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Review

Gels as vaginal drug delivery systems

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Abstract

The vagina has been used as a mucosal drug delivery route for a long time. Its single characteristics can be either limitative or advantageous when drug delivery is considered. Gels are semi-solid, three-dimensional, polymeric matrices comprising small amounts of solid, dispersed in relatively large amounts of liquid, yet possessing more solid-like character. These systems have been used and are receiving a great deal of interest as vaginal drug delivery systems. Gels are versatile and have been used as delivery systems for microbicides, contraceptives, labour inducers, and other substances. Although somewhat neglected in clinical studies, pharmaceutical characterization of vaginal gels is an important step in order to optimize safety, efficacy and acceptability. Indeed, the simple formulation of a gel can lead to different performances of systems containing the same amount of active substances. Therefore, this paper discusses and summarizes current use and research of vaginal drug delivery systems based in gels.

Keywords: Vagina; Gels; Microbicides; Contraceptives; Labour inducers

Contents

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1. Introduction

The vagina has been used for a long time as a route for drug delivery, traditionally with the purpose of obtaining a local pharmacological effect, although some systemic drug absorption was observed. Several drug classes have been administered through the vaginal mucosa such as antimicrobials, labour inducers, spermicides, and sexual hormones. Until the 1920s the vagina was considered to be an organ incapable of absorbing drugs systematically. Although most are indicated for the treatment of local conditions, a number of them can achieve sufficient serum levels to have systemic effects (Alexander et al., 2004; Song et al., 2004). Actually, the vaginal mucosa permeability proved to be higher to some substances such as water, 17-β-estradiol, arecoline, and arecaidine, when compared to the intestinal mucosa (van der Bijl and van Eyk, 2003). The permeation mechanism to most substances is simple diffusion, where hydrophobic substances are preferentially absorbed by intracellular route, while hydrophilic ones are preferentially absorbed by pores present in the vaginal mucosa (Sassi et al., 2004).

Ideal vaginal drug delivery systems should be easy to use, discreet, of reversible application, painless to the patient, cost-effective, widely available, and safe for continuous administration. It should also allow self-administration, with minimal interference with body functioning and daily life, and obtain high bioavailability with other medications (Alexander et al., 2004; Garg et al., 2003b). The advantages of administration by the vaginal route are the avoidance of hepatic first-pass metabolism, a reduction in the incidence and severity of gastrointestinal side effects, a decrease in hepatic side effects of drugs such as steroids, and overcoming of pain, tissue damage, and probable infection observed with parental routes (Vermani and Garg, 2000).

2. Vaginal anatomy, histology and physiology

The vagina is the feminine genital organ with functions related with sexual intercourse, conception and menstruation discharge. The vagina is a tubular, fibromuscular organ that extends from the cervix of the uterus to the vaginal vestibule measuring in length about 9 cm (Van De Graaff, 2001). Histologically the vagina is composed by four distinct layers (Fig. 1): stratified squamous epithelium, lamina propria, muscular layer, and adventicia (Burkitt et al., 1994). The mucosal layer forms a series of transverse folds called rugae which dramatically increases its surface. Although considered a mucosal tissue, the normal vagina does not have glands, the vaginal secretion being a mixture of fluids from a number of sources (Stenchever et al., 2002). This mucus coating has several important physiological functions, playing an important role in drug absorption or action (Khanvilkar et al., 2001). It is also noteworthy that vaginal characteristics change with the menstrual cycle, particularly pH and vaginal fluid: the normal pH value is around 4.5–5.5, while vaginal fluid varies largely in volume, composition and rheological properties. Epithelial thickness is about 200–300 μm, and is not significantly affected with the sexual cycle (Song et al., 2004). The vaginal pH is maintained by *Lactobacilli* present in the

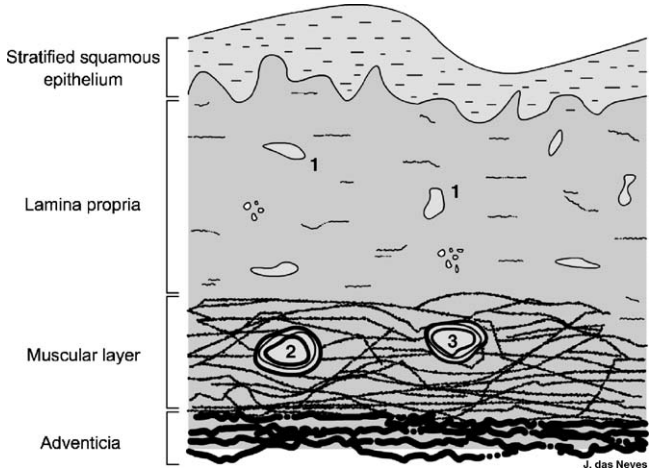

Fig. 1. Schematic drawing of the vaginal mucosa. 1: capillary vessels; 2: artery; 3: vein.

healthy vagina, playing an important role in the control of infection by common pathogens. These organisms also produce other bactericidal compounds such as hydrogen peroxide, bacteriocin-like substances and possibly biosurfactants (Boris and Barbés, 2000). Vaginal pH is an important parameter concerning the efficacy of drug delivery systems, as demonstrated by Ramsey et al. This group studied the influence of vaginal pH on the efficacy of a dinoprostone gel for cervical ripening/labour induction in 32 women. Results showed that the efficacy of this gel was significantly associated with the pH of the vagina (Ramsey et al., 2002). Post-menopausal women experience important changes in the vaginal physiology such as progressive atrophy of vaginal epithelium, elevation of vaginal pH (6.0–7.5), and a decrease in the quantity of vaginal secretions (Caillouette et al., 1997; Greendale et al., 1997). These features can significantly change the performance of drug delivery systems.

3. Gels as vaginal drug delivery systems

Vaginal drug delivery systems include a large variety of pharmaceutical forms such as semi-solids, tablets, capsules, pessaries, liquid preparations, vaginal films, vaginal rings, foams, and tampons. Most widely used semi-solid preparations for vaginal drug delivery include creams, ointments, and gels (Prista et al., 1996; Vermani and Garg, 2000). Beside their consistency properties, the capability of adhesion to surfaces for a reasonable period of time, before being removed by washing or by natural factors, is the common feature to these preparations (Lachman et al., 2001). The main advantages of semi-solid preparations are acceptability, feasibility, and low cost. On the other hand, messiness, discomfort, and leakage are its main disadvantages (Hussain and Ahsan, 2005). In fact, one of the problems presented by conventional vaginal drug delivery systems is rapid removal from the application site (Garg et al., 2003b).

Gels are semi-solid systems comprising small amounts of solid, dispersed in relatively large amounts of liquid, yet possessing more solid-like character (Justin-Temu et al., 2004). These systems form a three-dimensional, polymeric matrix in which a high degree of physical (or sometimes, chemical) reticulation

has been comprised (Lachman et al., 2001). They are formed of long, disordered chains that are connected at specific points, but the connections must be reversible. The molecular mechanisms of gelation are poorly understood, but researchers are attempting to design and enhance molecules with these properties (Goodsell, 2004). Gels can present several advantages over other vaginal drug delivery systems such as higher bioavailability, safety, versatility, and economical savings (Justin-Temu et al., 2004). It is well known that the choice of dosage formulation can influence the disposition of an active substance. This fact was well demonstrated by Cunningham et al. in a pharmacokinetic study of a vaginal gel containing metronidazole. The performance of the studied gel differed from earlier work, this fact being attributed to formulation differences (Cunningham et al., 1994).

3.1. Environmentally sensitive gels

Environmentally sensitive gels are systems that can dramatically alter their physical characteristics as a result of exposure to environmental changes. Of particular importance to the vaginal administration are thermosensitive gels. These gels are systems capable of gelling in response to temperature change, generally from ambient to body temperature. Usually, the gelation temperatures have been considered suitable if they are in the range of 25–37 °C (Chang et al., 2002b). The thermogelation mechanisms include partial crystallization, coil-to-helix transition, hydrophobic association, and micelle packing, which serves as reversible physical cross-linking points to form a gel (Jeong et al., 2002). The thermoreversible properties of these gels can be evaluated by rheological parameters such as the shear stress changes upon shear rates, sol–gel transition temperature, and viscoelasticity moduli (Chang et al., 2002a). In response to the increase of environmental temperatures, and at a specific value (lower critical solution temperature), some polymers undergo abrupt changes in solubility. This phase is generally viewed as a phenomenon governed by the balance of hydrophilic and hydrophobic moieties on the polymer change and the free energy of mixing ($\Delta G = \Delta H - T\Delta S$, where ΔG is the free energy, ΔH the enthalpy, T the temperature, and ΔS is the entropy). Commonly used polymers include polysaccharides, *N*-isopropylacrilamide copolymers, poloxamers and its copolymers, poly(ethylene oxide)/poly(D,L-lactic acid-co-glycolic acid) copolymers, and some liposome-based systems. Poloxamer hydrogels perhaps represent the most extensively studied systems, while polysaccharides usually demonstrate good biocompatibility and/or biodegradability, their solutions being thermosensitive at low polymeric concentrations (Ruel-Gariepy and Leroux, 2004).

In recent years, some studies were carried out by Chang et al. in order to obtain vaginal gels with thermosensitive behaviour containing clotrimazole. Results indicate that these thermosensitive gels are potential candidates for safe, convenient, and effective treatment of vaginal candidiasis. These gels, that also presented mucoadhesive behaviour, were prepared with mixtures of poloxamers and polycarbophil (Chang et al., 2002a,b). In fact, recent studies confirmed that the combination of a thermosensitive polymer like poloxamer, and a bioadhesive polymer like polycarbophil, appears to be promising from a pharmaceutical viewpoint (Tirnaksiz and Robinson, 2005).

4. Pharmaceutical characterization of vaginal gels

As far as vaginal drug delivery is concerned, optimization of formulations will require more attention, particularly characteristics such as hydrophilicity, distribution, viscosity and bioadhesion (Keller et al., 2003). Gel characterization should be performed throughout the time of storage (Santos et al., 2002). It is known that gel characteristics that influence its performance can change, such as the amount of solvent present, or lost, in the system can alter the migration of active molecules through the gel (Gallagher et al., 2003).

4.1. Evaluation of drug release and permeability studies

In the pharmaceutical industry, drug release evaluation is very important in drug development and quality control. The choice of the correct in vitro method may not necessarily closely imitate the in vivo environment, but it should still test the key performance indicators of the formulation (Siewert et al., 2003). Thus, Franz cell diffusion system is generally considered the most appropriate in vitro method for evaluating drug release from topical gels for vaginal use (FDA, 1997; Siewert et al., 2003). When formulating vaginal gels, several factors, such as pH and osmolarity, should be assessed, since they can affect the diffusion and permeation of delivered drugs through the gel and vaginal mucus (Owen et al., 1999). For vaginal gels intended to achieve systemic absorption, permeability tests must also be performed. Human and animal vaginal mucosa has been used as permeability models for in vitro studies. Although several animal models, such as porcine vaginal mucosa, are generally considered appropriate to assess the degree of absorption through the vaginal wall, these permeability studies do not always correspond closely to the ones performed with human samples (van Eyk and van der Bijl, 2005). When using human vaginal mucosa, proper treatment of samples should be performed, in order to obtain consistent and homogeneous permeability results. An Ussing chamber technique has been proposed, and results showed that this method can be considered useful for in vitro studies of vaginal permeability (Bechgaard et al., 1994).

4.2. Mechanical studies: rheological and textural properties

Composition of a gel can strongly influence its rheological properties, and even only one different constituent can lead to significantly different rheological behaviour (Bahia, 2001; Owen et al., 2001). The rheological properties can be determined by oscillatory or flow rheometry, although oscillatory are preferable to flow measurements as it allows a complete characterization of both the elastic and viscous components of the systems in study (Madsen et al., 1998; Jones et al., 2003). The ideal viscosity value of vaginal gels is hard to define. Since gels always present non-Newtonian flow behaviour, a single measurement

of the viscosity at a defined shear rate is not sufficient to characterize the product distinctly. Instead, a multipoint measurement capable of decompose the rheological behaviour into individual viscous and elastic components is required. Also, more important than a specific range of viscosity values, vaginal gels should retain their viscosity when higher shear rates are applied or after dilution with fluids present in the vagina (Garg et al., 2001a).

Assessing the rheological properties before a clinical study is an important step. The formulation to be tested and the placebo should have similar rheological properties, differing only in the presence or absence of the active compound (Owen et al., 2001). The gel optimization should not focus simply upon rheology of undiluted material, but also include a selection of macromolecules that produce desired interactions with the vaginal environment. Temperature, pH, and interactions of formulations with fluids in the vagina cause changes in viscosity that should be taken into account in formulation design (Owen et al., 2003). Thus, rheological measurements should be determined before and after dilution with vaginal or cervical fluid simulant, as this mixture often presents different behaviour (Owen et al., 2000). Synthetic substitutes have been used mainly because human and animal vaginal fluids are hard to get and highly degradable (Burruano et al., 2002). A few simulants have been proposed by some authors (Owen and Katz, 1999; Burruano et al., 2002). Also, gels may encounter semen during their residence within the vagina, and the resulting interactions can affect the rheological performance of the vaginal formulation. Consequently, as for vaginal fluids, semen simulants can be used in rheological studies, such as the one proposed by Owen and Katz (2005). In addition, these rheological tests should be performed throughout the time of storage, as alterations in the structure of gels may occur (Tamburic and Craig, 1996).

Texture profile analysis (TPA) has been used as an interesting technique in order to determine the mechanical properties of semi-solids, particularly of vaginal gels. As for rheological measurements, these determinations should be performed in the gel and in the mixture gel/vaginal fluids (Jones et al., 2003). TPA can provide some important parameters such as hardness, compressibility/spreadability, and adhesiveness of a pharmaceutical preparation. Lack of direct rheological significance of TPA parameters and a unified experimental instrumentation and/or methods were the two main problems in the comparison of different studies, but recently developed dimensional analysis of TPA data can overcome these limitations (Jones et al., 2002).

4.3. Toxicological evaluation

Gel bases can be toxic to vaginal mucosa. Thus, mucosal toxicity potential of these formulations should be assessed (Van Damme et al., 2000). Although a gel base can prove to be safe, it also should protect the vaginal mucosa when it is used as a drug delivery system of an irritant drug such as nonoxynol-9 (Amaral et al., 1999). In vitro reconstituted human vaginal epithelial cells or tissue has been used to test mucosal toxicity of gels. Alterations in the normal histoarchitecture of exposed epithelial cells can reveal toxicity (D'Cruz et al., 2004). Many animal species have been used as models to predict the in vivo mucosal toxicity of vaginal gels in preclinical studies. Rabbits remain the standard animal model, although others like pigs have been studied as alternatives (Catalone et al., 2004; D'Cruz et al., 2005). Also, alternative models to vertebrates such as slug mucosal irritation tests have been proposed to monitor irritation potential of vaginal gels, particularly in early stages of the development process (Dhondt et al., 2005). In vivo tests in women are the most reliable, and should be performed in phase I of the clinical development of a gel. After single and multiple exposure genital irritation is assessed by findings of epithelial changes seen on pelvic examination and colposcopy, as well as self reported symptoms by women (Mauck et al., 2004). Determination of inflammatory cytokines in vaginal flow (Paternoster et al., 2004), and of alterations in vaginal microflora (Patton et al., 1999) can also be of value in the assessment of vaginal irritation. Comparative phase I studies between new formulations and commercially available ones have been used and showed usefulness in establishing whether the new formulations are suitable in terms of vaginal irritation, safety, vaginal leakage, acceptability, and other characteristics (Mauck et al., 2001).

4.4. Vaginal distribution and retention

Vaginal distribution and retention of gels are important parameters to evaluate in order to achieve efficacy. Thus, a vaginal gel must distribute and maintain an epithelial coating layer (Geonnotti et al., 2005). Optimally, each specific gel should be assessed to characterize these two parameters (Barnhart et al., 2004). Factors such as a higher volume of application (2.5 ml versus 3.5 ml), ambulation, and sexual intercourse seem important in order to improve spread and surface contact of gels when applied in the vagina (Barnhart et al., 2005b). Sexual intercourse is an important factor in gel distribution and retention. It is believed that sexual activity may dramatically increase the spread throughout the vagina, although it is possible that concentration of the gel in certain areas may occur. Simulated intercourse with a plastic phallus has proven to be an acceptable substitute for live intercourse in determining the effect of sexual activity in vaginal spreading and retention of gels (Pretorius et al., 2002).

Initially, in vitro studies are used to evaluate if a preparation has the potential to suitably distribute and be retained in the vagina. Several in vitro methods have been developed in order to achieve this goal. These tests should expose gels to vaginal fluid, cervical mucus, semen, and simulating coital shearing activity (Geonnotti et al., 2005). A simple in vitro method to study the gravity-induced coating flows can be used to pre-evaluate the distribution of vaginal gels. This simple technique based on the flow of gels in inclined surfaces, together with mechanistic mathematical models, can help in the selection of primary candidate formulations before in vivo tests commence (Kieweg et al., 2004).

Rheological properties are important to the critical functions of spreading and retention of gels over the vaginal surface, which are fundamental to their efficacy (Owen et al., 2000; El-Gizawy and Aglan, 2003). The selection of the correct viscosity of a gel can be determinant to select the adequate retention and

distribution in the vagina. These simple characteristics can be determinant in order to achieve a therapeutical effect, particularly in the case of microbicides (Di Fabio et al., 2003).

Recently, Vermani et al. developed a simple test to measure bioadhesion in 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. These investigators used as a membrane either cellophane hydrated with simulated vaginal fluid or sheep vaginal mucosa. Both tensile strength and shear stress tests should be performed because they may provide different results for the same gel, due to the different types of forces involved (Vermani et al., 2002b).

Imaging studies remain the most reliable methods of in vivo assessment of drug distribution of local methods of delivery, such as vaginal administration (Berridge et al., 2003). Magnetic resonance imaging can be used to evaluate vaginal spreading of gels (Barnhart et al., 2001, 2005a,b). In fact, this technique can provide objective in vivo comparison of coverage at different times or between competing products, and also monitor the migration of substances from the vagina to the endocervix (Barnhart et al., 2001, 2004).

Another imaging technique that has been used to monitor vaginal distribution and retention is gamma scintigraphy. Although it requires to be used only in post-menopausal women, this radioactive tracer method has proven to be useful in assessing vaginal retention and distribution of vaginal dosage forms (Brown et al., 1997; Chatterton et al., 2004).

4.5. Mucoadhesion

Bioadhesion may be defined as the state in which two materials, at least one of which is biological in nature, are held together for extended periods of time by interfacial forces. If this attachment is due to a mucus coating, the phenomenon is sometimes referred to as mucoadhesion. The vaginal route appears to be highly appropriate for bioadhesive drug delivery systems, to retain systems for treating largely local (although some systemic) conditions, or for use in contraception (Smart, 2004). Gels are one of the most commonly studied mucoadhesive formulations for vaginal drug delivery (Edsman and Hagerstrom, 2005). The main advantage of mucoadhesive systems is the possibility of increasing the time of residence in situ, thus reducing the number of applications. Ideally, the formulation will be retained at the biological surface and the drug will be released close to the absorptive membrane, with a consequent enhancement of bioavailability (Woodley, 2001).

Although several theories try to explain mucoadhesion, none of them can be applied alone to all different pharmaceutical formulations, but several can be combined to obtain a picture of the mucoadhesion process. These include electronic theory, adsorption theory, wetting theory, mechanical theory, fracture theory, and diffusion or interpenetration theory. This last theory is one of the most accepted and has been the focus of substantial research. Generally, it can be described in two steps: creation of intimate contact between the dosage form and the mucosa, and interpenetration of the components of both systems (Fig. 2). The interpenetration of polymer chains across the mucus layer that coats the vaginal mucosa can result in adhesion. The depth of interpenetration depends on the diffusion coefficient and the time of contact, influencing the strength of the adhesive bond (Smart, 2005). In the case of a gel system, it is the chain ends and smaller molecular weight chains that contribute to the interpenetration process. Also, an adequate solubility of the bioadhesive in the mucus is essential for good mucoadhesion (Huang et al., 2000; Edsman and Hagerstrom, 2005). Adhesive bond can be classified as non-specific (most synthetic polymers) or specific (lectins and other biological molecules). Mechanism of non-specific bioadhesion is not well understood, but physicochemical processes like electrostatic forces, hydrophobic interactions, hydrogen bonding, and Van Der Wall's interactions play important roles (Woodley, 2001).

Mucoadhesion is usually obtained by using both synthetic and natural bioadhesive polymers. To date the most used mucoadhesive polymers are synthetic polyacrylates, although some others such as chitosan, carragenan or sodium alginate will likely gain more importance in the near future (Valenta, 2005). Recently, thyolated polymers have been explored as new mucoadhesive molecules. In a comparative study these polymers showed higher adhesion time and higher total work of adhesion than frequently used polymers (Grabovac et al., 2005).

Polymer properties such as swelling state, bioadhesive properties and the effect on the medium pH have significant importance for bioadhesive vaginal formulations (Baloglu et al., 2003). Some of the polymers may exhibit site-specific bioadhesive properties; thus, the mucoadhesive properties should be evaluated and optimized with reference to physiological environments (Garg et al., 2003a).

Mucoadhesion force can be studied by several in vitro and in vivo methods (Edsman and Hagerstrom, 2005). The rheology of gels seems to be correlated with mucoadhesion (Tamburic and Craig, 1995). Rheological studies of vaginal gels should be performed over mixtures of the formulations and mucus, which can provide some information relating to the interaction between these two fluids, and consequently of the potential mucoadhesive properties of the vaginal formulation (Burruano et al., 2004). A rheological synergism can be found within a certain concentration of some polymers, which can give rise to a gel-strengthening effect when mixed with mucus (Madsen et al., 1998).

5. Excipients used in vaginal gels

Compliance is one of the main attributes that excipients should provide to vaginal drug delivery systems. Classes of excipient usually added to vaginal gels include gelling agents, humectants, preservatives and vehicles (Garg et al., 2001b). Polymers commonly used as gelling agents in the formulation of vaginal hydrophilic gels are presented in Table 1.

By definition, excipients are usually chosen from among the materials noted for being very nearly pharmaco-toxicologically inert (Pifferi and Restani, 2003). Consequently, excipients are deprived of therapeutical activity. Nonetheless, this is not always true, and sometimes can be an advantage or even a goal in the development of pharmaceutical systems. As an example, some sulphated polymers such as carrageenans that are usually used

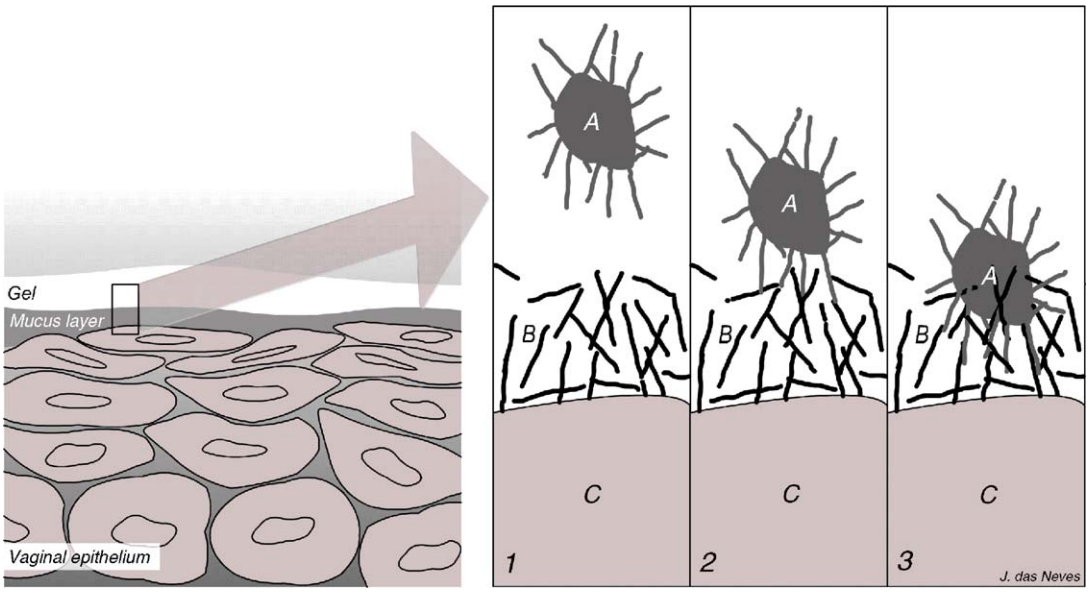

Fig. 2. Schematic drawing of the steps involved in the interpenetration theory. (1) Polymer chains approaching the mucus layer. (2) Interpenetration of polymer and mucin chains. (3) Consolidation of adhesion forces (A: polymer particle; B: mucus layer; C: epithelial cell lining).

Table 1
Polymers commonly used in vaginal hydrophilic gels

Carbopol® (974P, 980, 971P and 943)
Gelatin
Hydroxyethylcellulose
Hydroxypropylcellulose
Hydroxypropylmethylcellulose
Methylcellulose
Polycarbophil
Polyethylene glycol
Polysaccharide hyaluronic
Polyvinylpyrrolidone
Sodium alginate
Sodium carboxymethylcellulose
Starch

as gelling agents, present themselves as one of the most promising classes of potential microbicidal compounds (Coggins et al., 2000). Carrageenans formulations may be effective against HIV infection because they seem to prevent the trafficking of HIV-infected mononuclear cells from vaginal fluids to the mucosa. These polymers form a negatively charged coat around HIV-infected mononuclear cells preventing their adhesion to the vaginal epithelial surface (Perotti et al., 2003). Also, data from a phase I preliminary safety study indicates that a Carbopol® gel containing carrageenans as microbicidal agent do not cause significant irritation to the female reproductive tract, which can be an advantage over another microbicides that can be irritant to the vaginal mucosa (Coggins et al., 2000).

Vaginal drug delivery systems used to obtain systemic effects need to facilitate mucosal penetration of active substances. In order to improve drug capability of permeating skin and mucosa, several penetration enhancers have been tested and referred to in literature reviews (Finnin and Morgan, 1999; Thomas and Finnin, 2004; Ting et al., 2004). Chitosan is commonly used to obtain pharmaceutical systems for mucosal delivery, particularly of biotechnology drugs (Issa et al., 2005). In fact, chitosan is currently receiving a great deal of interest for medical and pharmaceutical application, particularly because of its penetration enhancement capability, alongside with other intrinsic properties such as biocompatibility, biodegradability, bioadhesivity, and bacteriostatic effect (Berger et al., 2004). The mechanism of penetration enhancement appears to occur because chitosan is able to bind to negatively charged tissues and cell surfaces, thus facilitating the permeability of epithelial barriers (Issa et al., 2005). The penetration enhancement seems to be higher for high molecular weight chitosans, being 5-methyl-pyrrolidinone chitosan considered the most promising chitosan derivative to enhance the hydrophilic drug absorption via the vaginal mucosa (Sandri et al., 2004).

Before usage, excipients used to formulate vaginal drug delivery systems must prove their biocompatibility. These tests are usually performed in cell culture models and biocompatibility can be determined by cell growth/cell viability assays, cell proliferation assay, cytotoxicity assay, nitrite assay, and by protein assay and identification (Warrier et al., 2004). Also, differential scanning calorimetry (DSC) can also be used in animal models to elucidate a direct relationship between vaginal membrane stability and the degree of disorder of the lipid layers upon exposure to polymer-based drug delivery systems, and consequently, biocompatibility (Wang and Lee, 2002).

6. Clinical usage and potential of vaginal gels

6.1. Vaginal gels as microbicides

Currently, most of the research on vaginal drug delivery focuses on the prevention of HIV infection and sexually transmitted diseases. Vaccines for prevention of HIV transmission are not likely to become available soon (Garber et al., 2004). Microbicides circumvent many of the immunological difficul-

ties associated with HIV vaccine development and make topical gels a more realistic goal to pursue, especially in the short term (Greenberger, 2001; Markel, 2005). On the other hand, condoms are not always accepted and correctly used by men, in order to prevent sexually transmitted diseases (Elias and Heise, 1994; Langenberg, 2004; Mukenge-Tshibaka et al., 2005). Additionally, unlike condoms, microbicides are controlled by women, and do not require the cooperation, consent or even knowledge of the partner. Microbicides can provide an option to thousands of women who are at risk of HIV but are unable to negotiate condom use because of gender inequality, illiteracy, cultural resistance and poverty (Joshi et al., 2005). Thus, microbicides can be a solution to the sexual transmission of HIV.

Microbicides are chemical agents topically used by women within the vagina, in order to prevent infection by HIV and potentially by other enveloped viruses, as well as sexually transmitted pathogens such as *Chlamydia trachomatis* and *Treponema pallidum* (Pilcher, 2004; Weber et al., 2005). Several substances have been studied for their microbicidal properties, including viral entry inhibitors monoclonal antibodies (Veazey et al., 2003), reverse transcriptase inhibitors (Di Fabio et al., 2003), plant products (Bourne et al., 1999; Vermani and Garg, 2002a; Dezzutti et al., 2004; Whaley and Zeitlin, 2005), ionic surfactants (Roy et al., 2001), dendrimers (Bourne et al., 2000; McCarthy et al., 2005), sulphated and sulphonated polymers (Cheshenko et al., 2004), zinc salts (Bourne et al., 2005), and bile salts (Herold et al., 1999). Recently obtained data from the macaque vaginal model showed that the topical use of monoclonal antibodies can also be effective in preventing HIV transmission. These agents could also be contraceptive, although most of the currently potential microbicides are not. Microbicides can be classified as first generation or surfactants (e.g. nonoxynol-9), second generation or blocking HIV binding (e.g. naphthalene sulphonate polymer, carageenan, and cellulose sulphate), and third generation or topical antiretrovirals (e.g. tenofovir, UC781, and TMC120) (Weber et al., 2005).

Barnhart et al. observed that after the vaginal insertion of topical microbicides there was migration of the active substances to the cervix, uterus, and tubes. These findings may represent an important unrecognized microbicidal mechanism of action (Barnhart et al., 2001). It is noteworthy that although several clinical trials are in course (see Table 2), at present, an effective microbicide is not available. Nonoxynol-9, once considered one of the most promising microbicides, has been slowly abandoned. Studies indicate that this molecule is not as effective in vivo as in vitro against commonly sexually transmitted pathogens (Roddy et al., 2002; Wilkinson, 2002).

Although microbicides can be formulated as creams, films or pessaries, gel formulations appear to be preferred among researchers. Microbicide gels are likely to be only partially effective, but that can make a big difference in places such as Africa, where the fact that most gels can be produced at reasonable costs is also an advantage (Pilcher, 2004). The properties of the gel in which microbicides are included are known to affect the mucosal permeation rate of microbicides and their microbicidal properties. A gel (Carbopol® 974P 1.0–1.5% and hydroxipropylmethylcellulose 1.0–1.5%) showed to be a good gel base to microbicide delivery, being able to pose itself as an ideal approach for vaginal controlled drug delivery system of microbicides against sexually transmitted diseases (Wang and Lee, 2002). Also, the development of a "universal" placebo gel is required in order to assure that the control preparation does not distort either safety or efficacy assessments of microbicides. Recently, a hydroxyethylcellulose placebo gel has been proposed as an adequate "universal" placebo, demonstrating to be safe and sufficiently inactive for use in the clinical study of investigational microbicides (Tien et al., 2005).

Beside vaginal delivery of commonly used or studied microbicides, other active substances and strategies based on gels have been experimented. Gels containing monocaprin (1-monoglyceride of capric acid) have been tested as potential microbicides, presenting good characteristics to be used in the future in the prevention of sexual transmitted diseases (Neyts et al., 2000). In vitro studies showed that gels containing monocaprin, formulated using as gel-forming agents, either sodium carboxymethylcellulose and polyvinylpyrrolidone (pH 7.0) or carbomer and hydroxypropylmethylcellulose (pH 5.0), are highly virucidal (HSV-1) and less cytotoxic than nonoxynol-9 (Kristmundsdottir et al., 1999). In another study, a step forward was taken when in vitro data showed that these gels containing monocaprin are also effective against other pathogens such as HIV-1, and common bacteria that can cause vaginitis. Also, the gels presented spermicidal properties, suggesting potential contraceptive activity (Thormar et al., 1999). In vivo studies in mice demonstrated that these gels present no irritation or toxicity potential following application in the vagina (Neyts et al., 2000).

Sulphated polysaccharides have been tested as potential microbicides. An iota-carrageenan gel formulation (PC 213) showed to be effective and safe in preventing HIV infection in a phase I trial. The formulation contained 2% iota-carrageenan, benzyl alcohol as preservative, and hydrochloric acid in order to adjust pH to 6.0 (Elias et al., 1997).

Poly(sodium 4-styrene sulphonate) (T-PSS) gel formulations have promising in vivo activity as topical microbicides, showing themselves as alternatives to nonoxynol-9. T-PSS acts by blocking pathogen cell binding. T-PSS gel formulations tested contained 5–10% T-PSS, hydroxyethyl cellulose, glycerine, propylene glycol, benzoic acid, methylparaben, and sodium hydroxide (Bourne et al., 2003). Also, this gel showed to be effective and safe as a contraceptive in rabbits (Zaneveld et al., 2002).

As previously referred, thermoreversible gels are recognized to allow advantageous application of active substances in the vagina. A thermoreversible gel composed of polyoxypropylene and polyoxyethylene in citrate buffer (pH 4.0) presents a phase transition temperature of 28 °C, demonstrating to be an ideal vehicle to deliver microbicidal substances, such as sodium lauryl sulphate or *n*-lauroylsarcosine, when applied in a murine model of herpes simplex type 2 (Roy et al., 2001).

Also, dendrimers have been proposed as potential microbicides. These substances are large highly branched macromolecules synthesized from a polyfunctional core (Bourne et al., 2000). SPL7013 emerged as the most promising dendrimer after pre-clinical studies, and has been formulated as a gel (VivaGel®)

Table 2
Several recent clinical trials on microbicides

Clinical trial	Formulation	Phase	Purpose	Status
Safety and Tolerability of the Vaginal Gel PRO 2000/5®	Naphthalene 2-sulphonate (polymer) gel	Phase I	Determine whether the vaginal gel PRO 2000/5® causes irritation when used daily	Completed
Safety and Acceptability of PRO 2000/5® Vaginal Gel in HIV Uninfected Women in India	PRO 2000/5® gel	Phase I	Determine the safety and acceptability when used by women; examine what women and men think about using PRO 2000® Gel	In progress
Safety and Acceptability of the Anti-Microbe Vaginal Gel, PMPA Gel	Tenofovir PMPA gel	Phase I	Evaluate the PMPA gel in HIV-infected and HIV-uninfected women	In progress
BufferGel® and PRO 2000/5®: Vaginal Gels to Prevent HIV Infection in Women	BufferGel® and PRO 2000/5® gel	Phase II	Determine the safety and effectiveness of these gels	In progress
Safety and Acceptability of a Vaginal Microbicide	1% Tenofovir gel	Phase II	Determine the safety and acceptability in HIV uninfected sexually active women	Not yet started
Effectiveness of 851B Gel for the Treatment of High-Risk Cervical Human Papillomavirus (HPV) Infection	851B gel	Phase II	Determine the effectiveness compared to placebo by assessing clearance of the HPV infection on the cervix	In progress
Effectiveness of BufferGel® as a Vaginal Contraceptive	BufferGel®	Phases II and III	Compare BufferGel® to Gynol II®, a currently available contraceptive gel	In progress
Randomized Controlled Trial of SAVVY® and HIV	1.0% C31G SAVVY® vaginal gel	Phase III	Determine the effectiveness and safety for the prevention of male-to-female transmission of HIV among women at high risk	In progress
Cellulose Sulphate (CS) Gel and HIV in Nigeria	6% cellulose sulphate vaginal gel	Phase III	Determine the effectiveness and safety for the prevention of HIV infection	In progress
A Study of Nonoxynol-9 (N-9) and HIV Infection	Nonoxynol-9 (N-9) gel	Phase III	Determine if it can prevent the spread of HIV	In progress

Source: http://www.clinicaltrials.gov.

who is already in clinical trialling (McCarthy et al., 2005). Bernstein and colleagues showed that a 5% Carbopol® gel (pH 4.5) can also be a suitable vehicle for the intravaginal administration of SPL7013 (Bernstein et al., 2003).

6.2. Vaginal gels as contraceptives

As already mentioned, gels used as microbicides, such as those containing nonoxynol-9, can be effective as contraceptive agents. Also, some vaginal gels have been specifically developed with the purpose of contraception, particularly those containing spermicides. Although not as effective as other contraceptive methods, these gels are still in the dawn of their development, and some appear to have great potential. Some new classes of contraceptive agents and innovative approaches have been tested in recent years.

Bis(cyclopentadienyl) complexes of vanadium (IV) or vanadocenes are a potential new class of contraceptive agents. In animal studies, results of intravaginal use of vanadocene dithiocarbamate via a gel microemulsion showed clinical potential as a safe alternative to currently used detergent-type contraceptives (D'Cruz and Uckun, 2005).

As in the case of microbicides, the formulation of a gel can be sufficient to assure contraceptive effect. Acid-buffering gels are good examples. An in vitro study showed that ACIDFORM® gel has good acid-buffering properties when mixed with semen, thus presenting spermicidal activity. Additionally, this gel allows inclusion of active ingredients that are water soluble and acid stable (Garg et al., 2001a). The potential spermicidal effect of this gel was confirmed in a phase I, blinded, randomized, crossover clinical study. Maintenance of its activity when inserted up to 10 h before coitus is an interesting feature (Amaral et al., 2004). In another study, the acid-buffering capacity of a thermosensitive gel (polyoxypropylene, polyoxyethylene, citrate buffer) has a synergic effect with sodium lauryl sulphate in inhibiting sperm motility. Thus, this gel could represent a potential candidate to be used as a topical vaginal contraceptive (Haineault et al., 2003).

Rheological properties of gels have considerable influence in the contraceptive success. As the consistency of the applied product increases, its efficacy may also increase as a result of becoming more tenacious and more resistant to sperm migration, and consequently decreasing the capability of sperm to reach the site of fertilization (El-Gizawy and Aglan, 2003).

6.3. Vaginal gels as labour inducers

Intravaginal gels containing prostaglandins are known to be efficient labour inducers and abortifacients for a long time (MacKenzie and Embrey, 1977; Mackenzie et al., 1978; Taylor et al., 1999). Several gel formulations based in polymers such as starch (Harris et al., 1980), hydroxyethylcellulose (Gauger, 1984), methylcellulose (Gauger, 1983), or hydroxyethylmethylcellulose (Calder et al., 1977) have been used and cited in the literature as vehicles for prostaglandins.

The vaginal route can even be more effective in inducing labour than other routes. In a clinical trial, the administration of a prostaglandin E2 gel showed to be more efficacious in inducing labour, compared to intracervical administration of the same preparation (Seeras, 1995). Also, Bartusevicius et al. in a comparative review demonstrated that vaginal misoprostol appears to be more effective than the equivalent dosage administered by mouth (Bartusevicius et al., 2005). However, the vaginal route can be associated with a higher risk of uterine hyperstimulation and has the disadvantage of being less convenient for both patients and doctors (Bartusevicius et al., 2005; Uludag et al., 2005). Also, higher price and the necessity of refrigeration for storage can be limiting factors for gel usage as labour inducers, particularly in developing countries (Gregson et al., 2005).

In comparison with another vaginal dosage forms, Shetty et al. observed in a retrospective analysis that there were no significant differences in labour or neonatal outcomes between prostaglandin E2 vaginal gel and vaginal tablet used in the induction of labour, although cervical dilatation with the gel was significantly more, with fewer women requiring oxytocin augmentation (Shetty et al., 2004).

Gels containing prostaglandins have also showed to be useful in other labour related conditions. In a randomized, double blind, placebo-controlled trial a gel containing prostaglandin E2 proved to be effective and safe in resolving dystocia in women with spontaneous labour at term (Oppenheimer et al., 2005).

6.4. Other applications of vaginal gels

As in the case of sexually transmitted diseases, intravaginal gels can be used in the treatment of infections by non-sexually transmitted pathogens. In fact, vaginal gels containing antimicrobial agents demonstrated that they can be as effective as oral regimens in the treatment of bacterial vaginosis (Sweet, 1993). Also, gels can be a convenient dosage form for vaginal administration of liposomes (phosphatidylcholine or distearoylphosphatidylcholine) containing antibacterial drugs such as clotrimazole, metronidazole, and chloramphenicol for the treatment of vaginal infections (Pavelic et al., 1999). Controlled release of antimicrobials can be achieved using hydrophilic gels as vehicles. The use of a sustained release miconazole gel showed to be able to reduce the number of applications, and even allow single dose treatments (Mandal, 2000).

A vaginal hydrophilic gel (hydroxyethylcellulose 1%) containing 5-fluorouracil (1%) has been tested for the treatment of intravaginal warts. The clinical results demonstrated that 5-fluorouracil in a vaginal hydrophilic gel is safe, tolerable and significantly more effective than placebo (Syed et al., 2000). In another study, investigators evaluated the capacity of several pharmaceutical hydrophilic gels to topically deliver granulocyte-macrophage colony-stimulating factor (GM-CSF) on human papillomavirus-associated cervicovaginal pre-neoplastic lesions. A mouse xenograft model demonstrated that polycarbophil gels (1% w/w) were suitable to deliver GM-CSF to the vaginal mucosa, with the advantage of stabilizing the protein. Also, this stabilization was dependent on the gel pH: at pH 5.5 GM-CSF was stabilized, whereas at pH 6.9 a dramatically loss of bioactivity was observed (Hubert et al., 2004).

The simple formulation of a drug delivery system can lead to gel bases with intrinsic microbicidal activity. Fiorilli et al. published a double blind, placebo controlled, 12-week trial study, where a mucoadhesive vaginal gel with acidic-buffering properties showed to be effective in the treatment of bacterial vaginosis. This gel was formed by two polymers, polycarbophil (responsible for the acidic-buffering properties) and Carbopol® (Fiorilli et al., 2005). The maintenance of the vaginal pH at around 4.5 can be enough to prevent infection. Additionally, this acidic-buffering vaginal gel can be of value when used in combination with oral regimens of antibacterial drugs, in order to treat bacterial vaginosis (Milani et al., 2003). As acidic-buffering gels maintain the normal pH of the vagina, they allow the reestablishment of the normal physiology (Robinson and Bologna, 1994). Bachman and colleagues also reported that an acidic-buffering polycarbophil based gel is effective in the treatment of vaginal dryness, particularly in post- and peri-menopausal women (Bachmann et al., 1991). These gels can also be useful in the reduction of symptoms associated with post-menopausal vaginal atrophy, particularly in women who cannot use systemic estrogens (Greendale et al., 1997).

Also, liposomes containing antimicrobial drugs demonstrated potential to be applied in the vagina in order to treat vaginitis, although their behaviour in this organ is still not understood (Pavelic et al., 1999). Polyacrylate gels showed to improve the stability of liposomes (phosphatidylcholine and phosphatidylglycerol-sodium, 9:1) containing calcein, and thus, proving to be suitable vehicles for vaginal drug delivery. These bioadhesive gels may be able to provide sustained and controlled release of appropriate drugs for local vaginal therapy (Pavelic et al., 2001). Recent studies by Pavelic et al. confirmed the applicability of this type of gels as adequate liposome vaginal delivery systems, being able to provide localized and sustained release of active substances, namely acyclovir, clotrimazole and metronidazole for the treatment of genital herpes, as well as vaginitis (Pavelic et al., 2005a,b).

Insulin administration through the vaginal mucosa has been tested using polyacrylic acid aqueous gel bases. Results showed that when these gels were administered to rats and rabbits, the plasma insulin reached a peak, and the hypoglycaemic effects were sustained for 30 min. However, sustained release improvement is necessary in order to achieve longer time of hypoglycaemia (Morimoto et al., 1982). Recently, Degim et al. developed vaginal chitosan gels as carriers for insulin. Studies were performed in rabbits and results showed that chitosan gels, containing 5% dimethyl-β-cyclodextrin as a penetration enhancer, may provide longer insulin release. Thus, these gels based on a natural polymer may offer an alternative to the parental route (Degim et al., 2005).

6.5. Currently marketed vaginal gels

Although many of the vaginal products based in gels are still under development, there are a few that have been on the market for a few years. Table 3 summarizes some of these gels, includ-

ing their qualitative composition and intended uses. Efforts are being made to launch new products in the market since clinical results of some vaginal gels look promising, particularly those concerning to microbicides and contraceptives. Features such as vaginal retention, distribution, and mucoadhesion are key points to the success of most of these gels. Nonetheless, a long way has to be made until excellence can be achieved.

7. Acceptability of gels for vaginal administration

Understanding the factors that determine the acceptability of a gel, and thus, its use is critically important since a product can only be effective when used regularly. While many products are being tested for safety and effectiveness, clinical trials generally overlook acceptability evaluation. Indeed, as a vaginal product approaches phase I testing, acceptability assessments should be performed (Bentley et al., 2000).

Taboos and preconceived notions regarding the application of a product in the vagina can limit acceptability (Hardy et al., 2003). Also, factors like formulation, applicator and packaging are determinant to achieve compliance during usage (Hardy et al., 1998). Although there are only a few acceptability studies in literature about vaginal drug delivery systems, most of them demonstrate that gels are among women's preferences, concerning to vaginal formulation of choice (Coggins et al., 1998; Hardy et al., 1998; Bentley et al., 2000; Morrow et al., 2003; Vandebosch et al., 2004). Gels possess lubricating properties and that can be perceived during sexual intercourse. The

Table 3
Some of the marketed vaginal gels

Brand name	Gelling agents	Other excipients	Active substances	Intended uses	Comments	Company
Acid Jelly®[a]	Tragacanth gum and acacia gum	Egg albumen, glycerin, perfume, potassium bitartrate, potassium hydroxide, propylparaben, stannous chloride, water	Oxyquinoline sulphate, ricinoleic acid, acetic acid	Maintenance of vaginal acidity, antiseptic	pH 3.9–4.1	Hope Pharmaceuticals
Advantage-S®[b]	Polycarbophil and Carbopol® 974P	Glycerin, hydrogenated palm oil glyceride, methylparaben, mineral oil, sodium hydroxide, sorbic acid, water	Nonoxynol-9	Contraceptive	Bioahesive	Columbia Laboratories
Conceptrol®	Sodium carboxymethylcellulose	Lactic acid, methylparaben, povidone, propylene glycol, sorbic acid, sorbitol solution, water	Nonoxynol-9	Contraceptive		Advanced Care Products
Gynol II®	Sodium carboxymethylcellulose	Lactic acid, methylparaben, povidone, propylene glycol, sorbic acid, sorbitol solution, water	Nonoxynol-9	Contraceptive	Discontinued in the UK (January 2006)	Janssen–Cilag
K-Y®	Hydroxyethylcellulose	Chlorhexidine gluconate, gluconolactone, glycerin, methylparaben, sodium hydroxide, water	–	Vaginal lubrication		Johnson & Johnson
Metrogel Vaginal®[c]	Carbopol® 974P	EDTA, methylparaben, propylene glycol, propylparaben, sodium hydroxide, water	Metronidazole	Bacterial vaginosis	pH 4.0	3M Pharmaceuticals
Crinone®[d]	Polycarbophil and Carbopol® 974P	Glycerin, hydrogenated palm oil glyceride, mineral oil, sodium hydroxide, sorbic acid, water	Progesterone	Infertility, secondary amenorrhea	Bioahesive, sustained release	Serono
Prostin E2®[e]	Colloidal silicon dioxide	Triacetin	Dinoprostone	Labour inducer		Pharmacia
Replens®	Polycarbophil and Carbopol® 974P	Glycerin, hydrogenated palm oil glyceride, methylparaben, mineral oil, sodium hydroxide, sorbic acid, water	–	Vaginal moisturizer	Bioadhesive	LDS Consumer Products

[a] Available in some countries by the trade name Aci-Jel® (Janssen–Cilag).
[b] Available in some countries by the trade name Advantage 24® (Lake Pharmaceutical).
[c] Available in some countries by the trade name Zidoval® (3M Pharmaceuticals).
[d] Available in some countries by the trade name Prochieve® (Columbia Laboratories).
[e] Available in some countries by the trade name Prepidil® (Pharmacia).

degree of lubrication provided by a product will likely be an important determinant of its acceptability and use (Braunstein and Van de Wijgert, 2005).

Taylor and Armour compared a prostaglandin E2 vaginal gel and a non-vaginal method (amniotomy plus intravenous oxytocin) in labour inducement. This willingness-to-pay study showed that the intravaginal gel method was preferred (Taylor and Armour, 2000). Following this study, these investigators confirmed the preference of the gel over more invasive methods, although the shorter time to induction of the amniotomy plus intravenous oxytocin can be seen as a limitation of the vaginal gel (Taylor and Armour, 2003).

8. Conclusion

Although most of the times regarded as an alternative to more conventional routes of drug delivery, vaginal drug administration has proven to be useful and even advantageous in some particular cases. Gels have been used for quite a long time as drug carriers and recent advances in gel and polymer technology attracted researchers' interest to these polymeric systems. The vaginal route was no exception. In these review we summarized the continuing interest, and discussed the current research in this field. Nonetheless, future work is required in order to optimize pharmaceutical performance of vaginal gels and allow excellence of clinical outcome.

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References

Alexander, N.J., Baker, E., Kaptein, M., Karck, U., Miller, L., Zampaglione, E., 2004. Why consider vaginal drug administration? Fertil. Steril. 82, 1–12.

Amaral, E., Faundes, A., Zaneveld, L., Waller, D., Garg, S., 1999. Study of the vaginal tolerance to Acidform, an acid-buffering, bioadhesive gel. Contraception 60, 361–366.

Amaral, E., Perdigão, A., Souza, M.H., Mauck, C., Waller, D., Zaneveld, L., Faúndes, A., 2004. Postcoital testing after the use of a bio-adhesive acid buffering gel (ACIDFORM) and a 2% nonoxynol-9 product. Contraception 70, 492–497.

Bachmann, G.A., Notelovitz, M., Gonzalez, S.J., Thompson, C., Morecraft, B.A., 1991. Vaginal dryness in the menopausal woman: clinical characteristics and non-hormonal treatment. Clin. Pract. Sex. 7, 1–8.

Bahia, M.F., 2001. Reologia de Medicamentos e de Cosméticos. In: Castro, A.G., Covas, J.A., Diogo, A.C. (Eds.), Reologia e suas Aplicações Industriais. Instituto Piaget, Lisboa, pp. 185–218.

Baloglu, E., Özyazici, M., Hizarcioglu, S.Y., Karavana, H.A., 2003. An in vitro investigation for vaginal bioadhesive formulations: bioadhesive properties and swelling states of polymer mixtures. Farmaco 58, 391–396.

Barnhart, K.T., Stolpen, A., Pretorius, E.S., Malamud, D., 2001. Distribution of a spermicide containing nonoxynol-9 in the vaginal canal and the upper female reproductive tract. Hum. Reprod. 16, 1151–1154.

Barnhart, K.T., Pretorius, E.S., Timbers, K., Shera, D., Shabbout, M., Malamud, D., 2004. In vivo distribution of a vaginal gel: MRI evaluation of the effects of gel volume, time and simulated intercourse. Contraception 70, 498–505.

Barnhart, K.T., Pretorius, E.S., Timbers, K., Shera, D., Shabbout, M., Malamud, D., 2005a. Distribution of a 3.5-mL (1.0%) C31G vaginal gel using magnetic resonance imaging. Contraception 71, 357–361.

Barnhart, K.T., Pretorius, E.S., Shaunik, A., Timbers, K., Nasution, M., Mauck, C., 2005b. Vaginal distribution of two volumes of the novel microbicide gel cellulose sulfate (2.5 and 3.5 mL). Contraception 72, 65–70.

Bartusevicius, A., Barcaite, E., Nadisauskiene, R., 2005. Oral, vaginal and sublingual misoprostol for induction of labor. Int. J. Gynaecol. Obste. 91, 2–9.

Bechgaard, E., Riis, K.J., Jørgensen, L., 1994. The development of an Ussing chamber technique for isolated human vaginal mucosa, and the viability of the in vitro system. Int. J. Pharm. 106, 237–242.

Bentley, M.E., Morrow, K.M., Fullem, A., Chesney, M.A., Horton, S.D., Rosenberg, Z., Mayer, K.H., 2000. Acceptability of a novel vaginal microbicide during a safety trial among low-risk women. Fam. Plann. Perspect. 32, 184–188.

Berger, J., Reist, M., Mayer, J.M., Felt, O., Gurny, R., 2004. Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. Eur. J. Pharm. Biopharm. 57, 35–52.

Bernstein, D.I., Stanberry, L.R., Sacks, S., Ayisi, N.K., Gong, Y.H., Ireland, J., Mumper, R.J., Holan, G., Matthews, B., McCarthy, T., Bourne, N., 2003. Evaluations of unformulated and formulated dendrimer-based microbicide candidates in mouse and guinea pig models of genital herpes. Antimicrob. Agents Chemother. 47, 3784–3788.

Berridge, M.S., Heald, D.L., Lee, Z., 2003. Imaging studies of biodistribution and kinetics in drug development. Drug Dev. Res. 59, 208–226.

Boris, S., Barbés, C., 2000. Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect. 2, 543–546.

Bourne, K.Z., Bourne, N., Reising, S.F., Stanberry, L.R., 1999. Plant products as topical microbicide candidates: assessment of in vitro and in vivo activity against herpes simplex virus type 2. Antiviral Res. 42, 219–226.

Bourne, N., Stanberry, L.R., Kern, E.R., Holan, G., Matthews, B., Bernstein, D.I., 2000. Dendrimers, a new class of candidate topical microbicides with activity against herpes simplex virus infection. Antimicrob. Agents Chemother. 44, 2471–2474.

Bourne, N., Zaneveld, L.J., Ward, J.A., Ireland, J.P., Stanberry, L.R., 2003. Poly(sodium 4-styrene sulphonate): evaluation of a topical microbicide gel against herpes simplex virus type 2 and *Chlamydia trachomatis* infections in mice. Clin. Microbiol. Infect. 9, 816–822.

Bourne, N., Stegall, R., Montano, R., Meador, M., Stanberry, L.R., Milligan, G.N., 2005. Efficacy and toxicity of zinc salts as candidate topical microbicides against vaginal herpes simplex virus type 2 infection. Antimicrob. Agents Chemother. 49, 1181–1183.

Braunstein, S., Van de Wijgert, J., 2005. Preferences and practices related to vaginal lubrication: implications for microbicide acceptability and clinical testings. J. Womens Health (Larchmt.) 14, 424–433.

Brown, J., Hooper, G., Kenyon, C.J., Haines, S., Burt, J., Humphries, J.M., Newman, S.P., Davis, S.S., Sparrow, R.A., Wilding, I.R., 1997. Spreading and retention of vaginal formulations in post-menopausal women as assessed by gamma scintigraphy. Pharm. Res. 14, 1073–1078.

Burkitt, H.G., Young, B., Heath, J.W., 1994. Histologia Funcional, 3rd ed. Guanabara Koogan, Rio de Janeiro, RJ, p. 353.

Burruano, B.T., Schnaare, R.L., Malamud, D., 2002. Synthetic cervical mucus formulation. Contraception 66, 137–140.

Burruano, B.T., Schnaare, R.L., Malamud, D., 2004. In vitro test to evaluate the interaction between synthetic cervical mucus and vaginal formulations. AAPS PharmSciTech. 5, E17.

Caillouette, J.C., Sharp Jr., C.F., Zimmerman, G.J., Roy, S., 1997. Vaginal pH as a marker for bacterial pathogens and menopausal status. Am. J. Obstet. Gynecol. 176, 1270–1275.

Calder, A.A., Embrey, M.P., Tait, T., 1977. Ripening of the cervix with extra-amniotic prostaglandin E2 in viscous gel before induction of labour. Br. J. Obstet. Gynaecol. 84, 264–268.

Catalone, B.J., Kish-Catalone, T.M., Budgeon, L.R., Neely, E.B., Ferguson, M., Krebs, F.C., Howett, M.K., Labib, M., Rando, R., Wigdahl, B., 2004. Mouse model of cervicovaginal toxicity and inflammation for preclinical evaluation of topical vaginal microbicides. Antimicrob. Agents Chemother. 48, 1837–1847.

Chang, J.Y., Oh, Y.K., Kong, H.S., Kim, E.J., Jang, D.D., Nam, K.T., Kim, C.K., 2002a. Prolonged antifungal effects of clotrimazole-containing mucoadhesive thermosensitive gels on vaginitis. J. Control. Release 82, 39–50.

Chang, J.Y., Oh, Y.K., Choi, H., Kim, Y.B., Kim, C.K., 2002b. Rheological evaluation of thermosensitive and mucoadhesive vaginal gels in physiological conditions. Int. J. Pharm. 241, 155–163.

Chatterton, B.E., Penglis, S., Kovacs, J.C., Presnell, B., Hunt, B., 2004. Retention and distribution of two 99mTc-DTPA labelled vaginal dosage forms. Int. J. Pharm. 271, 137–143.

Cheshenko, N., Keller, M.J., MasCasullo, V., Jarvis, G.A., Cheng, H., John, M., Li, J.H., Hogarty, K., Anderson, R.A., Waller, D.P., Zaneveld, L.J., Profy, A.T., Klotman, M.E., Herold, B.C., 2004. Candidate topical microbicides bind herpes simplex virus glycoprotein B and prevent viral entry and cell-to-cell spread. Antimicrob. Agents Chemother. 48, 2025–2036.

Coggins, C., Elias, C.J., Atisook, R., Bassett, M.T., Ettiègne-Traoré, V., Ghys, P.D., Jenkins-Woelk, L., Thongkrajai, E., VanDevanter, N.L., 1998. Women's preferences regarding the formulation of over-the-counter vaginal spermicides. AIDS 12, 1389–1403.

Coggins, C., Blanchard, K., Alvarez, F., Brache, V., Weisberg, E., Kilmarx, P.H., Lacarra, M., Massai, R., Mishell Jr., D., Salvatierra, A., Witwatwongwana, P., Elias, C., Ellertson, C., 2000. Preliminary safety and acceptability of a carrageenan gel for possible use as vaginal microbicide. Sex. Transm. Infect. 76, 480–483.

Cunningham, F.E., Kraus, D.M., Brubaker, L., Fischer, J.H., 1994. Pharmacokinetics of intravaginal metronidazole gel. J. Clin. Pharmacol. 34, 1060–1065.

D'Cruz, O.J., Waurzyniak, B., Uckun, F.M., 2004. Mucosal toxicity studies of a gel formulation of native pokeweed antiviral protein. Toxicol. Pathol. 32, 212–221.

D'Cruz, O.J., Erbeck, D., Uckun, F.M., 2005. A study of the potential of the pig as a model for the vaginal irritancy of benzalkonium chloride in comparison to the nonirritant microbicide PHI-443 and the spermicide vanadocene dithiocarbamate. Toxicol. Pathol. 33, 465–476.

D'Cruz, O.J., Uckun, F.M., 2005. Vaginal contraceptive activity of a chelated vanadocene. Contraception 72, 146–156.

Degim, Z., Degim, T., Acarturk, F., Erdogan, D., Ozogul, C., Koksal, M., 2005. Rectal and vaginal administration of insulin-chitosan formulations: an experimental study in rabbits. Drug Target. 13, 563–572.

Dezzutti, C.S., James, V.N., Ramos, A., Sullivan, S.T., Siddig, A., Bush, T.J., Grohskopf, L.A., Paxton, L., Subbarao, S., Hart, C.E., 2004. In vitro comparison of topical microbicides for prevention of human immunodeficiency virus type 1 transmission. Antimicrob. Agents Chemother. 48, 3834–3844.

Dhondt, M.M., Adriaens, E., Roey, J.V., Remon, J.P., 2005. The evaluation of the local tolerance of vaginal formulations containing dapivirine using the Slug Mucosal Irritation test and the rabbit vaginal irritation test. Eur. J. Pharm. Biopharm. 60, 419–425.

Di Fabio, S., Van Roey, J., Giannini, G., van den Mooter, G., Spada, M., Binelli, A., Pirillo, M.F., Germinario, E., Belardelli, F., de Bethune, M.P., Vella, S., 2003. 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.

Edsman, K., Hagerstrom, H., 2005. Pharmaceutical applications of mucoadhesion for the non-oral routes. J. Pharm. Pharmacol. 57, 3–22.

El-Gizawy, S.A., Aglan, N.I., 2003. Formulation and evaluation of metronidazole acid gel for vaginal contraception. J. Pharm. Pharmacol. 55, 903–909.

Elias, C.J., Heise, L.L., 1994. Challenges for the development of female-controlled vaginal microbicides. AIDS 8, 1–9.

Elias, C.J., Coggins, C., Alvarez, F., Brache, V., Fraser, I.S., Lacarra, M., Lahteenmaki, P., Massai, R., Mishell Jr., D.R., Phillips, D.M., Salvatierra, A.M., 1997. Colposcopic evaluation of a vaginal gel formulation of iota-carrageenan. Contraception 56, 387–389.

FDA Guidance for Industry: SUPAC-SS Nonsterile Semisolid Dosage Forms. Scale-Up and Post Approval Changes: Chemistry, Manufacturing, and Controls; in Vitro Release Testing and in Vivo Bioequivalence Documentation. May 1997.

Finnin, B.C., Morgan, T.M., 1999. Transdermal penetration enhancers: applications, limitations, and potential. J. Pharm. Sci. 88, 955–958.

Fiorilli, A., Molteni, B., Milani, M., 2005. Successful treatment of bacterial vaginosis with a polycarbophil-carbopol acidic vaginal gel: results from a randomised double-blind, placebo-controlled trial. Eur. J. Obstet. Gynecol. Reprod. Biol. 120, 202–205.

Gallagher, S.J., Trottet, L., Heard, C.M., 2003. Ketoprofen: release from, permeation across and rheology of simple gel formulations that simulate increasing dryness. Int. J. Pharm. 268, 37–45.

Garber, D.A., Silvestri, G., Feinberg, M.B., 2004. Prospects for an AIDS vaccine: three big questions, no easy answers. Lancet Infect. Dis. 4, 397–413.

Garg, S., Anderson, R.A., Chany II, C.J., Waller, D.P., Diao, X.H., Vermani, K., Zaneveld, L.J., 2001a. Properties of a new acid-buffering bioadhesive vaginal formulation (ACIDFORM). Contraception 64, 67–75.

Garg, S., Tambwekar, K.R., Vermani, K., Garg, A., Kaul, C.L., Zaneveld, L.J.D., 2001b. Compendium of pharmaceutical excipients for vaginal formulations. Pharm. Technol. 25, 14–24.

Garg, S., Kandarapu, R., Vermani, K., Tambwekar, K.R., Garg, A., Waller, D.P., Zaneveld, L.J.D., 2003a. Development pharmaceutics of microbicide formulations. Part I. Preformulation considerations and challenges. AIDS Patient Care STDs 17, 17–32.

Garg, S., Tambwekar, K.R., Vermani, K., Kandarapu, R., Garg, A., Waller, D.P., Zaneveld, L.J.D., 2003b. Development pharmaceutics of microbicide formulations. Part II. Formulation, evaluation, and challenges. AIDS Patient Care STDs 17, 377–399.

Gauger, L.J., 1983. Extemporaneous preparation of a dinoprostone gel for cervical ripening. Am. J. Hosp. Pharm. 40, 2195–2196.

Gauger, L.J., 1984. Hydroxyethylcellulose gel as a dinoprostone vehicle. Am. J. Hosp. Pharm. 41, 1761–1762.

Geonnotti, A.R., Peters, J.J., Katz, D.F., 2005. Erosion of microbicide formulation coating layers: effects of contact and shearing with vaginal fluid or semen. J. Pharm. Sci. 94, 1705–1712.

Goodsell, D.S., 2004. Bionanotechnology. Lessons from Nature. Wiley-Liss, Hoboken, NJ, p. 186.

Grabovac, V., Guggi, D., Bernkop-Schnurch, A., 2005. Comparison of the mucoadhesive properties of various polymers. Adv. Drug. Deliv. Rev. 57, 1713–1723.

Greenberger, P., 2001. News from the Society for Women's Health Research: seeking effective topical microbicides. J. Womens Health Gend. Based Med. 10, 99–100.

Greendale, G.A., Lee, N.P., Arriola, E.R., 1997. The menopause. Lancet 353, 571–580.

Gregson, S., Waterstone, M., Norman, I., Murrells, T., 2005. A randomized controlled trial comparing low dose vaginal misoprostol and dinoprostone vaginal gel for inducing labour at term. BJOG 112, 438–444.

Haineault, C., Gourde, P., Perron, S., Desormeaux, A., Piret, J., Omar, R.F., Tremblay, R.R., Bergeron, M.G., 2003. Thermoreversible gel formulation containing sodium lauryl sulfate as a potential contraceptive device. Biol. Reprod. 69, 687–694.

Hardy, E., Jiménez, A.L., de Pádua, K.S., Zaneveld, L.J.D., 1998. Women's preferences for vaginal antimicrobial contraceptives. III. Choice of a formulation, applicator, and packaging. Contraception 58, 245–249.

Hardy, E., de Pádua, K.S., Hebling, E.M., Osis, M.J.D., Zaneveld, L.J.D., 2003. Women's preferences for vaginal antimicrobial contraceptives. V. Attitudes of brazilian women to the insertion of vaginal products. Contraception 67, 391–395.

Harris, A.S., Kirstein-Pedersen, A., Stenberg, P., Ulmsten, U., Wingerup, L., 1980. Preparation, characterization, and stability of new prostaglandin E2 gel for local administration. J. Pharm. Sci. 69, 1271–1273.

Herold, B.C., Kirkpatrick, R., Marcellino, D., Travelstead, A., Pilipenko, V., Krasa, H., Bremer, J., Dong, L.J., Cooper, M.D., 1999. Bile salts: natural detergents for the prevention of sexually transmitted diseases. Antimicrob. Agents Chemother. 43, 745–751.

Huang, Y., Leobandung, W., Foss, A., Peppas, N.A., 2000. Molecular aspects of muco- and bioadhesion: tethered structures and site-specific surfaces. J. Control. Release 65, 63–71.

Hubert, P., Evrard, B., Maillard, C., Franzen-Detrooz, E., Delattre, L., Foidart, J.M., Noel, A., Boniver, J., Delvenne, P., 2004. Delivery of granulocyte-macrophage colony-stimulating factor in bioadhesive hydrogel stimulates migration of dendritic cells in models of human papillomavirus-associated (pre)neoplastic epithelial lesions. Antimicrob. Agents Chemother. 48, 4342–4348.

Hussain, A., Ahsan, F., 2005. The vagina as a route for systemic drug delivery. J. Control. Release 103, 301–313.

Issa, M.M., Köping-Höggård, M., Artursson, P., 2005. Chitosan and the mucosal delivery of biotechnology drugs. Drug Discov. Today 2, 1–6.

Jeong, B., Kim, S.W., Bae, Y.H., 2002. Thermosensitive sol–gel reversible hydrogels. Adv. Drug Deliv. Rev. 54, 37–51.

Jones, D.S., Lawlor, M.S., Woolfson, A.D., 2002. Examination of the flow rheological and textural properties of polymer gels composed of poly(methylvinylether-co-maleic anhydride) and poly(vinylpyrrolidone): rheological and mathematical interpretation of textural parameters. J. Pharm. Sci. 91, 2090–2101.

Jones, D.S., Lawlor, M.S., Woolfson, A.D., 2003. Rheological and mucoadhesive characterization of polymeric systems composed of poly(methylvinylether-co-maleic anhydride) and poly(vinylpyrrolidone), designed as platforms for topical drug delivery. J. Pharm. Sci. 92, 995–1007.

Joshi, S., Solomon, S., Mayer, K., Mehendale, S., 2005. Preparing for efficacy trials of vaginal microbicides in Indian women. Indian J. Med. Res. 121, 502–509.

Justin-Temu, M., Damian, F., Kinget, R., Van Den Mooter, G., 2004. Intravaginal gels as drug delivery systems. J. Womens Health (Larchmt.) 13, 834–844.

Keller, M.J., Klotman, M.E., Herold, B.C., 2003. Rigorous pre-clinical evaluation of topical microbicides to prevent transmission of human immunodeficiency virus. J. Antimicrob. Chemother. 51, 1099–1102.

Khanvilkar, K., Donovan, M.D., Flanagan, D.R., 2001. Drug transfer through mucus. Adv. Drug. Deliv. Rev. 48, 173–193.

Kieweg, S.L., Geonnotti, A.R., Katz, D.F., 2004. Gravity-induced coating flows of vaginal gel formulations: in vitro experimental analysis. J. Pharm. Sci. 93, 2941–2952.

Kristmundsdottir, T., Arnadottir, S.G., Bergsson, G., Thormar, H., 1999. Development and evaluation of microbicidal hydrogels containing monoglyceride as the active ingredient. J. Pharm. Sci. 88, 1011–1015.

Lachman, L., Lieberman, H.A., Kanig, J.L., 2001. Teoria e Prática na Indústria Farmacêutica, vol. 2. Fundação Calouste Gulbenkian, Lisboa, p. 907.

Langenberg, A., 2004. Interrupting herpes simplex virus type 2 transmission: the role of condoms and microbicides. Herpes 11, 147A–154A.

MacKenzie, I.Z., Embrey, M.P., 1977. Cervical ripening with intravaginal prostaglandin E2 gel. Br. Med. J. 2, 1381–1384.

Mackenzie, I.Z., Embrey, M.P., Davies, A.J., Guillebaud, J., 1978. Very early abortion by prostaglandins. Lancet 1, 1223–1226.

Madsen, F., Eberth, K., Smart, J.D., 1998. A rheological examination of the mucoadhesive/mucus interaction: the effect of mucoadhesive type and concentration. J. Control. Release 50, 167–178.

Mandal, T.K., 2000. Swelling-controlled release system for the vaginal delivery of miconazole. Eur. J. Pharm. Biopharm. 50, 337–343.

Markel, H., 2005. The search for effective HIV vaccines. N. Engl. J. Med. 353, 753–757.

Mauck, C., Weiner, D.H., Ballagh, S., Creinin, M., Archer, D.F., Schwartz, J., Pymar, H., Lai, J.J., Callahan, M., 2001. Single and multiple exposure tolerance study of cellulose sulfate gel: a Phase I safety and colposcopy study. Contraception 64, 383–391.

Mauck, C.K., Weiner, D.H., Ballagh, S.A., Creinin, M.D., Archer, D.F., Schwartz, J.L., Pymar, H.C., Lai, J.J., Rencher, W.F., Callahan, M.M., 2004. Single and multiple exposure tolerance study of polystyrene sulfonate gel: a phase I safety and colposcopy study. Contraception 70, 77–83.

McCarthy, T.D., Karellas, P., Henderson, S.A., Giannis, M., O'Keefe, D.F., Heery, G., Paull, J.R., Matthews, B.R., Holan, G., 2005. Dendrimers as drugs: discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention. Mol. Pharm. 2, 312–318.

Milani, M., Barcellona, E., Agnello, A., 2003. Efficacy of the combination of 2 g oral tinidazole and acidic buffering vaginal gel in comparison with vaginal clindamycin alone in bacterial vaginosis: a randomized, investigator-blinded, controlled trial. Eur. J. Obstet. Gynecol. Reprod. Biol. 109, 67–71.

Morimoto, K., Takeeda, T., Nakamoto, Y., Morisaka, K., 1982. Effective vaginal absorption of insulin in diabetic rats and rabbits using polyacrylic acid aqueous gel bases. Int. J. Pharm. 12, 107–111.

Morrow, K., Rosen, R., Richter, L., Emans, A., Forbes, A., Day, J., Morar, N., Maslankowski, L., Profy, A.T., Kelly, C., Abdool Karim, S.S., Mayer, K.H., 2003. The acceptability of an investigational vaginal microbicide, PRO 2000 Gel, among women in a phase I clinical trial. J. Womens Health (Larchmt.) 12, 655–666.

Mukenge-Tshibaka, L., Alary, M., Geraldo, N., Lowndes, C.M., 2005. Incorrect condom use and frequent breakage among female sex workers and their clients. Int. J. STD AIDS 16, 345–347.

Neyts, J., Kristmundsdottir, T., De Clercq, E., Thormar, H., 2000. Hydrogels containing monocaprin prevent intravaginal and intracutaneous infections with HSV-2 in mice: impact on the search for vaginal microbicides. J. Med. Virol. 61, 107–110.

Oppenheimer, L.W., Labrecque, M., Wells, G., Bland, E.S., Fraser, W.D., Eason, E., Brisson-Carroll, G., Yetisir, E., 2005. Prostaglandin E vaginal gel to treat dystocia in spontaneous labour: a multicentre randomised placebo-controlled trial. BJOG 112, 612–618.

Owen, D.H., Katz, D.F., 1999. A vaginal fluid simulant. Contraception 59, 91–95.

Owen, D.H., Dunmire, E.N., Plenys, A.M., Katz, D.F., 1999. Factors influencing nonoxynol-9 permeation and bioactivity in cervical mucus. J. Control. Release 60, 23–34.

Owen, D.H., Peters, J.J., Katz, D.F., 2000. Rheological properties of contraceptive gels. Contraception 62, 321–326.

Owen, D.H., Peters, J.J., Katz, D.F., 2001. Comparison of the rheological properties of Advantage-S and Replens. Contraception 64, 393–396.

Owen, D.H., Peters, J.J., Lavine, M.L., Katz, D.F., 2003. Effect of temperature and pH on contraceptive gel viscosity. Contraception 67, 57–64.

Owen, D.H., Katz, D.F., 2005. A review of the physical and chemical properties of human semen and the formulation of a semen simulant. J. Androl. 26, 459–469.

Paternoster, D.M., Tudor, L., Milani, M., Maggino, T., Ambrosini, A., 2004. Efficacy of an acidic vaginal gel on vaginal pH and interleukin-6 levels in low-risk pregnant women: a double-blind, randomized placebo-controlled trial. J. Matern. Fetal Neonatal Med. 15, 198–201.

Patton, D.L., Kidder, G.G., Sweeney, Y.C., Rabe, L.K., Hillier, S.L., 1999. Effects of multiple applications of benzalkonium chloride and nonoxynol 9 on the vaginal epithelium in the pigtailed macaque (*Macaca nemestrina*). Am. J. Obstet. Gynecol. 180, 1080–1087.

Pavelic, Z., Skalko-Basnet, N., Jalsenjak, I., 1999. Liposomes containing drugs for treatment of vaginal infections. Eur. J. Pharm. Sci. 8, 345–351.

Pavelic, Z., Skalko-Basnet, N., Schubert, R., 2001. Liposomal gels for vaginal delivery. Int. J. Pharm. 219, 139–149.

Pavelic, Z., Skalko-Basnet, N., Filipovic-Grcic, J., Martinac, A., Jalsenjak, I., 2005a. Development and in vitro evaluation of a liposomal vaginal delivery system for acyclovir. J. Control. Release 106, 34–43.

Pavelic, Z., Skalko-Basnet, N., Jalsenjak, I., 2005b. Characterisation and in vitro evaluation of bioadhesive liposome gels for local therapy of vaginitis. Int. J. Pharm. 301, 140–148.

Perotti, M.E., Pirovano, A., Phillips, D.M., 2003. Carrageenan formulation prevents macrophage trafficking from vagina: implications for microbicide development. Biol. Reprod. 69, 933–939.

Pifferi, G., Restani, P., 2003. The safety of pharmaceutical excipients. Farmaco 58, 541–550.

Pilcher, H., 2004. Starting to gel. Nature 430, 138–140.

Pretorius, E.S., Timbers, K., Malamud, D., Barnhart, K., 2002. Magnetic resonance imaging to determine the distribution of a vaginal gel: before, during, and after both simulated and real intercourse. Contraception 66, 443–451.

Prista, L.N., Alves, A.C., Morgado, R., 1996. Tecnologia Farmacêutica, vol. III, 4th ed. Fundação Calouste Gulbenkian, Lisboa, pp. 1585–1594.

Ramsey, P.S., Ogburn, P.L., Harris, D.Y., Heise, R.H., Ramin, K.D., 2002. Effect of vaginal pH on efficacy of the dinoprostone gel for cervical ripening/labor induction. Am. J. Obstet. Gynecol. 187, 843–846.

Robinson, J.R., Bologna, W.J., 1994. Vaginal and reproductive system treatments using a bioadhesive polymer. J. Control. Release 28, 87–94.

Roddy, R.E., Zekeng, L., Ryan, K.A., Tamoufe, U., Tweedy, K.G., 2002. Effect of nonoxynol-9 gel on urogenital gonorrhea and chlamydial infection: a randomized controlled trial. JAMA 287, 1117–1122.

Roy, S., Gourde, P., Piret, J., Desormeaux, A., Lamontagne, J., Haineault, C., Omar, R.F., Bergeron, M.G., 2001. Thermoreversible gel formulations containing sodium lauryl sulfate or *n*-Lauroylsarcosine as potential topical microbicides against sexually transmitted diseases. Antimicrob. Agents Chemother. 45, 1671–1681.

Ruel-Gariepy, E., Leroux, J.C., 2004. In situ-forming hydrogels – review of temperature-sensitive systems. Eur. J. Pharm. Biopharm. 58, 409–426.

Sandri, G., Rossi, S., Ferrari, F., Bonferoni, M.C., Muzzarelli, C., Caramella, C., 2004. Assessment of chitosan derivatives as buccal and vaginal penetration enhancers. Eur. J. Pharm. Sci. 21, 351–359.

Santos, D.F.G., Bahia, M.F.G., Silva, J.A.L., 2002. Influencia de las condiciones de almacenaje en la plasticidad y la liberación de fentiazac a partir de una formulación tópica. Anal. Real Acad. Nac. Farm. 68, 251–265.

Sassi, A.B., McCullough, K.D., Cost, M.R., Hillier, S.L., Rohan, L.C., 2004. Permeability of tritiated water through human cervical and vaginal tissue. J. Pharm. Sci. 93, 2009–2016.

Seeras, R.C., 1995. Induction of labor utilizing vaginal vs. intracervical prostaglandin E2. Int. J. Gynaecol. Obstet. 48, 163–167.

Shetty, A., Livingston, I., Acharya, S., Templeton, A., 2004. Vaginal prostaglandin E2 gel versus tablet in the induction of labour at term – a retrospective analysis. J. Obstet. Gynaecol. 24, 243–246.

Siewert, M., Dressman, J., Brown, C.K., Shah, V.P., 2003. FIP/AAPS guidelines to dissolution/in vitro release testing of novel/special dosage forms. AAPS PharmSciTech. 4, E7.

Smart, J.D., 2004. Bioadhesion. In: Bowlin, G.L. (Ed.), Encyclopedia of Biomaterials and Biomedical Engineering. Marcel Dekker, New York, pp. 62–71.

Smart, J.D., 2005. The basics and underlying mechanisms of mucoadhesion. Adv. Drug Deliv. Rev. 57, 1556–1568.

Song, Y., Wang, Y., Thakur, R., Meidan, V.M., Michniak, B., 2004. Mucosal drug delivery: membranes, methodologies and applications. Crit. Rev. Ther. Drug Carrier. Syst. 21, 195–256.

Stenchever, M.A., Droegemueller, W., Herbst, A.L., Mishell Jr., D.R., 2002. Comprehensive Gynecology, 4th ed. Mosby, St. Louis, MO, pp. 43–44.

Sweet, R.L., 1993. New approaches for the treatment of bacterial vaginosis. Am. J. Obstet. Gynecol. 169, 479–482.

Syed, T.A., Qureshi, Z.A., Ahmad, S.A., Ali, S.M., 2000. Management of intravaginal warts in women with 5-fluorouracil (1%) in vaginal hydrophilic gel: a placebo-controlled double-blind study. Int. J. STD AIDS 11, 371–374.

Tamburic, S., Craig, D.Q., 1995. An investigation into the rheological, dielectric and mucoadhesive properties of poly(acrylic acid) gel systems. J. Control. Release 37, 59–68.

Tamburic, S., Craig, D.Q., 1996. The effects of ageing on the rheological, dielectric and mucoadhesive properties of poly(acrylic acid) gel systems. Pharm. Res. 13, 279–283.

Taylor, S.J., Peat, J.K., Armour, C.L., 1999. An evaluation of prostaglandin E2 vaginal gel use in practice. J. Clin. Pharm. Ther. 24, 303–310.

Taylor, S.J., Armour, C.L., 2000. Measurement of consumer preference for treatments used to induce labour: a willingness-to-pay approach. Health Expect. 3, 203–216.

Taylor, S., Armour, C., 2003. Consumer preference for dinoprostone vaginal gel using stated preference discrete choice modelling. Pharmacoeconomics 21, 721–735.

Thomas, B.J., Finnin, B.C., 2004. The transdermal revolution. Drug Discov. Today 9, 697–703.

Thormar, H., Bergsson, G., Gunnarsson, E., Georgsson, G., Witvrouw, M., Steingrimsson, O., De Clercq, E., Kristmundsdottir, T., 1999. Hydrogels containing monocaprin have potent microbicidal activities against sexually transmitted viruses and bacteria in vitro. Sex. Transm. Infect. 75, 181–185.

Tien, D., Schnaare, R.L., Kang, F., Cohl, G., McCormick, T.J., Moench, T.R., Doncel, G., Watson, K., Buckheit, R.W., Lewis, M.G., Schwartz, J., Douville, K., Romano, J.W., 2005. In vitro and in vivo characterization of a potential universal placebo designed for use in vaginal microbicide clinical trials. AIDS Res. Hum. Retroviruses 21, 845–853.

Ting, W.W., Vest, C.D., Sontheimer, R.D., 2004. Review of traditional and novel modalities that enhance the permeability of local therapeutics across the stratum corneum. Int. J. Dermatol. 43, 538–547.

Tirnaksiz, F., Robinson, J.R., 2005. Rheological, mucoadhesive and release properties of pluronic F-127 gel and pluronic F-127/polycarbophil mixed gel systems. Pharmazie 60, 518–523.

Uludag, S., Salihoglu Saricali, F., Madazli, R., Cepni, I., 2005. A comparison of oral and vaginal misoprostol for induction of labor. Eur. J. Obstet. Gynecol. Reprod. Biol. 122, 57–60.

Valenta, C., 2005. The use of mucoadhesive polymers in vaginal delivery. Adv. Drug. Deliv. Rev. 57, 1692–1712.

van Eyk, A.D., van der Bijl, P., 2005. Porcine vaginal mucosa as an in vitro permeability model for human vaginal mucosa. Int. J. Pharm. 305, 105–111.

Van Damme, L., Wright, A., Depraetere, K., Rosenstein, I., Vandersmissen, V., Poulter, L., McKinlay, M., Van Dyck, E., Weber, J., Profy, A., Laga, M., Kitchen, V., 2000. A phase I study of a novel potential intravaginal microbicide, PRO 2000, in healthy sexually inactive women. Sex. Transm. Infect. 76, 126–130.

Van De Graaff, K., 2001. Human Anatomy, 6th ed. McGraw-Hill Education, Europe, pp. 736–737.

van der Bijl, P., van Eyk, A.D., 2003. Comparative in vitro permeability of human vaginal, small intestinal and colonic mucosa. Int. J. Pharm. 261, 147–152.

Vandebosch, A., Goetghebeur, E., Ramjee, G., Alary, M., Ettiegne-Traore, V., Chandeying, V., Van Damme, L., 2004. Acceptability of COL-1492, a vaginal gel, among sex workers in one Asian and three African cities. Sex. Transm. Infect. 80, 241–243.

Veazey, R.S., Shattock, R.J., Pope, M., Kirijan, J.C., Jones, J., Hu, Q., Ketas, T., Marx, P.A., Klasse, P.J., Burton, D.R., Moore, J.P., 2003. Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. Nat. Med. 9, 343–346.

Vermani, K., Garg, S., 2000. The scope and potential of vaginal drug delivery. Pharm. Sci. Technol. Today 3, 359–364.

Vermani, K., Garg, S., 2002a. Herbal medicines for sexually transmitted diseases and AIDS. J. Ethnopharmacol. 80, 49–66.

Vermani, K., Garg, S., Zaneveld, L.J., 2002b. Assemblies for in vitro measurement of bioadhesive strength and retention characteristics in simulated vaginal environment. Drug Dev. Ind. Pharm. 28, 1133–1146.

Wang, Y., Lee, C.H., 2002. Characterization of a female controlled drug delivery system for microbicides. Contraception 66, 281–287.

Warrier, B.K., Kostoryz, E., Lee, C.H., 2004. Biocompatibility of components of a female controlled drug delivery system. J. Biomed. Mater. Res. A 71, 209–216.

Weber, J., Desai, K., Darbyshire, J., 2005. The development of vaginal microbicides for the prevention of HIV transmission. PLoS Med. 2, e142.

Whaley, K.J., Zeitlin, L., 2005. Preventing transmission: plant-derived microbicides and mucosal vaccines for reproductive health. Vaccine 23, 1819–1822.

Wilkinson, D., 2002. Nonoxynol-9 fails to prevents STDs, but microbicide research continues. Lancet 360, 962–963.

Woodley, J., 2001. Bioadhesion: new possibilities for drug administration? Clin. Pharmacokinet. 40, 77–84.

Zaneveld, L.J., Waller, D.P., Anderson, R.A., Chany 2nd, C., Rencher, W.F., Feathergill, K., Diao, X.H., Doncel, G.F., Herold, B., Cooper, M., 2002. Efficacy and safety of a new vaginal contraceptive antimicrobial formulation containing high molecular weight poly(sodium 4-styrenesulfonate). Biol. Reprod. 66, 886–894.