



Review

The vagina as a route for systemic drug delivery

Alamdar Hussain, Fakhrul Ahsan*

*Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University, Health Sciences Center,
1300 Coulter Drive, Amarillo, TX 79106, United States*

Received 25 August 2004; accepted 29 November 2004

Available online 13 January 2005

Abstract

Exhaustive efforts have been made toward the administration of drugs, via alternative routes, that are poorly absorbed after the oral administration. The vagina as a route of drug delivery has been known since ancient times. In recent years, the vaginal route has been rediscovered as a potential route for systemic delivery of peptides and other therapeutically important macromolecules. However, successful delivery of drugs through the vagina remains a challenge, primarily due to the poor absorption across the vaginal epithelium. The rate and extent of drug absorption after intravaginal administration may vary depending on formulation factors, vaginal physiology, age of the patient and menstrual cycle. Suppositories, creams, gels, tablets and vaginal rings are commonly used vaginal drug delivery systems. The purpose of this communication is to provide the reader with a summary of advances made in the field of vaginal drug delivery. This report, therefore, summarizes various vaginal drug delivery systems with an introduction to vaginal physiology and factors affecting drug absorption from the vaginal route.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Vagina; Drug delivery; Contraception; Vaginal enzymes; Bioadhesion; Peptides and proteins

Contents

1. Introduction	302
2. Anatomy and physiology of the vagina	302
3. Factors affecting the vaginal absorption of drugs	303
3.1. Physiological factors	303
3.2. Physicochemical properties of drugs	303
4. Vaginal enzymes in different species	304
5. Drug delivery systems for vaginal administration	304

* Corresponding author. Tel.: +1 806 356 4015x335; fax: +1 806 356 4034.

E-mail address: fakhrul.ahsan@ttuhsc.edu (F. Ahsan).

5.1. Creams and gels	305
5.2. Suppositories and vaginal tablets	307
5.3. Vaginal rings.	308
5.4. Bioadhesive delivery systems	308
6. Vaginal immunization	309
7. Conclusions	310
References	310

1. Introduction

Currently, there is a huge interest in the scientific community and drug industry to exploit various mucosal routes of delivering drugs, which are poorly absorbed after oral administration. Based on the numbers of scientific papers published in pharmaceutical journals over the last decade, it is apparent that the human vagina remains to be a relatively unexplored route of drug delivery despite its potential as a non-invasive route of drug administration. The presence of dense network of blood vessels has made the vagina an excellent route of drug delivery for both systemic and local effect. The main advantages of vaginal drug delivery over conventional drug delivery are the ability to by-pass first pass metabolism, ease of administration and high permeability for low molecular weight drugs. However, several drawbacks, including cultural sensitivity, personal hygiene, gender specificity, local irritation and influence of sexual intercourse, need to be addressed during the design of a vaginal formulation. Further, considerable variability in the rate and extent of absorption of vaginally administered drugs is observed by changes in thickness of vaginal epithelium. The objective of this paper is to provide an overview of various vaginal drug delivery systems currently in developmental stages or available in the market, immunization via the vagina and special emphasis on the challenges and difficulties associated with systemic delivery of drugs via the vaginal route.

2. Anatomy and physiology of the vagina

In the pharmaceutical literature, human vagina is often described as slightly S-shaped fibromuscular collapsible tubes between 6 and 10 cm long extending

from cervix of the uterus [1,2]. The vaginal wall consists of three layers: the epithelial layer, the muscular coat and the tunica adventia [3]. During the menstrual cycle, the thickness of the vaginal epithelial cell layer changes by approximately 200–300 μm [4]. The surface of the vagina is composed of numerous folds, which are often called rugae. The rugae provide distensibility, support and an increased surface area of the vaginal wall. The vagina has an excellent elasticity because of the presence of smooth elastic fibers in the muscular coat. Loose connective tissue of tunica adventia further increases the elasticity of this organ. The network of blood vessels that supply blood to the vagina include a plexus of arteries extending from the internal iliac artery, uterine, middle rectal and internal pudental arteries. In fact, arteries, blood vessels and lymphatic vessels are abundant in the walls of the vagina. Drugs absorbed from the vagina does not undergo first-pass metabolism because blood leaving the vagina enters the peripheral circulation via a rich venous plexus, which empties primarily into the internal iliac veins [5]. There is some drainage to the hemorrhoidal veins as well. The lower part of the vagina receives its nerve supply from the pudental nerve and from the inferior hypogastric and uterovaginal plexuses [2].

Although the vagina does not possess any gland, it secretes a large amount of fluid [6]. Cervical secretion and transudation from the blood vessels with desquamated vaginal cells and leucocytes mainly constitute the vaginal fluid [7]. Secretions from the endometrium and fallopian tubes also contribute to the vaginal fluid [6]. Like the thickness of the vaginal epithelium, the amount and composition of the vaginal fluid also changes throughout the menstrual cycle. Women of reproductive age produce fluid at a rate of 3–4 g/4 h, while the discharge produced by postmenopausal women is reduced by 50% compared to that produced

by women of reproductive age [8]. The human vaginal fluid may contain enzymes, enzyme inhibitors, proteins, carbohydrates, amino acids, alcohols, hydroxylketones and aromatic compounds [9]. Sexual arousal may affect the volume and composition of vaginal fluids [10] and that can alter the drug release pattern from vaginal delivery system [11]. 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. During menstruation, the pH of vaginal fluid increases and frequent acts of coitus may also cause an increase in the vaginal pH because both ejaculate and vaginal transudate are alkaline. The presence of cervical mucus and the amount of vaginal transudate may also alter vaginal pH [1]. The vaginal epithelium has a high activity of enzymes that could potentially affect short- and long-term stability of intravaginal delivery systems and devices.

3. Factors affecting the vaginal absorption of drugs

Like other mucosal routes of administration, drugs administered via vaginal route are absorbed (i) transcellularly via concentration dependent diffusion through the cells, (ii) paracellularly mediated by tight junctions and (iii) vesicularly or receptor mediated transport as pointed out by Richardson and Illum [5]. Absorption of drug from vaginal delivery systems occurs in two main steps: drug dissolution in vaginal lumen and membrane penetration. Any biological or formulation factor that affects drug dissolution and membrane transport could potentially affect the absorption profile from vaginal drug delivery systems. Overall, vast and multifarious factors and processes are involved in drug absorption from the vaginal route [1].

3.1. Physiological factors

As mentioned above, cyclic changes in thickness of vaginal epithelium, fluid volume and composition, pH and sexual arousal could potentially affect drug release from intravaginal delivery systems. For example, the vaginal absorption of steroids is affected by the thickness of the vaginal epithelium [12]. Vidarabine has been shown to have a 5–100 times higher permeability coefficient during the early

dioestrous stage than during the oestrus stage in guinea pigs [13]. Vaginal absorption of estrogen has been shown to be higher in postmenopausal women compared to premenopausal women [14]. There have been some conflicting reports as to the change in drug absorption with the increase in vaginal epithelium. Studies have shown that the vaginal absorption of steroids is influenced by the thickness of vaginal epithelium and the epithelial thickness is therefore reduced by long-term estrogen therapy [12]. However, the vaginal progesterone absorption in estrogen-deficient women who were receiving vaginal estrogen therapy was found to be increased [15], although prior estradiol therapy should have caused an increase in the vaginal epithelium thickness. This anomalous finding was explained by the fact that the absorption of progesterone was increased with increased vascularity of the vagina. Further cervical mucus of the vagina, which is a glycoprotein gel [16], could possibly be exploited for bioadhesive drug delivery [17]. However, the presence of cervical mucus could also serve as a permeability barrier to prospective drug candidates.

The volume, viscosity and pH of vaginal fluid may have either negative or positive impact on vaginal drug absorption. The absorption of drug that is poorly water-soluble may be increased when the fluid volume is higher. However, the presence of overly viscous cervical mucus may present a barrier to drug absorption and increased fluid volume may remove the drug from vaginal cavity and subsequently reduce absorption. Since many drugs are weak electrolytes, the pH may change their degree of ionization and affect the absorption of drug. In vitro study has showed that release of PGE₂ from vaginal preparations may vary depending on the pH of the media [18]. Any change in the vaginal pH may affect the release profiles of pH sensitive drugs from vaginal drug delivery systems [19].

3.2. Physicochemical properties of drugs

Physicochemical properties such as molecular weight, lipophilicity, ionization, surface charge, chemical nature can influence vaginal drug absorption. For example, the vaginal permeability of straight chain aliphatic alcohols increases in a chain length dependent manner [20]. Similarly, vaginal permeability is

much greater to lipophilic steroid such as progesterone and estrone than to hydrophilic steroid such as hydrocortisone and testosterone [21]. However, it is generally accepted that low molecular weight lipophilic drugs are likely to be absorbed more than large molecular weight lipophilic or hydrophilic drugs. A study on vaginal absorption of polyvinyl alcohol suggested that the molecular weight cut-off above which compounds are not absorbed may be higher for the vagina than other mucosal surfaces [22]. Since vaginal fluid contains a large amount of water, any drug intended for vaginal delivery require a certain degree of solubility in water. In fact, data on the human vaginal permeability to drugs with different physicochemical properties is very limited; much work needs to be done on the effects of physicochemical parameters of drug on vaginal absorption.

4. Vaginal enzymes in different species

The external cell layers and the basal cell layers of the vagina retain most of the enzyme activity [1,23,24]. Among the enzymes present, proteases are likely to be the prominent barrier for the absorption of intact peptide and protein drugs into the systemic circulation. It has also been reported that the rat vaginal smears have trypsin-like activity, which reaches a maximum level during the proestrus stage [25]. Lee [26] has suggested that most of the exopeptidases and endopeptidases, which digest the peptides and proteins are present in the vaginal epithelium. The various enkephalins studied in rabbit vaginal epithelium suggest the presence of at least three peptidases viz. aminopeptidase, dipeptidyl peptidase and dipeptidyl carboxypeptidase, which play a vital role in metabolism of enkephalins [27]. Among these enzymes, aminopeptidases were the main enzymes responsible for methionine and leucine enkephalin metabolism, while dipeptidyl carboxypeptidase was the main enzyme for D-ala-met-enkephalin metabolism. Sayani et al. [28] reported the existence of aminopeptidases in rabbit nasal, rectal and vaginal extracts. The highest concentration of these enzymes was in the vaginal extract (0.045 U/ml) of the rabbit. A specific study [29] dealing with the comparison of enzymatic activities of four different aminopeptidases (aminopeptidase N, leucine aminopeptidase, amino-

peptidase A and aminopeptidase B) in vaginal homogenates of various species report that the enzyme activity in rat, rabbit and human was significantly lower than that of sheep and guinea-pig. Overall, the aminopeptidase activity in the species showed the following order of activity: sheep > guinea-pig > rabbit \geq human \geq rat. The authors conclude that the rat and the rabbit could be used as potential model animals for vaginal enzymatic activity studies and for the determination of degradation of protein and peptide drugs in the vagina.

5. Drug delivery systems for vaginal administration

Traditionally, solutions, suppositories, gels, foams and tablets have been used as vaginal formulations. More recently, vaginal ring has been introduced for hormone replacement and contraceptive therapy. Table 1 enlists few formulation systems intended for vaginal delivery of different therapeutic agents. In general, based on the drug delivery system or formulations used, drug absorption, distribution and residence time in the vagina may vary. In fact, early work in this field by Johnson and Masters [30] showed that the drug distribution and coverage of vaginal tissue varies considerably with the nature of the delivery system; solution, suspension and foam showing greater superiority over tablet dosage form. Ideally, a vaginal drug delivery system that is intended for local effect should distribute uniformly throughout the vaginal cavity. Ideally, the choice of vaginal drug administration depends on the applicability of the intended effect; whether a local or topical effect is required? For a local effect to occur, semi-solid or fast dissolving solid system will be required. For a topical effect, generally, a bioadhesive dosage form or intravaginal ring system would be more preferable.

However, by far, it had been difficult to quantitatively measure the distribution of a drug after an intravaginal administration and also it is uncertain if the administered formulation coated the whole organ. In this regard, an interesting study by Chatterton et al. [31], evaluating the distribution of two radio labeled vaginal products, proves helpful. The authors describe the retention and distribution of ^{99m}Tc -DTPA labeled

Table 1
Formulation systems experimented for vaginal drug delivery

Therapeutic drug	Intended use	Dosage form	Animal model	Comments	Ref.
Nonoxynol-9	Spermicide/topical contraceptive	Gel, foam, cream	Rabbit	Detergent type spermicide, irritation and increased risk of infections	[38,62,78]
Miconazole nitrate	Anti-fungal	Cream, suppository, swelling controlled release system	In vitro	–	[36]
Prostaglandin E ₂	Cervical ripening	Crosslinked PEG hydrogel, suppository	In vitro	Onset of labor not always predictable	[79,80]
Lactobacilli strains	Urogenital tract infections	Bi-layered tablet	In vitro	Restoration of normal vaginal flora, good bacterial viability in tablets	[81]
Progestin, levonorgestrel, norethindrone acetate	Contraceptives	Vaginal ring	Human	Uterine bleeding, hormonal side effects, expulsions	[58]
Estradiol	Hormone replacement therapy	Vaginal ring	Human	Risk of endometrial proliferation	[82]
Relaxin	Cervical ripening	Gel	Human	Decreased incidence of cesarean deliveries, reduced maternal–fetal morbidity	[83]
LHRH	Hormone-dependent mammary tumors, fertility control	Suppository	Rat	Chronic administrations suppress secretion of ovarian steroids	[5]
Leuprolide	Ovulation inducing activity	Solution, suppository, jelly	Rat	Activity increased by 5 times with addition of absorption enhancers	[84]
Insulin	Diabetes mellitus	Solution, gel	Rat, rabbit	Low bioavailability	[85,86]

vaginal cream (reference product) and gel (experimental) dosage form. Such studies are useful in assessing and comparing different vaginal dosage forms with regard to retention and distribution. Vaginal delivery may be designed for administration of drugs by using an applicator or specifically designed systems for intravaginal administration. Further, vaginal formulations may be designed to produce local effect such as spermicidal or antibacterial effect or to produce a systemic effect by continuous release of drugs such as contraceptives. Readers further interested in vaginal drug delivery are directed to a recent review by Alexander et al. [32]. Few of the commonly used marketed preparations with their intended use are tabulated in Table 2.

5.1. Creams and gels

Creams and gels are used for topical delivery of contraceptives and anti-bacterial drugs. These vaginal dosage forms are messy to apply, uncomfortable and sometimes embarrassing when they leak into the undergarments. Further, creams and gels may not

provide an exact dose because of nonuniform distribution and leakage. The desirable properties of vaginally administered cream or gel against microbicides are acceptability and feasibility. They must be easy to use, non-toxic and non irritating to the mucus membrane. In the treatment of bacterial vaginosis, metronidazole and clindamycin vaginal cream are found to be nearly as effective as orally administered drugs [33]. To evaluate the efficacy of an anti-bacterial vaginal cream in the treatment of bacterial vaginosis, Lamont et al. [34] carried out a randomized, placebo controlled 3-day course study during the second trimester of pregnant women. They found that the clindamycin vaginal cream was well tolerated and more efficacious than placebo in the treatment. In the absence of an effective prophylactic anti-HIV vaccine or therapy, current efforts are aimed at developing topical intravaginal formulations of anti-HIV agents or microbicides to reduce the mucosal and perinatal virus transmission [35].

Vaginal creams and gels could be based on the principle of emulsion or hydrogel based drug delivery. During the past few years, considerable work has been

Table 2
Few of the commonly used marketed vaginal products

Therapeutic drug (brand name)	Intended use	Dosage form	Comments	Company
Etonogestrel+ethinyl estradiol (NuvaRing®)	Contraception	Vaginal ring	Commonly reported adverse events are vaginitis, leukorrhea, weight gain	Organon
Progesterone (Prochieve®)	Infertility, secondary amenorrhea	Bioadhesive vaginal gel	Possible side effects are constipation, pain around vaginal area, breast pain	Fleet Laboratories
Desogestrel+ethinyl estradiol (Desogen®)	Contraception	Vaginal tablet	Adverse effects may include shortness of breath, headache, flushing	Organon
Metronidazole (Metrogel-vaginal®)	Bacterial infection	gel	Side effects are vaginal discharge, vulvovaginal irritation, cervicitis	3M Pharmaceuticals
Dinoprostone (Cervidil®)	Induction of labor	suppository	Other effects include abdominal cramps, diarrhea, fever	Controlled Therapeutics
Estradiol (Estring®)	Hormone therapy	ring	Frequently reported side effect is increased vaginal secretions	Pharmacia and Upjohn
Tioconazole (Vagistat-1®)	Anti-fungal, vaginal candida infection	Ointment	Possible side effects are shortness of breath; swelling of lips, face, or tongue	Bristol Myers Squibb
Clotrimazole (Trivagizole®)	Anti-fungal	Cream	Partners may experience minor skin irritation	Taro Pharmaceuticals
Estradiol tablets (Vagifem®)	Atrophic vaginitis	Vaginal tablet	Mild adverse effects are vaginal spotting, vaginal discharge, allergic reaction	Novo Nordisk

done on the development of hydrogel controlled release drug delivery systems. These hydrogels, when placed in an aqueous environment, swell and retain large volumes of water in their swollen structure and release drug in a controlled fashion. A swelling controlled release hydrogel delivery system for intravaginal administration of an antifungal drug, miconazole, has been reported [36]. Hydrogels are hydrophilic polymers that have been cross-linked by means of covalent bonds. A 3% alginate gel of nonoxynol-9 has been investigated for intravaginal spermicide delivery [37]. In the study, it was shown that spermicidal activity and diffusion of the agent changes with pH and osmolarity of the formulation. Recently, gel-microemulsions have been proposed as a nontoxic vaginal formulation [38]. A gel microemulsion based formulation of a spermicide with anti-HIV effect, phenyl phosphate derivative of zidovudine, has been developed [39]. Multiple intravaginal application of this drug as microemulsion gel formulation did not cause any damage in the vaginal epithelium in rabbit model. Vaginal gel has also been used for intravaginal vaccine delivery. Intravaginal delivery of cholera vaccine showed a greater mucosal

response in female genital tract compared to oral administration of the vaccine [40].

Antibacterial agents and drugs for cervical ripening and induction of labor are also available as vaginal gel form. Oxytocin, dinoprostone and misoprostol are commonly used drugs for cervical ripening and induction of labor. Recently, Shetty et al. [41] studied the efficacy of dinoprostone (prostaglandin E2) vaginal gel versus vaginal tablet in the induction of labor. Their retrospective analysis was performed to compare the labor outcomes between women who received dinoprostone vaginal gel (1–2 mg) over a 3-month period and women who were receiving a dinoprostone vaginal tablet (3 mg) over the following 3 months. The authors observed no statistically significant differences in labor outcomes between dinoprostone vaginal gel and tablet use in the induction of labor. However, in their analysis, the authors did not compare the safety between the two dosage forms. In another similar study, the efficacy and safety of dinoprostone vaginal insert with vaginal tablet was compared [42]. Women who were requiring labor induction were randomly assigned to receive either a 10 mg dinoprostone vaginal insert or 3 mg

dinoprostone tablet twice at six-hour intervals. The complications for the two dosage forms were tested by the occurrence of uterine hyper stimulation, abnormal fetal heart rate patterns, use of β -2 adrenergic drugs and fetal outcome. The interval from insertion of the induction agent to the onset of regular uterine contractions was similar between the two groups. In seven of eight patients from the group who were receiving the insert and experienced uterine hyper stimulation, removal of the insert was sufficient to stop the hyper stimulation. However, in the group that was receiving tablet, eight out of nine subjects needed medical intervention to end hyper stimulation.

An interesting study by Danielian et al. [43] compares the efficacy of vaginal misoprostol and dinoprostone vaginal gel for labor induction. The principal outcome measures were oxytocin requirement in labor, necessity of analgesia, mode of delivery, time for induction to delivery and neonatal outcome. In misoprostol administered group, a reduced need for oxytocin in labor, but a highly significant reduction in time for induction to delivery was observed compared to the dinoprostone administered group. However, no significant differences in the requirement of analgesia, mode of delivery, or neonatal outcome were noticed between the two cohorts. In another recent randomized control study involving dinoprostone suppository, vaginal misoprostol administration was found to be more efficacious than prostaglandin F₂- α gel and dinoprostone suppository [44]. Nevertheless, compared to PGF₂- α gel, both misoprostol and dinoprostone suppositories showed reduced need for oxytocin and shorter labor duration.

Since the inception of misoprostol in 1993 for labor induction [45], the intravaginal administration of this drug has been studied extensively. Recently, there have been several citations in the literature comparing the effectiveness of oral versus vaginal misoprostol delivery [46–50]. The dose required for the oral delivery of misoprostol is usually 4 times than that of intravaginal dose. However, there have been few conflicting reports too with respect to the efficacy of the route of misoprostol administration. For example, Hall et al. [48] reported that oral misoprostol had the potential to induce labor as safely and effectively as that produced by vaginal misoprostol, whereas a study by Shetty et al. [47] found that vaginal administration of the drug was more efficacious than the oral route.

Although the oral (100 μ g) and vaginal dose (25 μ g) as well as the intervals of drug administration were the same in both these studies, the results were not similar. This disparity in their observation could be attributed to their principal outcome criterion, which was assessed in each of these studies. In the former study, the key outcome measurement was the time for start of induction to vaginal delivery, while in the latter study the chief outcome measurement was the number of women who went on to deliver vaginally within 24 h of initiation of the first dose of misoprostol. In a specific study evaluating the safety and efficacy of oral versus vaginal misoprostol administration, the investigators found that, although oral misoprostol had similar effects as the vaginal form, the oral administration was associated with higher frequency of high uterine contractility and intervention [49]. In an interesting report, concerning the sublingual use of misoprostol in first-trimester surgical abortion, the authors found that sublingual delivery of misoprostol was an effective alternative to vaginal administration for cervical priming [50]. Although a greater incidence of side effects was observed, the patient acceptability was quite high. From an analysis of different studies performed employing the oral and vaginal routes of misoprostol administration, it appears that the currently recommended vaginal misoprostol dose (25 μ g) is efficacious and safer than the 100 μ g oral dose. Also, as rightly noticed by İnal et al. [51], different methods of misoprostol administration may not be equivalent with regard to efficacy and safety.

5.2. Suppositories and vaginal tablets

A large number of vaginal medications are available in the form of tablets or suppositories. Some authors use the terms pessaries and suppositories interchangeably and consider vaginal tablets as a separate dosage form. These vaginal formulations are designed to melt in the vaginal cavity and release the drug for several hours. Suppository systems are now most commonly used to administer drugs for cervical ripening prior to child birth and local delivery of drugs. Drugs that are administered as suppository include dehydroepiandrosterone sulphate [52] for ripening effect on the uterine cervix, miconazole for vaginal candidiasis [53,54] and progesterone for hor-

monal replacement therapy. Vaginal tablets may contain binders, disintegrant and other excipients that are used to prepare conventional oral tablets. It has the advantage of ease of manufacture and insertion. Mucoadhesive polymers are sometimes used in vaginal tablet formulation to increase vaginal residence time. Drugs that are administered as vaginal tablets include itraconazole, clotrimazole and prostaglandins. Presence of hydrophobic and release retarding materials may decrease the absorption of a drug from a vaginal formulation. Too hydrophobic drugs may not be suitable for vaginal tablets. Presence of penetration enhancers such as surfactants, bile salts can significantly enhance absorption.

5.3. Vaginal rings

Vaginal rings are circular ring type drug delivery devices designed to release drug in a controlled fashion after insertion in the vagina. Advantages of vaginal ring are that it is user controlled, does not interfere with coition, does not require daily intake of pills and allows continuous delivery of low dose steroids. They are approximately 5.5 cm diameter with a circular cross section diameter of 4–9 mm and the ring is inserted in the vagina. In simple vaginal rings, drug is homogeneously dispersed within a polymeric ring. Drug at the surface of the ring is released faster than drug in the inner layer of the ring. Sometimes, drugs in the outermost layer provide an initial burst release. To obtain a constant release of drug from vaginal ring, sandwich or reservoir type rings have been developed. Sandwich type devices consist of a narrow drug-containing layer located below the surface of the ring and positioned between a non-medicated central core and a nonmedicated outer band. In reservoir type rings, drugs are dispersed in a centralized core, which is then encapsulated by a drug free layer of polymer. In a single ring, it is possible to have several cores of different drugs and thereby allowing administration of several drugs from the same device. Rate of drug release can be modified by changing the core diameter or thickness of the nonmedicated coating. The material for making vaginal ring is usually polymeric in nature. Much of the vaginal ring literature relates to commonly used polymer, poly(dimethylsiloxane) or silicone devices, although other elastomeric polymers such as ethylene

vinyl acetate and styrene butadiene block copolymer have been tested in recent years [55,56]. Ethylene vinyl acetate polymers are classified by the content of vinyl acetate. The addition of vinyl acetate units in the polyethylene provides the following advantages: increased flexibility, improved optical properties, greater adhesion, and increased impact and puncture resistance. Further, the clinical acceptability of rings made of ethylene vinyl acetate is very high [55,57]. In a study by Roumen and Dieben [55], evaluating the tolerability of ethylene vinyl acetate nonmedicated vaginal ring of diameter 54 mm, the acceptability percent among the subjects involved in the study was 91%. The ring was to remain inserted for 21 consecutive days after insertion, permitting temporary removal during coition. Most of the women judged the ring easy to insert and remove. No adverse effects were experienced among the test group during the study period.

Vaginal rings are used for contraceptive and hormone replacement therapy [58–60]. For most contraceptive applications, the rings are placed in vagina for 21 days followed by a week of ring free period. NuvaRing® is the only combined contraceptive vaginal ring available in the US market. NuvaRing® is a flexible, transparent, contraceptive vaginal ring containing two active components, etonogestrel and ethinyl estradiol. The ring releases 120 µg/day of etonogestrel and 15 µg/day of ethinyl estradiol over a 3-week period of use. Clinical trials show that NuvaRing® is an effective contraceptive ring with good cycle control and user acceptability [57]. Femring® and Estring® are estrogen releasing rings used for estrogen therapy. Femring®, which is made up of silicone elastomer, contains acetate derivate of estradiol, which is placed in the vagina once every trimester. Estradiol acetate is hydrolyzed to estradiol after being released from the delivery device. Estring® is made of silicone polymers and when inserted in the vagina releases 7.5 µg of estradiol per day.

5.4. Bioadhesive delivery systems

Conventional vaginal formulations are associated with disadvantages of low retention to the vaginal epithelium, leakage and messiness thereby causing inconvenience to the user. To circumvent these

problems, bioadhesive drug delivery systems are being propagated. Bioadhesive polymers that have been used for vaginal formulation include polycarboxyl, hydroxypropylcellulose and polyacrylic acid. A bioadhesive polycarboxyl gel, Replens[®], is available in the market, which is used to retain moisture and lubricate vagina. The formulation remains in the vagina for 2–3 days and maintains the vagina at healthy, acidic pH. Various peptide and protein drugs have also been attempted to administer via bioadhesive microparticulate vaginal delivery system. Hyaluronic acid based intravaginal delivery of calcitonin, a polypeptide used in the treatment of postmenopausal osteoporosis, have shown promise for intravaginal administration of drugs for systemic effect [61].

A mucoadhesive controlled release drug delivery system for nonoxynol-9, a spermicidal agent, has been reported [62]. This gel type system consisting of varying levels of nonoxynol-9 and EDTA, a chelating agent, were formulated using carbopol 934P polymer [63,64]. The carbopol 934P polymer system provided a high burst release of nonoxynol-9 in the first 2 min and controlled release for 6 h. Gel type dosage form has the advantage over the tablet type dosage form, in that the former has greater surface contact and less irritation [65,66]. In one study [67], a new mucoadhesive vaginal dosage form for the antimycotic agent, clotrimazole, was developed by incorporating bioadhesive polymers viz. polycarboxyl, hydroxypropylmethylcellulose and hyaluronic sodium salt into suppositories made of semi-synthetic solid triglycerides. The authors argue that these polymers hold the suppositories in the vaginal tract for longer period of time without adverse effects, thereby prolonging the permanence of the drug on the vaginal epithelium. The presence of mucoadhesive polymers largely modulated the behavior of suppositories in terms of adhesive force, liquefaction time and permanence of the drug in the simulated application site; however, their presence did not alter the release of the drug. The developed formulations showed good technological and adhesion properties and the ability to hold the dosage form at the application site.

Assemblies for *in vitro* measurement of bioadhesive strength and retention characteristics of a polymer in a vaginal delivery system have been reported [68]. A modified simulated vaginal fluid was used to

simulate vaginal conditions for bioadhesion. Isolated lamb vaginal epithelium and cellophane saturated with simulated vaginal fluid were used as model membranes. The principle of bioadhesion is based on the measurement of tensile strength or shear stress required to break the adhesive bond between a model membrane and test formulation. The delivery system is placed between two model membranes fixed on flexible supports in the assemblies for a certain period of time. After the adhesive bond is formed, the force required to separate the bond is measured and calculated as bioadhesive strength. Such assemblies are useful for comparative evaluation of various polymers for bioadhesion and retention properties *in vitro*.

6. Vaginal immunization

In recent years, there have been several reports of successful immunization with DNA vaccines administered via various mucosal routes [69–71]. Mucosal sites, including the vaginal route, represent the primary site of entry of pathogens into the human body. Most of the conventional vaccines are administered via the oral or parenteral route resulting in systemic rather than mucosal immunity. On the other hand, mucosal immunization causes mucosal as well as systemic immunity [72]. In this regard, several vaginal vaccine formulations are being continuously researched against a variety of pathogens, including the human immunodeficiency virus (HIV). A recent study reports the development of a novel HIV-CCR5 receptor vaccine for the control of mucosal simian (SIV) and human forms of the virus. The vaccine, which targets both the virus and its CCR5 receptor, was administered in female rhesus monkey either by the vaginal route or by targeting the proximity of the draining iliac lymph nodes [73]. This immunization strategy through the vagina was found to significantly inhibit SIV/HIV infection in the animal model and shows promise for a novel approach in the prevention of HIV transmission. In another study [74], intravaginal infection of mice with influenza A virus resulted in mucosal and systemic immunity against HIV type 1 epitope. DNA vaccines represent a newer approach to the control of infectious diseases.

A recent study [75] demonstrates the formulation and application of plasmid DNA vaccine to mucosal inductive tissues, including the vagina. The female genital tract has the capacity to produce humoral and cellular immune responses against locally encountered antigens [75]. Vaginal immunization of rodents, human and non-human primates has been shown to elicit serum and secretory IgA and IgG responses in cervico-vaginal washes [76]. Further, this route of immunization was ineffective at eliciting immune responses in other mucosal compartments. Rather than simple application of vaccine formulation at the target site, an example of vaccine administration via vagina specific dosage form could best be illustrated by a seminal paper on mucosal immunity by Loehr et al. [77]. In an attempt to induce immunity against bovine herpes virus-1 in cattle, the researcher's immunized cows intravaginally with suppositories containing plasmid coding for the glycoprotein D. The level of immunity obtained, as assessed by the level of IgG in serum and IgA in both serum and nasal fluids, was of sufficient magnitude to minimize weight loss and significantly reduce the duration of virus shedding. Such successful noninvasive DNA immunization in large animals could open avenues for a new area of mucosal immunization in humans.

7. Conclusions

The vagina still remains to be an underutilized route of drug delivery. Although the human vagina is used as a route for local action in the cervico-vaginal region, its adoption for systemic delivery of macromolecules still needs to be accomplished. Various therapeutically important drugs such as insulin, calcitonin and sex hormones have been attempted to deliver via vaginal route but there is not much success in the development of safe and viable vaginal formulations for these macromolecular drugs. Among the drug delivery systems available for this route, intravaginal gels for labor induction have been found to be potential vaginal drug delivery systems mainly because of their bearing on childbirths. Bioadhesive vaginal formulations are likely to emerge as new vaginal formulations for both local and systemic delivery. With the increasing number of novel polymers each year, challenge remains to design appropriate bioadhesive vaginal

formulations. Vaginal rings have shown significant promise and are well accepted within female population. Several combination vaginal contraceptive rings have been found to provide excellent contraceptive efficacy with little risk of adverse effects. More sophisticated and programmable vaginal rings could be developed in the near future for systemic delivery of therapeutically important macromolecules.

Another area that needs to be investigated in detail is the application of immunization via the vagina. With the pandemic increase in the number of HIV infected individuals every year worldwide, the development of an effective vaginal vaccine rendering local immunity becomes imperative. One of the real challenges for future vaginal drug delivery will be to recognize ways to subjugate the complex biological barriers that limit the delivery of small and macromolecular drugs.

References

- [1] A.D. Woolfson, R.K. Malcolm, R. Gallagher, Drug delivery by the intravaginal route, *Crit. Rev. Ther. Drug Carr. Syst.* 17 (2000) 509–555.
- [2] N. Washington, C. Washington, C.G. Wilson, Vaginal and intrauterine drug delivery, in: N. Washington, C. Washington, C.G. Wilson (Eds.), *Physiological pharmaceuticals: barriers to drug absorption*, Taylor and Francis, London, 2001, pp. 271–281.
- [3] W. Platzner, S. Poisel, E.S.E. Hafez, Functional anatomy of the human vagina, in: E.S.E. Hafez, T.N. Evans (Eds.), *Human reproductive medicine: the human vagina*, North Holland Publishing, New York, 1978, pp. 39–54.
- [4] I. Sjoberg, S. Cajander, E. Rylander, Morphometric characteristics of the vaginal epithelium during the menstrual cycle, *Gynecol. Obstet. Invest.* 26 (1988) 136–144.
- [5] J.L. Richardson, L. Illum, Routes of drug delivery: case studies (8) The vaginal route of peptide and protein drug delivery, *Adv. Drug Deliv. Rev.* 8 (1992) 341–366.
- [6] J. Paavonen, Physiology and ecology of the vagina, *Scand. J. Infect. Dis., Suppl.* 40 (1983) 31–35.
- [7] M.H. Burgos, C.E. Roig de Vargas-Linares, Ultrastructure of the vaginal mucosa, in: E.S.E. Hafez, T.N. Evans (Eds.), *Human reproductive medicine: the human vagina*, North Holland Publishing, New York, 1978, pp. 63–93.
- [8] P.A. Bergh, Vaginal changes with aging, in: J.L. Breen (Ed.), *The gynecologist and the older patient*, Aspen Publishers, Maryland, 1988, pp. 299–311.
- [9] G. Wagner, R.J. Levin, Vaginal fluid, in: E.S.E. Hafez, T.N. Evans (Eds.), *Human reproductive medicine: the human vagina*, North Holland Publishing, New York, 1978, pp. 121–137.

- [10] W.H. Masters, V.E. Johnson, Human sexual response, Little Brown, Boston, 1966.
- [11] A.A. Deshpande, C.T. Rhodes, M. Danish, Intravaginal drug delivery, *Drug Dev. Ind. Pharm.* 18 (1992) 1225–1279.
- [12] K. Carlstrom, H. Pschera, N.O. Lunell, Serum levels of estrogens, progesterone, follicle-stimulating hormone and sex-hormone-binding globulin during simultaneous vaginal administration of 17- β -oestradiol and progesterone in the pre- and post-menopause, *Maturitas* 10 (1988) 307–316.
- [13] M.J. Durani, A. Kusai, A.F.H. Ho, Topical vagina drug delivery in the guinea pig: I. Effect of estrous cycle on the vaginal membrane permeability of vidarabine, *Int. J. Pharm.* 24 (1985) 209–218.
- [14] H. Pschera, A. Hjerpe, K. Carlstrom, Influence of the maturity of the vaginal epithelium upon the absorption of vaginally administered estradiol-17- β and progesterone in postmenopausal women, *Gynecol. Obstet. Invest.* 27 (1989) 204–207.
- [15] B. Villanueva, R.F. Casper, S.S.C. Yen, Intravaginal administration of progesterone: enhanced absorption after estrogen treatment, *Fertil. Steril.* 35 (1981) 433–437.
- [16] D.F. Katz, E.N. Dunmire, Cervical mucus. Problems and opportunities for drug delivery via the vagina and cervix, *Adv. Drug Deliv. Rev.* 11 (1993) 385–401.
- [17] J. Woodley, Bioadhesion: new possibilities for drug adhesion? *Clin. Pharmacokinet.* 40 (2001) 77–84.
- [18] T.A. Johnson, I.A. Greer, R.W. Kelly, A.A. Calder, The effect of pH on release of PGE2 from vaginal and endocervical preparations for induction of labour: an in-vitro study, *Br. J. Obstet. Gynaecol.* 99 (1992) 877–880.
- [19] S. Hwang, E. Owada, L. Suhardja, N.F.H. Ho, G.L. Flynn, W.I. Higuchi, Systems approach to vaginal delivery of drugs: IV. Methodology for determination of membrane surface pH, *J. Pharm. Sci.* 66 (1977) 778–781.
- [20] S. Hwang, E.O. Wada, T. Yotsuanagi, I. Suhardja, N.F.H. Ho, G.L. Flynn, W.I. Higuchi, Systems approach to vaginal delivery of drugs: II. In situ vaginal absorption of unbranched aliphatic alcohols, *J. Pharm. Sci.* 65 (1977) 1574–1578.
- [21] L. Brannon-Peppas, Novel vaginal drug release applications, *Adv. Drug Deliv. Rev.* 11 (1992) 169–177.
- [22] J.M. Sanders, H.B. Matthews, Vaginal absorption of polyvinyl alcohol in Fischer 344 rats, *Human Exp. Toxicol.* 9 (1990) 71–77.
- [23] E.H. Schmidt, F.K. Beller, Biochemistry of the vagina, in: E.S.E. Hafez, T.N. Evans (Eds.), *Human reproductive medicine: the human vagina*, vol. 2, North-Holland Publishing, New York, 1978, pp. 139–149.
- [24] C.P. Wendel-Smith, P.M. Wilson, The vulva, vagina, and urethra and the musculature of the pelvic floor, in: E. Philipp, M. Setchell, J. Ginsburg (Eds.), *Scientific foundations of obstetrics and gynecology*, Butterworth Heinemann, London, 1991, p. 84.
- [25] R.T. Havran, G. Oster, Trypsin-like activity in the vaginal epithelial cells of the rat, *J. Histochem. Cytochem.* 25 (1977) 1178–1186.
- [26] V.H.L. Lee, Enzymatic barriers to peptide and protein absorption, *CRC Crit. Rev. Ther. Drug Carr. Syst.* 5 (1988) 69–98.
- [27] S.D. Kashi, V.H.L. Lee, Enkephalin hydrolysis in homogenates of various absorptive mucosae of the albino rabbit: similarities in rates and involvement of aminopeptidases, *Life Sci.* 38 (1986) 2019–2028.
- [28] A.P. Sayani, I.K. Chun, Y.W. Chien, Transmucosal delivery of leucine enkephalin: stabilization in rabbit enzyme extracts and enhancement of permeation through mucosae, *J. Pharm. Sci.* 82 (1993) 1179–1185.
- [29] F. Acartürk, Z.I. Parlatan, Ö.F. Saracoglu, Comparison of vaginal aminopeptidase enzymatic activities in various animals and in humans, *J. Pharm. Pharmacol.* 53 (2001) 1499–1504.
- [30] V.E. Johnson, W.H. Masters, Intravaginal contraceptive study: Phase I. Anatomy, *West. J. Surg., Obstet. Gynecol.* 70 (1962) 202–207.
- [31] B.E. Chatterton, S. Penglis, J.C. Kovacs, B. Presnell, B. Hunt, Retention and distribution of two ^{99m}Tc-DTPA labeled vaginal dosage forms, *Int. J. Pharm.* 271 (2004) 137–143.
- [32] N.J. Alexander, E. Baker, M. Kaptein, U. Karck, L. Miller, E. Zampaglione, Why consider vaginal drug administration? *Fertil. Steril.* 82 (2004) 1–12.
- [33] L. DuBouchet, J.A. McGregor, M. Ismail, W.M. McCormack, A pilot study of metronidazole vaginal gel versus oral metronidazole for the treatment of trichomonas vaginalis vaginitis, *Sex. Transm. Dis.* 25 (1998) 176–179.
- [34] R.F. Lamont, B.M. Jones, D. Mandal, P.E. Hay, M. Sheehan, The efficacy of vaginal clindamycin for the treatment of abnormal genital tract flora in pregnancy, *Infect. Dis. Obstet. Gynecol.* 11 (2003) 181–189.
- [35] O.J. D’Cruz, F.M. Uckun, Clinical development of microbicides for the prevention of HIV infection, *Curr. Pharm. Des.* 10 (2004) 315–336.
- [36] T.K. Mandal, Swelling-controlled release system for the vaginal delivery of miconazole, *Eur. J. Pharm. Biopharm.* 50 (2000) 337–343.
- [37] D.H. Owen, E.N. Dunmire, A.M. Planys, D.F. Katz, Factors influencing nonoxynol-9 permeation and bioactivity in cervical mucus, *J. Control. Release* 60 (1999) 23–34.
- [38] O.J. D’Cruz, F.M. Uckun, Gel microemulsions as vaginal spermicides and intravaginal drug delivery vehicles, *Contraception* 64 (2001) 113–123.
- [39] O.J. D’cruz, Z.H. Zhu, S.H. Yiv, C.L. Chen, B. Waurzyniak, F.M. Uckun, WHI-05, a novel bromo-methoxy substituted phenyl phosphate derivative of zidovudine, is a dual-action spermicide with potent anti-HIV activity, *Contraception* 59 (1999) 319–331.
- [40] L. Wassen, K. Schon, L. Holmgren, M. Jerborn, N. Lycke, Local intravaginal vaccination of the female genital tract, *Scand. J. Immunol.* 44 (1996) 408–414.
- [41] A. Shetty, I. Livingston, S. Acharya, A. Templeton, Vaginal prostaglandin E2 gel versus tablet in the induction of labour at term—a retrospective analysis, *J. Obstet. Gynaecol.* 24 (2004) 243–246.
- [42] M. Rabl, E.A. Joura, Y. Yucel, C.A. Egarter, A randomized trial of vaginal prostaglandin E2 for induction of labor, insert vs. tablet, *J. Reprod. Med.* 47 (2002) 115–119.
- [43] P. Danielian, B. Porter, N. Ferri, J. Summers, A. Templeton, Misoprostol for induction of labour at term: a more effective

- agent than dinoprostone vaginal gel, *Br. J. Obstet. Gynaecol.* 1106 (1999) 793–797.
- [44] F. Majoko, M. Zwizwai, L. Nystrom, G. Lindmark, Vaginal misoprostol for induction of labour: a more effective agent than prostaglandin F2 alpha gel and prostaglandin E2 pessary, *Cent. Afr. J. Med.* 48 (2002) 123–128.
- [45] L. Sanchez-Ramos, A.M. Kaunitz, G.O. del Valle, I. Delke, P.A. Schroeder, D.K. Briones, Labor induction with the prostaglandin E1 methyl analogue misoprostol versus oxytocin: a randomized trial, *Obstet. Gynecol.* 81 (1993) 332–336.
- [46] S.A. Fisher, V.P. Mackenzie, G.A. Davies, Oral versus vaginal misoprostol for induction of labor: a double-blind randomized controlled trial, *Am. J. Obstet. Gynecol.* 185 (2001) 906–910.
- [47] A. Shetty, I. Livingstone, S. Acharya, P. Rice, P. Danielian, A. Templeton, Oral misoprostol (100 µg) versus vaginal misoprostol (25 µg) in term labor induction: a randomized comparison, *Acta Obstet. Gynecol. Scand.* 82 (2003) 1103–1106.
- [48] R. Hall, M. Duarte-Gardea, F. Harlass, Oral versus vaginal misoprostol for labor induction, *Obstet. Gynecol.* 99 (2002) 1044–1048.
- [49] S.J. Carlan, S. Bouldin, D. Blust, W.F. O'Brien, Safety and efficacy of misoprostol orally and vaginally: a randomized trial, *Obstet. Gynecol.* 98 (2001) 107–112.
- [50] H. Hamoda, P.W. Ashok, G.M.M. Flett, A. Templeton, A randomized controlled comparison of sublingual and vaginal administration of misoprostol for cervical priming before first-trimester surgical abortion, *Am. J. Obstet. Gynecol.* 190 (2004) 55–59.
- [51] M.M. İnal, K. Ertopçu, A. Arici, İ. Özelmaz, The effect of oral versus vaginal misoprostol on cervical dilatation in first-trimester abortion: a double-blind, randomized study, *Eur. J. Contracept. Reprod. Health Care* 8 (2003) 197–202.
- [52] A. Yamashita, S. Oshima, K. Matsuo, K. Ito, A. Ito, Y. Mori, Pharmacological studies of intravaginally applied dehydroepiandrosterone sulfate, *Folia Pharmacol. Jpn.* 98 (1991) 31–39.
- [53] R.A. Vukovich, A. Heald, A. Darragh, Vaginal absorption of two imidazole anti-fungal agents, econazole and miconazole, *Clin. Pharmacol. Ther.* 21 (1977) 121–125.
- [54] L.S. Abrams, H.S. Weintraub, Disposition radioactivity following intravaginal administration of ³H-miconazole nitrate, *Am. J. Obstet. Gynecol.* 147 (1983) 970–971.
- [55] F.J.M.E. Roumen, T.O.M. Dieben, Clinical acceptability of an ethylene-vinyl-acetate nonmedicated vaginal ring, *Contraception* 59 (1999) 59–62.
- [56] J.A.H. van Laarhoven, M.A.B. Krufft, H. Vromans, In vitro properties of etonogestrel and ethinyl estradiol from a contraceptive vaginal ring, *Int. J. Pharm.* 232 (2002) 163–173.
- [57] A. Novák, C. de la Loge, L. Ebetz, E.A. van der Meulen, The combined contraceptive vaginal ring, NuvaRing: an international study of user acceptability, *Contraception* 67 (2003) 187–194.
- [58] B. Harwood, D.R. Mishell, Contraceptive vaginal ring, *Semin. Reprod. Med.* 19 (2001) 381–390.
- [59] S.A. Ballagh, Vaginal ring hormone delivery systems in contraception and menopause, *Clin. Obstet. Gynecol.* 44 (2001) 106–113.
- [60] G. Dezarnaulds, I.S. Fraser, Vaginal ring delivery of hormone replacement therapy—a review, *Expert Opin. Pharmacother.* 4 (2002) 201–212.
- [61] J.L. Richardson, T.I. Armstrong, Vaginal delivery of calcitonin by hyaluronic acid formulations, in: E. Mathiowitz, D.E. Chickering III, C.M. Lehr (Eds.), *Bioadhesive drug delivery systems: fundamentals, novel approaches and development*, Marcel Dekker, New York, 1999, pp. 563–599.
- [62] C.H. Lee, Y.W. Chien, In vitro permeation study of a mucoadhesive drug delivery for controlled delivery of non-oxynol-9, *Pharm. Dev. Technol.* 1 (1996) 135–145.
- [63] C.H. Lee, M. Anderson, Y.W. Chien, The characterization of in vitro spermicidal activity of chelating agent on human sperm, *J. Pharm. Sci.* 85 (1996) 649–654.
- [64] C.H. Lee, R. Bagdon, Y.W. Chien, Comparative in vitro spermicidal activity and synergistic effect of chelating agents with nonoxynol-9 on human sperm functionality, *J. Pharm. Sci.* 85 (1996) 91–95.
- [65] K. Knuth, M. Amiji, J.R. Robinson, Hydrogel delivery systems for vaginal and oral applications: formulation and biological consideration, *Adv. Drug Deliv. Rev.* 11 (1993) 137–164.
- [66] M. Ishida, N. Nambu, T. Nagai, Ointment-type oral mucosal dosage form of carbopol containing prednisolone for treatment of aphtha, *Chem. Pharm. Bull.* 31 (1983) 1010–1014.
- [67] G.C. Ceschel, P. Maffei, S.L. Borgia, C. Ronchi, S. Rossi, Development of a mucoadhesive dosage form for vaginal administration, *Drug Dev. Ind. Pharm.* 27 (2001) 541–547.
- [68] K. Vermani, S. Garg, L.J.D. Zaneveld, Assemblies for in vitro measurement of bioadhesive strength and retention characteristics in simulated vaginal environment, *Drug Dev. Ind. Pharm.* 28 (2002) 1133–1146.
- [69] T.S. Kanellos, D.K. Byarugaba, P.H. Russell, C.R. Howard, C.D. Partidos, Naked DNA when co-administered intranasally with heat-labile enterotoxin of *Escherichia coli* primes effectively for systemic B- and T-cell responses to the encoded antigen, *Immunol. Lett.* 74 (2000) 215–220.
- [70] L.S. Klavinskis, M. Daheshia, K. Karem, E. Manickan, B.T. Rouse, Intranasal immunization with plasmid DNA-lipid complexes elicits mucosal immunity in the female genital and rectal and rectal tracts, *J. Immunol.* 1 (1999) 254–262.
- [71] N. Kuklin, M. Daheshia, K. Karem, E. Manickan, B.T. Rouse, Induction of mucosal immunity against herpes simplex virus by plasmid DNA immunization, *J. Virol.* 71 (1997) 3138–3145.
- [72] P.L. Ogra, J. Mestecky, M.E. Lamm, W. Strober, J.R. Mcghee, J. Bienenstock, *Handbook of mucosal immunology*, Academic press, CA, 1994, pp. 1–766.
- [73] W.M.J.M. Bogers, L.A. Bergmeier, J.Ma.H. Oostermeijer, Y. Wang, C.G. Kelly, P. ten Haaf, M. Singh, J.L. Heeney, T. Lehner, A novel HIV-CCR5 receptor vaccine strategy in the control of mucosal SIV/HIV infection, *AIDS* 18 (2004) 25–36.
- [74] B. Garulli, Y. Kawaoka, M.R. Castrucci, Mucosal and systemic immune responses to a human immunodeficiency virus type 1 epitope induced upon vaginal infection with a recombinant influenza A virus, *J. Virol.* 78 (2004) 1020–1025.

- [75] P. Hobson, C. Barnfield, A. Barnes, L.S. Klavinskis, Mucosal immunization with DNA vaccines, *Methods* 31 (2003) 217–224.
- [76] C. Czerkinsky, F. Anjuere, J.R. McGhee, A. George-Chandy, J. Holmgren, M.P. Kieny, K. Fujiyashi, J.F. Mestecky, V. Pierrefite-Carl, C. Rask, J.B. Sun, Mucosal immunity and tolerance: relevance to vaccine development, *Immunol. Rev.* 170 (1999) 197–222.
- [77] B.I. Locher, R. Rankin, R. Pontarollo, T. King, P. Willson, L.A. Babiuk, S. van Drunen Littel-van den Hurk, Suppository-mediated DNA immunization induces mucosal immunity against bovine herpesvirus-1 in cattle, *Virology* 289 (2001) 327–333.
- [78] F. Acatürk, J.R. Robinson, Effect of the spermicide, non-oxynol 9, on vaginal permeability in normal and ovariectomized rabbits, *Pharm. Res.* 13 (1996) 950–951.
- [79] A.V.G. Taylor, J. Boland, I.Z. Mackenzie, The concurrent in vitro and in vivo release of PGE₂ from a controlled-release hydrogel polymer pessary for cervical ripening, *Prostaglandins* 40 (1990) 89–98.
- [80] W.F. Rayburn, R.J. Wapner, V.A. Barss, E. Spitzberg, R.D. Molina, N. Mandsager, M.L. Yonekura, An intravaginal controlled-release prostaglandin E₂ pessary for cervical ripening and initiation of labor at term, *Obstet. Gynecol.* 79 (1992) 374–379.
- [81] L. Maggi, P. Mastromarino, S. Macchia, P. Brigidi, F. Pirovano, D. Matteuzzi, U. Conte, Technological and biological evaluation of tablets containing different strains of lactobacilli for vaginal administration, *Eur. J. Pharm. Biopharm.* 50 (2000) 389–395.
- [82] D. Grady, T. Gebretsadik, K. Kerlikowske, V. Ernster, D. Petitti, Hormone replacement therapy and endometrial cancer risk: a meta-analysis, *Obstet. Gynecol.* 85 (1995) 304–313.
- [83] S.A. Chen, A.J. Perlman, N. Spanski, C.M. Peterson, S.W. Sanders, R. Jaffe, M. Martin, T. Yalcinkaya, R.C. Cefalo, N.C. Chescheir, M.K. Menard, J. Mordenti, The pharmacokinetics of recombinant human relaxin in nonpregnant women after intravenous, intravaginal, and intracervical administration, *Pharm. Res.* 10 (1993) 834–838.
- [84] H. Okada, I. Yamazaki, Y. Ogawa, S. Hirai, T. Yashiki, H. Mima, Vaginal absorption of a potent luteinizing hormone-releasing hormone analog (leuprolide) in rats: I. Absorption by various routes and absorption enhancement, *J. Pharm. Sci.* 71 (1982) 1367–1371.
- [85] K. Morimoto, T. Takeeda, Y. Nakamoto, K. Morisaka, Effective vaginal absorption of insulin in diabetic rats and rabbits using polyacrylic acid aqueous gel bases, *Int. J. Pharm.* 12 (1982) 107–111.
- [86] J.L. Richardson, L. Illum, N.W. Thomas, Vaginal absorption of insulin in the rat: effect of penetration enhancers on insulin uptake and mucosal histology, *Pharm. Res.* 9 (1992) 878–883.