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RESEARCH PAPER

Assemblies for In Vitro Measurement of Bioadhesive Strength and Retention Characteristics in Simulated Vaginal Environment

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ABSTRACT

The vaginal route of administration offers a promising option for local and systemic delivery of drugs. Conventional vaginal formulations are associated with limitations of poor retention, leakage, and messiness, thereby causing inconvenience to users. To overcome these limitations, formulations that adhere to the vaginal mucosa for a sufficient period of time need to be developed. Bioadhesion and retention are desirable characteristics of a vaginal formulation to achieve desired efficacy. These properties can be built in during formulation development by the use of bioadhesive polymers. In the present study, assemblies for in vitro measurement of bioadhesive strength and retention characteristics of vaginal formulations have been developed. A modified simulated vaginal fluid (SVF_M) was used to simulate vaginal conditions for bioadhesion studies. Cellophane hydrated with SVF_M and isolated sheep vaginal mucosa were used as model membranes. The bioadhesive potential of various polymers and their combinations was evaluated. Among the polymers evaluated, xanthan gum (XG), sodium alginate (SA), Polycarbophil (PC), and their combinations (XG + SA and XG + PC) were found to possess

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significant bioadhesive strength. In retention experiments, XG, SA, and combinations (XG + SA and XG + PC) were retained in isolated sheep vaginal tissue, while PC exhibited poor retention under experimental conditions. Based on the results of the study conducted, XG, SA, and combinations (XG + SA and XG + PC) have been proposed as potential candidates for developing bioadhesive vaginal drug delivery systems.

Key Words: *Bioadhesion; Bioadhesive polymers; In vitro bioadhesion measurement; Retention; Simulated vaginal fluid; Vagina*

INTRODUCTION

The vagina has been studied as a favorable site for local and systemic delivery of drugs that are used specifically for the treatment of female-related conditions. The vaginal cavity has traditionally been used for local delivery of drugs such as prostaglandins, steroids, antibiotics, antifungal, antiprotozoal, antichlamydial, antiviral, and spermicidal agents.[1] Recently, there has been increased interest in vaginally administered agents and formulations known as "microbicides," which provide effective contraception and protection against transmission of various sexually transmitted infections (STDs), including acquired immuno deficiency syndrome (AIDS).[2] In addition, vaginal preparations include products meant for the maintenance of reproductive hygiene, enhancement of sexual pleasure, and moisturizing the vaginal mucosa in case of dry vagina in postmenopausal women.

Several kinds of vaginal dosage forms have been developed, such as sponges, rings, suppositories, tablets, tampons, foams, films, gels, and creams. Most of these are associated with a number of problems, including leakage and messiness, causing discomfort to users and expulsion due to the self-cleansing action of the vaginal tract. These limitations lead to poor patient compliance and failure of the desired therapeutic effects. In addition to providing the desired therapeutic effect, vaginal products need to be designed for women's convenience. A product's aesthetic qualities are important to ensure proper compliance and regular use. There is a need to advance vaginal formulation technology to fulfill certain functions, such as product dispersion throughout the vagina, retention for intended intervals, physicochemical interaction with the vaginal environment, release of the active ingredients, and effects on target organisms.[3] Retention of the dosage form in the vaginal cavity for a sufficient period of time is highly desirable, which can be achieved by the use of bioadhesive drug delivery systems.[4]

Bioadhesion and retention at the site of application for a sufficient period of time can be achieved by incorporating bioadhesive polymers in the formulations. Some of the polymers reported to possess bioadhesive properties are Polycarbophil, Carbopol 934P, chitosan, sodium carboxymethylcellulose (SCMC), hydroxypropylcellulose (HPC), hydroxypropyl methylcellulose (HPMC), and sodium alginate.[5] In most vaginal preparations, that claim to be bioadhesive, either Carbopol or Polycarbophil has been used as bioadhesive polymer.[4,6,7]

Extensive research has been carried out on bioadhesive delivery systems for buccal and gastrointestinal administration, because of the strong interest in drug delivery by these routes. On the other hand, to date, only a limited number of studies have been reported on bioadhesive drug delivery systems for vaginal administration. Several in vitro methods have been described to study bioadhesion in the former, including methods based on measurement of shear stress,[8] tensile strength,[9–11] adhesion weight method,[12] fluorescent probe method,[13] colloidal gold staining,[14] rheological method,[15] flow channel method,[16] and falling liquid film method.[17,18] Only a few in vitro studies[7,19] have been reported to measure bioadhesion in the vaginal environment.

The present work was undertaken with an objective to rationalize the selection of the "right" kind of polymers so as to achieve the above-mentioned characteristics in vaginal formulations. Various polymers reported to possess bioadhesive properties at different mucosal sites were selected. These include Polycarbophil, Carbopol 934P NF, xanthan gum, chitosan, and sodium alginate.

For measuring bioadhesion in simulated vaginal conditions (in vitro), two assemblies have been developed. These work on the principle of application of tensile and shear stresses to break the adhesive bond

Table 1

Comparison of the Simulated Vaginal Fluid (SVF) Described by Owen and Katz[20] with the Proposed Simulated Vaginal Fluid Containing Mucin (SVF_M)

Property	SVF	SVF_M
Mucin	Absent	Present (1.5% w/v)
Color	Colorless	Buff color
Odor	Odorless	Characteristic odor of mucin
pH	Adjusted to 4.2 during preparation	Adjusted to 4.2 during preparation
Viscosity[a] at 37°C	2.23 cP	3.23 cP

[a]Viscosity measured as described in text.

between the test sample and a model membrane. To simulate vaginal conditions, a synthetic membrane (cellophane hydrated with modified simulated vaginal fluid) and sheep vaginal mucosa were used as model membranes. Using the assemblies developed, selected polymers were evaluated for bioadhesive characteristics in a simulated vaginal environment. An in vitro method to measure the retention of gels in the vaginal cavity has also been developed, and used to compare the retention characteristics of selected polymer gels.

MATERIALS AND METHODS

Materials

Carbopol 934P NF and Noveon™ 75 (Polycarbophil) were obtained as gift samples from BF Goodrich (Cleveland, OH, USA). Xantural™ (xanthan gum) of pharmaceutical grade was obtained as a free gift from Monsanto Pharmaceutical Ingredients (Norristown, PA, USA).

Chitosan (from crab shells, min. 85% deacetylated) and sodium azide were purchased from Sigma (St. Louis, MO). Sodium alginate LR and lactic acid (about 75% lactic acid plus 10% lactide) were purchased from SD Fine Chemicals Limited (Boisar, India). Disodium edetate was purchased from Loba Chemie Private Limited (Mumbai, India).

Chemicals used in the preparation of SVF_M were obtained from commercial sources. Mucin (type III, partially purified, from porcine stomach) was purchased from Sigma (St. Louis, MO). Demineralized water (prepared by ion-exchange process, resistivity 0.769 MΩ cm at 25°C) was used in the preparation of simulated vaginal fluid (SVF_M).

Methods

Preparation of Modified Simulated Vaginal Fluid

The vaginal environment was simulated to study the bioadhesion of formulations using a modified simulated vaginal fluid (SVF_M). Simulated vaginal fluid (SVF) proposed by Owen and Katz[20] was modified by incorporating 1.5% w/v mucin and designated as modified simulated vaginal fluid (SVF_M). The latter was then compared with SVF in terms of appearance, pH, and viscosity (Table 1). Viscosity was measured using a Brookfield RVDV III+ Programmable Rheometer (Brookfield Engineering, Middleboro, MA, USA) with coaxial cylinders and UL Adapter (measuring spindle: ULA spindle, sample volume 16 mL, temperature 37±0.5°C), by rotating the spindle at a shear rate of 305.75 sec^{-1} for 1 min.

Model Membranes

Cellophane hydrated with SVF_M was used as a synthetic model membrane.[21] The extent of cellophane hydration was studied by soaking the cellophane in SVF_M and measuring the weight gain as a function of time. Hydration reached a maximum in a period of 1 hr. Hence, cellophane was hydrated with SVF_M for an hour before use, in bioadhesion experiments.

Sheep vaginal mucosa has been reported as a biological substrate to study bioadhesion in the vaginal environment.[19] Hence sheep (*Ovis aries*, non-descriptive local breed) tissue was selected as the model biological membrane for bioadhesion and retention studies. Sheep vaginal tissue was obtained immediately after the sacrifice of animals at a slaughter house. Vaginal tissue was cleaned, separated from the supporting muscular and connective

Table 2

Characteristics of Polymer Combination Gels on the Day of Preparation

Combination	Polymers	Viscosity[a] (cP)	pH	Physical Appearance
C1	Xanthan gum and sodium alginate	71,184	3.55	No physical change
C2	Xanthan gum and chitosan	32,368	4.31	Precipitation on mixing
C3	Xanthan gum and polycarbophil	18,120	7.43	No physical change
C4	Sodium alginate and chitosan	64,804	3.76	Precipitation (after 3 days)
C5	Sodium alginate and polycarbophil	2,000	3.81	Liquification of gel
C6	Chitosan and polycarbophil	5,552	4.51	Precipitation on mixing, liquification of gel

[a]Viscosity measured as described in text.

tissues, taking care to maintain the integrity of its tubular structure. The isolated tissue was frozen at −20°C till further use. Before experiments, sheep vaginal tissue was thawed in normal saline containing 0.1% w/v sodium azide as preservative. For bioadhesion studies, the vaginal tube was incised longitudinally just before the experiments after thawing. In retention experiments, vaginal tissue was used as an intact tubular structure. Required clearance from the institutional animal ethics committee was obtained for the experiments involving sheep tissue.

Preparation of Test Samples

For measuring bioadhesion, polymers (alone and in combination) were used in the form of aqueous gels. Concentrations of the polymers were selected so that all the gels have viscosity in the same range (30,000 cP±10%), since both bioadhesion and retention of a gel in the vagina are greatly affected by its viscosity. The viscosity of gels (sample volume 150 mL gel in a 250-mL plastic beaker with a diameter and height of 7.15 cm and 9.5 cm, respectively) was measured with Brookfield DV III+ Programmable Rheometer using a disc-shaped spindle RV 05 (without guard leg) rotated at 10 rpm for 2 min at room temperature (28–30°C). The viscosity of gels was measured at five different positions in the beaker, and the mean viscosity recorded. The viscosity range was selected on the logic of studying gels with acceptable aesthetic appeal. The concentrations of polymers used were: xanthan gum (4% w/w), sodium alginate (4.1% w/w), chitosan (3.5% w/w), Carbopol 934P (0.4% w/w), and Polycarbophil (0.3% w/w).

Xanthan gum gel was prepared by soaking the polymer in water followed by stirring. Gels of Carbopol 934P and Polycarbophil were prepared by neutralization of the aqueous dispersion of polymer with 1 N NaOH (1 g of Carbopol/Polycarbophil was neutralized with 0.4 g of NaOH).[22] Sodium alginate gel was prepared by adding lactic acid (2.94% w/w) to the viscous aqueous solution of sodium alginate. Chitosan gel was prepared by dissolving the polymer in an aqueous solution of disodium edetate (2.34% w/w) and lactic acid (2.83% w/w). Sodium alginate and chitosan form gels in acidic conditions. Since lactic acid is an important constituent of vaginal fluid,[23] it was used to impart the required acidity in sodium alginate and chitosan gels.

Polymer Combinations

Polymers showing significant bioadhesive strength under test conditions were selected to explore the possibility of synergistic effect. Various polymer combinations (Table 2) were prepared by mixing the gels of individual polymers in a ratio of 1:1 by weight. Viscosity, physical appearance, and pH of gel combinations were observed. Viscosity of combination gels was measured using a Brookfield Rheometer as described previously. The pH of a 10% w/v solution of gels in demineralized water was measured with a Titroline alpha digital pH meter.

The compatability of the polymer gels in combination was studied by observing visual changes in physical appearance (color, liquification, precipitation), pH, and viscosity of the combination gel. Combination gels showing any liquification or precipitation were eliminated from further studies.

Only the compatible polymer combinations, i.e., the combinations showing no physical changes on mixing, were studied for bioadhesion and retention. All experiments were performed in replicates of five, and the mean bioadhesive strength of each polymer combination was determined and compared to the bioadhesive strength of the individual polymer gels.

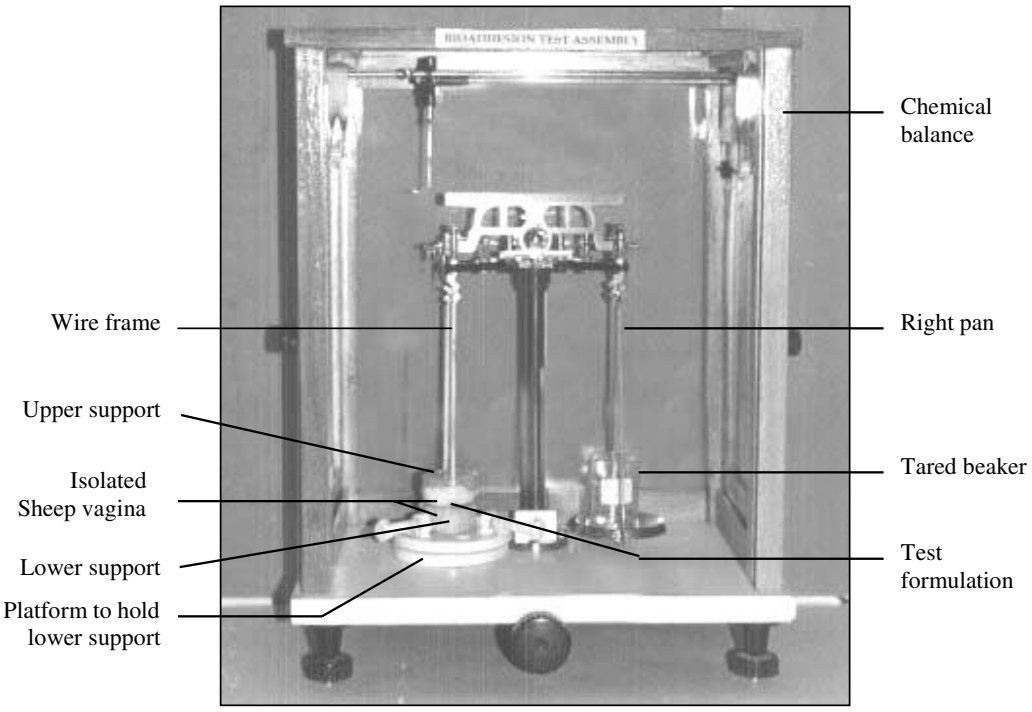

Figure 1. Bioadhesive assembly based on tensile strength.

Bioadhesion Measurement

The assemblies developed for in vitro measurement of bioadhesive strength in a simulated vaginal environment are a modification of the bioadhesion test assembly[10] designed to measure bioadhesion in a buccal environment. The method is based on the measurement of tensile strength or shear stress required to break the adhesive bond between a model membrane and the test formulation. The test formulation is sandwiched between two model membranes fixed on flexible supports in the assemblies for a sufficient period of time. After the adhesive bond has formed, the force (weight) required to separate the bond was measured and calculated as bioadhesive strength.

Bioadhesion Assembly Based on Tensile Strength (Tensile Test)

A chemical balance (Modern Balance Works, Varanasi, India) was modified as a bioadhesion assembly based on tensile strength (Fig. 1). The left pan of the balance was removed and replaced with a wire frame designed to hold the upper support. The lower support was fixed on a platform below the upper support. The supports were made of circular plastic caps (3.8 cm outer diameter and 1.7 cm height) of specimen bottles (37 mm × 50 mm, Tarsons Products, Calcutta, India) filled with vaseline and covered with a cellophane membrane. The caps were filled with a semisolid non-aqueous base (Vaseline) to provide a flexible backing support for model membranes. This was done in order to simulate the flexibility of vaginal muscular tissue under in vivo conditions. The model membrane was fixed to the flexible surface of both supports. A small plastic beaker was placed on the right pan and balanced.

Cellophane was soaked in SVF_M for 1 hr and then tied on both supports with a thread. In experiments using sheep vagina as membrane, the mucosa with mucosal side exposed was tied to both the supports with the help of a thread. Gel (0.5 g) was mixed with SVF_M (0.25 mL) and applied uniformly over the membrane on the lower support (12 cm^2). Both sides of the assembly were balanced. An extra weight (10 g) was placed above the upper support for 5 min to establish the contact of gel with membranes and allow the formation of an adhesive bond. Before measurement, this extra weight was removed. The two sides were in a balanced position

and the supports were in contact, due to adhesion of the gel with the membranes. Water was added dropwise in the beaker on the right side until the two supports separated due to breaking of the adhesive bond. The time required for addition of water ranged from 1 to 2 min depending on the quantity of water required. The amount of water added was weighed, the force required to break the adhesive bond per unit area was calculated, and considered as the bioadhesive strength of the test sample. All experiments were performed in replicates of five (sheep vaginal tissue) or six (hydrated cellophane). The Student Newman Keuls (SNK) test and unpaired *t*-test (software SigmaStat 2.0) at $P < 0.05$ were used to compare the results to determine any significant difference in the bioadhesive strength of polymers.

Bioadhesion Assembly Based on Shear Stress (Shear Test)

A physical balance (200 g capacity) was used to fabricate the bioadhesion assembly based on shear stress (Fig. 2). The right pan was replaced with the pan used in a horizontal assembly and a small plastic beaker was placed on it. On the left side, one support was suspended in a vertical position with the help of a frame made of copper wire. The fixed support was attached at the same level as that of the first support when both sides were in a balanced position. A screw was fixed on the backside of the hanging support so as to maintain proper contact between the gel and the membranes on both supports. Before measuring the bioadhesive strength, the screw was brought back to the previous position to ensure the absence of any resistance, followed by the addition of water in the beaker. The rest of the experimental methodology was the same as before.

Retention Measurement

Another desirable property of vaginal formulations is retention in the vaginal cavity for a desired period of time. An assembly was designed to measure retention in which the test gel, placed inside a vertically suspended excised sheep vagina, was allowed to fall under the influence of gravity. The weight of gel falling down as a function of time was recorded.

An intact tubular piece of sheep vagina (cut to 3 in long; thawed in normal saline containing 0.1% w/v

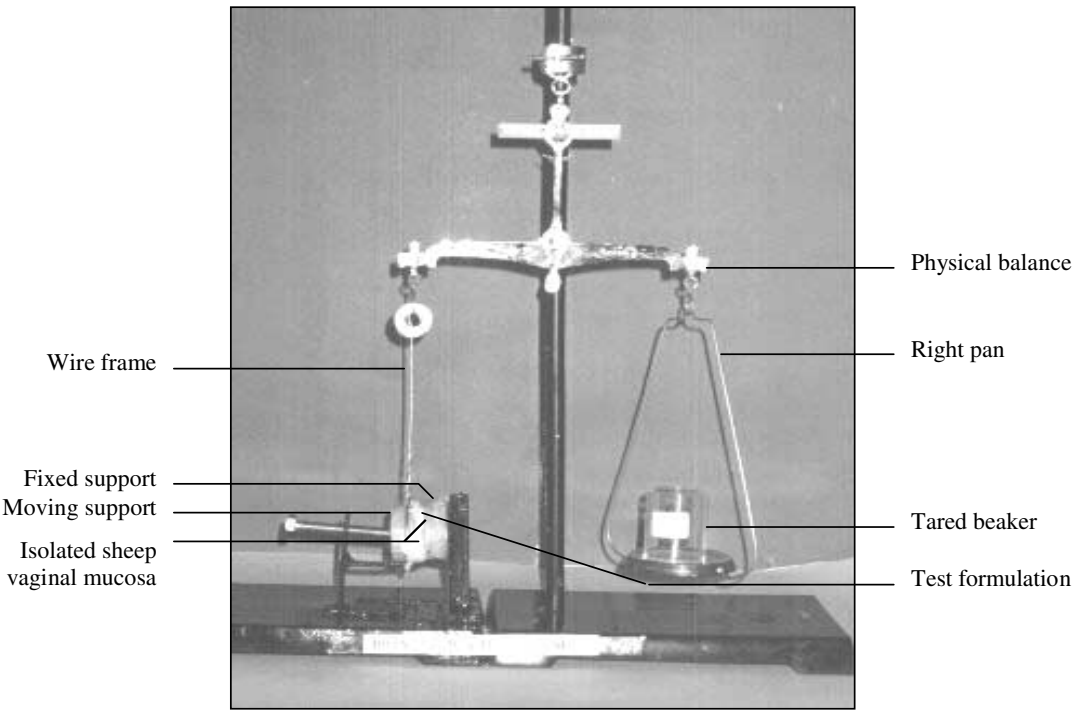

Figure 2. Bioadhesive assembly based on shear stress.

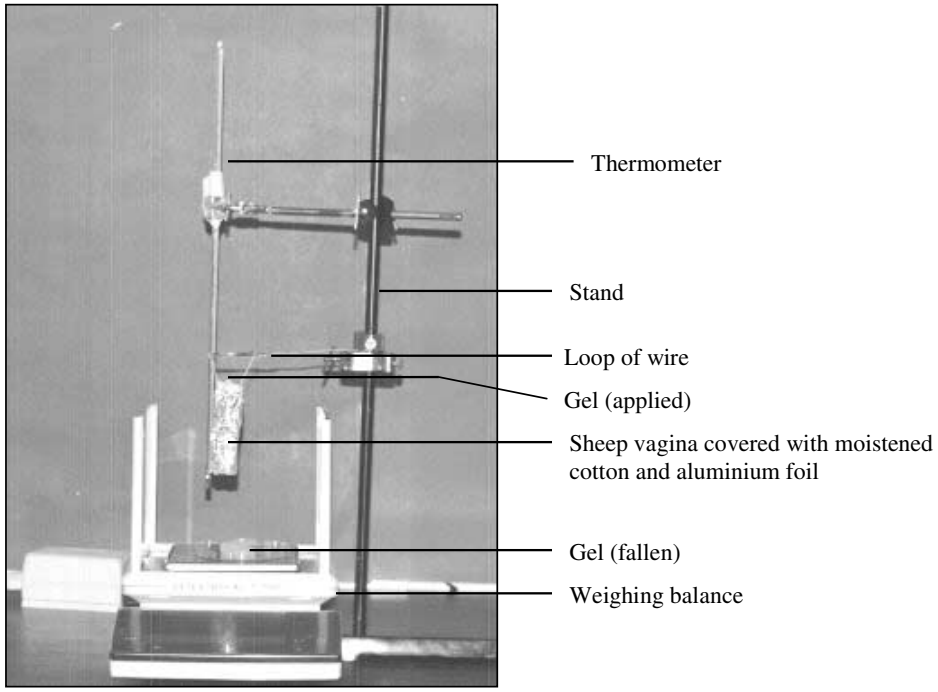

Figure 3. Retention assembly.

sodium azide) was suspended vertically with the help of a loop of wire and a stand (Fig. 3). The tissue was surrounded with a cotton pad moistened with normal saline, further surrounded by aluminum foil in order to keep the tissue moist for the duration of the experiment. A balance (Mettler-Toledo GmbH, PR2003 DeltaRange® d−0.01 g/0.001 g, Switzerland) was placed below the suspended tissue to measure the weight of gel falling down. The room temperature was maintained at 37±2°C. A test sample (4 mL) was introduced into the isolated vaginal tube with the help of a 10 mL syringe (without needle), taking care to avoid spillage. The expulsion of gel from the lower end was then recorded for 2 hr as a measure of retention.

RESULTS AND DISCUSSION

Preparation of Modified Simulated Vaginal Fluid

Several researchers have defined different compositions of a simulated fluid that represent the vaginal environment and can be used for in vitro testing of vaginal formulations. One of the compositions reported possesses the same physical and chemical properties, as vaginal fluid under normal physiological conditions. This has been proposed for research in the area of contraception and prophylactic drug delivery to study the permeation and bioactivity of active agents.[20] In another study, an artificial vaginal fluid has been used to observe the binding and release characteristics of solutions of all-*trans*-retinoic acid to evaluate the efficacy of collagen-based contraceptive sponges.[24] A chemically defined medium simulating female genital tract secretions, that can support the growth of vaginal microflora, has also been developed.[25]

The vagina itself does not possess goblet cells, and hence there is no direct supply of mucus, but it contains mucins from cervical mucus.[4] Mucins are secreted by mucosal cells and play an important role in adhesion of the formulations to the mucosa.[9,26] Human cervical mucus has been reported to contain 1.5% w/v of mucins.[27] None of the above-mentioned compositions contain mucin. Simulated vaginal fluid (SVF) described by Owen and Katz[20] was modified by incorporating 1.5% w/v mucin, for bioadhesion and retention studies. The resulting SVF_M possesses a characteristic odor of mucin, buff color, and viscosity greater than SVF, owing to the presence of mucin (Table 1).

Bioadhesion Assemblies

Under normal physiological conditions, the residence time of formulations in the vaginal cavity is influenced by a number of variables, including vaginal and cervical secretions, smooth muscle contractions, intercourse, and physical posture of the individual. The effect of forces due to these variables may be exerted in different directions. In order to simulate these variables in vitro, forces were applied in both directions (perpendicular and parallel) to the membranes using tensile strength and shear stress-based bioadhesion test assemblies. The two assemblies differ in the modes of separation of two membranes containing the gel in between and the forces applied (Fig. 4). Tensile strength was applied in the former and shear stress in the latter case.

The assemblies developed offer distinct advantages over the previously reported methods for bioadhesion measurement in the vaginal environment.[7,19] The flexible supports used to hold the membranes (in place of rigid metal/glass supports) and the membrane on both sides of the formulation (instead of only on one side) provide a better simulation of vaginal conditions. Vaginal mucosa of other species such as bovine, rabbit, or human vaginal mucosa can also be used as model membranes, as and when required.

Bioadhesive Strength of Polymers

The gels of Carbopol 934P and Polycarbophil were found to liquify when mixed with SVF_M. These polymers form gels at a pH of 6–7; addition of SVF_M (pH 4.2) reduced the pH and caused the liquification of gels. All other polymer gels retained their consistency when mixed with SVF_M. The bioadhesive strength of all the polymers determined with both the assemblies using cellophane hydrated with SVF_M as model membrane is given in Fig. 5.

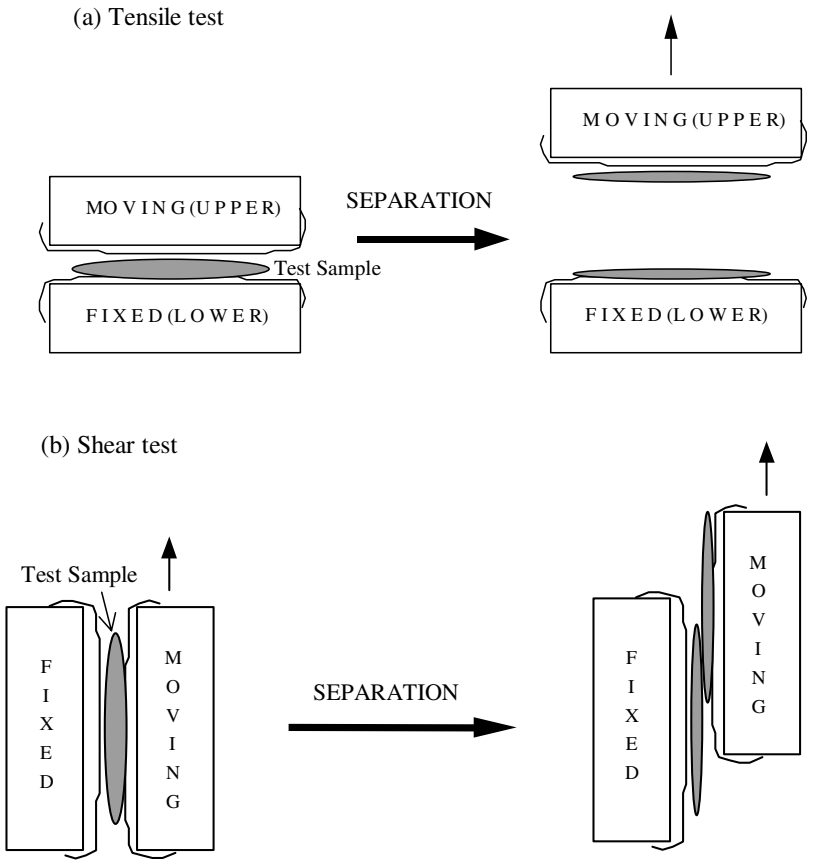

Figure 4. Mechanism of separation of membranes in bioadhesion tests based on (a) tensile strength and (b) shear stress.

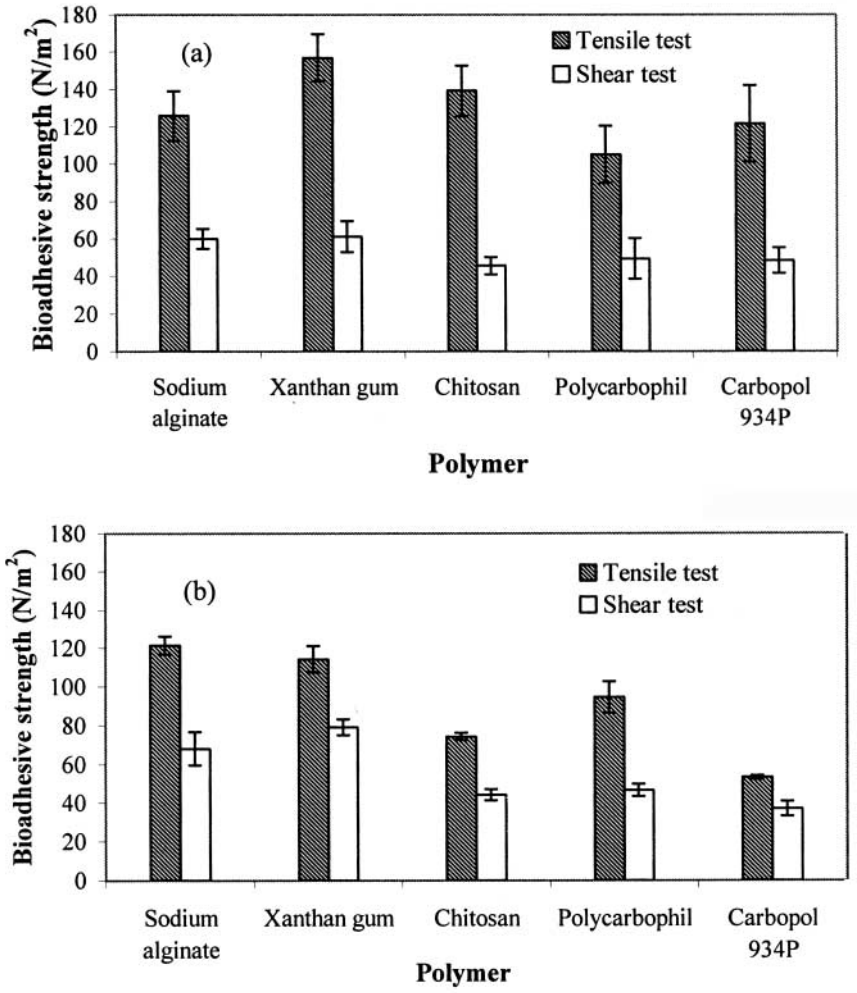

Figure 5. Bioadhesive strength of different polymer gels as measured by application of tensile strength and shear stress using (a) cellophane hydrated with SVF_M and (b) sheep vaginal mucosa.

Xanthan gum was found to possess maximum bioadhesive strength ($P < 0.05$, SNK test) among all polymer gels using cellophane as model membrane in both the tensile and shear tests. In case of the tensile test using sheep vaginal mucosa as model membrane, the bioadhesive strength of xanthan gum was greater than and statistically significant at $P < 0.05$ (SNK test) from that of other polymer gels except sodium alginate. When shear stress was applied to separate the test sample from sheep vaginal mucosa, the bioadhesive strength of sodium alginate was higher and statistically different from that of xanthan gum and other polymer gels ($P < 0.05$, SNK test). Sodium alginate gel was also found to possess good bioadhesive properties in the vaginal environment, as is evident from its bioadhesive strength measured by both assemblies and both model membranes.

Among the various polymers studied, xanthan gum followed by sodium alginate was found to be the best polymer in the terms of bioadhesive strength in the vaginal environment, under experimental conditions. In addition, Polycarbophil and chitosan also exhibit good bioadhesive strength when compared to other polymers. Polymers showing significant bioadhesive strength (xanthan gum, sodium alginate, chitosan, and Polycarbophil) were selected for further studies in combination with each other.

Polymer Combinations

Viscosity, physical characteristics, and pH of the polymer combinations prepared by mixing individual polymer gels are shown in Table 2. In C2 and C6, precipitation occurred on mixing the gels of different polymers. Precipitation occurred in C4 after three days of storage at 40°C. In combinations C5 (sodium alginate and Polycarbophil) and C6 (chitosan and Polycarbophil), liquification of gel occurred. Sodium alginate forms gel in acidic medium, whereas Polycarbophil forms gel on neutralization with a base. On mixing the two, liquification occurred due to a change in pH. A change in pH is also responsible for the liquification of combination C6 containing Polycarbophil and chitosan (which gels in acidic medium). Xanthan gum, sodium alginate, and Polycarbophil present in combinations C2, C4, and C6, respectively are anionic polymers,[22] whereas chitosan in C2, C4, and C6 is cationic in nature. Differences in the ionic nature of polymers present in combinations are a possible reason for precipitation. Out of six combinations, only two (C1 and C3) that have not shown any physical incompatibility on mixing during preparation were selected for further studies.

Bioadhesive Strength of Polymer Combinations

The bioadhesive strength of gel combinations (C1 and C3) measured with both the assemblies using both model membranes is shown in Fig. 6. No

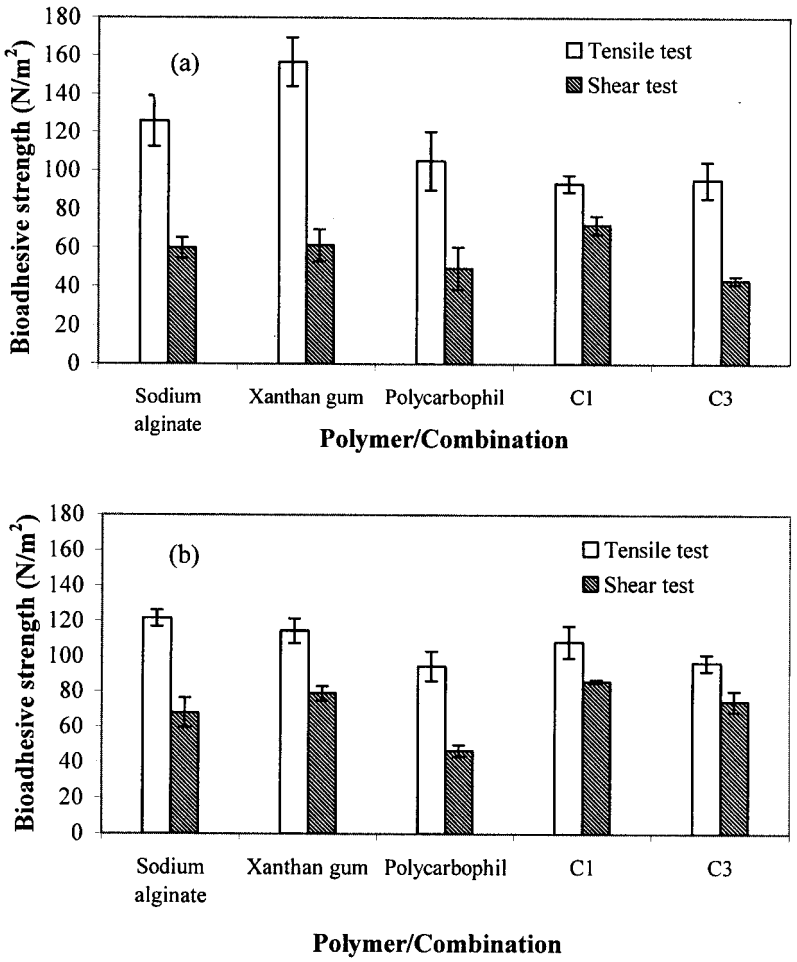

Figure 6. Comparison of polymer gels and their combinations based on bioadhesive strength measured by application of tensile strength and shear stress using (a) cellophane hydrated with SVF_M and (b) sheep vaginal mucosa.

statistically significant difference in bioadhesive strength of C1 and C3 ($P = 0.05$, t-test) was observed when tested by applying tensile strength using cellophane treated with SVF_M. The bioadhesive strength of combination C1 was found to be significantly greater than that of C3 ($P = 0.05$, t-test) when tested by shear stress. The bioadhesive strength of combination C1 was greater than that of C3 ($P = 0.05$, t-test), when tested by both the assemblies using sheep vaginal mucosa.

The bioadhesive strength of combination gels was compared with that of the individual polymer gels (Fig. 6). In case of the tensile test, a statistically significant reduction ($P < 0.05$, SNK test) in bioadhesive strength of sodium alginate and xanthan gum was found on mixing the two polymers, when cellophane membrane was used. When sheep vaginal mucosa was used as a model membrane, the bioadhesive strength of C1 was found to be significantly less than that of xanthan gum and equal to that of sodium alginate ($P < 0.05$, SNK test). The bioadhesive strength of the gel combination of xanthan gum and Polycarbophil, measured with both the assemblies, was significantly less than that of xanthan gum and equal to that of Polycarbophil ($P < 0.05$, SNK test). In case of the shear test, using both the membranes, a statistically significant increase ($P < 0.05$, SNK test) in bioadhesive strength of sodium alginate and xanthan gum was found when these polymers were mixed to form a gel combination (C1).

The bioadhesive strength of combination C3 was significantly less than one of its constituents, xanthan gum, and equal to that of Polycarbophil ($P < 0.05$, SNK test) when cellophane was used. On the other hand, when sheep vaginal mucosa was used, the bioadhesive strength of C3 was found to be equal to that of xanthan gum and greater than that of Polycarbophil ($P < 0.05$, SNK test).

The effect of combining sodium alginate and xanthan gum on bioadhesive strength, when measured by the two assemblies, was different. In the tensile test, the bioadhesive strength was found to increase, while it decreased when measured by applying shear stress. The bioadhesive strength of combination C3 was found to be less than that of xanthan gum and equal to that of Polycarbophil.

The bioadhesive strength of all the polymers and combinations as measured in the tensile test was found to be greater ($P < 0.05$, SNK test) than that measured in the shear test by using both cellophane hydrated with SVF_M and sheep vaginal mucosa as membranes. These differences may have occurred due to the effect of different factors, such as hydrodynamic forces (tensile and shear stress) and gravitational force in the case of the shear test. In the tensile test, the bioadhesive strength is a consequence of polymer–membrane and polymer–mucin interactions. However in the vertical assembly, in addition to the above-mentioned interactions, viscoelastic properties of the test sample and gravity also influence the bioadhesion.

No statistically significant difference ($P < 0.05$, SNK test) was observed in some cases, such as the bioadhesive strength of sodium alginate and Polycarbophil measured using cellophane hydrated with SVF_M and sheep vaginal mucosa. However in the case of xanthan gum and combination C1, the bioadhesive strength was found to be significantly different when cellophane and sheep vaginal tissue were used as model membranes. This suggests that the cellophane membrane hydrated with SVF_M, having acidic pH and containing mucin, has potential as an alternative to animal tissues for the measurement of bioadhesive properties, especially in preliminary screening studies. But before using it as a substitute for animal tissue, its use needs to be further studied and validated.

Retention of Gels in the Vagina

Retention of gels of xanthan gum, sodium alginate, chitosan, Polycarbophil, and combination gels (C1 and C3) in the vagina was assessed as described earlier. The total weight of gel expelled from the vertically suspended sheep vagina was considered as 100%, and the percentage of weight fallen with time was recorded (Fig. 7). Gels of xanthan gum and sodium alginate were retained in the sheep vagina suspended in the assembly for the entire period of observation (2 hr). Each experiment was repeated four times. A very small amount (0.213 g) of sodium alginate gel fell down in 106 min only in one experiment, whereas in all other cases there was complete retention. Both polymer combinations (C1 and C3) were also retained for a period of 2 hr.

Xanthan gum, sodium alginate, their combination gel, and the combination of xanthan gum and Polycarbophil showed good retention under experimental conditions, whereas the gels of chitosan and Polycarbophil were found to possess poor retention.

From the results obtained, it can be concluded that among the polymers evaluated, xanthan gum

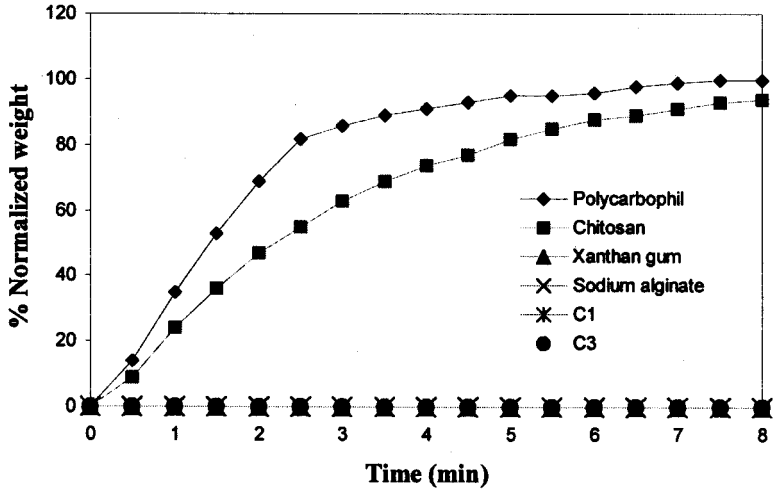

Figure 7. Retention of polymer gels in vertically suspended isolated sheep vaginal tube (100% polycarbophil gel and 94% chitosan gel have fallen down in 8 min, whereas other polymer gels did not fall even in 2 hr; retention was studied for a period of 2 hr, the graph is shown up to 8 min only).

followed by sodium alginate was found to be the best polymer, possessing maximum bioadhesive strength and good retention properties, under the conditions used.

Though Polycarbophil has been used as a bioadhesive polymer[4,7,28] in many vaginal preparations, its bioadhesive strength measured by both assemblies using cellophane hydrated with SVF_M and sheep vaginal mucosa was found to be less (statistically significant at $P < 0.05$, SNK test) as compared to xanthan gum and sodium alginate under the experimental conditions specified. This observation can provide valuable clues about selecting the polymers during development of bioadhesive vaginal formulations.

Bioadhesion and retention are desirable characteristics of a vaginal formulation. In order to develop a vaginal formulation, some optimization has to be carried out with the above-mentioned characteristics. In the present study, the bioadhesive strength of polymers in simulated vaginal environment was studied and compared. Drugs and other excipients may also have some effect on these characteristics of the formulations. Detailed studies need to be done on actual formulations during formulation development to determine the effect of drugs and excipients on these characteristics in the concentrations used.

In vivo studies are further required to supplement the data obtained from in vitro studies on bioadhesion and retention characteristics. Once in vitro–in vivo correlation is established, the in vitro methods mentioned in this paper can replace the expensive and laborious in vivo studies. This will facilitate the development and optimization of vaginal formulations with desired features. Thus, bioadhesion and retention measurement studies will help evaluate the performance of vaginal preparations in terms of acceptability, patient compliance, and clinical efficacy.

CONCLUSIONS

The present paper describes the use of two simple in vitro assemblies for measurement of bioadhesive strength based on application of tensile strength and shear stress to detach a test sample from the membrane. Tensile and shear tests give different results for the same test because of the different types of forces involved. Hence the effects of both tensile strength and shear stress need to be studied during vaginal formulation development. The use of cellophane membrane hydrated with SVF_M needs to be further optimized and validated before using it as an alternative to animal tissue in bioadhesion studies. An in vitro method of evaluating retention of a formulation in simulated vaginal environment has also been described. This has been reported for the first time. Although in vivo assessment of the above-mentioned parameters is the real

test of formulation characteristics and effectiveness, the assemblies designed herewith can be of great help in initial screening during formulation development and optimization.

The assemblies developed have been used for comparative evaluation of various polymers for bioadhesion and retention characteristics in the vaginal environment. Among the polymers evaluated, xanthan gum and sodium alginate were found to exhibit maximum bioadhesive strength and longest retention time. These polymers can further be used to develop bioadhesive vaginal formulations to overcome the major problem, i.e., leakage of formulation from the vaginal cavity.

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