

Strategies to Prolong the Intravaginal Residence Time of Drug Delivery Systems

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ABSTRACT

The vagina has been studied as a suitable site for local and systemic delivery of drugs. There are a large number of vaginal medications on the market. Most of them, however, require frequent applications due to their short vaginal residence time. A prolonged vaginal residence time of formulations is therefore a key parameter for improved therapeutic efficacy. Promising approaches for prolonging the residence time base on mucoadhesion, were in-situ sol-to-gel transition and mechanical fixation. Mucoadhesive drug delivery systems can be tailored to adhere to the vaginal tissue. In-situ gelling systems offer the advantage of increased viscosity in vaginal cavity and consequently reduce outflow from the vagina. Mechanical fixation needs specially shaped drug delivery systems and reduce the frequency of administration significantly. In this review, an overview on these different strategies and systems is provided. Furthermore, the techniques to evaluate the potential of these systems for prolonged vaginal residence time are described.

1. INTRODUCTION

The vaginal route is commonly used for the administration of locally acting drugs such as antimicrobials, labor-inducing agents, spermicidal agents, prostoglandins and steroids (1). Moreover, the administration of drugs for systemic effects via the vagina is also feasible.

Compared with other mucosal application sites, the vagina has the following noteworthy advantages as listed below:

- The vagina may serve as a better route for the delivery of drugs due to the paucity of drug metabolism and the avoidance of the liver first-past effect (1).
- A reduction in the incidence and severity of gastrointestinal side effects.
- A reduction in hepatic side effects of steroids used in hormone replacement therapy or contraception period.
- Avoidance of the inconvenience caused by pain, tissue damage and risk of infections which are associated with parenteral routes.
- Ease of self-insertion and removal of the dosage form is possible (2).

In comparison to all other mucosal membranes, the vaginal mucosa offers the advantage that drug delivery systems can remain for the longest time period at the site of application. Robinson and Bologna, for instance,

have reported that a polycarbophil gel is capable of remaining on the vaginal mucosa for 3 to 4 days (3). Moreover; vaginal rings can remain on the vaginal mucosa even for months, which renders this route of non-invasive administration unique and provides promising opportunity for more efficient and convenient therapies.

The full potential of these drug delivery systems which provide a prolonged residence time, however, has yet to be realized. An overview of state-of-the-art vaginal retentive delivery systems shall be given within this review. It shall provide the basis for the development of novel more effective vaginal formulations guaranteeing prolonged residence times.

2. VAGINAL ANATOMY, HISTOLOGY and PHYSIOLOGY

The vagina is a muscular, tubular organ which plays a major role in reproduction. As shown in Figure 1, it connects the cervix (the opening of the uterus) and the vulva (the external genitalia).

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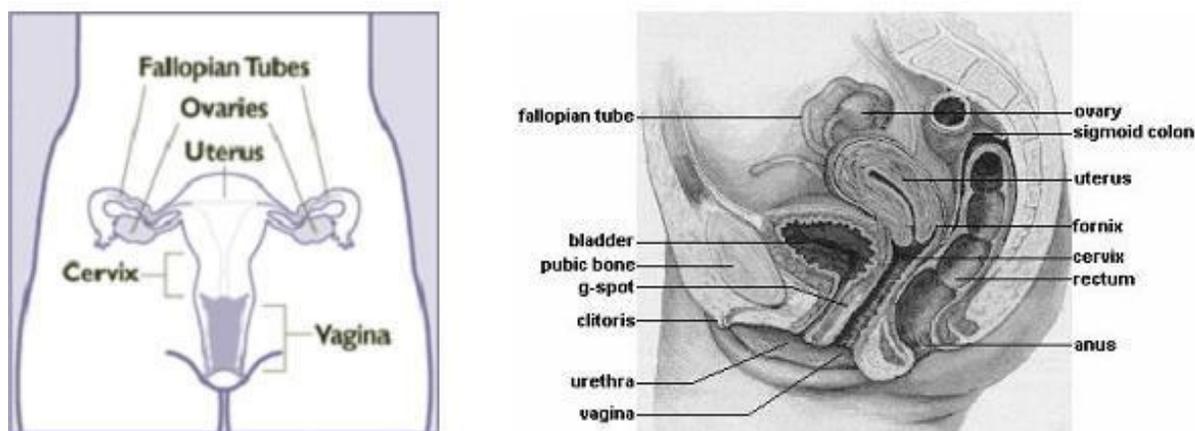


Figure 1. Schematic drawing of the vagina (7)

Table 1. Influence of age on the variation of pH, length, and width of human vagina (1,15)

Changes of vagina	pH	Length of vagina (cm)	Width of vagina (cm)
Before puberty	7	4.5-6	1-1.5
Reproductive age	4-5	10	2.5
Adult premenopause	4-5	7-8	2
Postmenopause	4-7	4.5-6	1-1.5

It is positioned between the rectum, bladder and urethra (4). The vagina is a slightly S-shaped fibromuscular collapsible tube and its dimensions range from 8.4 to 11.3 cm in length and 2.1 to 5.0 cm in diameter (5,6).

The vaginal wall consists of three layers: the epithelial layer, the muscular coat and the tunica adventia (8). The main blood supply to the vagina is through the vaginal branch of the uterine artery (4). The vaginal mucosa has no goblet cells but it secretes a large amount of fluid containing enzymes, enzyme inhibitors, proteins, carbohydrates, amino acids and alcohols (9). Among these secreted compounds are mucins, in particular MUC4, MUC5A/C and MUC8, covering the vaginal wall with a mucus layer (10). The enzymatic activity in the vagina is comparatively lower than in the gastrointestinal tract but there is still a wide range of enzymes present such as nucleases, lysozymes and esterases (11,12). Women of reproductive age produce fluid at a rate of approximately 6 mL/day, with 0.5-0.75 mL continually present in the vagina. The discharge produced by postmenopausal women is reduced by 50% compared to that produced by women of reproductive age (9,13,14). At the time of ovulation, mucus secretion increases and it becomes clear, thin and alkaline. At other times

the mucus produced is scanty and viscous (15). The ecology of the vagina is influenced by factors such as pH, hormonal levels and trauma during sexual intercourse, birth-control method, age and antimicrobial treatment. In the vaginal microflora, *Lactobacillus* (Döderlein's bacilli) is the most prevalent organism together with other facultative and obligate aerobes and anaerobes (1). The vaginal pH of healthy women of reproductive age is acidic. Lactic acid produced from glycogen by the *Lactobacillus acidophilus* present in the vagina acts as a buffer to maintain the vaginal pH between 3.8 and 4.2. As listed in Table 1 the pH changes with age, stages of menstrual cycle, infections and sexual arousal. During menstruation, the pH of vaginal fluid is comparatively higher (16). Menstrual, cervical and uterine secretions and semen act as alkalinizing agents and increase pH (2).

3. DRUGS FOR VAGINAL APPLICATION

The vagina has been studied as a favorable site for local and systemic delivery of drugs for female-related conditions (1,17). Traditionally, the vaginal cavity has been used for the delivery of locally acting drugs such as antibacterial, antifungal, antiprotozoal, antiviral, labor-inducing and spermicidal agents, prostoglandins, steroids,

metronidazole (18), lactic acid (19), itraconazole (20), polystyrene sulfonate (21), stampidine (22), 5-fluorouracil (23), clotrimazole (24), fluconazole (26), cisplatin (25), and bleomycine (27).

The vagina has also great potential for systemic delivery because of its large surface area, rich blood supply and permeability to a wide range of compounds including peptides and proteins. It offers a favorable alternative to the parenteral route for some drugs such as bromocriptine (28,29), bromocriptine mesylate (30), propranolol (31), oxytocin (32), misoprostol (33), calcitonin (34), LHRH agonists (35) and human growth hormone (36). The vaginal route has furthermore potential for new molecules of biotechnological origin such as monoclonal antibodies, vaccines and the uterine targeting of active agents (1).

Nyende et al. compared the efficacy of vaginal and oral misoprostol in women. It was reported that the vaginal misoprostol was more effective in a shorter time than oral misoprostol. Vaginal administration of the drug also provided significantly less side effects than oral route (37). It is believed that vaginal therapy can be significantly improved if a delivery system can keep the drug at the site of administration for longer than the conventional dosage forms can (38).

The mean plasma concentration of misoprostol after oral and vaginal administration was shown in Figure 2. The plasma concentration of misoprostol peaked in all subjects receiving the drug orally. Although, peak plasma levels were reached more slowly and were slightly lower with vaginal administration, the greater bioavailability of vaginal misoprostol was obtained. Due to the

hepatic metabolism, vaginal misoprostol was found more effective than oral misoprostol (39).

4. INTRAVAGINAL DRUG DELIVERY SYSTEMS

It is necessary to incorporate drugs in adequate vehicles in order to be administered intravaginally. Their design should address anatomical and physiological features of the vagina. Traditionally available dosage forms include solutions, emulsions, suspensions, vaginal tablets, suppositories and semi-solid formulations such as creams and ointments (1, 40-43).

Liquid formulations are inappropriate for controlled drug release due to their short residence time in the vaginal cavity. Vaginal tablets and inserts are convenient, simple to manufacture and a sustained drug release can be achieved over several hours. Vaginal inserts are also extensively used especially in the veterinary field (40-44).

A large number of vaginal medications are available in form of suppositories. Although solid formulations are easy and inexpensive to manufacture and their application is simple, the vaginal residence time is still poor, making frequent application necessary (43-46).

The main advantages of semi-solid preparations are their acceptability, feasibility and low cost. However, they can be messy to apply, uncomfortable and sometimes embarrassing if they leak into the undergarment (46). Moreover they may not provide an exact dose because of non-uniform distribution (47).

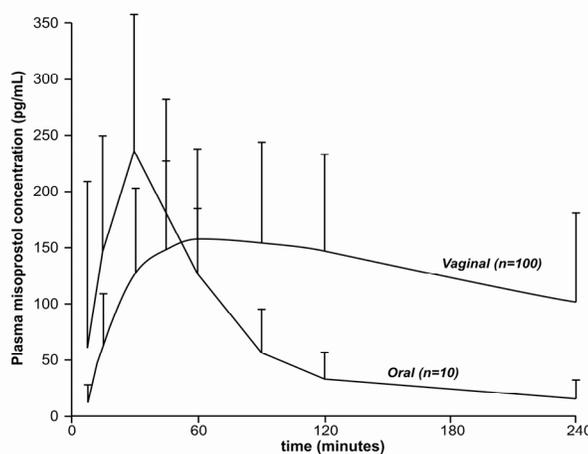


Figure 2. Mean plasma concentration of misoprostol over time with oral (solid line) and vaginal (dotted line) administration [Reproduced from Ref. 39 with permission].

Vaginal formulations, although generally perceived as safe, have historically been undesirable for night-time administration and multiple dosing. The delivery of the active agent plays a critical role in the overall success of the therapy. In order to overcome the mentioned shortcomings of traditional vaginal drug delivery systems, the development of novel, more potent vaginal retentive delivery systems is highly on demand (48).

Several aesthetic and functional qualities must be incorporated into such intravaginal formulations which need to be designed for a specific prolongation of residence time and also for desirable distribution and delivery of the active substance for an extended period at a predictable rate, retention and release characteristics (1). Generally a prolonged vaginal residence time can be achieved by the use of mucoadhesive and/or in situ gelling polymers and by mechanical fixation (3,49,50).

4.1 Mucoadhesive Drug Delivery Systems

Mucoadhesion is a topic of interest in the design of drug delivery systems. Various types of formulations have been widely used to prolong the residence time of the dosage form at the site of application (50). They were introduced in 1947 and recent reports have suggested that the market share of mucoadhesive drug delivery systems has been increasing (51). Mucoadhesive formulations play a key role in the release of the drug through their attachment to the vaginal mucosa and are currently being utilized for controlled release (15).

4.1.1. Dosage Forms

4.1.1.1. Mucoadhesive Gel Formulations

Mucoadhesive semi-solid formulations are able to facilitate intimate contact with the underlying absorption surface and improve the bioavailability of drugs. Rheological properties of gels are important for their retention on the vaginal surface, which are fundamental to their efficacy. The remarkable elastic character and the improvement of the rheologic properties of the mucoadhesive gel prolong the residence time at the application site (52). For these kinds of formulations, selection of correct viscosity of the formulation is important in order to provide adequate retention and distribution in the vagina.

These simple characteristics can be determinants in order to achieve a therapeutic effect (53,54).

Mucoadhesive polymers in gel formulations such as polycarbophil, hydroxypropylcellulose or polyacrylic acid are used to eliminate the effect of vaginal discharge which shortens the residence time of vaginal formulations (55). It was shown that a polycarbophil gel remains on vaginal tissue for 3-4 days and hence has the potential to serve as a platform for vaginal drug delivery (55).

Chitosan, another well-known mucoadhesive polymer, is used for the vaginal administration of drugs as a stable and mucoadhesive gel base (19). Degim et al. prepared and used the chitosan gel formulation for the vaginal administration of insulin and found it useful as a carrier for longer release (56).

Sodium alginate, hydroxypropylmethyl cellulose (Methocel K4M) and Carbopol ETD 2020 have been evaluated for their ability to gel fluconazole microemulsions for topical treatment of vaginal candidiasis. Only Carbopol ETD 2020 renders clear gel without disturbing the microstructure of the microemulsion and provided higher residence time in the vagina compared to conventional gels (25).

Clomiphene citrate has been used in the treatment of infections owing to human papilloma virus. Its mucoadhesive gel preparation with carbomers and their thiolated derivatives was prepared to increase the retention time of the drug at the application site and the results showed higher treatment efficiency with a lower dose (57).

Carbopol resins were used to prepare a polyherbal mucoadhesive drug delivery system for the local treatment of aerobic vaginitis. The relation between mucoadhesive performance and the rheologic property of gel was investigated. In addition, the effect of the vaginal fluid's dilution of the mucoadhesiveness of the gel was also evaluated. The mucoadhesive detachment force was almost unchanged or slightly higher with dilution which suggested that the dilution may provide better wetting of polymer and hence formation of stronger mucoadhesive bonds and prolongation of the residence time (58).

ACIDFORM is a whitish/colorless mucoadhesive vaginal gel and is able to remain more than 12 h after administration (59). It has some advantages over presently marketed vaginal delivery systems such as acid-buffering and viscosity-retaining properties. Furthermore it has been shown to exhibit greater intra-vaginal retention than other similar gel products

(Conceptrol, Advantage S, Replens, Aci-Jel, and K-Yjelly) (60,61).

Table 2 provides an overview on mucoadhesive vaginal formulations which are available on the market.

Currently, mucoadhesive gel formulations are used in antimicrobial therapy due to the dual-function of polymers such as chitosan with potential therapeutic activity (62,63). The maximum duration of drug release for intravaginal microbicidal drug delivery systems is 6 h for vaginal gels (64,65). The potential for developing controlled release formulations for long-term intravaginal delivery of microbicides has recently gained momentum and the recent mucoadhesive formulations under clinical research are shown in Table 3.

These mucoadhesive gel formulations are preferred over conventional intravaginal gel formulations because of their prolonged retention to the vaginal epithelium.

4.1.1.2. Mucoadhesive Tablet Formulations

Vaginal mucoadhesive tablet formulations are important alternatives to conventional vaginal formulations and are particularly useful for the therapy of insistent vaginal infections as they reduce the required dose frequency, provide easy application and therefore increase patient compliance.

Table 2. Some of the currently available vaginal mucoadhesive formulations

Product	Active Substance	Mucoadhesive Polymer	Company	Administration frequency/effect
Aci-Jel	Glacial acetic acid Oxyquinoline sulfate	Tragacanth (Acacia)	Janssen-Cilag	Twice daily administration
Crinone	Progesterone	Carbomer	Serono	Once daily administration
Zidoval	Metronidazole	Carbomer	3M	Once daily administration
Miphil	Acid Buffer	Polycarbophil Carbopol	Mipharm	Reduces vaginal pH for 72 h
Replens	-	Polycarbophil	Colombia Lab.	Relieves vaginal dryness up to 72 h
Conceptrol	Nonoxynol- 9	Sodium carboxymethylcellulose Sodium	Walgreens	
Gynol-II	Nonoxynol- 9	carboxymethylcellulose Polyvinyl pyrrolidone	Janssen-Cilag	
Advantage 24	Nonoxynol- 9	Carbomer	Colombia Lab.	Contraceptive protection for up to 24 h

Table 3. Mucoadhesive gel formulation under development used for prevention of sexually transmitted infections (27).

Gel Formulation	Active Substance	Polymer
Ushercell (Phase III)	Poly(sodium 4-styrene sulfonate) and Cellulose sulphate	Hydroxyethylcellulose
Pro 2000 gelTM (Phase III)	Naphthalene sulfonate	Carbomer
Buffer gelTM (Phase III)	Lactic acid and Hydrogen peroxide	Polyacrylic acid
CarraguardTM (Phase III)	Carrageenan	Carrageenan
PC-815 (pre-clinic)	Carrageenan and MIV150	Carrageenan Sodium carboxymethylcellulose
Monocaprin hydrogel (pre-clinic)	Monocaprin	+ Polyvinylpyrrolidone or Carbomer + Hydroxypropylmethylcellulose

The first mucoadhesive tablets contained hydroxypropylcellulose and polyacrylic acid as mucoadhesive polymers and were used to treat cancerous lesions with bleomycin (55).

A mucoadhesive vaginal tablet comprising ornidazole has been prepared with various mucoadhesive polymers such as Carbopol 934, pectin, hydroxypropylmethylcellulose, sodium carboxymethylcellulose and guar gum. All of them have shown mucoadhesive properties to the bovine vaginal mucosa. The drug release has been followed for 24 h and no histological damage is observed 24 h after administration (42).

Chitosan is used in mucoadhesive tablets for vaginal delivery of metronidazole. The polymer matrix containing a mixture of 6% chitosan, 24% sodium alginate, 30% sodium carboxymethylcellulose, 20% microcrystalline cellulose provides an adequate release of metronidazole for 8 h and maximum adhesion was also obtained with minimum pressure applied (18).

Polycarboxophil and sodium carboxymethylcellulose were chosen as mucoadhesive polymers for acid-buffering mucoadhesive vaginal tablets. Ex vivo retention studies justified the prolong retention of the tablet inside the vaginal tract and it was found more effective than existing vaginal drug delivery systems (66).

4.1.1.3. Mucoadhesive Suppositories

Among the solid vaginal preparations, suppositories have advantages as they facilitate the application due to their slippery and smooth surface. To provide a long term therapeutic concentration of miconazole following a single dose, hydrogel based suppositories were prepared with polyvinyl alcohol and the effect of the time of the freezing and thawing cycles was investigated vis-a-vis the swelling property of the hydrogels outcome. It was observed that an increase in the number of freeze-thaw cycles reduced the equilibrium swelling of polyvinyl alcohol hydrogel. This reduction in equilibrium swelling was due to an increase in the degree of crystallinity of the hydrogel. The release of the drug was continued beyond three days and the Fickian diffusion mechanism of release was found predominant (67).

4.1.1.4. Mucoadhesive Particulate Formulations

Although several studies have focused on mucoadhesive drug delivery systems in the form

of tablets, films, patches, and gels for mucosal routes, very few reports on mucoadhesive microparticles are available (50).

Hyaluronan esters (HYAFF) have opened new avenues for mucoadhesive vaginal formulations. Due to their biocompatibility and controllable degradation rate HYAFF microspheres have been used for localized drug delivery of steroids, analgesics, anti-inflammatory and anti-infectives (17). In various studies bioadhesive vaginal drug delivery systems containing various drugs such as insulin, nerve growth factor and salmon calcitonin were prepared with HYAFF polymers and satisfactory results were observed (17,68,69). Hyalurane benzyl esters are highly mucoadhesive polymers and can be processed into microspheres that can effectively deliver incorporated drugs by closely adhering to the mucosal surface.

Recent studies have indicated that a number of polymeric delivery systems such as mucoadhesive microspheres possess significant potential for the development of vaginally administered vaccines (70,71).

4.1.1.5. Mucoadhesive Film Formulations

Thin films have been found to be suitable physical forms for vaginal drugs and peptide delivery (72,73). To develop an efficient female controlled drug delivery system against sexually transmitted diseases, polymeric films containing sodium dodecyl sulfate were prepared with various compositions of Carbopol 934P, hydroxypropylmethylcellulose and polyethyleneglycol. It was demonstrated that the films made of Carbopol, hydroxypropylmethylcellulose and polyethyleneglycol were colorless, thin and soft, also had sufficient strength to withstand mechanical damage during production, handling and application for a female controlled drug delivery system. An increase in Carbopol content elevated the tensile strength and swelling ratio of the films but decreased the contact angle, erosion rate and sodium dodecyl sulfate release rate from the films. The films containing 0.25% (w/v) polyethyleneglycol as well as 0.75% (w/v) of Carbopol / Hydroxypropylmethylcellulose remained on the vaginal tissue for up to 6 hours (74).

4.1.2. Techniques to Determine the Residence Time of Mucoadhesive Intravaginal Formulations

The effectiveness of mucoadhesion to prolong the residence time at the site of administration is an important parameter for the mucoadhesive formulation. An increase of mucoadhesive force, between the formulation and the mucosal surface, successfully retain the drug delivery system and retard the natural clearance processes (75,76). Therefore, the quantification of the mucoadhesive forces between polymeric formulations and mucosal tissue is a useful indicator for residence time. In vitro techniques which measure mucoadhesion can provide information about the retention time of the formulation on the vaginal tissue.

4.1.2.1. Wilhelmy plate surface technique

It is a simple method which allows the analysis of mucoadhesion under different environmental conditions. The device consists of a glass plate which is dipped into a beaker of mucus. The mucoadhesive characteristic of the test compound is evaluated according to the work required to remove the plate from the mucus (77).

In some studies, modified Wilhelmy plate surface technique was also used (Figure 3). The modified device itself basically consists of a glass plate (which is laden with the polymer to be studied) suspended from a microbalance. The

polymer-coated plate was then slowly dipped into a beaker of mucus. This technique had the advantage of allowing the analysis of mucoadhesion under different environmental conditions via simple modification of instrumental setup. Subsequently though Mikkos and Peppas pointed out the short comings of such a technique due to the possible dissolution of the polymer upon testing (78). They suggested that this effect may be limited if polymer plates of the candidate material were used instead of polymer-coated glass plates. Further shortcomings were also detailed by Wong et al. who noted that the lack of biological tissue in such a setup may not represent true mucoadhesion (79). Despite the simplicity and efficiency of such a technique, tensile tests provide an incomplete picture of the process of mucoadhesion.

4.1.2.2. Bioadhesive force determination device

In this method as outlined in Figure 4, the mucosal tissue is secured with a glass vial using a rubber band and an aluminum cap. One vial with a section of tissue is connected to the balance and the other vial is placed on a height-adjustable pan. 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 (80).

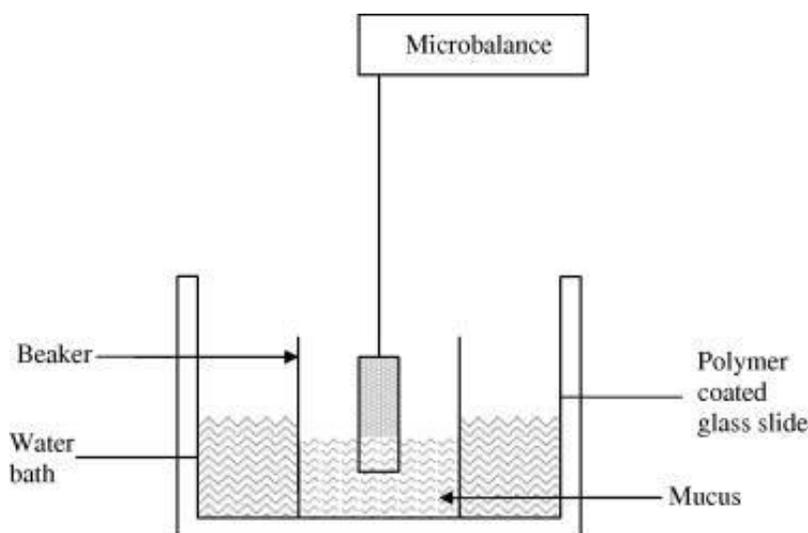


Figure 3. Illustrating the modified Wilhelmy plate surface technique, for mucoadhesion determination [Modified from Ref. 61 with permission].

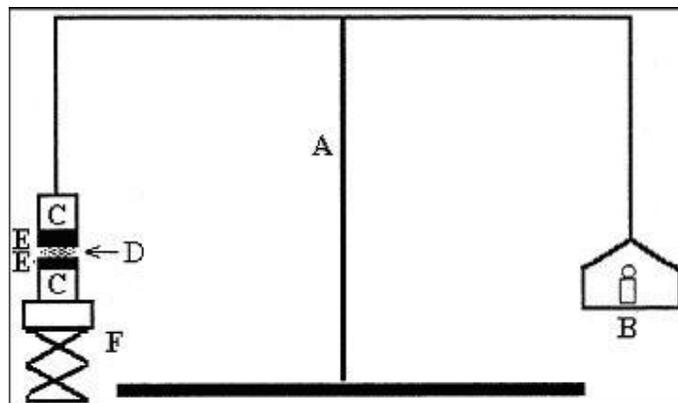


Figure 4. Mucoadhesive force-measuring device, (A) modified balance; (B) weights; (C) glass vial; (D) formulation; (E) tissue; (F) height-adjustable pan [Reproduced from Ref. 80 with permission].

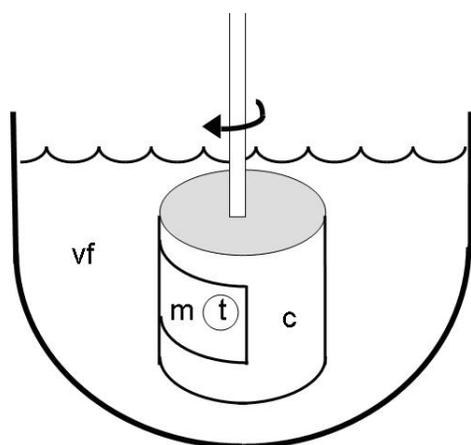


Figure 5. Schematic presentation of the test system used to evaluate the mucoadhesive properties of tablets. vf: vaginal fluid, c: cylinder, m: vaginal mucosa, t: tablet [Modified from Ref. 83 with permission].

4.1.2.3. Electromagnetic force transducer (EMFT) technique

EMFT which is a remote sensing instrument has unique ability to record remotely and simultaneously the tensile force of mucoadhesive interactions under simulated physiological conditions. The EMFT measures tissue adhesive forces by monitoring the magnetic force required to exactly oppose the mucoadhesive force. The primary advantage of EMFT is that no physical attachment is required between the force transducer and the formulation. This makes it possible to perform accurate mucoadhesive measurements on small microspheres. In this method briefly, the tissue sample is mounted in a special chamber with the microspheres positioned directly under the magnet tip. The stage is slowly moved away from the tip, and the video camera is used to detect motion of the sphere. As the sphere moves away from the magnet tip, the

control system increases the magnet current accordingly. The change in magnetic field strength results in a force that pulls the magnetic sphere back into its original position. This process is repeated until the sphere is pulled free from the tissue (81). This technique can also be used to evaluate the mucoadhesion of polymers to specific cell types and hence can be used to develop mucoadhesive drug delivery systems to target-specific tissues such as vaginal mucosa (17).

4.1.2.4. Rotating cylinder technique

To evaluate the binding to the mucosa, a new method has been established by Bernkop at al. As shown in Figure 5 tablets are attached on the freshly excised vaginal mucosa which has been fixed on a stainless steel cylinder. Thereafter, the cylinder is placed in a dissolution apparatus according to the USP containing simulated

vaginal fluid. The fully immersed cylinder is agitated and the detachment time of the tablets is evaluated visually (82,83).

4.1.2.5. Texture analyzer

A texture analyzer is used as a simple test which is able to measure mucoadhesion under simulated vaginal conditions, based on the principle of application of tensile strength and shear stress to break the adhesive bond between the test sample and a model membrane. The experimental set-up is illustrated in Figure 6. Several surface substrates such as hydrated cellophane with simulated vaginal fluid, mucin tablet, sheep vaginal mucosa, bovine vaginal mucosa have been used as a model membrane using texture analyzer (57, 84-87).

The measurement of mucoadhesion with texture analyser could be influenced by the instrumental parameters and test conditions such as pre-hydration time of polymer disc, contact time, contact force, test speed of probe withdrawal, gastrointestinal tissue and test medium.

Thirawong et al. examined mucoadhesive performance of various pectins with different degrees of esterification and molecular weights with mucosal tissue, using a texture analyzer equipped with mucoadhesive platform. The instrumental parameters and test conditions such as pre-hydration time of pectin disc, contact time, contact force, test speed of probe withdrawal, mucosal tissue and test medium were also studied. Their results indicated that degree of hydration of pectin disc affected the mucoadhesive properties. The mucoadhesion of pectin increased with the

increased contact time and contact force, but not by the increased probe withdrawal speed (76).

For mucoadhesion studies of tablets and film formulations, vaginal mucosa free from supporting tissues or mucin tablet which is moistened is held on the lower platform of the texture analyzer. The formulation is applied to the upper probe with the help of a double-sided adhesive tape. For gel formulations, vaginal mucosa or mucin tablet is held on the upper platform of the texture analyzer. The formulation, in small cylindrical vessel, is placed across to the upper probe. The force required to detach the probes is determined as the peak value in a force-time graph.

4.1.2.6. Lowering Platform

A lowering platform was used to measure the force of detachment of mucoadhesive tablet formulation (74,88). Yoo et al. 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 Figure 7.

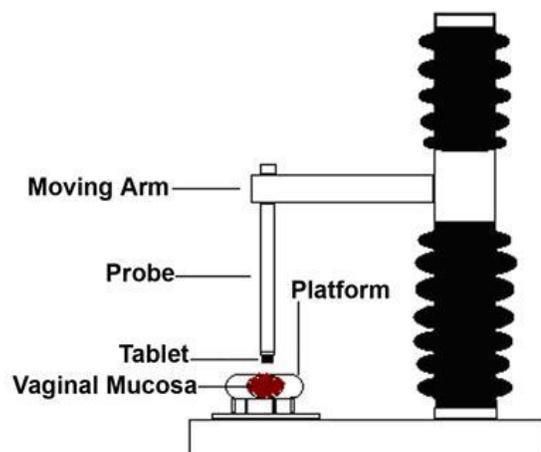


Figure 6. Outline diagram of texture analyzer showing the set up of in vitro mucoadhesion experiments for tablets [Reproduced from Ref. 66 with permission].

4.1.2.7. Modified Setnikar-Fanteli Technique

This is a technique for *ex vivo* mucoadhesion/retention studies as outlined in Figure 8. Distilled water is circulated through the two small side arms into the glass cell with a pump (66).

Ceschel et al. evaluated the adherence of a new dosage form for clotrimazole comprising 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 (89).

4.1.2.8. Modified Dual Tensiometer Method

Despite the simplicity and efficiency of tensile tests, they provide an incomplete picture of the process of mucoadhesion because most mucoadhesive delivery systems will tend to exhibit other mechanical forces, such as the shear stresses exhibited within application site. The effect of both these important forces on measuring the adhesive bond was made possible via the use of a dual tensiometer such as the one used by Leung et al. (Figure 9). The vertical tensile stress of copolymers to mucosal tissues was studied using a modified tensiometer method, and was found to correlate closely with their degree of hydration. Degree of hydration is related to the expanded nature of the polymer network, which in turn is related to charge density, and this contributes significantly to the strength of mucoadhesion (90).

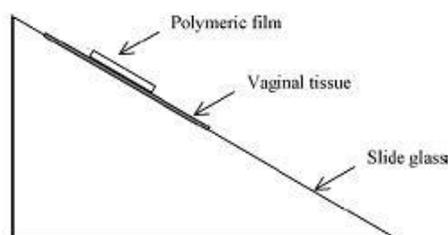


Figure 7. Lowering platform to measure the detachment force of mucoadhesive film [Modified after Yoo et al. (74) with permission].

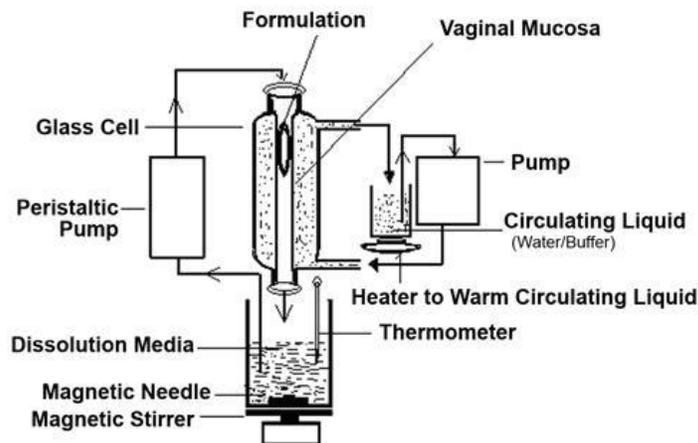


Figure 8. Apparatus for *ex vivo* experiments for mucoadhesion [Reproduced from Ref. 66 with permission].

In order to study the contribution of anionic polymer structural features to mucoadhesion, a series of 0.2% cross-linked copolymers (acrylic acid-methyl methacrylate) were synthesized using free-radical polymerization. Both infrared spectroscopy and acridine orange binding were used to characterize the polymers. The vertical tensile stress of these copolymers to tissue was studied using a modified tensiometer method, and was found to correlate closely with their degree of hydration. Degree of hydration is related to the expanded nature of the polymer network, which in turn is related to charge density, and this contributes significantly to the strength of mucoadhesion.

A dual tensiometer apparatus was also developed to study the shear stress of mucoadhesives. The mucin-mucin shear stress was found to increase with applied weight to a limiting plateau value. The shear stress was found to decrease with addition of calcium and increase with addition of EDTA. It seems that the expanded nature of both the interacting mucus and polymer networks influences the strength of mucoadhesion (61).

4.1.2.9. Agar Plate Method

In the method reported by Bachav et al. an agar plate (1%, w/w) is prepared in pH 4.5 citrated

phosphate buffer. The test sample is placed on the center 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 $37 \pm 1^\circ\text{C}$. The sample on the plate is immersed into 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 (91). The experimental set-up of this method is illustrated in Figure 10.

4.1.2.10. Mucin- Gold Staining Method

In this method, red colloidal gold particles are conjugated with mucin. Upon interaction with mucin-gold conjugates, mucoadhesive hydrogels develop a red color on the surface. The mucoadhesive properties of hydrogels can be compared quantitatively by measuring the intensity of the red color (92).

4.1.2.11. Glass Spheres Method

A simple, quantitative and realistic in situ method is used to evaluate the mucoadhesive potential of polymers.

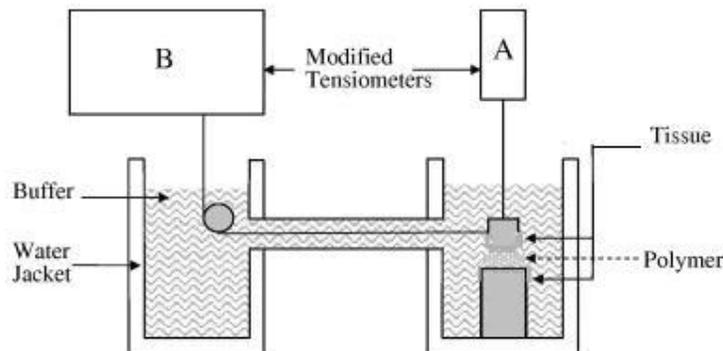


Figure 9. Illustrating the modified dual tensiometer [Modified from Ref. 90 with permission].

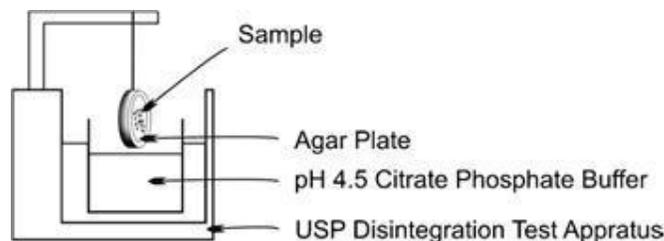


Figure 10. The schematic configuration of the apparatus used for agar plate method [Reproduced from Ref. 91 with permission].

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 proper buffer solution at a constant rate. The percent of particles retained on the tissue is considered as an index of mucoadhesion (93).

4.1.2.12. Flowing Liquid Film Technique

The flowing liquid film technique is quantitatively sensitive method and, consequently, allows one to focus on the mechanism of the approach of micron-size particles to the mucous surface without the major concern of particle flocculation in the bulk liquid. In this method, the adsorption of particles and concurrent steady-state flow of a dilute suspension from an infinite reservoir over the mucous surface of mucosal strips are quantitatively studied. Thin falling liquid film system was developed wherein an excised mucosal tissue cut lengthwise is spread on a plastic flute and positioned at an incline, and a suspension is allowed to flow down the mucosal strip. The Coulter counter is used to determine the concentration of the particles which enters the segment from the dilute suspension reservoir and leaves the intestinal segment. Thus, the steady state fraction of particles adsorbed is quantified (94).

4.1.2.13. Rheological Methods

The analytical techniques such as creep analysis, flow rheometry, oscillatory rheometry, dielectric spectroscopy and gel strength/shear and compression analysis are used to investigate the product performance evaluating their structural and rheological properties which may be related with the product performance. These techniques cannot describe the textural properties of the formulation which is directly related with the retention of the product at the application site. In the development of topical mucosal adhesive dosage forms, it is useful to quantify the interaction between the dosage form and mucosal substrate. There are few methods available that may be employed to quantify the strength of interaction of gel systems with either mucous epithelia or mucin. Jones et al., used both non-destructive rheology to convey information concerning both the physical structure of the gels and to predict the effects of the stresses encountered under physiological conditions on

their structural properties, and also texture profile analysis to characterise the mechanical and textural properties of gel systems (86).

The rheological method was used to measure the mucoadhesive force between the mucin and polymers. The effect of the ionic charge on the mucoadhesive force was evaluated and the technique was validated. Viscosity of mucin was measured with a Brookfield viscometer in the absence of neutral, anionic and cationic polymers and the force of mucoadhesion (F) was calculated using Equation 1.

$$F = \eta \sigma \text{ (Equation 1)}$$

σ : shear rate (s^{-1}), η : viscosity

These two parameters give a direct estimate of the polymer-mucin interactions occurring in mucoadhesion (95). It is clear that the force of mucoadhesion is dependent on the initial viscosity of the polymer (76,86,96).

The rheological behaviour of a mucus-carbopol 934P gel was evaluated using mechanical spectroscopy. This gel strengthening was markedly affected by pH (i.e., it was minimal at pH values below 4.5 and above 8), while temperatures up to 45°C did not break down this gel. It was concluded that molecular interpenetration resulting in strengthening of the layer between the mucoadhesive/mucosal surface may offer an explanation for the large forces required to break a mucoadhesive joint (97).

4.1.2.14. Gamma Scintigraphy Method

More quantitative information can be obtained with in vivo methods based on imaging techniques for the residence time. Measuring the retention of the formulation on the administered site has been difficult. Nuclear medicine techniques have proven valuable in assessing the retention of pharmaceuticals. Mucoadhesive vaginal formulations can be evaluated with scintigraphic techniques (98).

The retention of a commercially available vaginal clotrimazole cream labeled with ^{99m}Tc -DTPA was assessed by gamma scintigraphy for 24 h after administration of the products. Images were obtained and geometric mean counts were recorded. The data were then converted to the remaining amount of the vaginal formulation (99).

Technetium-labeled HYAFF microspheres and a dry powder formulation which were administered intravaginally to the sheep were

dispersed along the length of the vagina and were retained at this site for the duration of the study. 12 hours after administration, between 60 and 80% of the radioactivity remained within the vagina with retention of the microspheres being slightly higher for the dry powder formulation than for the vaginal pessary (100).

The gamma-scintigraphy methodology was not only used to monitor mucoadhesion of intravaginal formulation in animals but also in humans. Brown et al. reported that gamma scintigraphy is a valuable technique with which to assess novel formulations aimed at optimizing retention in the vagina for topical or systemic drug delivery (45).

Despite the disadvantage that gamma scintigraphy is used only on post menopausal women, this radioactive tracer method has proven to be useful in assessing vaginal retention of the vaginal dosage forms (41,99).

4.1.2.15. Visualization with dyes

Dyes are used to determine 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. At 1 h after administration, mice were sacrificed and the retention of the mucoadhesive delivery systems at the administered sites was visualized by the blue color of the dye (101).

4.1.2.16. Analytical methods

Thus far, to our knowledge no analytical methods that enables *in vitro* determination of the residence time of systems that are based on mechanical fixation have been reported.

4.2. In-Situ Gelling Systems

In situ gelling drug delivery systems that release drugs in response to various environmental conditions such as temperature, access to oxygen, pH and ionic strength. Therefore, they provide sufficient coverage of the vagina and longer retention of the formulation on the mucosal tissue.

The *in-situ* gelling properties of polymers can be determined by simple rheological measurements of aqueous polymer gels.

Rheological investigations are performed before, during and after changing the conditions in a way that *in-situ* gelation is initiated such as change of temperature, pH or ionic strength (102).

4.2.1. Types of *in situ* gelling systems

4.2.1.1. Thermoplastic systems

Thermoplastic graft copolymers are well-known *in situ* sol-to-gel transition polymers which are sensitive to the changes of temperature. Non-aqueous solutions of the copolymer in hydrophilic excipients undergo *in situ* gelation shortly after application (103).

Recent studies confirmed that the combination of a thermosensitive polymer like poloxamer and a mucoadhesive polymer like polycarbophil, appears to be promising for vaginal formulations (104).

Chang et al. prepared both thermosensitive and mucoadhesive gels of clotrimazole and evaluated the effect of the rheologic properties on the formulation characteristics in order to strengthen the adhesivity and spreadability of the formulation on the vaginal mucosa. Within this study the mixture of Poloxamer 407/ Poloxamer 188/ Polycarbophil (15/20/0.2) was found to be the most suitable as an intravaginal delivery system for clotrimazole (102,105). In other studies, it was also observed that the rheology of gels seems to correlate with mucoadhesion (106,107).

Baloglu et al. prepared various mucoadhesive gel formulations of miconazole nitrate and econazole nitrate with different ratios of poloxamer 188 and 407 in order to gain appropriate mechanical properties and to provide prolonged retention on the vaginal mucosa. The results showed that the thermosensitive gel formulation consisting of 20% Poloxamer 188 and 10% Poloxamer 407 provided a proper gelation temperature for the vaginal administration. Furthermore, the sol-to-gel transition temperature determined to be $38 \pm 0.3^\circ\text{C}$ was not affected by the addition of 1% of miconazole (108).

Bilensoy et al. formulated a vaginal gel with clotrimazole using the thermosensitive polymer Pluronic F127 together with mucoadhesive polymers such as Carbopol 934 and hydroxypropylmethylcellulose. The gelation temperature, rheological behavior and *in vitro* release profile of the formulations were determined. The optimum formulation for a

controlled-release thermosensitive and mucoadhesive vaginal gel was determined to be based on 0.2% hydroxypropylmethylcellulose in Pluronic F127 gel (20%) providing continuous and prolonged release of active material (24).

Pluronic F127 was also used in combination with mucoadhesive polymers such as, hyaluronic acid, Carbopol 934 and hydroxypropyl-methylcellulose to prepare a thermosensitive vaginal gel for treatment of human papilloma virus-induced cervical cancers (23).

Topical treatment is the most common method of administration because of the systemic toxicity of most antifungal drugs used for therapy of vaginal yeast infections. To be most effective, antifungal agents need to remain at the site of infection for prolonged periods. Conventional over-the-counter gel formulations do not remain at the site for extended periods. To provide high patient compliance, the frequency of administration must be reduced. Mucoadhesive formulation with prolonged antifungal activity based on a combination of poloxamers and polycarbophil were reported. The formulation exhibited significantly prolonged activity over a currently available formulation. It was also shown that cell viability and morphology of the vaginal tissue support the safety of mucoadhesive thermosensitive gels (109).

Recently, it has been suggested that intravaginal thermosensitive and mucoadhesive gels are promising for the induction of vaginal antibody response. Generally, phosphate-buffered saline solution are mostly used as vehicle, dosing and the rapid leakage of the free-flowing solution type vaccines from the vaginal cavity limits the immunogenicity of these intravaginally administered vaccines. Prolonged retention of antigens on the vaginal mucosa can be achieved by the development of in situ gelling mucoadhesive formulations which are able to adhere to the vaginal mucosa for a prolonged time period. An in situ gelling mucoadhesive delivery system for hepatitis B surface antigen being based on poloxamer and polycarbophil showed for instance a prolonged retention on the vaginal tissue. Results showed that thermosensitive and mucoadhesive polymer-based vaginal delivery systems might be useful in enhancing the mucosal and systemic immune response (101). Generally the number of studies on intravaginal vaccination has increased significantly (70,110).

4.2.1.2. Oxidizing Systems

Access to oxygen is another factor which induces the in situ sol-to-gel transition. Thiolated polymers show in situ gelling properties. Due to the oxidation of thiol groups at physiological pH values disulfide bonds are formed leading to extensively crosslinked polymer gels. This oxygen dependent sol-to-gel transition property can be exploited to obtain liquid and semi-solid drug formulations of favorable viscoelastic properties which stabilize themselves once applied at the site of delivery and exhibit limited clearance with prolonged residence (111).

4.2.1.3. pH Sensitive Systems

The pH sensitive polymers can be classified as acidic weak polyelectrolytes and basic weak polyelectrolytes according to the method of ionization, i.e., donating or accepting protons. Corresponding to the pH variation range in vivo, weak polyelectrolytes with the pKa between 3 to 10 are suitable candidates for biomedical applications. Typical acidic pH-sensitive polymers are based on polymers containing carboxylic groups such as polyacrylic acid, poly(methacrylic acid) (PMAA), poly(L-glutamic acid) (PLG), and polymers containing sulfonamide groups (112).

Both temperature and pH sensitive systems named intelligent polymers based on poly(N-substituted acrylamide)/poly electrolyte block copolymer were also studied (112).

4.2.1.4. Ionic Strength Depending Systems

Ionic strength can modify the rheological properties of polymers such as those of Poloxamer (113), gellan gum (114) and Xantan gum. Xantan gum which is a natural polymer interacts with mucin and its viscosity increases due to the ions present (115). The viscosity of pectin gels can also considerably increase with increasing ionic strength. As the ionic strength is raised, the polyanionic charges are likely to be suppressed and this leads to the significant increase in viscosity. Highly methoxylated pectin showed an even greater interaction resulting also in higher mucoadhesive properties (76). Sodium-deoxycholate (Na-DOC) is a naturally occurring bile salt and it is able to form gels. Na-DOC forms a viscous thixotropic gel when in contact with excess of ions (116).

4.3. Mechanical Fixation

The mechanical fixation of drug delivery systems in the vagina can provide high patient compliance and can lower side effects by reducing the frequency of administration.

4.3.1. Formulations

4.3.1.1. Tablets and discs

Voorspoels et al. designed a specially shaped tablet to increase its adherence to the genital tract. The tablet has a diameter of 20 mm, a flat bottom and a concave upper surface, aiming to better fit the uterine cervix (Figure 11). The results showed that higher cure rates might be obtained but more research is needed in relation to vaginal hydrodynamics and tablet shape and the relation with tablet residence time and erosion rate (117,118).

Discs may be another alternative in the near future for therapeutics derived from biotechnology and antibody-based drugs due to their ability to fix on the vaginal wall (50,119,120).

Vaginal discs prepared with ethylene-co-vinyl acetate (EVAc) were used to examine systemic antibody distribution during prolonged vaginal administration. Polymer matrices were fabricated by dispersing antibody particles in EVAc using a solvent evaporation process. Discs were fixed to mice vaginal wall with single suture. Mucus samples were collected for 30 days. Antibodies were slowly released from EVAc matrices. Even IgM molecules were released continuously from the polymer discs for

a long period. In all cases, detectable amounts of antibody were present in the vaginal lumen up to 30 days after insertion. The results provided important information for the design of controlled antibody delivery devices for vaginal use and long term therapy (121).

Livingston et al., presented DNA vaccines to induce mucosal immunity via vaginal route using the gold-DNA coated tubing which were cut into $\frac{1}{2}$ in pieces and were applied on the vaginal mucosa and abdominal skin. It was found that the vaginal immunization yielded higher titer cervicovaginal antibodies than the skin route of immunization. IgA and IgG antibody titers to HGH were sustained for at least 14 weeks. It showed that the vaginal mucosal route is more effective than systemic immunization via the skin (36).

Many researchers have been working to overcome the difficulties of immunizing the female genital tract and related problems (122-124).

Shen et al. has worked on controlled delivery of a DNA vaccine via the vaginal route. The DNA-loaded EVAc discs were produced by solvent evaporation and were inserted into the vaginal tract of mice. In this work, the discs triggered the immune system and induced specific IgA to LDH-C4 in the vaginal secretions. The gene expression in the vagina was evaluated up to 28 days showing the highest expression at day 6 and then a slowly decrease. At day 28 there was still detectable gene expression in the vagina. The immune response to the expressed protein was also sustained.

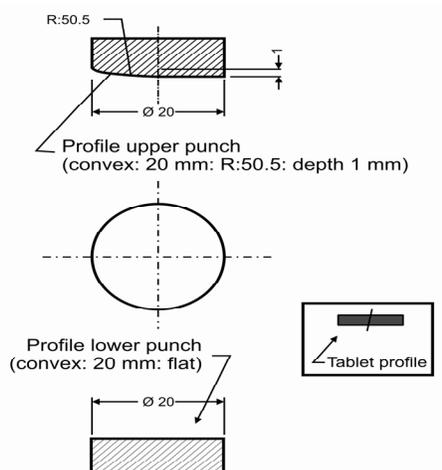


Figure 11. Upper and lower punch geometry for the production of a vaginal tablet with a diameter of 20 mm and a concave upper side [Reproduced from Ref. 117 with permission].

The results demonstrated that the vaginal device was an efficient and a convenient vehicle for delivering DNA to the vaginal tract providing long-term local immunity (125).

4.3.1.2. Intravaginal rings

Several drugs have been approved for vaginal administration either locally or systemically. The intravaginal ring technology allows non-daily, low and continuous dosing, achieves lower side effects, makes drug administration easy and discrete for patients and improves patient compliance. Intravaginal rings are doughnut-shaped polymeric devices as illustrated in Figure 12 and are designed to provide a controlled release of drugs to the vagina for extended periods of time.

Vaginal rings are easily inserted and removed. The vaginal walls hold them in place. Although their exact location within the vagina is not critical for clinical efficacy, rings commonly reside next to the cervix. Intravaginal rings deliver active substances, mostly hormones, at uniform concentrations and over a longer period of time; they allow lower doses to be used, and

can still be user controlled. They also allow accurate, controlled administration of drugs for up to one year and an earlier removal is possible. Although some women hesitate to use intravaginal rings out of fear of them getting lost, the intravaginal rings offer a novel approach and also have increasing acceptability among women (127).

Intravaginal devices are particularly suitable drug delivery device to provide controlled release and to enhance both patient compliance and minimize fluctuations in the level of estrogen. Figure 13 showed the mean plasma concentration of 17 β -estradiol 84-day period with an intravaginal ring (128).

Intravaginal ring delivery systems are usually based upon silicone elastomers with an inert inner ring which is coated with another layer of elastomer containing drug. An outer rate controlling elastomer layer may be added as a third to prevent an initial burst release in various rings. The rings are 6 cm in diameter and 4-7 mm in cross-section. The rings are left for 21 days and can deliver drugs at a consistent ratio with approximately zero-order kinetics (55).



Figure 12. A matrix-type silicone an intravaginal ring. Dimensions: 54 mm external diameter, 9 mm cross-sectional diameter [Reproduced from Ref. 126 with permission].

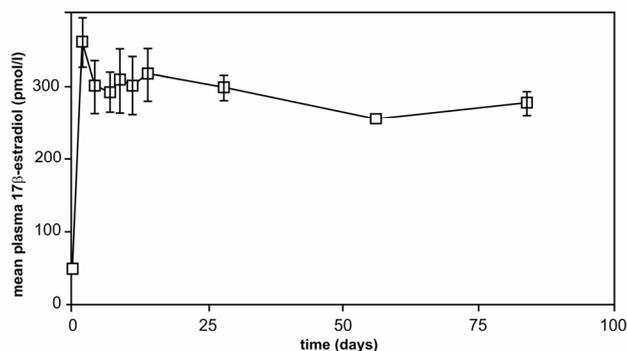


Figure 13. Mean plasma 17 β -estradiol concentrations in twelve healthy postmenopausal human volunteers treated over an 84-day period with a intravaginal rings having a nominal release rate of 100 mg/day estradiol (as its 3-acetate ester) in vitro under sink conditions [Reproduced from Ref. 128 with permission].

Nuvaring is a doughnut-shaped combined contraceptive (etonogestrel/ ethinyl oestradiol) vaginal ring marketed in different countries. It is worn vaginally for 3 weeks and removed for one week. Its contraceptive efficacy is 99.4% and the ring has been well tolerated with good compliance (129).

Nesterone-containing vaginal rings for contraception include Nesterone and ethinyl estradiol. The vaginal ring is made of dimethylsiloxane/ vinylmethylsiloxane copolymer and is supported with medical adhesives. The steroids are contained in cores within the ring body. The rings are designed for 1-year efficacy (Figure 14) (130).

Melt extrusion represents an efficient pathway for the manufacturing of vaginal rings. A contraceptive vaginal ring containing etonogestrel and ethinyl estradiol has been prepared by melt extrusion. Coaxial fibers have been composed to the main structure of the intravaginal rings with varying steroid concentrations in the polymer (131).

In order to provide a longer retention of medication in the vagina and assure a more constant delivery of active ingredients to the tissue, several types of vaginal rings have been patterned. For contraception, a patented vaginal ring was designed with an elliptical shape and was impregnated or covered with lectins which are able to immobilize sperm (132). Although, lectins are generally used in contraception and in the therapy of sexually transmitted diseases, they are also used as a specific cyto-adhesion agent due to their cell-specific characteristics. Lectin-mediated drug delivery is proposed as the next generation of mucoadhesives. There are various studies with lectins in the literature related to their ability to provide long term fixation directly to the surface of cells, independent of mucus turnover (61,133-135).

The development of ring delivery systems would also be an alternative and have an importance for efficient therapy with microbicides against HIV-1, in contrast the poor vaginal retention time of conventional semi-solid formulations are inadequate for optimal protection against HIV infection (136,137).

5. FUTURE TRENDS

5.1. Thiolated Polymers

Since the concept of mucoadhesion was pioneered, numerous modifications have been investigated to improve the adhesive properties of

polymers. However, most of the attempts have been based on the formation of non-covalent bonds and have provided only weak adhesion. A recent approach to improve the adhesiveness of polymers is about the immobilization of thiol groups onto polymer systems and forming covalent bonds with the mucus layer. "Thiolated polymers" or "thiomers" are the second generation of mucoadhesive polymers which display thiol bearing side chains. Figure 15 shows the chemical structure of thiolated polymers (138).

Based on thiol/disulfide exchange reactions and/or a simple oxidation process as shown in Figure 16, disulfide bonds are formed between thiomers and cysteine-rich subdomains of mucus glycoproteins. Hence, thiomers mimic the natural mechanism of secreted mucus glycoproteins, which are covalently anchored to the mucus layer by the formation of disulfide bonds and their mucoadhesive properties are significantly improved (138).

For example, the mucoadhesive properties of chitosan can be significantly improved by the immobilization of thiol groups on a polymer. A degree of modification of 25-250 μmol thiol groups per gram chitosan leads to the highest improvement in mucoadhesive properties (139). Thiomers are capable of forming intra- and interchain disulfide bonds within the polymeric network leading to strongly improved cohesive properties and the stability of drug delivery systems such as matrix tablets can also be improved with thiomers. Strong cohesive properties are highly advantageous because the loss of parts of disintegrated tablets or the leakage of a semisolid system cause a dislike for vaginally applied systems and therefore a limited compliance (83). Furthermore, thiolated polymers display in situ-gelling features and provide an almost zero order release of drugs (140).

To design a novel carrier system based on a mucoadhesive polymer exhibiting improved properties concerning drug delivery to the vaginal mucosa, polyacrylic acid-cysteine conjugate (NaC974P-Cys) was synthesized. Valenta et al. formulated and evaluated a mucoadhesive vaginal tablet of progesterone with this thiolated polymer. The thiolated tablets had good mucoadhesive properties compared to tablets with unmodified polymer. As a result of formation of disulfide bonds between the thiolated polymer and the mucus layer, the adhesive strength of thiolated tablets was an up to 2.28-fold increase.

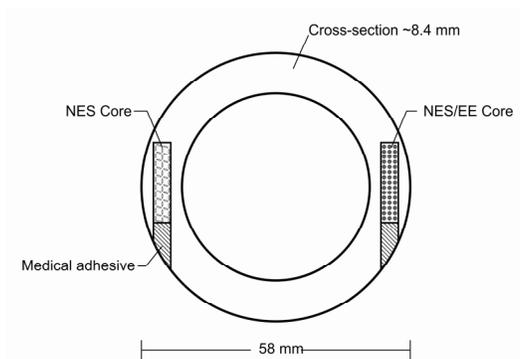


Figure 14. Cross-section of nesterone/ethinyl estradiol ring [Reproduced from Ref. 130 with permission].

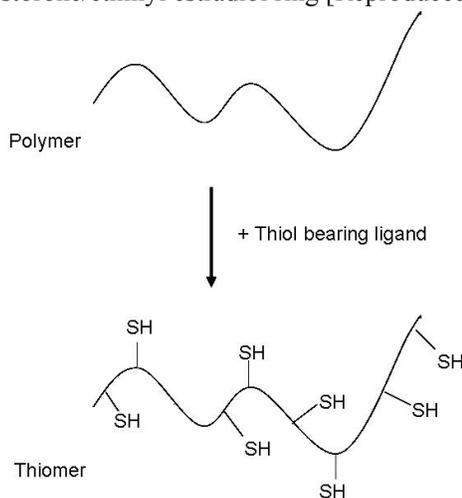


Figure 15. Chemical structure of thiomers [Reproduced from Ref. 138 with permission from Elsevier].

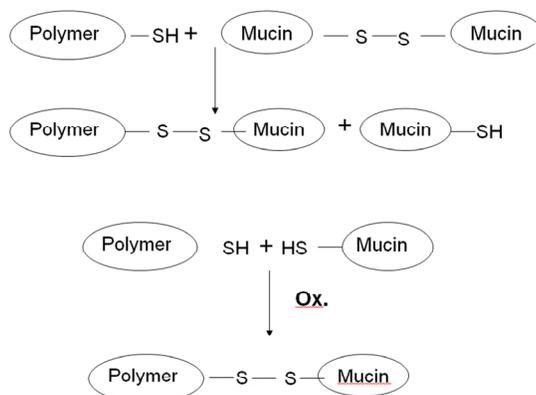


Figure 16. Hypothetical schema representing the formation of covalent bonds between thiolated polymers and mucin glycoproteins (A) via thiol/disulphide exchange reaction (B) via an oxidation process [Modified from Ref. 138 with permission].

It was observed that the higher the amount of cysteine covalently bound to the polymer, the higher work of adhesion was obtained. In addition, a controlled progesterone release was

demonstrated from thiolated polymer. The release rates with unmodified polymer and thiolated polymer were 0.58% per hour and 0.12% per hour, respectively. The thiolated tablet caused

almost zero-order release which guaranteed a sustained blood level of progesterone (139).

Another study based on vaginal tablets for clotrimazole with thiomers was performed by Kast et al. In this study, chitosan was used as a cationic polymer and it was modified by thioglycolic acid (TGA). The water uptake, disintegration behavior, release properties and bioadhesive properties utilizing the rotating cylinder method were evaluated. As a result, the disintegration time was prolonged up to 100-fold and the adhesion time was 26-times longer for chitosan-TGA conjugate tablets. In addition, chitosan-TGA tablets showed a slower release from the tablets prepared with unmodified polymer (80). Chitosan-thioglycolic acid conjugates also seem to be very promising new excipients for liquid or semisolid formulations which should stabilize themselves once applied on the site of drug delivery and might be advantageous in vaginal delivery (141).

Not only thiolated chitosan but also modified chitosan such as 5-methyl-pyrrolidinone-chitosan (MPCS) increase the adherence of the formulations' retention thus prolonging drug-mucosa contact time. MPCS increases gel mucoadhesion force and improves the performance of vaginal gels. Their application requires only a few daily administrations guaranteeing a better patient compliance (142).

5.2. VagiSite Bioadhesive Technology

The VagiSite technology comprises a high-internal-phase ratio, water-in-oil emulsion. As such, the phase of the emulsion acts as the carrier of the active drug. The drug-containing internal dispersed phase globules serve a dual purpose for both the sequestering and the controlled release of the active substance. The high internal emulsion containing disperse phase globules develops a high affinity for surfaces, especially mucosal tissues. This tenacious, mucoadhesive film acts as a drug delivery platform providing a controlled release of the drug into the lumen of the vaginal canal (143).

5.3. Dendrimers

Dendrimers are in the newest category of cationic polymers. These macromolecules contain a central core, interior branches, and terminal surface groups adapted to specific targets. Because of their size and multiple terminal surface groups, they possess the ability to bind to multiple locations on multiple cells. Dendrimers

are also well defined, versatile, monodisperse and stable molecular level nanostructures which are being studied based on the "dendritic state" architecture (144,145).

The first dendrimer, SPL7013 (Vivagel, Starphara Holdings Ltd., Melbourne, Australia), was to be formulated as a gel and was tested clinically. It has been tested in phase 1 trial (146). VivaGel is a current topical agent which contains poly(lysine) dendrimers and which has been found to be useful as antiviral drug against the herpes simplex virus. The general mode of action of antibacterial dendrimers is to adhere and damage the anionic bacterial membrane. VivaGel is safe and well tolerated when administered vaginally, twice daily for 14 days. The National Institutes of Health (NIH) supported trials extending the duration of use for VivaGel. Dendrimers are attractive molecules and their size, shape, density and surface functionality makes these compounds ideal carriers in biomedical application such as drug delivery (145).

Dendronized polymers are also attractive and chitosan-dendrimer hybrids may promise hope for novel mucoadhesives in the future (147).

5.4. Modified Theratron Syringe

Intravaginally located device for veterinary applications, as shown in Figure 17, was designed to control drug delivery electronically for cows. The results showed that the device is able to release the drug over an extended time period (148). It is thought that it may be an alternative for intravaginal administration for women who need long-term therapy and have problems with alternatives if the syringe is mini-fied and adapted to the vaginal cavity.

6. CONCLUSION

Short retention time of drug delivery systems due to the self cleansing action of vaginal tract is a problem for vaginal administration. The major challenge is to design vaginal drug delivery systems which remain for sufficient time on the mucosal tissue. Mucoadhesion, in situ sol-to-gel transition properties and the design of devices guaranteeing a mechanical fixation in the intravaginal cavity are the main strategies to prolong the residence time of vaginal drug delivery systems. Moreover, the further improvement in techniques to determine the formulations' vaginal residence time will have a

great impact on the development of more effective systems. Having the great potential of delivery systems providing prolonged intravaginal residence time in mind and taking all the opportunities ahead into consideration, such systems will certainly further alter the landscape

of drug delivery towards more efficient therapeutic systems. This review should encourage and motivate scientists in academia and industry to move in or intensive their activities in this promising research field.

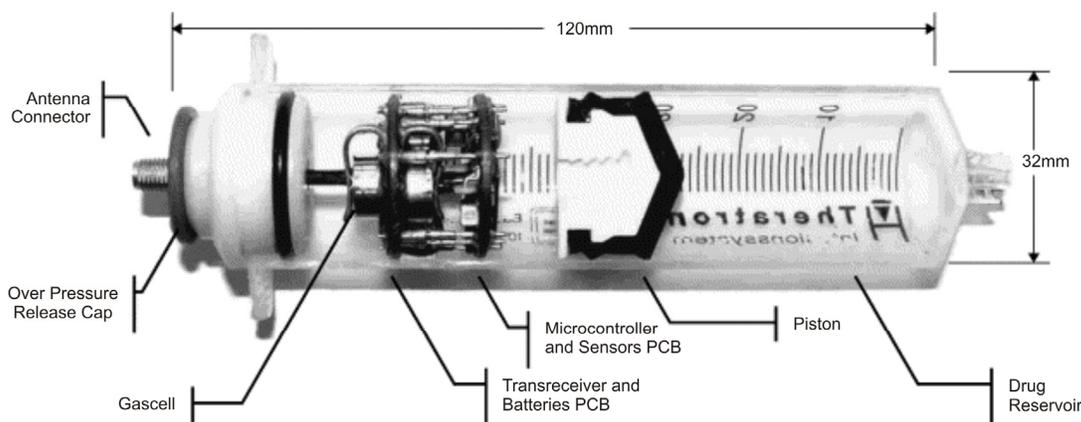


Figure 17. Modified Theratron syringe, sectioned for display, showing the device layout and location of electronics package [Reproduced from Ref. 148 with permission].

REFERENCES

- Vermani K, Garg S. The scope and potential of vaginal drug delivery. *Pharm Sci Technol Today*, 3:359-364, 2000.
- Richardson JL, Illum L. Routes of delivery: Case studies: The vaginal route of peptide and protein drug delivery. *Adv Drug Deliv Rev*, 8:341-366, 1992.
- Robinson JR, Bologna WJ. Vaginal reproductive system treatments using a bioadhesive polymer. *J Control Release*, 28:87-94, 1994.
- Valenta C. The use of mucoadhesive polymers in vaginal delivery. *Adv Drug Deliv Rev*, 57:1692-1712, 2005.
- Pendergrass PB, Belovicz MW, Reeves CA. Surface area of the human vagina as measured from vinyl polysiloxane casts. *Gynecol Obstet Invest*, 55:110-113, 2003.
- Weber AE, Walters MD, Schover LR. Vaginal anatomy and sexual function. *Obstet Gynecol*, 86:946-949, 1995.
- Vagina, *The Free Histology Encyclopedia*, Available at: <http://www.ihcworld.com/histowiki/doku.php?id=vagina>
- Platzner W, Poisel S, Hafez ESE. Functional anatomy of the human vagina. In: Hafez ESE, Evans TN (eds). *Human reproductive medicine: the human vagina*. North Holland Publishing, New York, pp 39-54, 1978.
- Wagner G, Levin RJ. Vaginal fluid. In: Hafez ESE, Evans TN (eds). *Human reproductive medicine: the human vagina*. North Holland Publishing, New York, pp 121-137, 1978.
- Bernkop-Schnürch A. Mucoadhesive Polymers. In: Dumitriu S (eds). *Polymeric Biomaterials*. Marcel Dekker, New York, pp 147-165, 2002.
- Muir GG. Multiple enzyme studies in the vaginal fluid in relation to benign and malignant gynaecological conditions. *Br J Cancer*, 20:448-462, 1966.
- Goldberg DM, Hart DM, Watts C. Distribution of enzymes in human vaginal fluid: relevance to the diagnosis of cervical cancer. *Cancer*, 21:964-973, 1968.
- Paavonen J. Physiology and ecology of the vagina. *Scand J Infect Dis*, 40:31-35, 1983.
- Owen DH, Katz DF. A vaginal fluid stimulant. *Contraception*, 9:91-95, 1999.
- Justin-Temu M, Damian F, Kinget R, Van Den Mooter G. Intravaginal gels as drug delivery systems. *J Womens Health*, 13:834-844, 2004.
- Woolfson AD, Malcolm RK, Gallagher R. Drug delivery by the intravaginal route. *Crit Rev Ther Drug Carr Syst*, 17:509-555, 2000.
- Vasir JK, Tambwekar K, Garg S. Bioadhesive microspheres as a controlled drug delivery system. *Int J Pharm*, 255:13-32, 2003.
- El-Kamel A, Sokar M, Naggat V, Al-Gamal S. Chitosan and sodium alginate-based bioadhesive vaginal tablets. *AAPS PharmSci*, 4:44, 2002.

19. Bonferoni MC, Giunchedi P, Scalia S, Rossi S, Sandri G, Caramella C. Chitosan gels for the vaginal delivery of lactic acid: relevance of formulation parameters to mucoadhesion and release mechanisms. *AAPS PharmSciTech*, 7:104, 2006.
20. Francois M, Snoeckx E, Putteman P, Wouters F, De Proost E, Delaet U, Peeters J, Brewster ME. A mucoadhesive, cyclodextrin-based vaginal cream formulation of itraconazole. *AAPS PharmSci*, 5:5, 2003.
21. Garg S, Vermani K, Garg A, Anderson RA, Rencher WB, Zaneveld LJD. Development and characterization of bioadhesive vaginal films of sodium polystyrene sulfonate (PSS), a novel contraceptive antimicrobial agent. *Pharm Res*, 22:584-595, 2005.
22. D'Cruz OJ, Samuel P, Waurzyniak B, Uckun FM. Development and evaluation of a thermoreversible ovule formulation of stampidine, a novel nonspermicidal broad-spectrum anti-human immunodeficiency virus microbicide. *Biol Reprod*, 69:1843-1851, 2003.
23. Bilensoy E, Çirpanlı Y, Şen M, Doğan AL, Çalış S. Thermosensitive mucoadhesive gel formulation loaded with 5-Fu:cyclodextrin complex for HPV-induced cervical cancer. *J Incl Phenom Macrocycl Chem*, DOI 10.1007/s10847-006-9259-y.
24. Bilensoy E, Rouf MA, Vural I, Şen M, Hıncal AA. Thermosensitive, prolonged-release vaginal gel for clotrimazole:β-cyclodextrin complex. *AAPS PharmSciTech*, 7:38, 2006.
25. Keskar V, Mohanty PS, Gemeinhart EJ, Gemeinhart RA. Cervical cancer treatment with a locally insertable controlled release delivery system. *J Control Release*, 115:280-288, 2006.
26. Ndesendo VMK, Pillay V, Choonara YE, Buchmann E, Bayever DN, Bachav LYG, Patravale VB. Microemulsion based vaginal gel of fluconazole: formulation, in vitro and in vivo evaluation. *Int J Pharm*, 365:175-179, 2009.
27. Meyer C. A review of current intravaginal drug delivery approaches employed for the prophylaxis of HIV/AIDS and prevention of sexually transmitted infectious. *AAPS PharmSciTech*, 9:505-519, 2008.
28. Vermesh M, Fossum GT, Kletzky OA. Vaginal bromocriptine: pharmacology and effect on serum prolactin in normal women. *Obstet Gynecol*, 72:693-698, 1988.
29. Jasonni VM, Raffelli R, March A, Frank G, Flamigni C. Vaginal bromocriptine in hyperprolactinemic patients and puerperal women. *Acta Obstet Gynecol Scand*, 70:493-495, 1991.
30. Darwish AM. Clinical assessment of unique buccal vs vaginal mucoadhesive bromocriptine mesylate discs for treating hyperprolactinemic women *Fertil Steril*, 84:159-160, 2005.
31. Patel LG. Propanolol concentration in plasma after insertion into the vagina. *Br Med J*, 287:1247-1248, 1984.
32. Christensen FC, Tehranifar M, Gonzales JL, Qualls CR, Rappaport VJ, Rayburn WF. Randomized trial of concurrent oxytocin with a sustained-release dinoprostone vaginal insert for labor induction at term. *Am J Obstet Gynecol*, 186:61-65, 2002.
33. Agarwal N, Gupta A, Kriplani A, Bhatla NP. Six hourly vaginal misoprostol versus intracervical dinoprostone for cervical ripening and labor induction. *J Obstet Gynaecol Res*, 29:147-151, 2003.
34. Richardson JL, Trevor TI. Vaginal delivery of calcitonin by hyaluronic acid formulations. *Drugs Pharm Sci*, 98:563-599, 1999.
35. Han K, Park JS, Chung YB, Jeong NJP, Park HB, Robinson JR. Development of luteinizing hormone releasing hormone (LH-RH) delivery systems for vaginal mucosal route. *Arch Pharm Res*, 18:325-331, 1995.
36. Livingston JB, Lu S, Robinson H, Anderson DJ. Immunization of the female genital tract with a DNA-based vaccine. *Infect Immun*, 66:322-329, 1998.
37. Nyende L, Towobola OA, Mabina MH. Comparison of vaginal and oral misoprostol, for the induction of labour in women, with intra-uterin foetal death. *East Afr Med J*, 81:179-182, 2004.
38. Bilensoy E, Rouf MA, Vural I, Şen M, Hıncal AA. Mucoadhesive, Thermosensitive, Prolonged-Release Vaginal Gel for Clotrimazole: β-Cyclodextrin Complex. *AAPS PharmSciTech*, 7:E1-E7, 2006.
39. Ziemann M, Fong SK, Benowitz NL, Bankster D, Darney PD. Absorption kinetics of misoprostol with oral or vaginal administration. *Obstet Gynecol*, 90:88-92, 1997.
40. Prista LN, Alves AC, Morgado R, Tecnologia Farmaceutica. *Fundacao Calouste Gulbenkian*. Lisboa, 4th Edition, 3:1585-1594, 1996.
41. Neves J, Bahia MF. Gels as vaginal drug delivery systems. *Int J Pharm*, 318:1-14, 2006.
42. Baloğlu E, Özyazıcı M, Hızarcıoğlu SY, Şenyiğit T, Özyurt D, Pekçetin Ç. Bioadhesive Controlled Release Systems of Ornidazole for Vaginal Delivery. *Pharm Dev Technol*, 11:477-484, 2006.
43. Bernkop-Schnürch A, Hornof M. Intravaginal drug delivery systems design, challenges, and solutions. *Am J Drug Deliv*, 1:241-254, 2003.

44. Lucy MC, Billings HJ, Butler WR. Efficacy of an intravaginal progesteron insert and an injection of PGF2 alpha for synchronizing estrus and shortening the interval to pregnancy in post partum beef cows, peripubertal beef heifers and dairy heifers. *J Anim Sci*, 79:982-995, 2001.
45. Brown J, Hooper G, Kenyon CJ. Spreading and retention of vaginal formulations in post-menopausal women as assessed by gamma scintigraphy. *Pharm Res*, 14:1073-1078, 1997.
46. Hussain A, Ahsan F. The vagina as a route for systemic drug delivery. *J Control Release*, 103:301-313, 2005.
47. Garg S, Tambwekar KR, Vermani K, Kandarapu R, Garg A, Waller DP, Zaneveld LJD. Development pharmaceuticals of microbicide formulations, Part II: Formulation, evaluation and challenges. *AIDS Patient Care STDs*, 17:377-399, 2003.
48. Merabet J, Thompson D, Saul Levinson R. Advancing vaginal drug delivery. *Expert Opin Drug Deliv*, 2:769-777, 2005.
49. Iyer V, Bendgude N, Poddar SS. Vaginal drug delivery. Available at: <http://www.expresspharmaonline.com/20080715/research02.shtml>
50. Chowdary KPR, Rao YS. Design and in vitro and in vivo evaluation of mucoadhesive microcapsules of glipizide for oral controlled release: A technical note. *AAPS PharmSciTech*, 4(3):39, 2003.
51. Batchelor H. Novel bioadhesive formulations in drug delivery. *The Drug Delivery Companies Report, Autumn/Winter, Technology/Industry Overviews*, 16- 19, 2004.
52. Bonacucina G, Martelli S, Palmieri GF. Rheological, mucoadhesive and release properties of Carbopol gels in hydrophilic cosolvents. *Int J Pharm*, 282:115-130, 2004.
53. Fabio S, Van Roey J, Giannini G, Mooter G, Spada M, Binelli A, Pirillo MF, Germinario E, Belardelli F, Bethune MP, Vella S. Inhibition of vaginal transmission of HIV-1 in hu-SCID mice by the non-nucleoside reverse transcriptase inhibitor TMC120 in a gel formulation. *AIDS*, 17:1597-1604, 2003.
54. Geonnotti AR, Peters JJ, Katz DF. Erosion of microbicide formulation coating layers: effects of contact and shearing with vaginal fluid or semen. *J Pharm Sci*, 94:1705-1712, 2005.
55. Washington N, Washington C, Wilson CG (eds). *Vaginal and intrauterine drug delivery, Physiological Pharmaceutics: Barriers to Drug Absorption*, CRC Press, London, pp 272-281, 2000.
56. Değim Z, Değim T, Acartürk F, Erdoğan D, Özoğul C, Köksal M. Rectal and vaginal administration of insulin-chitosan formulations: An experimental study in rabbits. *J Drug Target*, 13:563-572, 2005.
57. Cevher E, Taha MAM, Orulu M, Araman A. Evaluation of Mechanical and Mucoadhesive Properties of Clomiphene Citrate Gel Formulations Containing Carbomers and Their Thiolated Derivatives. *Drug Deliv*, 15:57-67, 2008.
58. Chopra S, Motwani SK, Iqbal Z, Talegaonkar S, Ahmad FJ, Khar RK. Optimisation of polyherbal gels for vaginal drug delivery by Box-Behnken statistical design. *Eur J Pharm Biopharm*, 67:120-131, 2007.
59. Garg S, Anderson RA, Chany CJ, Waller DP, Diao XH, Vermani K, Zaneveld LJD. Properties of a new acid-buffering bioadhesive vaginal formulation (Acidform). *Contraception*, 64:67-75, 2001.
60. Amaral E, Perdigao A, Souza MH, Mauck C, Waller D, Zaneveld L, Faundes A. Vaginal safety after use of a bioadhesive, acid-buffering, microbicidal contraceptive gel (Acidform) and a 2% nonxynol-9 product. *Contraception*, 73:542-547, 2006.
61. Andrews GP, Lavery TP, Jones DS. Mucoadhesive polymeric platforms for controlled drug delivery. *Eur J Pharm Biopharm*, 71:505-518, 2009.
62. Balicka-Ramisz A, Wojtasz-Pajak A, Pilarczyk B, Ramisz A, Laurans L. Antibacterial and antifungal activity of chitosan. *ISAH*, 2:406-408, 2005.
63. Seyfarth F, Schliemann S, Elsner P, Hipler UC. Antifungal effect of high- and low-molecular-weight chitosan hydrochloride, carboxymethyl chitosan, chitosan oligosaccharide and N-acetyl-d-glucosamine against *Candida albicans*, *Candida krusei* and *Candida glabrata*. *Int J Pharm*, 353:139-148, 2008.
64. Microbicide Research and Development Database, 2005, Alliance for Microbicide Development. Accessed July 21, 2007 Available at: <http://secure.microbicide.org/NetReports/ClinicalTrialsOngoingByProduct.aspx>
65. Mitchel D. Focus renewed on HIV microbicides. *International Health Conference, Canada*, 2006.
66. Alam MA, Ahmad FJ, Iqbal ZI, Khan ZI, Khar RK, Ali M. Development and evaluation of acid-buffering bioadhesive vaginal tablet for mixed vaginal infections. *AAPS PharmSciTech*, 8:109, 2007.
67. Mandal TK. Swelling-controlled release system for the vaginal delivery of miconazole. *Eur J Pharm Biopharm*, 50:337-343, 2000.
68. Ghezze E, Benedetti L, Rochira N, Biviano F, Callegaro L. Hyaluronane derivative microsphere as NGF delivery device:

- Preparation methods and in vitro release characterization. *Int J Pharm*, 87:21-29, 1992.
69. Richardson JL, Armstrong TI. Vaginal delivery of calcitonin by hyaluronic acid formulations. In: Mathiowitz E, Chickering DE, Lehr CM (eds). *Bioadhesive Drug Delivery Systems-Fundamentals, Novel approaches and Development*. Marcel Dekker, New York, pp 563-599, 1999.
70. O'Hagan DT, Raffery D, Wharton S, Illum L. Intravaginal immunization in sheep using a bioadhesive microsphere antigen delivery system. *Vaccine*, 11:660-664, 1993.
71. O'Hagan DT. Microparticles and polymers for the mucosal delivery of vaccines. *Adv Drug Deliv Rev* 34:305-320, 1998.
72. Esposito E, Menegatti E, Cortesi R. Hyaluronan-based microspheres as tools for drug delivery: a comparative study. *Int J Pharm*, 288:35-49, 2005.
73. Bonucci E, Ballanti P, Ramires A, Richardson JL, Benedetti LM. Prevention of ovariectomy osteopenia in rats after vaginal administration of hyaff 11 microspheres containing salmon calcitonin. *Calcif Tissue Int*, 56:274-279, 1995.
74. Yoo JW, Dharmala K, Lee CH. The physico-dynamic properties of mucoadhesive polymeric films developed as female controlled drug delivery system. *Int J Pharm*, 309:139-145, 2006.
75. Choy YB, Park JH, Prausnitz MR. Mucoadhesive microparticles engineered for ophthalmic drug delivery. *J Phys Chem Solids*, 69:1533-1536, 2008.
76. Thirawong N, Kennedy RA, Sriamornsak P. Viscometric study of pectin-mucin interaction and its mucoadhesive bond strength. *Carbohydr Polym*, 71:170-179, 2008.
77. Wu N, Dai J, Micale FJ. Dynamic surface tension measurement with a dynamic Wilhelmy Plate technique. *J Colloid Interf Sci*, 215:258-269, 1999.
78. Mikos AG, Peppas NA. Systems for controlled release of drugs V: Bioadhesive systems. *STP Pharma Sci*, 19:705-715, 1986.
79. Wong C, Yuen K, Peh K. An in-vitro method for buccal adhesion studies: importance of instrument variables. *Int J Pharm*, 180:47-57, 1999.
80. Yong CS, Choi JS, Quan QZ, Rhee JD, Kim CK, Lim SJ, Kim KM, Oh PS, Choi HG. Effect of sodium chloride on the gelation temperature, gel strength and bioadhesive force of poloxamer gels containing diclofenac sodium. *Int J Pharm*, 226:195-205, 2001.
81. Hertzog BA, Mathiowitz E. Novel magnetic technique to measure bioadhesion. In: Mathiowitz E, Chickering DE, Lehr CM (eds). *Bioadhesive Drug Delivery Systems-Fundamentals, Novel Approaches and Development '98*. Marcel Dekker, New York, pp 147-171, 1999.
82. Kast CE, Valenta C, Leopold M, Bernkop-Schnürch A. Design and in vitro evaluation of a novel bioadhesive vaginal drug delivery system for clotrimazole. *J Control Release*, 81:347-354, 2002.
83. Bernkop-Schnürch A, Steininger S. Synthesis and characterization of mucoadhesive thiolated polymers. *Int J Pharm*, 194:239-247, 2000.
84. Bruschi ML, Jones DS, Panzeri H, Gremia MPD, Freitas O, Lara EHG. Semisolid systems containing propolis for the treatment of periodontal disease: In vitro release kinetics, syringeability, rheological, textural, and mucoadhesive properties. *J Pharm Sci*, 96:2074-2089, 2007.
85. Accili D, Menghi G, Bonacucina G, Martino P, Palmieri GF. Mucoadhesion dependence of pharmaceutical polymers on mucosa characteristics. *Eur J Pharm Sci*, 22:225-234, 2004.
86. Jones DS, Woolfson AD, Brown AF. Textural, viscoelastic and mucoadhesive properties of pharmaceutical gels composed of cellulose polymers. *Int J Pharm*, 151:223-233, 1997.
87. Garga S, Anderson RA, Chany CJ, Waller DP, Diao XH, Vermani K, Zaneveld LJD. Properties of a new acid-buffering bioadhesive vaginal formulation (ACIDFORM). *Contraception*, 64:67-75, 2001.
88. Alur HH, Pather SI, Mitra AK, Johnston TP. Transmucosal sustained-delivery of chlorpheniramine maleate in rabbits using a novel, natural mucoadhesive gum as an excipient in buccal tablets. *Int J Pharm*, 188:1-10, 1999.
89. Ceschel GC, Maffei P, Borgia SL, Ronchi C, Rossi S. Development of a mucoadhesive dosage form for vaginal administration. *Drug Dev Ind Pharm*, 27:541-547, 2001.
90. Leung SH, Robinson JR. The contribution of anionic polymer structural features to mucoadhesion. *J Control Release*, 5:223-231, 1988.
91. Bachav YG, Patravale VB. Microemulsion based vaginal gel of fluconazole: Formulation, *in vitro* and *in vivo* evaluation. *Int J Pharm*, 365:175-179, 2009.
92. Park K. A new approach to study mucoadhesion: colloidal gold staining. *Int J Pharm*, 53:209-217, 1989.
93. Ranga Rao KV, Buri P. A novel in situ method to test polymers and coated microparticles for bioadhesion. *Int J Pharm*, 52:265-270, 1989.
94. Teng CLC, Ho NFH. Mechanistic studies in the simultaneous flow and adsorption of polymer-coated latex particles on intestinal mucus I: Methods and physical model

- development. *J Control Release*, 6:133-149, 1987.
95. Hassan EE, Gallo JM. A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. *Pharm Res*, 7:491-495, 1990.
96. Jones DS, Muldoon BCO, Woolfson AD, Andrews GP, Sanderson FD. Physicochemical Characterization of Bioactive Polyacrylic Acid Organogels as Potential Antimicrobial Implants for the Buccal Cavity. *Biomacromolecules*, 9:624-633, 2008.
97. Mortazavi SA, Carpenter BG, Smart JD. An investigation of the rheological behaviour of the mucoadhesive/mucosal interface. *Int J Pharm*, 83:221-225, 1982.
98. Mauck CK, Katz D, Sandefer EP, Nasution MD, Henderson M, Digenis GA, Su I, Page R, Barnhart K. Vaginal distribution of Replens® and K-Y® Jelly using three imaging techniques. *Contraception*, 77:195-204, 2008.
99. Chatterton BE, Penglis S, Kovacs JC, Presnell B, Hunt B. Retention and distribution of two ^{99m}Tc-DTPA labelled vaginal dosage forms. *Int J Pharm*, 271:137-143, 2004.
100. Richardson JL, Whetstone J, Fisher AN, Watts P, Farraj NF, Hinchcliffe M, Benedetti L, Illum L. Gamma-scintigraphy as a novel method to study the distribution and retention of a bioadhesive vaginal delivery system in sheep. *J Control Release*, 42:133-142, 1996.
101. Oh YK, Park JS, Yoon H, Kim CK. Enhanced mucosal and systemic immune responses to a vaginal vaccine coadministered with RANTES-expressing plasmid DNA using in situ-gelling mucoadhesive delivery system. *Vaccine*, 21:1980-1988, 2003.
102. Chang JY, Oh YK, Choi H, Kim YB, Kim CK. Rheological evaluation of thermosensitive and mucoadhesive vaginal gels in physiological conditions. *Int J Pharm*, 241:155-163, 2002.
103. Ruel-Gariépy E, Leroux JC. In situ-forming hydrogels-review of temperature-sensitive systems. *Eur J Pharm Biopharm*, 58:409-426, 2004.
104. Trnaksız F, Robinson JR. Rheological, mucoadhesive and release properties of pluronic F-127 gel and pluronic F-127/polycarbopil mixed gel systems. *Pharmazie*, 60:518-523, 2005.
105. Chang JY, Oh YK, Kong HS, Kim EJ, Jang DD, Nam KT, Kim CK. Prolonged antifungal effects of clotrimazole-containing mucoadhesive thermosensitive gels on vaginitis. *J Control Release*, 82:39-50, 2002.
106. Madsen F, Eberth K, Smart JD. A rheological examination of the mucoadhesive/mucus interaction: the effect of mucoadhesive type and concentration. *J Control Release*, 50:167-178, 1998.
107. Tamburic S, Craig DQ. An investigation into the rheological, dielectric and mucoadhesive properties of poly(acrylic acid) gel systems. *J Control Release*, 37:59-68, 1995.
108. Baloğlu E, Karavana SY, Ay Z, Güneri T. Texture Analysis of Vaginal Bioadhesive Gel Formulations of Miconazole Nitrate. *Pharmaceutical Sciences World Congress, Amsterdam, DD-P-005*, 2007.
109. Weidner J. Improved therapy for yeast infection through use of mucoadhesive thermosensitive gels. *Drug Discov Today*, 7:1230-1231, 2002.
110. Thapar MA, Parr EL, Bozzola JJ, Parr MB. Secretory immune responses in the mouse vagina after parenteral or intravaginal immunization with an immunostimulating complex (ISCOM). *Vaccine*, 9:129-133, 1991.
111. Mourya VK, Inamdar NN. Chitosan-modifications and applications: opportunities galore. *React Funct Polym*, 68:1013-1051, 2008.
112. He C, Kim SW, Lee DS. In situ gelling stimuli-sensitive block copolymer hydrogels for drug delivery. *J Control Release*, 127:189-207, 2008.
113. Alexandridis P, Holzwarth JF. Differential Scanning Calorimetry Investigation of the Effect of Salts on Aqueous Solution Properties of an Amphiphilic Block Copolymer (Ploxamer). *Langmuir*, 13:6074-6082, 1997.
114. Ding S. Recent developments in ophthalmic drug delivery. *PSTT*, 1:328-335, 1998.
115. Ceulemans J, Vinckier I, Ludwig A. The use of xanthan gum in an ophthalmic liquid dosage form: rheological characterization of the interaction with mucin. *J Pharm Sci*, 91:1117-1127, 2002.
116. Valenta C, Nowack E, Bernkop-Schnürch A. Deoxycholate-hydrogels: Novel drug carrier systems for topical use. *Int J Pharm*, 185:103-111, 1999.
117. Voorspoels J, Casteels M, Remon JP, Temmerman M. Local treatment of bacterial vaginosis with a bioadhesive metronidazole tablet. *Eur J Obstet Gynecol Reprod Biol*, 105:64-66, 2002.
118. Brannon-Peppas L. Novel vaginal drug release applications. *Adv Drug Deliv Rev*, 11:169-177, 1993.
119. Daugherty AL, Mersny RJ. Formulation and delivery issues for monoclonal antibody therapeutics. *Adv Drug Deliv Rev*. 58:686-706, 2006.
120. Almeida A, Souto E. Solid lipid nanoparticles as a drug delivery system for peptides and proteins. *Adv Drug Deliv Rev*, 59:478-490, 2007.
121. Saltzman WM, Sherwood JK, Adams DR, Haller P. Long-term vaginal antibody delivery:

- delivery systems and biodistribution. *Biotechnol Bioeng*, 67:253-264, 2000.
122. Parr MB, Kepple L, McDermott MR, Drew MD, Bossola JJ, Parr EL. A mouse model for studies of mucosal immunity to vaginal infection by herpes simplex virus type 2. *Lab Invest*, 70:369-380, 1994.
123. Wira CR, Rossoll RM. Antigen-presenting cells in the female reproductive tract: Influence of sex hormones on antigen presentation in the vagina. *Immunology*, 84:505-508, 1995.
124. Wira CR, Rossoll RM, Kaushic C. Antigen-presenting cells in the female reproductive tract: Influence of estradiol on antigen presentation by vaginal cells. *Endocrinology*, 141:2877-2885, 2000.
125. Shen H, Goldberg E, Saltzman WM. Gene expression and mucosal immune responses after vaginal DNA immunization in mice using a controlled delivery matrix. *J Control Release*, 86:339-348, 2003.
126. Malcolm RK, McCullagh S, Woolfson AD, Catney M, Tallon P. A dynamic mechanical method for determining the silicon elastomer solubility of drugs and pharmaceutical excipients in silicon intravaginal drug delivery rings. *Biomaterial*, 23:3589-3594, 2002.
127. Alexander NJ, Baker E, Kaptein M, Karck U, Miller L, Zampaglione E. Why consider vaginal drug administration? *Fertil Steril*, 82:1-12, 2004.
128. Woolfson AD, Elliott G RE, Gilligan CA, Passmore CM. Design of an intravaginal ring for the controlled delivery of 17 β -estradiol as its 3-acetate ester. *J Control Release*, 61:319-328, 1999.
129. Gupta S. Non-oral hormonal contraception. *Curr Obstet Gynaecol*, 16:30-38, 2006.
130. Sitruk-Ware R, Small M, Kumar N, Tsong Y, Sundaram K, Jackanicz T. Nestorone[®]: clinical applications for contraception and HRT. *Steroids*, 68:907-913, 2003.
131. Breitenbach J. Melt extrusion: from process to drug delivery technology. *Eur J Pharm Biopharm*, 54:107-117, 2002.
132. Nutter NM. Method of using lectins for contraception. Patent no: 5766632. Available at: <http://www.freepatentsonline.com/5766632.html>
133. Lehr CM. Lectin-mediated drug delivery: the second generation of bioadhesives. *J Control Release*, 65:19-29, 2000.
134. Wirth M, Gerhardt K, Wurm C, Gabor F. Lectin-mediated drug delivery: influence of mucin on cytoadhesion of plant lectins in vitro. *J Control Release*, 79:183-191, 2002.
135. Haltner E, Easson JH, Lehr CM. Lectins and bacterial invasion factors for controlling endo- and transcytosis of bioadhesive drug carrier systems. *Eur J Pharm Biopharm*, 44:1-13, 1997.
136. Woolfson AD, Malcolm RK, Morrow RJ, Toner CF, McCullagh SD. Intravaginal ring delivery of the reverse transcriptase inhibitor TMC 120 as an HIV microbicide. *Int J Pharm*, 325:82-89, 2006.
137. Malcolm RK, Woolfson AD, Toner C, Morrow RJ, McCullagh SD. Long-term, controlled release of the HIV microbicide TMC120 from silicon elastomer vaginal rings. *J Antimicrob Chemother*, 56:954-956, 2005.
138. Bernkop-Schnürch A. Thiomers: a new generation of mucoadhesive polymers. *Adv Drug Deliver Rev*, 57:1569-1582, 2005.
139. Valenta C, Kast CE, Harich I, Bernkop-Schnürch A. Development and in vitro evaluation of a mucoadhesive vaginal delivery system for progesterone. *J Control Release*, 77:323-332, 2001.
140. Leitner VM, Walker GF, Bernkop-Schnürch A. Thiolated polymers: evidence for the formation of disulphide bonds with mucus glycoproteins. *Eur J Pharm Biopharm*, 56:207-214, 2003.
141. Hornof MD, Kast CE, Bernkop-Schnürch A. In vitro evaluation of the viscoelastic properties of chitosan-thioglycolic acid conjugates. *Eur J Pharm Biopharm*, 55:185-190, 2003.
142. Perioli L, Ambrogi V, Venezia L, Pagano C, Ricci M, Rossi C. Chitosan and a modified chitosan as agents to improve performances of mucoadhesive vaginal gels. *Colloids Surf B Biointerfaces*, 66:141-145, 2008.
143. Levinson RS, Thompson DJ. VagiSite Bioadhesive Technology. In: Rathbone M, Hadgraft J, Roberts MS (eds). *Modified-release Drug Delivery Technology*. Marcel Dekker, New York, pp 801-806, 2003.
144. Boas U, Heegaard MH. Dendrimers in drug research. *Chem Soc Rev*, 33:43-63, 2004.
145. Svenson S, Tomalia DA. Dendrimers in biomedical applications-reflections on the field. *Adv Drug Deliv Rev*, 57:2106-2129, 2005.
146. Cutler B, Justman J. Vaginal microbicides and the prevention of HIV transmission. *The Lancet Infect Dis*, 8:685-697, 2008.
147. Sashiwa H, Aiba S. Chemically modified chitin and chitosan as biomaterials. *Prog Polym Sci*, 29:887-908, 2004.
148. Cross PS, Künemeyer R, Bunt CR, Carneige DA, Rathbone MJ. Control, communication and monitoring of intravaginal drug delivery in dairy cows. *Int J Pharm*, 282:35-44, 2004.