusion theory and the dehydration theory. According to the diffusion theory; the mucoadhesive molecules and the glycoproteins of the mucus mutually interact by means of interpenetration of their chains and the building of secondary bonds. For this to take place, the mucoadhesive device has features favouring both chemical and mechanical interactions. For example, molecules with hydrogen bond building groups(-OH,-COOH), an anionic surface charge, high molecular weight, flexible chains and surface active properties, which help in spreading throughout the mucus layer can present mucoadhesive properties [4].

MUCOADHISIVE THEORIES

Mucoadhesive is a complex process and numerous theories have been proposed to explain the mechanism involved. These theories include mechanical interlocking,

- Wetting theory
- Diffusion theory
- Fracture theory
- Electrostatic theory
- Absorption theory

Wetting Theory

The wetting theory applies to liquids systems which present affinity to the surface in order to spread over it. This affinity can be found by using measuring techniques such as the contact angel, the greater is the affinity (figure 3). The contact angle should be equal or close to zero to provide adequate Spreadability. The Spreadability coefficient S_{AB}, can be calculated from the difference between the surface energies γB and γA and the interfacial energy γAB as indicated in an equation given below. This theory explains the importance of contact angle and reduction of surface and interfacial energies to achieve good amount of mucoadhesion [3].

$$S_{AB} = \gamma_B - \gamma_A - \gamma_{AB}$$

Diffusion Theory

Diffusion theory describes the interpenetration of both polymer and mucin chains to a sufficient depth to create a semi-permanent adhesive bond (fig4). It is believed that the adhesion force increases with the degree of penetration of the polymer chains. This penetration rate depends on the diffusion coefficient, flexibility and nature of the mucoadhesive chains, mobility and contact time. According to the literature, the depth of interpenetration required to produce an efficient bioadhesive bond lies to the range 0.2-.5μm. This interpenetration depth of polymer and mucin chains can be estimated by the following equation.

$$l = (tD_b)^{1/2}$$

Where t is the contact time and D is the diffusion coefficient of the mucoadhesion material in the mucus. The adhesion strength for a polymer is reached when the depth of penetration is approximately equivalent to the polymer chain size, In order for diffusion to occur, it is important that the components involved have good mutual solubility, that is both the bioadhesive and the mucus have similar chemical structures. The greater the structural similarity, the better is the mucoadhesive bond [3].

Fracture Theory

This is perhaps the most used theory in studies on the mechanical measurement of mucoadhesion. It analyzes the force required to separate two surfaces after adhesion is established. This force S_m is frequently calculated in tests of resistance to rupture by the ratio of maximal detachment force F_m and the total surface area A_o involved in the adhesive interaction.

$$S_{m=\frac{F_m}{A_o}}$$

Since the fracture theory (fig 5) is concerned only with the force required to separate the parts, it does not take into account the interpenetration or diffusion of polymer chains. Consequently, it is appropriate for use in the calculations for rigid or semi-rigid bioadhesive materials, in which the polymer chain do not penetrate into the mucus layer [4].

The Electronic Theory

This theory describes adhesion occurring by means of electron transfer between the mucus and the mucoadhesive system, arising through differences in their electronic structures. The electron transfer between the mucus and the mucoadhesive results in the formation of double layer of electrical charges at the mucus and mucoadhesive interface. The net result of such process is the formation of attractive forces within the double layer [5].

Adsorption Theory

In this instance, adhesion is the result of various surface interactions (primary and secondary bonding) between the adhesive polymer and mucus substrate. Primary bonds due to chemisorptions result in adhesion due to ionic, covalent and metallic bonding, which is generally undesirable due to their permanency. Secondary bonds arise mainly due to vander walls forces, hydrophobic interactions and hydrogen bonding. Whilst these interactions require less energy to "break", they are the most prominent from of surface interaction in mucoadhesion process as they have the advantages of being semi-permanent bonds [6].

All these numerous theories should be considered as supplementary process involved in the different stages of the mucus/substrate interaction, rather than individual and alternative theories. Each and every theory is equally important to describe the mucoadhesion process. There is a possibility that there will be initial wetting of the mucin and then diffusion of the polymer into mucin layer, thus causing the fracture in the layers to effect the adhesion or electronic transfer or simple adsorption phenomenon that finally leads to the perfect mucoadhesion. The mechanism by which mucoadhesive bond is formed will depend on the nature of the mucus membrane and mucoadhesive material the type of formation, the attachment process and the subsequent environment of the bond. It is apparent that a single mechanism for mucoadhesion proposed in many texts is unlikely for all the different occasions when adhesion occurs [7].

Factors Affecting Mucoadhesion

Mucoadhesion may be affected by a number of factors, including hydrophilicity, molecular weight, cross-linking, swelling, pH, and the concentration of the active polymer. [7,8,9]

Hydrophilicity

Bioadhesive polymers possess numerous hydrophilic functional groups, such as hydroxyl and carboxyl. These groups allow hydrogen bonding with the substrate, swelling in aqueous media, thereby allowing maximal exposure of potential anchor sites. In addition, swollen polymers have the maximum distance between their chains leading to increased chain flexibility and efficient penetration of the substrate.

Molecular Weight

The interpenetration of polymer molecules is favoured by low-molecular-weight polymers, whereas entanglements are favoured at higher molecular weights. The optimum molecular weight for the maximum mucoadhesion depends on the type of polymer, with bioadhesive forces increasing with the molecular weight of the polymer up to 100,000. Beyond this level, there is no further gain [10].

Cross-Linking and Swelling

Cross-link density is inversely proportional to the degree of swelling [11]. The lower the cross-link density, the higher the flexibility and hydration rate; the larger the surface area of the polymer, the better the mucoadhesion. To achieve a high degree of swelling, a lightly cross-linked polymer is favoured. However, if too much moisture is present and the degree of swelling is too great, a slippy mucilage results and this can be easily removed from the substrate [12]. The mucoadhesion of cross-linked polymers can be enhanced by the inclusion in the formulation of adhesion promoters, such as free polymer chains and polymers grafted onto the preformed network [9].

Spatial Conformation

Besides molecular weight or chain length, spatial

conformation of a polymer is also important. Despite a high molecular weight of 19,500,000 for dextrans, they have adhesive strength similar to that of polyethylene glycol (PEG), with a molecular weight of 200,000. The helical conformation of dextran may shield many adhesively active groups, primarily responsible for adhesion, unlike PEG polymers, which have a linear conformation [7].

pH

The pH at the bioadhesive to substrate interface can influence the adhesion of bioadhesives possessing ionisable groups. Many bioadhesives used in drug delivery are polyanions possessing carboxylic acid functionalities. If the local pH is above the p*K* of the polymer, it will be largely ionized; if the pH is below the p*K* of the polymer, it will be largely unionized. The approximate pK^a for the poly(acrylic acid) family of polymers is between 4 and 5. The maximum adhesive strength of these polymers is observed around pH 4–5 and decreases gradually above a pH of 6. A systematic investigation of the mechanisms of mucoadhesion clearly showed that the protonated carboxyl groups, rather than the ionized carboxyl groups, react with mucin molecules, presumably by the simultaneous formation of numerous hydrogen bonds [13].

Concentration of Active Polymer

Ahuja [8] stated that there is an optimum concentration of polymer corresponding to the best mucoadhesion. In highly concentrated systems, beyond the optimum concentration the adhesive strength drops significantly. In concentrated solutions, the coiled molecules become solvent-poor and the chains available for interpenetration are not numerous. This result seems to be of interest only for more or less liquid mucoadhesive formulations. It was shown by Duchene [14] that, for solid dosage forms such as tablets, the higher the polymer concentration, the stronger the mucoadhesion.

Drug/Excipient Concentration

Drug/excipient concentration may influence the mucoadhesion. BlancoFuente [15] studied the effect of propronolol hydrochloride to Carbopol® (a lightly cross-linked poly (acrylic acid) polymer) hydrogels adhesion. Author demonstrated increased adhesion when water was limited in the system due to an increase in the elasticity, caused by the complex formation between drug and the polymer. While in the presence of large quantities of water, the complex precipitated out, leading to a slight decrease in the adhesive character. Increasing toluidine blue O (TBO) concentration in mucoadhesive patches based on Gantrez® (poly(methylvinylether/maleic acid) significantly increased mucoadhesion to porcine cheek tissue.[16] This was attributed to increased internal cohesion within the patches due to electrostatic interactions between the cationic drug and anionic copolymer.

Other Factors Affecting Mucoadhesion

Mucoadhesion may be affected by the initial force of application. [17] Higher forces lead to enhanced interpenetration and high bioadhesive strength. In addition, the greater the initial contact time between bioadhesive and substrate, the greater the swelling and interpenetration of polymer chains.[18] Physiological variables can also affect mucoadhesion. The rate of mucus turnover can be affected by disease states and also by the presence of a bioadhesive device. [19] In addition, the nature of the surface presented to the bioadhesive formulation can vary significantly depending on the body site and the presence of local or systemic disease.

Benefits of Vaginal Drug Administration

In the vagina, arteries and veins form a dense network which provides a rich blood supply and consequently the vagina is well suited for the rapid and steady uptake of hormones. Drugs administered via the vagina are not subject to the first-pass effect and gastrointestinal interferences with absorption of medication are avoided. This has been demonstrated by the greater bioavailability of misoprostol following vaginal as opposed to oral administration. Vaginal administration often minimizes side effects associated with the oral route. An example is the administration of bromocriptine vaginally in treatment of hyperprolactinemia in women who suffer from nausea and vomiting following oral administration.

Bioadhesive vaginal delivery systems have several advantages when compared to conventional dosage forms. Firstly, the bioadhesive vaginal formulations are readily localized in the region of application thus improving the bioavailability of drugs. Greater bioavailability of insulin, calcitonin, progesterone and estrogen was observed from bioadhesive vaginal formulations. Secondly, these delivery systems provide intimate contact of the formulation with the underlying absorption surface. This allows for modification of tissue permeability for absorption of macromolecules such as proteins and peptides. Thirdly, it permits continuous and prolonged residence of the dosage form at the site of application. Lastly, it reduces side effects due to avoidance of repeated administration of the drug [2].

Vaginal Anatomy and Physiology With Respect To Drug Delivery

The vagina is a fibromuscular tube approximately 10 cm in length comprised of three distinct layers namely an outer adventitial layer, a middle muscularis layer and an innermost mucosal layer. The vaginal rugae and micro ridges on the epithelial cell surface permit the vagina to expand, allow the placement of vaginal formulations and increase the surface area of the vagina thus enhancing drug absorption. The vagina has remarkable features in terms of vaginal secretion, pH, enzyme activity and micro flora.

These factors affect formulation spreading and retention as well as absorption and drug release in vagina [2].

Vaginal Secretions

The vaginal discharge is a mixture of multiple secretions that collect in the vagina from peritoneal, follicular tubal, uterine, Bartholin's and Skene's glands. In presence of moisture, solid dosage formulations should ideally disperse in the vaginal canal immediately after insertion to avoid inconvenience to the users.

Enzyme Activity

The specific enzymatic activity of four different amino peptidases in vaginal homogenates decreases in the order: sheep > guinea pig > rabbit ≥ human ≥ rat. The human genital tract has lower enzymatic activity leading to less degradation of protein and peptide drugs in the vagina than the gastrointestinal tract [20].

Vaginal pH

The pH of the healthy female genital tract is acidic (pH 3.5–4.5) and is maintained within that range by bacterial conversion of glycogen from exfoliated epithelial cells to lactic acid.

Vaginal Routes of Drug Absorption

The drug is delivered in the vagina mainly via two routes: intravaginally to the vaginal epithelium or transvaginally through the vaginal mucosa to uterus and systemic circulation [20]. Vagina has specific blood flow characteristics, either by a portal type circulation or by venous and lymphatic channels that allow bypassing the gastrointestinal tract absorption and liver detoxification and permit preferential transport of drug molecules from the vagina to the uterus and systemic circulation. Several physical models have been devised to study the vaginal permeability of drugs. Many therapeutic compounds have been shown to be absorbed through the vaginal mucosa. Antifungal agents such as tioconazole, clotrimazole and miconazole are topically administered to treat vaginal yeast infections. On the basis of our knowledge of anatomical and physiological features of the vagina, it is likely that many other drugs will be formulated for vaginal administration in the future. [2]

Techniques for Evaluation of Mucoadhesion

[A] In Vitro Methods

1. Tensile strength measurement.
2. Shear strength measurement.
3. Modified physical balance.
4. Detachment force method.
5. Microbalance method.
6. Ex – vivo mucoadhesion.
7. Falling film method.
8. Swelling index.
9. Wash off method.
10. Colloidal gold staining.
11. Adhesion number.
12. Viscometric method.
13. Everted sac technique.
14. Drug permeation.
15. Fluorescent probe method.
16. Mucoadhesion time.
17. Surface pH study.
18. Scanning Electron microscopy. (SEM)
19. Novel Rheological Approach.
20. Texture analyzer.

[B] In Vivo Methods

1. Use of radioisotopes.
2. Use of gamma scintigraphy.
3. X-ray studies
4. In vivo evaluation of mucoadhesive studies
5. Isolated loop technique.

[C] In Vitro As Well As In Vivo Method

1. Biacore.

Techniques for Evaluating Bioadhesive Properties

Various in vitro and in vivo methods are used for testing the efficacy of the mucoadhesive nature of a polymer matrix. The methods used to evaluate mucoadhesion include the following:-

A. In vitro / Ex vivo methods

In vitro tests were initially designed to screen potential bioadhesion, because an evaluation of bioadhesive properties is fundamental to the development of new bioadhesives. There are various in vitro experimental setups which have evolved from simple measurements to more sophisticated and expensive setups [21]. The most commonly employed in vitro techniques are:-

1. Tensile Strength Measurement

***a)* Wilhelmy Plate Technique**

The Wilhelmy plate technique is traditionally used for the measurement of dynamic contact angles. The instrument measures the bioadhesive force between mucosal tissue and the dosage form. By using the CAHN software system, parameters such as fracture strength, deformation to failure and work of adhesion can be analysed [22].

Fracture strength

It is the maximum force per unit surface area required to break the adhesive bond.

Deformation to failure

It is the distance required to move the stage before complete separation occurs. This parameter is dependent

on the material stiffness and the intensity of strength of adhesion.

Work of adhesion

It is a function of both the fracture strength and the deformation to failure. It tends to be the strongest indicator of the mucoadhesive potential [23].

Method

A small glass plate (2×5cm) was coated with 1% w/v of the mucoadhesive agent. The mucus gel was taken from goat intestine kept in a suitable container, where the above mentioned glass plate can be kept in contact with gel in a balanced condition and the temperature was maintain at30°C. Nylon thread was attached at one end of the glass plate. Provision was given to raise the weight at the other end. At specified intervals, weight was added to detach the coated glass plate from gel and the force required to pull the plate out of the gel was determined under experimental condition. Six plates were tested for each material and the average weights required were calculated [24].

b) Tensile Tester

It is used to measure the adhesive force of the polymer complexes with a plastic (Polyvinylchloride) plate. Polymers and plastic plates were cut with the area 1 cm sq. (thickness: 0.8 mm). The polymer was pre wetted with water and placed on the surface of the plastic plate. They were kept in contact with the plate under the force of "finger tip for 2 min before the measurement. The peak force required to detach the polymer from the plastic plate was measured [25].

c) Electromagnetic Force Transducer (EMFT)

The electromagnetic force transducer (EMFT) is a remote sensing instrument that uses a calibrated electromagnet to detach a magnetic loaded polymer microsphere from a tissue sample. It has the unique ability to record simultaneously the tensile force information as well as high magnification video images of mucoadhesive interactions at near physiological conditions. The EMFT measures tissue adhesive forces by monitoring the magnetic force required to exactly oppose the mucoadhesive force [26].

2. Shear Stress Measurement

The shear stress measures the force that causes a mucoadhesive to slide with respect to the mucus layer in a direction parallel to their place of contact of adhesion.

Principle

Adhesion tests based on the shear stress measurement involve two glass slides coated with polymer and a film of mucous. Mucous forms a thin film between the two polymer coated slides, and the test measures the force required to separate the two surfaces. [23, 25, 27]

Method

Two smooth, polished plexi glass boxes were selected; one block was fixed with adhesive araldite on a glass plate, which was fixed on levelled table. The level was adjusted with the spirit level. To the upper block, a thread was tied and the thread was passed down through a pulley. At the end of the thread a pan was attached into which the weights can be added. [28]

3. Modified Physical Balance

A modified balance method was used to determine the ex vivo mucoadhesive strength. Fresh sheep buccal mucosa or goat stomach mucosa or rat stomach mucosa or porcine gastric mucosa was obtained from a local slaughterhouse and used within 2 hours of slaughter. The mucosal membrane was separated by removing underlying fat and loose tissues. The membrane was washed with distilled water and then with proper medium at 37°C.The sheep buccal mucosa was cut into pieces and washed with proper medium. A piece of buccal mucosa was tied to the glass vial, which was filled with phosphate buffer. The glass vial was tightly fitted into a glass beaker (filled with proper medium, at 37°C ± 1°C) so that it just touched the mucosal surface. The buccal tablet was stuck to the lower side of a rubber stopper with adhesive. The two sides of the balance were made equal before the study, by keeping a 5-g weight on the right-hand pan. A weight of 5 g was removed from the right-hand pan, which lowered the pan along with the tablet over the mucosa. The balance was kept in this position for 5 minutes contact time. The water (equivalent to weight) was added slowly with an infusion set (100 drops/min) to the right-hand pan until the tablet detached from the mucosal surface. This detachment force give the mucoadhesive strength of the buccal tablet in grams.[29] The weight of water required to detach mucoadhesive tablet from stomach mucosa was noted as mucoadhesive strength in grams. From the mucoadhesive strength following parameter was calculated.

$$Force\ of\ adhesion(N) = \frac{Bioadhesive\ strength(gm)}{1000} \times 9.8$$

$$Bond\ strength\left(\frac{N}{m^2}\right) = \frac{Force\ of\ adhesion(N)}{Surface\ area\ of\ tablet(m^2)}$$

4. Detachment Force Method

This method is based on the evaluation of mucoadhesive strength, i.e. the force required to break the binding between the model membrane and the mucoadhesive. Depending on the direction in which the mucoadhesive is separated from the substrate, it is possible to obtain the detachment, shear, and rupture tensile strengths [26].

Method

To characterize the mucoadhesive strength, the detachment force method was used. Mouth of a glass vial

fixed with a fresh section of animal tissue from fundus portion of goat intestine, facing mucosal side out and kept in simulated gastric fluid (pH 1.2) without pepsin. Kept another portion of mucus side of exposed tissue over a rubber stopper and secured with an aluminium cap. The mucoadhesive tablet placed on the exposed mucous layer is kept in contact with the former tissue which is connected with a pan in which the weight can be raised. At specific intervals, applied weight and the force required to detach was measured to determine mucoadhesive strength [24].

5. Microbalance Method

The microforce balance technique is used to measure the specific adhesion force of microparticles. This involves the use of a microtensiometer and a microforce balance, yielding both contact angle and surface tension. The mucous membrane is placed in a small mobile chamber with both pH and physiological temperature controlled. A unique microsphere is attached by a thread to the stationary microbalance. The chamber with the mucous membrane is raised until it comes into contact with the microsphere and, after contact time, is lowered back to the initial position [26]

6. Ex Vivo Mucoadhesive Strength Determination

This technique is specific for microspheres and in this technique four number of Albino rats were fasted overnight and then 25 numbers of microspheres (N0) were ingested to these rats through an oral feeding needle. These were then sacrificed at an interval of 0, 4, 8, 12 hours respectively to isolate their stomach and intestine region. The stomach and intestine regions are cut and opened longitudinally to note the number of microspheres adhering to these regions (NS). [30]This ultimately gives the adhesive strength of the formulation which is calculated using the formula given below

% adhesive strength = (Ns/No)*100.

No =Number of microspheres

Ns = Number of microspheres adhered [31].

7. Falling Liquid Film Technique

In this technique male Albino rats (200-250g) were sacrificed and their intestine region was isolated. Then from the intestine region, jejunum part was separated and cut longitudinally. This separated portion was placed on the semi cylindrical Plexi glass support, with a temperature controlled at 37 ºC and is washed with saline solution for 30 minutes at the rate of 30ml /minute. Then 25 numbers (N0) of counted mucoadhesive microspheres are hydrated with little amount of water and are dispersed on the mucosal tissue and left on it for 20 minutes for interaction with mucosal surface. During this period, 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 22ml / minute and the number of microspheres remaining on the mucosal surfaces (NS) are counted. The adhesive strength can be determined using the formula given below:- [23]

% adhesive strength = (Ns/No)*100

No =Number of microspheres

Ns = Number of microspheres adhered.

8. Swelling Index

Swelling of excipients of mucoadhesive dosage form involves the absorption of a liquid resulting in an increase in weight and volume. Liquid uptake by the particle may be due to saturation of capillary spaces within the particles or hydration of macromolecule. The liquid enters the particles through pores and bind to large molecule, breaking the hydrogen bond and resulting in the swelling of particle. The extent of swelling can be measured in terms of% weight gain by the mucoadhesive dosage form [32].

Method

One mucoadhesive dosage form is weighed and placed in a beaker containing 200 ml of buffer media. After each interval the dosage form is removed from beaker and weighed again up to 8 hours. The swelling index is calculated using following formula.

Swelling Index (S.I.) = (Wt-Wo)/Wo

Where, S.I. = Swelling index

Wt = Weight of the dosage form at time t

Wo = Weight of the dosage form before placing in the beaker [33]

9. Wash –Off Test

Wash-off test is used to determine the mucoadhesive property of dosage form. In this test, the mucosal tissue is attached onto a glass slide with the help of a double-sided cyanoacrylate tape. Thereafter, the dosage form is put on the surface of the tissue (exposed mucosal surface) with the subsequent vertical attachment of the system into the USP tablet disintegrator apparatus, which contains 1 L of physiological solution maintained at 370C. The operation of the equipment gives

Up-and-down movement to the tissue-delivery matrix system. In this study, the time for the complete detachment of the delivery system from the mucosal layer is determined [32].

% adhesive strength = (Ns/No)*100.

Where, No = Initial number of the dosage form spread over the mucosal surface.

Ns = Number of the dosage form detaching from the mucosal surface.

10. Colloidal Gold Staining Method

Colloidal gold staining was proposed in 1989 for bioadhesive hydrogels Interactions with mucingold conjugates resulted in the development of a red colour on the hydrogel surface. A direct staining method to evaluate

polymer adhesion to human buccal cells, following exposure to aqueous polymer dispersions, The polymer in a form of strip is incubated with colloidal gold –mucin conjugate and after a rinsing procedure the absorbance of strips is measured [34].

Method

This technique employs red colloidal gold particles, which are stabilized by the adsorbed mucin molecule by forming mucin–gold conjugates. Upon interaction with mucin– gold conjugates, bioadhesive hydrogels develop a red colour on the surface. Thus, the interaction between them can easily be quantified, either by the measurement of the intensity of the red colour on the hydrogel surface or by the measurement of the decrease in the concentration of the conjugates from the absorbance changes [27].

11. Adhesion number

Adhesion number for mucoadhesive microspheres is determined as the ratio of the number of particles attached to the substrate to the total number of applied particles, expressed as a percentage. The adhesion strength increases with an increase in the adhesion number [23].

12. Viscometeric method

A simple viscometric method is used to quantify mucin–polymer bioadhesive bond strength. Viscosities of 15 %w/v porcine gastric mucin dispersion in 0.1M HCl (pH 1) or 0.1M acetate buffer (Ph 5.5) is measured with a Brookfield viscometer in the absence or presence of selected neutral, anionic, and cationic polymers. Viscosity components and the forces of bioadhesion are calculated [27].

13. Everted sac technique

The everted gut sac technique is an example of an ex vivo method. It has been used since 1954 to study intestinal transport. This method is applied on mucoadhesion assays [26].

The everted intestinal sac technique is a passive test for mucoadhesion and involves polymeric microspheres and a section of the everted intestinal tissue. It is performed by using a segment of intestinal tissue excised from the rat, everted, ligated at the ends, and filled with saline. It is then introduced into a tube containing a known amount of the microspheres and saline, and agitated while incubating for 30 min. Sac is then removed, microspheres are washed and lyophilized, and the percentage of binding to the sac is calculated from difference in the weight of the residual spheres from the original weight of the microspheres [23]. The advantage of this technique is no external force is applied to the microspheres being tested. It is easy to reproduce and can be easily performed in laboratories.

14. Drug Permeation

The in vitro buccal drug permeation study of buccal tablet through the sheep buccal mucosa is performed by using Keshary-Chien type glass diffusion cell at 37°C ± 0.2°C. Fresh sheep buccal mucosa is mounted between the donor and receptor compartments. The buccal tablet is placed with the core facing the mucosa and the compartments clamped together. The donor compartment is filled with 1 mL of phosphate buffer pH 6.8. The receptor compartment (15-mL capacity) is filled with phosphate buffer pH 7.4, and the hydrodynamics in the receptor compartment is maintained by stirring with a magnetic bead at 50 rpm. A 1-mL sample is withdrawn at predetermined time intervals and analyzed for drug content at 290 nm using an UV spectrophotometer [29].

15. Fluorescent probe method

In this method the membrane lipid bilayer and membrane proteins are labelled with pyrene and fluorescein isothiocyanate, respectively. The cells are then mixed with candidate bioadhesive, and the changes in fluorescence spectra should be monitored. This gives a direct indication of polymer binding and its influence on polymer adhesion [35].

16. Mucoadhesion Time

It is measured by modified balance method. The ex vivo mucoadhesion time is performed (n = 3) after application of the buccal tablet on freshly cut sheep buccal mucosa. The fresh sheep buccal mucosa is tied on the glass slide, and a mucoadhesive core side of each tablet is wetted with 1 drop of phosphate buffer pH 6.8 and pasted to the sheep buccal mucosa by applying a light force with a fingertip for 30 seconds. The glass slide is then put in the beaker, which is filled with 200mL of the phosphate buffer pH 6.8, and kept at 37°C ± 1°C. After 2 minutes, a 50-rpm stirring rate is applied to simulate the buccal cavity environment, and tablet adhesion is monitored for 12 hours. The time for the tablet to detach from the sheep buccal mucosa is recorded as the mucoadhesion time [29].

17. Surface pH Study

The surface pH of the buccal tablets is determined in order to investigate the possibility of any side effects in vivo as an acidic or alkaline pH may cause irritation to the buccal mucosa. The method adopted, is used to determine the surface pH of the tablet. A combined glass electrode is used for this purpose. The tablet is allowed to swell by keeping it in contact with 1 mL of distilled water (pH 6.5 ± 0.05) for 2 hours at room temperature. The pH is measured by bringing the electrode in contact with the surface of the tablet and allowing it to equilibrate for 1 minute [29].

18. Scanning Electron Microscopy (SEM)

The micro beads are previously mounted on a brass stub using double-sided adhesive tape and then coated under vacuum with a thin layer of gold (3~5nm) for

75 sec and at 40W to make them electrically conductive. Afterwards, the stub containing the sample is placed in the scanning electron microscope chamber. The surface morphology of blank micro beads, drug loaded micro beads before and after dissolution are studied by photomicrographs at an excited voltage of 20 KV, specific chamber pressure (in mm Hg) under different magnification [32].

19. Novel Rheological Approach

The rheological properties of the mucoadhesive interface (i.e. of the hydrated gel) are influenced by the occurrence of interpenetration step in the process of mucoadhesion. 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 *invitro* model representative of the *invivo*behavior of mucoadhesive polymers. It has been reported that an optimum polymer concentration is required for rheology [27].

Rheological measurement of mucoadhesion

Rheological properties of film forming gel are evaluated on samples of gels with different percentages of water, which are obtained by interrupting the casting process at predetermined times. Rheological determinations are performed at 250C in a viscometer equipped with VT500/VT 3.01 software, and a NV sensor [37].

a) Oscillatory Rheometry

Oscillatory rheometry is performed by using Rheostressrheometer with plane-cone geometry of 35 mm diameter and a gap setting angle of 28. The rheological behaviour is measured by using the dynamic module GH and GHH as a function of frequency and torque. Where, GH is the storage (elastic) modulus GHH is the loss (viscous) modulus. Analysis of this behaviour gives information on the structure of samples, particularly in term of the rigidity, elasticity and deformability of the systems [37].

Torque sweep

Prior to carrying out oscillatory rheometry linear viscoelastic region is determined by carrying out a torque sweep experiment on the samples. A stress range of 0.4±50 Pa is applied and the values GH and GHH are determined at an intermediate frequency of 1 Hz. The equilibration time before starting the test is standardized at 1 min. and a temperature of 37.80C.

Frequency sweep

The storage modulus (GH) and loss modulus (GHH) are measured in a frequency range of 0.1±10 Hz, with a constant stress of 1 Pa ensuring that all samples remained in the viscoelastic region. These module provide direct evidence regarding the physical nature of the formulations. [38]

20. Texture Analyzer

The rupture tensile strength is evaluated by using the equipment known as texture analyzer or a universal testing machine. In addition to rupture tensile strength, the texture analyzer can also, evaluate the texture of the formulations and assess other mechanical properties of the system. In this test, the force required to remove the formulation from a model membrane is measured, which can be a disc composed of mucin, a piece of animal mucous membrane, generally porcine nasal mucus or intestinal mucus from rats. Based on results, a force-distance curve can be plotted which yields the force required to detach the mucin disc from the surface of the formulation. The tensile work (area under the curve during the detachment process), the peak force and the deformation to failure are also assessed. This method is more frequently used to analyze solid systems like microspheres, although there are also studies on semi-solid materials [37].

Method

A Mobile arm containing an analytical probe forces down into a sample held in a flask placed on the equipment's platform. Speed rate, time and depth are preset. From the resulting force-time and force-distance plots, it is possible to calculate the hardness (force required to reach a given deformation), compressibility (work required to deform the product during the compression), and adhesiveness (work required to overcome the attraction forces between the surfaces of sample and probe). Using this technique, it is possible to perform a previous evaluation of the material's adhesive capacity, evidencing mucoadhesion properties [38].

B. Measurement of Residence Time (In Vivo Methods)

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 the fluorescent labelling techniques [29].

1. Use of Radioisotopes

It is a simple procedure involving the use of radio-opaque markers, e.g. barium sulfate, encapsulated in mucoadhesive tablets 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. Mucoadhesive labelled with Chromium-51(Cr-51), Technitium-99 (Tc-99m), Indium-113(In-113m), or Iodine-123(I-123) have been used to study the transit of the tablets in the GI tract [27].

Method

Approximately 2 g of each formulation to be tested is radio labelled by the addition of 3-4 drops (20MBq) of technetium-99m DTPA (diethylenetriaminepentaacetic acid). The gel to be tested is then carefully and thoroughly mixed with the technetium. The average final activity per dose and per subject, at the time of administrations ranged from 0.92 to 1.14MBq.After the subject had been asked to swallow his saliva, an amount of approximately 100 mg accurately weighted of the formulation is applied topically with a syringe on the right lower premolar region and spread with a small Teflon spatula on an area of approximately 1 cm2 of the oral mucosa. Each test formulation is applied only once throughout the trial [39].

2. Gamma Scintigraphy Techniques

It is a valuable tool used in the development of pharmaceutical dosage forms. With this methodology, it is possible to obtain information non-invasively. This technique gives information 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. [27] Distribution and retention time of the mucoadhesive tablets can be studied using the gamma scintigraphy technique. The combination of the sheep model and the gamma scintigraphy method has been proved to be an extremely useful tool for evaluating the distribution, spreading, and clearance of administered stomach mucoadhesive tablets [23].

Method

Three groups of five healthy male volunteers are taken for gamma scintigraphy studies. A capsule containing the granules is administered with 180 ml of water, with the subject in a sitting position, at 8 a.m. or 12 p.m., after the volunteer had fasted overnight for at least 12 h. The volunteers are not allowed to eat or drink during the imaging period. One minute after administration gamma images, each of 1-min duration, are recorded continuously for 30 min, after which six images, each of 1-min duration, are recorded every 15 min for the next 3–4 h. During imaging each subject is in a supine position beneath the gamma camera. At all other times they are able to move freely .Gamma counts are detected using a dual-head gamma). Camera equipped with collimators [39].

3. *In vivo* bio adhesive study (X-ray studies)

To study the bioadhesive character and mean residence time of the natural polymer in the stomach, barium sulphate loaded tablet was used. Two healthy rabbits weighing 2.5 kg are selected and administered orally with the tablet. X-ray photograph is taken at different time intervals [24].

4. In vivo evaluation of gastric mucoadhesion of microspheres

Male Wistar rats, 200–250 g, are fasted for 24 h before the experiments, but are allowed free access to water. Labelled microspheres (2 mg) that are filled in capsules are administered to rats using a gastric sonde. Two hours after administration, the rats are sacrificed, and the stomach is removed and washed with phosphate-buffered saline (pH 7.4) to recover the remaining microspheres. The amount of labelled microspheres that remained in the stomach is determined.[30]

5. Rat gut loop studies of mucoadhesion

Male Wistar rats, with a mean weight about 300 g, are anesthetized and killed with an overdose of barbiturate. The small intestine is removed and washed with physiological saline with a syringe 5–10 ml/min for 10 min, then 20– 30 ml/min for about 20 min. At least 500 ml of the saline is used for cleaning the intestine. The cleaned tissues are used immediately or kept at −15°C until use. A required amount of microspheres are suspended in physiological saline and sonicated. The microsphere suspension is filled into lengths of small intestine (about 15 cm in length) and sealed. These tubes are incubated in saline at 37°C for 60 min. The microsphere suspension is then removed and the number of microspheres present in the suspension before and after the adhesion study is counted using a Coulter Counter method. The percentage of microspheres adhered to the tissue is calculated from the difference of the counts [40].

C. IN VIVO AS WELL AS IN VITRO TECHNIQUE
BIACORE Surface Plasmon Resonance (SPR)

Recently mucoadhesion studies have been reported by using BIACORE integrated chip (IC) systems. The method involves immobilization of the polymer (powder) on to the surface of the IC with the subsequent passage of the mucin solution over the same. This results in the interaction of the mucin with that of the polymer surface. The polymer-mucin interaction is measured by an optical phenomenon called Surface Plasmon Resonance (SPR), which measures the change in the refractive index when mucin binds on the polymer surface [21].

Principle

The BIACORE instrument is based on the principle underlying an optical phenomenon called Surface Plasmon Resonance (SPR). The SPR response is a measurement of the refractive index, which varies with the

solute content in a solution that contacts a sensor chip. When a detected molecule is attached to the surface of the sensor chip, or when the analyte binds to the detected molecule, the solute concentration on the sensor chip surface increases, leading to an SPR response.[41] The sensor chip consists of a glass surface coated in a thin layer of gold. This forms the basis for a range of specialized surfaces designed to optimize the binding of a variety of molecules. The most widely used sensor chip is CM5 (BIACORER) whose surface is modified with a carboxymethylated dextran layer. In general, the ligand can covalently bind to the sensor chip surface via carboxyl moieties on the dextran. Functional groups on the ligand that can be used for coupling include NH2, SH, CHO and COOH. [42]

In Vitro

In the detection of the mucoadhesive property of polymers using BIACORE, each polymer is immobilized on the surface of the sensor chip CM5 and the mucin suspension is passed through the sensor chip. When the analyte (mucin particle) binds to the ligand molecule (polymer) on the sensor chip surface, the solute concentration and the refractive index on that surface change, SPR response will increase, when they dissociate, the SPR response will fall. After that, the analyte can be removed from the ligand by using a regenerating reagent. The response will then turn back to the equilibrium state as the beginning step [23].

In Vivo

The in vivo experiments involve the administration of radioactive labelled delivery system with the subsequent measurement of radioactivity in the tissues, at regular intervals of time, where the delivery system is supposed to adhere. The higher the radioactivity, the higher is the mucoadhesive property of the designed delivery system [43].

The major advantages of the BIACORE instrument are:-

- Label-free detection of binding.
- The ability to monitor the change in response in real time [44].

Fig. 1 Instrument of Wilhelmy Plate Technique

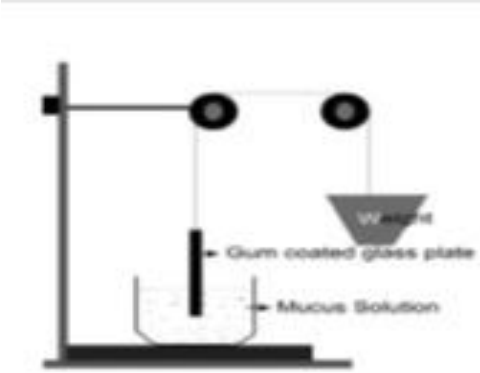


Fig. 2. Electromagnetic Force Transducer

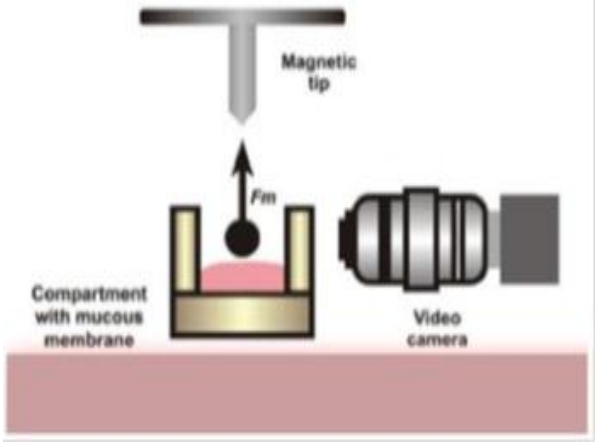


Fig. 3. Shear Stress Measurement

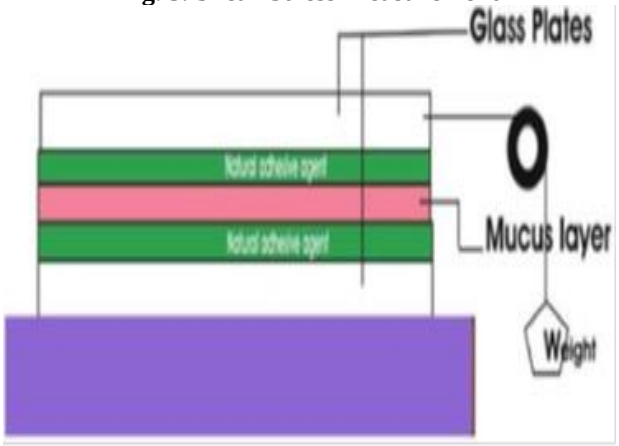


Fig. 4. Modified Physical Balance

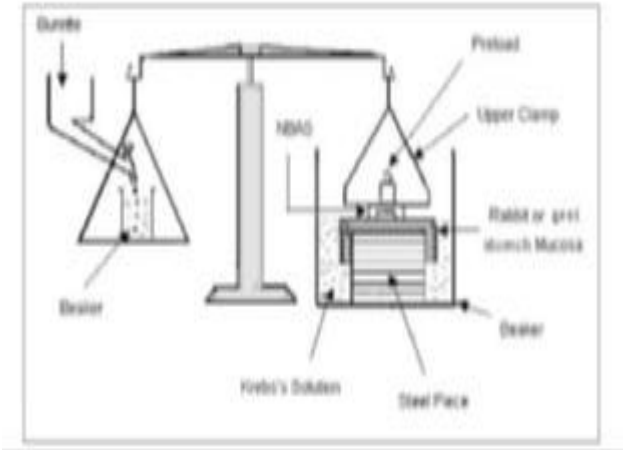


Fig. 5. Detachment Force Measurement

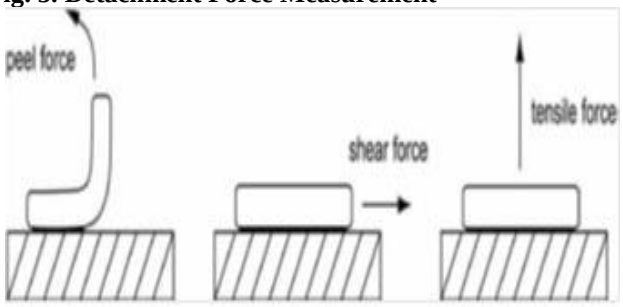


Fig. 6. Mucoadhesive testing system by the detachment force Method

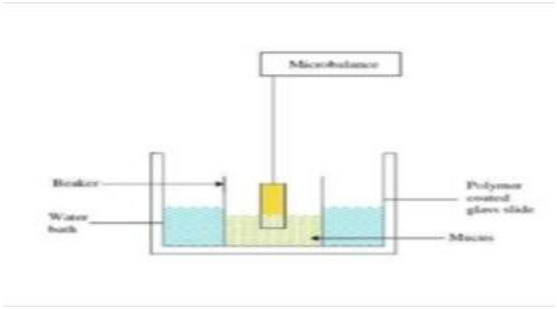


Fig. 7. Microbalance Method for Measuring Mucoadhesion

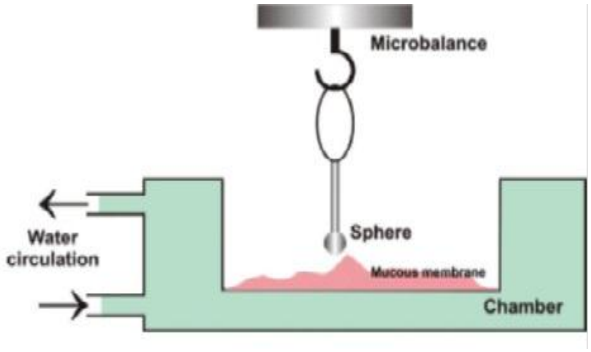


Fig. 8. Modified Dual Tensiometer for Measuring Mucoadhesion

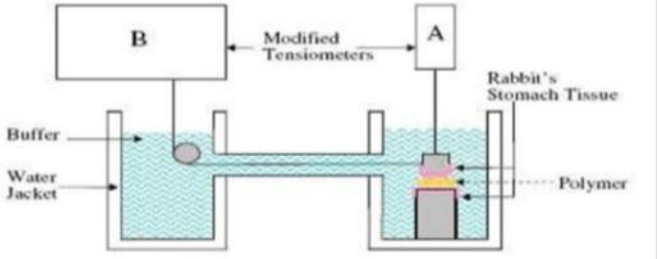


Fig. 9. Falling Liquid Film Technique

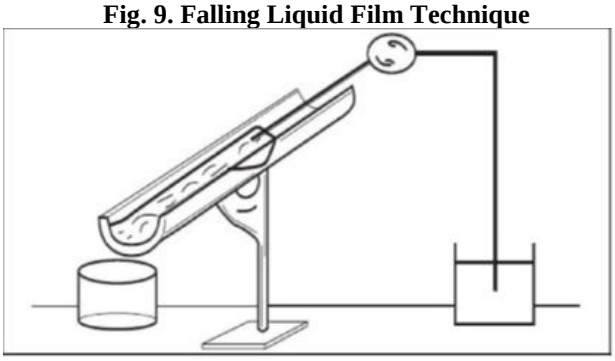


Fig. 10. Diagramatic Representation of Everted Gut Sac Technique

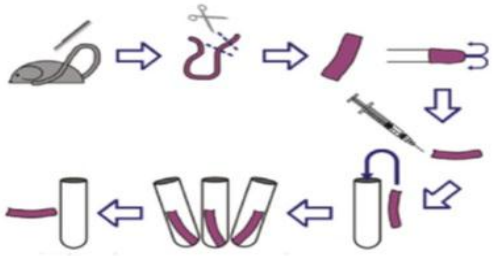


Fig. 11. Texture Analyzer

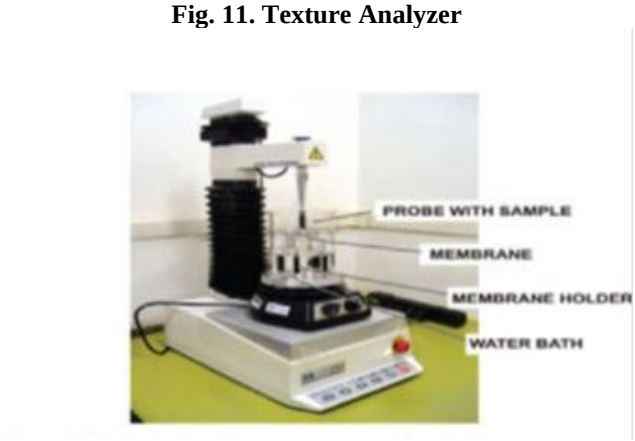
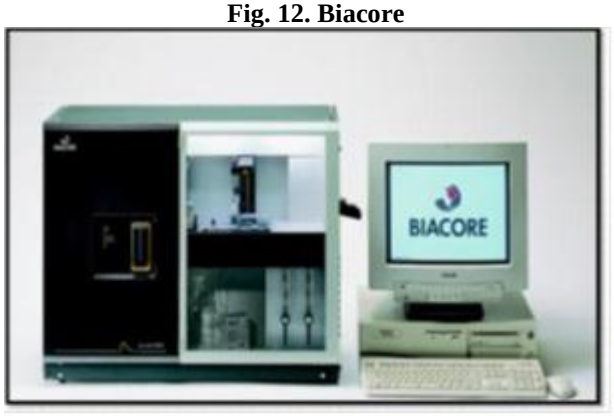


Fig. 12. Biacore



CONCLUSION

This overview about the mucoadhesive dosage forms might be useful tool for the efficient design of novel delivery system have applications from different angles, including development of novel mucoadhesive design of the device, mechanisms of mucoadhesion and permeation enhancement with theories of mucoadhesion and with various evaluation parameters for mucoadhesion. The vaginal route has been used for the local application of drugs but is now becoming a potential route for non-invasive controlled delivery of both local and systemic therapeutically active compounds. Novel vaginal delivery system overcomes some of the key limitations associated with conventional delivery of vaginal drugs. Vaginal drug delivery is a promising area for continued research on the delivery of microbicides that can prevent transmission of sexually transmitted diseases and HIV.

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