POLYMERIC GELS FOR INTRAVAGINAL DRUG DELIVERY

Michael T. Cook[1*] and Marc B. Brown[1,2]

[1]Department of Pharmacy, Pharmacology, and Postgraduate Medicine, University of Hertfordshire, Hatfield, AL10 9AB, United Kingdom

[2]MedPharm Ltd., 50 Occam Road, Surrey Business Park, Guildford, GU2 7AB, UK

*M.Cook5@Herts.ac.uk

Keywords: mucoadhesion, thermogelling materials, HIV PrEP, in vitro models, clinical trials, regulatory approval.

ABSTRACT

Intravaginal drug delivery can elicit a local effect, or deliver drugs systemically without hepatic first pass metabolism. There are a number of emerging areas in intravaginal drug delivery, but the vagina is a challenging route of administration, due to the clearance mechanisms present which result in poor retention of dosage forms, and the potential for irritation and other adverse reactions. Gel formulations are desirable due to the ease of application, spreading and that they cause little to no discomfort to the patient. However, these dosage forms, in particular, are poorly retained and traditional gels typically have little control over drug release rates. This has led to a large number of studies on improving the retention of vaginal gels and modulating the controlled release of drugs from the gel matrix.

This review outlines the anatomy and physiology of the vagina, focussing on areas relevant to drug delivery. Medical applications of vaginally administered medicines is then discussed, followed by an overview of polymeric gels in intravaginal drug delivery. The sensorial properties of intravaginal gels, and how these relate to user compliance are also summarised. Finally, some important barriers to marketing approval are described.

1. INTRODUCTION

Intravaginal administration involves the application of a substance into the body of the vagina, often via an applicator. The main advantage of this route of administration is that diseases and conditions of the vagina may be treated locally, providing a high concentration of drug at the site of disease and potentially limiting any adverse effects away from this region [1]. There are numerous diseases and conditions for which it is necessary to administer a drug intravaginally, as well as prophylactic and fertility-promoting applications, and several drug delivery devices are currently in use, including vaginal gels, pessaries, and intravaginal rings. This review will firstly discuss the anatomy of the vagina, followed by a description of applications of intravaginal medicines, a review of polymeric gels intended for intravaginal drug delivery, and a discussion of barriers to approval. There are several reviews

concerning other dosage forms, such as vaginal rings and nanoparticles [1–7], or those that focus on specific applications or disease states [2,8,9]. This review is intended as a broader overview of gels in intravaginal drug delivery and discussion of the translation of such formulations into approved medicines.

2. THE VAGINA – FEATURES RELEVANT TO DRUG DELIVERY.

2.1. ANATOMY AND PERMEABILITY OF THE VAGINA

The female reproductive tract (Figure 1) consists of the 'upper female reproductive tract', made up of ovaries, fallopian tubes, and the uterus, and the 'lower female reproductive tract', which includes the vagina, ectocervix, and external genitalia. The cervix is a narrowing portion of the uterus ending at the vagina in a conical bulge known as the ectocervix. The mucosal lining of the uterus is called the 'endometrium,' and the mucosa of the cervix is more specifically the 'endocervix' [10]. The endometrium sits within the myometrium, which consists mainly of smooth muscle cells. The upper female reproductive tract is lined with a layer of columnar epithelium possessing tight junctions [10]. The cervix is further coated with mucus, secreted by epithelial cells on the endocervical glands [11]. The thickness of the mucus layer and mucus consistency is dependent on the phase of the menstrual cycle [11].

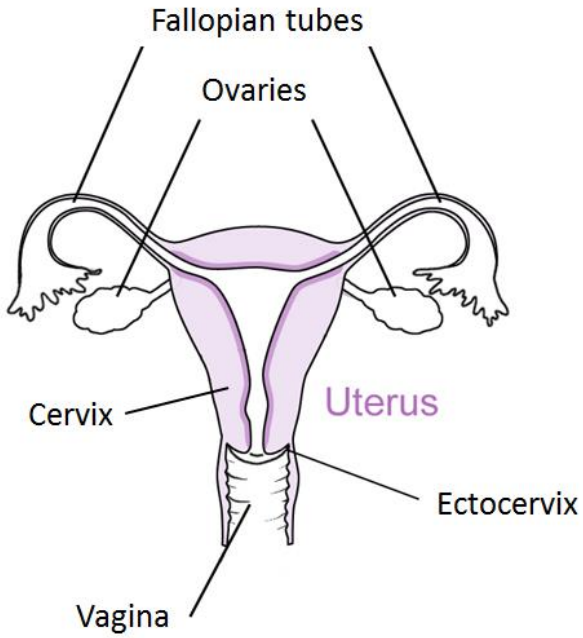

Figure 1: A schematic diagram of the female reproductive tract.

The 'lower' female reproductive tract consists of the ectocervix and vagina. The vagina is a muscular tube that extends from the ectocervix of the uterus to the vaginal opening, and sits between the urethra and rectum. The lining, or 'mucosa', of the vagina consists of a non-keratinising stratified squamous epithelium, attached to a basement membrane. The vaginal epithelium consists of approximately 28 cell layers on days 1-12 of the menstrual cycle, with a slight thinning to 26 on days 19-24 of the cycle [12]. The thickness of this epithelium has been measured as 261 ± 16 µm [13]. This stratified squamous epithelium is a main permeability barrier of the vaginal [14]. This barrier function is supported by the production and presence of amorphous intercellular lipids and membrane coating granules extruding ceramides, glucosyl-ceramides and cholesterol [15]. The vagina has been found to be 62.7 $\pm$ 11.25 mm in length, and 27.2 $\pm$ 4.79 mm in width at the mid-lower vagina from an MRI study

of 28 women [16]. The surface area of the vagina has also been measured from casts, and ranged from 65.73 to 107.07 cm^2 with a mean of 87.46 $\pm$ 7.80 cm^2 [17]. The diffusion of tritiated water through human cervical and vaginal tissue gave measured apparent permeability coefficients of 8×10^{-5} cm/s and 7×10^{-5} cm/s, respectively [18]. The permeation of tritiated water through human vaginal epithelium is not statistically different than the permeation through human *ex vivo* buccal mucosa [19]. The vagina is approximately 16-times more permeable to water than skin, using published values for skin permeability [20].

2.2. THE COMPOSITION AND VOLUME OF VAGINAL FLUID

The vagina is lubricated by vaginal fluid, which is a mixture of fluids from the upper reproductive tract, such as cervical mucus and endometrial fluids and transudate from the vaginal walls [21]. Bartholin's glands, positioned either side of the posterior section of the vaginal opening also serve to aid lubrication of the vagina, secreting relatively small amounts of fluid during sexual arousal [22]. Positioned either side of the anterior of the vaginal opening are Skene's glands, which perform a similar function. The constituents of vaginal fluid are water (90-95 %), mucins, carbohydrates, urea, salts, immunoglobulins, fatty acids, albumin, and other minor components [23]. Human cervicovaginal fluid also contains proteolytic enzymes, particularly serine and cysteine proteases [24].

Several studies have looked at the volume of vaginal fluid, providing useful estimates for pharmaceutical testing. Stone and Gambles' study of 113 healthy women, found a mean quantity of 0.76 g of fluid present [25]. The total vaginal fluid volume has been measured in 38 HIV-1 infected woman by Mitchell et al [26], and had a median value of 0.51 mL. The rate of fluid production was measured by Godley [27] as 1.55 g/8 h. This reached a peak of 1.96 g/8 h mid-cycle and was reduced to 1.38 and 137 g/8 h either side of ovulation. This cyclical variation was not seen in women taking the oral contraceptive pill [27]. These studies are consistent with the vagina having a low fluid volume, which is replenished during the course of the day. It is noteworthy that vaginal fluid volume increases substantially during sexual stimulation, which may be a consideration for some applications [21]. The composition and quantity of vaginal fluid is of great importance for intravaginal gel products, as these factors may affect the degree of swelling, retention and release rates. Although the United States Pharmacopeia (USP) has no standard recipe for simulated vaginal fluid, it references the simulated vaginal fluid of Marques et al [28] as a medium for drug release testing. Generally, simulated vaginal fluids typically have an acidic pH, of approximately pH 4, and may or may not include mucin.

2.3. THE VAGINAL MICROBIOME AND pH OF THE VAGINA

A healthy vagina has a diverse microbiome which maintains an acidic pH via the metabolism of glycogen to D/L lactic acid by bacteria, mainly of the genus Lactobacillus [29,30]. The bacteria of the vagina also have a protective role against pathogens, by reducing pH, producing bactericidal compounds and by competitive exclusion [30]. Women tend to have one of five microbial communities, dominated either by *L. iners, L. crispatus, L. gasseri, L. jensenii,* or a fifth community having lower proportions of lactic acid bacteria and higher proportions of strict anaerobes [30].

The pH of the vagina is typically between 3.5 and 5.5 [29]. During pregnancy, the pH of the vagina changes to 3.8-4.4; after menopause, the pH increases to 7.0–7.4, due to variation in glycogen content at these times [28]. The pH of the vagina is increased to approximately pH 7 during coitus by the infusion of seminal fluid [31]. Ethnicity also appears to affect vaginal pH. The average measured vaginal pH values of Hispanic (pH 5.0 ± 0.59) and black (pH 4.7 ± 1.04) women were higher than found for Asian (pH 4.4 ± 0.59) and white (pH 4.2 ± 0.3) women [30]. This ethnic difference is important to consider from a drug delivery standpoint, as formulations that require specific pH triggers may be less efficacious in certain ethnic groups of women and the absorption of ionisable drugs may be affected.

2.4. CERVICAL MUCUS – PRODUCTION, PROPERTIES, AND COMPOSITION.

As discussed previously, the vaginal fluid contains mucus from the cervix, the properties of which vary dependant on the menstrual cycle. The mucus produced in the cervix can be designated as type E or G, and is always a mixture of the two, the ratio of which depends on the time of the menstrual cycle. Type E mucus is controlled by estrogen dominance before and during ovulation, and is thin and watery. Type G mucus is controlled by progesterone dominance post-ovulation and is thick and sticky to stop the passage of sperm into the uterus. Type G mucus is dominant for women taking the combined oral contraceptive pill [11]. The primary components of cervical mucus are mucins. Mucins are glycoproteins coded for by the MUC gene family, from which they get their names and may be either transmembrane or gel-forming (secretory). Mucins have large molecular weights (up to tens of MDa) and are up to 80 % oligosaccharide by volume [32]. Carlstedt et al [33] analysed cervical mucus released from pregnant women prior to giving birth and found it to contain large amounts of proline, threonine and serine residues, consistent with mucins found elsewhere in the body. Additionally, cysteine residues were identified, which have been attributed as promoting gel formation via disulphide bridging [34]. Overall, the protein content of the mucin glycoproteins was 19.9 $\pm$ 0.8 % (w/w) [33]. The O-glycans adorning mucin contains both neutral (N-acetylgalactosamine, N-acetylglucosamine, galactose, and fucose) and acidic (sialic acid) sugar moieties [33]. Andersch-Björkman et al [35] found that cervical mucins isolated from 6 individuals contained three gel-forming (MUC5B, MUC5AC, and MUC6) and two transmembrane mucins (MUC16 and MUC1). It has also been found that mucus production in the cervix increases to 600 mg/day mid-cycle from 20-60 mg/day at other points of the menstrual cycle [36].

2.5 IMPROVING RETENTION IN THE VAGINA THROUGH MUCOADHESION

The retention of semi-solid dosage forms in the vagina is typically poor due to the presence of moisture, lubricative mucus and shear forces. There is often a need to attempt to enhance retention in the vagina using excipients which enhance adhesion to the vaginal mucosa. These so-called “mucoadhesive” excipients enhance retention by a number of different mechanisms, which have been extensively reviewed elsewhere and are briefly summarised here with emphasis placed on those relevant to semi-solid dosage forms [34,37–40].

The mucoadhesion process can be thought of as the result of a plethora of underlying mechanisms. Highly viscous materials are better able to resist the shear found in the vagina and the natural tendency to flow out of the vagina. The rheology of the dosage form also affects the spreading of the gel throughout the vagina; with thixotropic or pseudoplastic gels easily spread. Spreading is aided by the ability of the gels to wet across the mucosa. After this initial ‘contact’ stage, the gel can ‘consolidate’ its adhesion to the mucosa with further physical and chemical interactions. Hydrogen-bonding between excipients and mucosa is believed to play a great role, due to the oligosaccharide side-chains of the mucin, though other interactions such as electrostatic and ion-dipole interactions, may also play a part [34]. Hydrophobic interactions may also occur due to the loss of entropy of water molecules as they solvate hydrophobic regions of a macromolecule in solution. There are also mucoadhesive materials designed to form covalent bonds with mucins, such as “thiomers” which bond to thiol groups present in the mucin glycoproteins [41–43]. Consolidation of mucoadhesive interactions may also occur through polymer chain interpenetration into the secretory mucus gel found on the surface of mucosal membranes [44].

3. INTRAVAGINAL DOSAGE FORMS AND THEIR APPLICATIONS

3.1 INTRAVAGINAL DOSAGE FORMS

There are several common dosage forms used in intravaginal drug delivery. Gels and creams are common semisolid dosage forms for the delivery of drug over short periods, with repeated application. Suppositories/pessaries are also available, often marketed as "ovules", and are typically composed of glycerol-gelatin bases; as PEGs and fatty bases are likely to cause irritation. For drug delivery over longer periods, intrauterine devices or vaginal rings may be used. These may use "reservoir" delivery techniques for prolonged delivery over months/years. Vaginal rings may be composed of silicon or polymer elastomers, whereas intrauterine devices are often made of polymers with increased rigidity, such as ethylene-vinyl acetate copolymers. There are a number of less common dosage forms, such as films, sponges, and vaginal tablets.

3.2. VAGINAL INFECTIONS – BACTERIAL VAGINOSIS, CANDIDIASIS, AND TRICHOMONIASIS

Infections of the vagina typically result in inflammation, causing itching, discharge and pain while urinating or during coitus [45]. This 'vaginitis' also encompasses irritation arising from epithelial abnormalities and other conditions [46]. Vaginal infections/disorders are most commonly either bacterial vaginosis, candidiasis, and trichomoniasis. Medicines for vaginosis treatment are outlined in table 1. Bacterial vaginosis is the result of a shift in the composition of the vaginal microbiota from protective lactobacilli to pathogenic anaerobic bacteria. Antibiotics are administered for treating bacterial vaginosis, and can be taken either orally or intravaginally. It has been reported that intravaginally administered antibiotics are as efficacious as those orally administered and have the advantage of lower systemic effects [47]. Candidiasis is a vaginal infection arising from yeasts of the genus *Candida.* Candidiasis results in itching, burning and white discharge from the vagina [48]. Candidiasis can be treated locally and orally by the administration of antifungals [48]. Trichomoniasis is a disorder caused by the protozoan *Trichomonas vaginalis*. It often appears to be asymptomatic, but is associated with increased risk of human immunodeficiency virus infection and may cause women issues during pregnancy [49]. Trichomoniasis is typically treated with oral antiprotozoals, such as metronidazole or tinidazole [49]. There are, however, some intravaginally administered treatments [50].

Table 1: Intravaginally-administered medicines and their applications

Application	Medicine	Composition
Bacterial Vaginosis	Zidoval® Dalacin® Flagystatin®	0.75 % metronidazole gel [51] 2 % clindamycin cream [51] Suppository containing 500 mg metronidazole plus 10000 units nystatin [52]
Candidiasis	Clotrimazole Canesten®	Pessary with 500 mg clotrimazole [51] 1 % clotrimazole cream [51]
	Gyno-Daktarin®	Suppository containing 1.2 g miconazole nitrate [51]
Trichomoniasis	Neo-Penotran®	Suppository containing 750 mg of metronidazole plus 200 mg of miconazole [50]
Hormonal Contraception	NuvaRing® [53] Progering® [53]	Oestrogen and progestogen-containing intravaginal ring made of ethylene vinyl acetate. Progestogen-containing intravaginal ring made of silicone elastomer.

Spermicides	Vaginal Contraceptive Film® [54] Today® sponge [54]	Nonoxynol-9 - containing, dissolves in vaginal fluid to create a gel. Film contains poly(vinyl alcohol) [55]. Concomitantly blocks cervix and releases nonoxynol-9. Sponge made of polyurethane [55].
Cervical Ripening	Cervidil®/ Propess® Prepidil® / Prostin E2 vaginal gel Glandin-E2 Misodel®/Mysodelle®	Vaginal insert containing cross-linked polyethylene glycol. Controls release of dinoprostone over 24 h Dinoprostone gel formulation, containing colloidal silicon dioxide [55]. Vaginal tablet containing dinoprostone Vaginal insert containing misoprostol
Supplementation of endogenous bacteria	Purfem FloraFemme	Suppository containing two strains of lactobacilli Suppository containing various strains of lactobacilli and bifidobacteria

3.3. CONTRACEPTION USING HORMONES AND SPERMICIDES

Drugs can be administered intravaginally for contraceptive purposes (table 1). These drugs will be either hormones, to reduce fertility, or spermicides. Hormonal contraceptives delivered by the vaginal route are either combination oestrogen/progestogen or progestogen-only [56]. Progestogen and oestrogen are steroid hormones which can prevent ovulation and increase the thickening of cervical mucus, reducing the likelihood of conception. Spermicides aim to immobilize sperm so that they cannot fertilise. The most commonly marketed spermicide is nonoxynol-9, a surfactant which damages the membrane of spermatozoa.

3.4. CERVICAL RIPENING THROUGH PROSTAGLANDIN USE

In recent years there has been a greater number of labour inductions, largely due to patient choice [57]. Labour induction can achieved by chemical methods (table 1), often by the intravaginal administration of prostaglandins. Two intravaginally administered prostaglandins are dinoprostone, a trade name for prostaglandin E2, and misoprostol, a synthetic prostaglandin E1 analogue [58]. These prostaglandins have been formulated into vaginal gels and inserts, as summarised in table 1. It has been demonstrated that Prepidil, a vaginal gel, gave a shorter induction-to-vaginal delivery interval with less expense than the Cervidil implant, and had no greater occurrence of adverse events [59].

3.5. PRE-EXPOSURE HUMAN IMMUNODEFICIENCY VIRUS (HIV) PROPHYLAXIS

HIV is a sexually transmitted virus, with HIV-1 and HIV-2 subtypes. Male-to-female transmission of HIV occurs via seminal fluid, which contains infected cells as well as free virus. The HIV infection can be transmitted through the squamous epithelia found lining the vagina and ectocervix, as well as the columnar epithelia of the endocervix, followed by the infection of CD4 cells [60]. Whilst the use of condoms is effective in the prevention of HIV, there are groups of women who are often unable to negotiate their use, in particular, sex workers [61]. In order to provide HIV protection for those who are unable to negotiate condom use, there have been efforts to develop intravaginally administered HIV protection.

There have been several clinical trials looking at the efficacy of vaginally administered antiretrovirals in the prevention of HIV transmission. The progress of these trials for gel products is discussed later in the review. PrEP has also been attempted using intravaginal rings, which allow for the controlled release for over a month [2]. This reduces the necessity of patients' adherence to dosing regimens, and thus aims to improve prophylaxis. The efficacy in reducing HIV transmission of a silicone vaginal ring containing dapivirine, a non-nucleoside HIV-1 reverse-transcriptase inhibitor, was the subject of two phase III clinical trials, "the ring study" and "ASPIRE". ASPIRE found that HIV-1 infection was lower by 27 % in women using dapivirine intravaginal rings, relative to control [62]. Furthermore, a 37 % reduction in HIV transmission was found when excluding data from sites with low adherence and

retention. A 56 % reduction was found among women older than 21 years of age, who had greater levels of adherence. “The ring study”, conducted in 1959 women (1762 in South Africa and 197 in Uganda) over two years, found that there was a reduction in HIV transmission of 30.7 % in women using the dapivirine ring [63]. This figure was increased to 37.5 % in women over 21. The aforementioned clinical trials demonstrate the potential for pre-exposure HIV prophylaxis via intravaginal administration of antiHIV agents and is certain to increase interest in this field.

3.6. The supplementation of endogenous bacteria for prophylaxis and the treatment of bacterial vaginosis

It has been suggested that ‘probiotic’ bacteria administered vaginally may be able to promote vaginal health, and even treat bacterial vaginosis. This is due to the belief that the dominance of probiotic microorganisms in the healthy vagina maintains an environment in which pathogens are unable to grow to infectious levels. There are numerous medicines containing bacteria of the genii *Lactobacillus* and *Bifidobacterium* for vaginal administration. Published clinical trials claim efficacy of probiotic treatment for bacterial vaginosis [64,65], however literature reviews [66,67] and a Cochrane review [68] have cast doubt on the findings of these clinical trials, indicating that larger, better designed, clinical trials are required before any strong conclusions may be drawn. Additionally, it has been shown that women respond positively to probiotic treatment regardless of clinical response, meaning that any non-blinded studies may be highly misleading [69].

3.7 Applicators for vaginal gels

Gels are typically administered using an applicator to ensure effective insertion into the body of the vagina and to reduce messiness. For most products an applicator with a design analogous to a wide-bore syringe is used; an example is shown in reference [70]. The opening of the syringe may screw onto a tube containing the gel preparation to reduce messiness during filling of the applicator. Some semi-solid products are prepared in single-use pre-filled applicators. For instance, Canesten® intravaginal cream is prepared as a pre-filled barrel, into which a plunger is placed by the patient to initiate application of cream after insertion into the vagina. The traditional applicators described thus far administer gel most effectively to the upper vagina and cervix, and less of the dose is spread to the lower parts of vagina. Distributing the gel more homogeneously may aid systemic permeation or assure local action across the vagina. Omar et al [71] designed an applicator with multiple openings which aids spreading of gel in the vagina. This applicator was investigated for use in a spermicide product, the “Invisible Condom®” [72].

4.0 Polymeric gels in intravaginal drug delivery

Aqueous solutions of macromolecules may form macroscopic gels due to physical entanglement or chemical bonding between polymer chains. Polymer gels used for intravaginal drug delivery are typically viscous, aiding retention in the vagina and display pseudoplastic or thixotropic behaviour to aid spreading of the dosage form during application. A number of pharmaceutical grade excipients have been studied for intravaginal application, including polysaccharides and synthetic materials. Some common examples are shown in Figure 2. Within this review, polymeric gel products have been separated intro traditional gels, synthetic gels, gels containing particulates, and thermogelling materials.

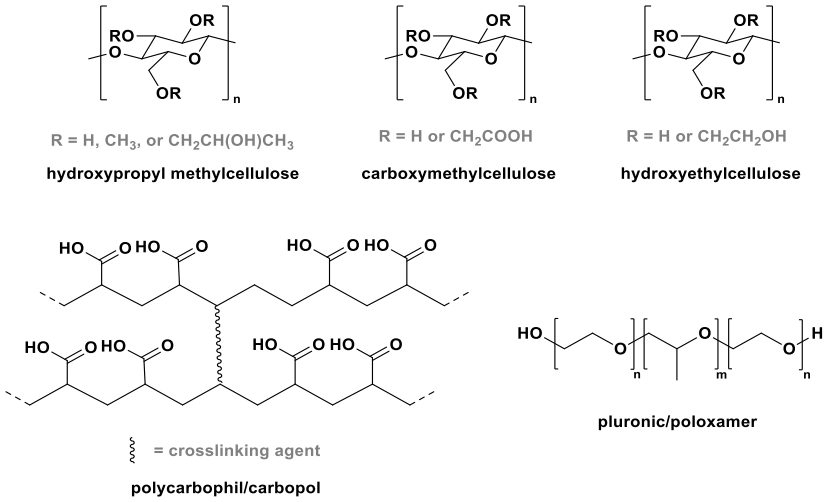

Figure 2. The chemical structure of some polymers commonly used in intravaginal drug delivery

4.1 TRADITIONAL GELS COMPRISED OF HYDROPHILIC POLYMER SOLUTIONS

This section discusses "traditional gel" products, i.e. those composed of natural or commercially-available polymers without stimuli-responsive behaviour. These medicines have the advantage of being simple to produce and many have a history of use in pharmaceuticals. Additionally, simple formulation approaches, such as multicomponent blending, allows for these traditional gel products to have altered properties without introducing any novel components with an associated regulatory risk.

Ahmad et al [73] studied the effect of various water-soluble polymer gels on the release of clotrimazole and metronidazole. The polymers hydroxypropyl methylcellulose (HPMC), sodium carboxymethyl cellulose (Na-CMC), xanthan gum, guar gum, sodium alginate, carbopol and polycarbophil were chosen as they are known bioadhesives. Carbopol and polycarbophil are both cross-linked poly(acrylic acid) derivatives, with the cross-linking agent and degree of cross-linking dependent on the grade. It was found that altering gel composition could change total release times from 1 to 5.5 h. Bioadhesion was determined on bovine vaginal mucosa using a texture analyser, which demonstrated that the force of bioadhesion was dependent on gel composition. Unfortunately, gel preparations were too complex to identify any obvious attributes leading to improved bioadhesion.

Polycarbophil and carbopol were used to deliver a poorly water-soluble salicylidene acylhydrazide compound by Pedersen et al [74]. A solubility enhancer, cremaphor, was used to allow the compound to be dissolved in the aqueous gel. The gel preparation retarded drug release by twenty-fold relative to a control of drug alone in a DMSO/buffer mixture. Furthermore, these formulations were efficacious in halting the transmission of *Chlamydia trachomatis* in a mouse model, which demonstrated a 68 % reduction in infection relative to control. Carbopol alone has been used by Gupta et al [75] to deliver sperm immobilising factor to produce a gel product which was able to immobilised spermatozoa within 20 s.

Kiser et al [76] have used a "design of experiments" method to optimise combination gels of Carbopol 974P and hydroxyethylcellulose (HEC) with respect to their rheology, in vitro drug release, and performance in permeability studies. Within the article they define two types of vaginal gel

rheologically; a “spreading” gel and a “bolus” gel. The spreading gel aims to provide ease of application by effective spreading into the vagina. However the bolus gel takes a different approach; by being more resistant to flow, the gel should exhibit greater retention in the vagina. Gel composition was related to the *in vitro* release rate of tenofovir and UC781 (a non-nucleoside reverse transcriptase inhibitor). Whilst the authors state that tenofovir release rate was largely independent of composition, the release of UC781 reached a maximum at low HEC levels and higher carbopol concentrations. Cumulative release plots revealed that tenofovir, which was soluble in the gels, was released rapidly *in vitro*, but the release of UC781, administered as a dispersion, occurred slowly over 8 h. The greater impact of gel composition on release of UC781 was related to the differences in viscosity of the gels affecting the dissolution of dispersed drug.

“Box-Behnken” statistical design was used by Chopra et al [77] to optimise gel formulations of Carbopol 934P, Carbopol 974P and Polycarbophil, in combination with honey as a bioactive and aerosil (fumed silica) as a thickener. Formulations were optimised for greatest gel strength and mucoadhesion. The release of honey from the gels occurred in a “burst” manner over 4 h, with release continuing over a 24 h period. The burst release phase was zero order, whilst the second phase was non-Fickian, as determined by the Korsmeyer–Peppas model. This non-Fickian diffusion was attributed to a combination of polymer hydration, drug dissolution and erosion.

Chitosan gels containing lactic acid for the maintenance of acidic vaginal pH have been studied [78]. It was also found that, despite its lower viscosity, a “medium” molecular weight chitosan had a greater mucoadhesion than “high”, as measured using a texture analyser on *ex vivo* vaginal mucosa. This was put down to the greater mobility of the lower molecular weight chitosan allowing for improved interpenetration between gel and mucosa. The release of lactic acid from these gels occurred over 7 h. Increasing chitosan molecular weight retarded release, whilst increased lactic acid loading accelerated release rates from the dosage form. In a separate study, the retention of chitosan in the vagina of live rats was studied using infrared imaging techniques [79]. It was found that 2 % chitosan gels of three molecular weights, 20, 200, and 800 kDa, were well retained over a 24 h time period. Texture analysis indicated that the work of adhesion of the 20, 200 and 800 kDa chitosan gels was 222.329 ± 5.199, 253.510 ± 38.041 and 426.538 ± 63.285 N mm s^{-1}, respectively, indicating an effect of molecular weight not seen *in vivo.* Chitosan, hydroxypropyl methylcellulose, and poloxamer 407 were used by Tugcu-Demiröz et al [80] as gel-formers to deliver oxybutynin, an antimuscarinic, via the vaginal route. Texture analysis demonstrated that HPMC was the most mucoadhesive. Using a diffusion chamber to measure permeation through vaginal tissue gave the rank order chitosan > HPMC> poloxamer 407. An *In vivo* study on rabbits demonstrated that administration of oxybutynin in chitosan or HPMC gels via the vaginal route gave a 79 % or 19 % improvement in bioavailability relative to the oral route, respectively.

4.2 “BESPOKE” SYNTHETIC POLYMERS FOR INTRAVAGINAL DRUG DELIVERY

Several novel synthetic materials have been reported for use in intravaginal drug delivery. Whilst novel materials have a greater regulatory risk, they often have improved functionality relative to traditional gel products. Within gel products, synthetic approaches usually aim to improve mucoadhesion to the vagina, either by increasing the strength of interaction between dosage form and mucosa, or allowing for gelation *in situ*.

Vivagel™ is a product for the treatment of bacterial vaginosis, and for STI prevention. Vivagel™ contains a generation 4 lysine dendrimer with naphthalene disulfonic acid surface groups contained in a Carbopol gel to enhance retention. Vivagel™ is able to bind to HIV surface proteins (gp120), inhibiting the binding of the virus to its cellular targets [81].

In situ gelling systems have been reported by Navath et al [82]. Dendrimers bearing thiol groups were able to form hydrogels within 10 seconds after mixing with 8-armed PEG thiol, due to disulphide bridge formation. This led to an injectable *in situ* gelator which was able to release amoxicillin over 10 days. The rate of release could be retarded by increasing the concentration of gel and no dose-dumping was found. An *In vivo* study using a pregnant guinea pig model over three days showed that gels were well tolerated by the animals and showed that there was no sign of changes in vaginal pH or skin-reddening. The gels showed some loss of retention after 2 days, moving away from the cervix into the vaginal cavity (figure 3).

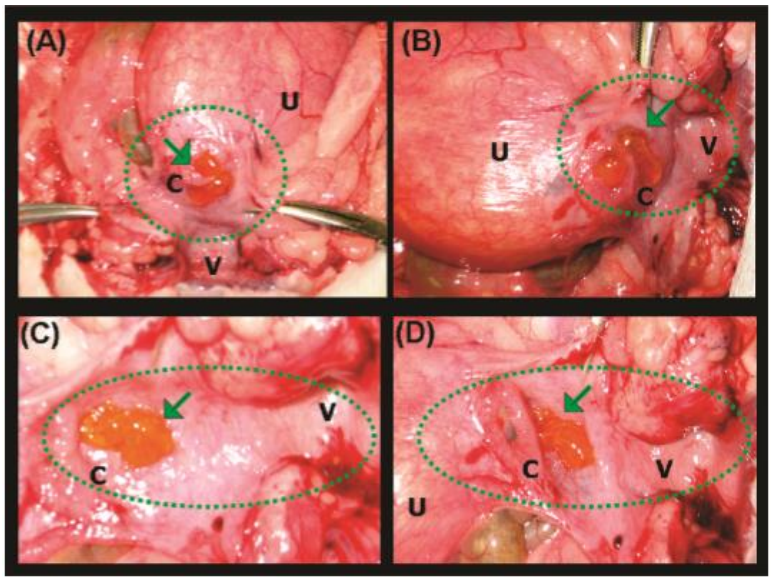

Figure 3. Dendrimer-based *in-situ* gelling hydrogel (orange) in the reproductive tract of guinea pigs after 5 h (A), 12 h (B), 2 days (C), and 3 days (D). C: cervix, V: vagina, U:Uterus [82]. Reprinted with permission [82]. Copyright 2011. American Chemical Society.

Mucoadhesive vaginal gels containing a mixture of chitosan, or its derivative 5-methyl-pyrrolidinone-chitosan and HEC were designed by Perioli et al [83] for the delivery of metronidazole. The gels produced with 5-methyl-pyrrolidinone-chitosan had improved mucoadhesion and released metronidazole over 90 minutes. The chitosan derivative also had lower viscosity than the constituent chitosan, which the authors suggest would allow for ease of extrusion from a syringe or applicator.

Friedl et al [84] report the use of a "preactived thiomer" for use in intravaginal drug delivery."Preactivated" thiomers are polymers bearing protected thiol groups, which are able to bind to cysteine residues of mucin. In the study, chitosan was coupled with thioglycolic acid using carbodiimide chemistry, followed by protection of thiol moieties with 2-mercaptonicotinic acid to give the preactivated thiomer. Mucoadhesion of polymer solutions was determined using a flow-through method, which demonstrated that the preactivated chitosan thiomer had improved retention on vaginal mucosa relative to commercial formulations. This improvement in mucoadhesion was also demonstrated using texture analysis and the rheological method of Hassan and Gallo [85]. Thiolated carbophil and chitosan have been explored by Cevher et al [86] for the intravaginal delivery of clomiphene citrate. Thiolation improved mucoadhesion, but it was also found to improve the cohesiveness and elasticity by texture profile analysis. The latter effect is potentially the result of internal disulphide bond formation enhancing cross-linking density. It was also found that carbophil and its thiolated derivative were able to sustain the release of clomiphene citrate over 72 h, whereas the chitosan gels delivered the drug over 12 h.

4.3 GELS CONTAINING PARTICULATES FOR MODULATED DRUG DELIVERY

The incorporation of particulates into vaginal gel formulations is often reported as a means of sustaining the release of bioactives. For water-soluble bioactives, gel preparations typically release their load over several hours, whereas microparticulates are capable of releasing drug over months. However, particulates are capable of causing mechanical irritation to the vagina, and it is generally recommended that particulates should be under 50 µm diameter to avoid discomfort [87].

Pavelic´ et al [88] incorporated liposomes into carbopol gels to form novel drug delivery systems which intended to combine the proposed controlled release properties of liposomes with the improved retention of gels. The "liposomal gels" slowed release of a hydrophilic fluorophore over 24 h relative to a control of carbopol, releasing approximately 16 % and 36-42 % of fluorophore, respectively. In a later publication, the authors used liposomes of egg phosphatidylcholine and egg phosphatidyl-glycerol–sodium in carbopol to deliver clotrimazole and metronidazole, releasing approximately 75 % and 60 % of their entrapped drug over 24 h. Acyclovir-containing liposomal gels were also studied, which again demonstrated controlled release of 65 % of encapsulated acyclovir over 24 h [89]. Chen et al [90] fabricated a dual pH- and temperature- responsive vaginal drug delivery system containing liposomes incorporated into a pluronic thermogelling material. Liposomes of methoxy polyethylene glycol 2000-hydrazonecholesteryl hemisuccinate were synthesised to create a pH-triggered delivery system. Arctigenin, a plant lignan, was encapsulated into these liposomes, which were subsequently dispersed in an aqueous pluronic mixture. These systems gave little to no release of arctigenin at pH 7.4 and pH 9.0, delivering ca. 5 % of their payload over 72 h. Once pH was reduced to 5.0, representative of vaginal fluid, arctigenin was released in a controlled manner over ca. 24 h. In addition to liposomes, solid particulates have been investigated as controlled release devices in vaginal gels. Chatterjee et al [91] have shown incorporating Zidovudine (an NRTI) into a bioadhesive carbopol/HPMC gel allowed for controlled release over 24 h. This was mirrored in plasma concentration measurements in rabbits. The authors also investigated the use of 50 um ethylcellulose microcapsules within these gels to control zidovudine release [92]. However, the particles used showed some irritancy *in vivo.* Solid Eudragit RS 100 and Eudragit S 100 nanoparticles measuring 200 nm were incorporated into a chitosan gel by Frank et al [93]. It was found that incorporating nanoparticles into a 2.5 % chitosan gel improved adhesion to porcine vaginal mucosa, as determined using a texture analyser. This improved mucoadhesion was not seen, however, using a flow-through method. It was also found that these nanoparticulates were able to aid the penetration of a fluorophore into vaginal tissue.

4.4 Thermogelling materials for intravaginal drug delivery

Thermogelling materials are polymeric solutions which undergo a sol-gel transition upon warming above a specific gelation temperature. Thermogelling materials with a gelation temperature between 25-37 °C are of particular interest in intravaginal drug delivery as they are able to flow through an applicator before forming a viscous gel in the vagina. This reduces the messiness of the application process, and enhances retention in the vaginal cavity due to the gel's greater resistance to shear and dilution. Thermogelling systems are usually block-copolymers which transition from hydrophilic to amphiphilic above a given temperature. Mechanistically, this may be caused by the self-assembly of micelles at raised temperature leading to "micelle-packing" (figure 4) or the dehydration of one block above its lower critical solution temperature, leading to physical cross-linking. The majority of thermogelling systems for intravaginal administration use poloxamer 407 (also known as pluronic F127) as the temperature-responsive component, as it is inexpensive and already in use in pharmaceuticals. The pluronics are poly(ethylene oxide) – poly(propylene oxide) – poly(ethylene oxide) triblock copolymers, with differing block lengths.

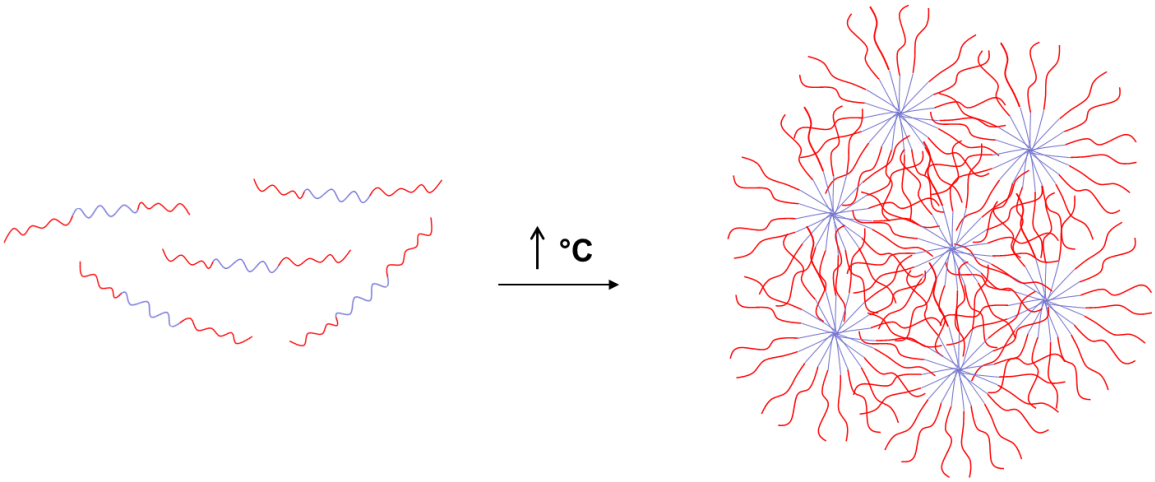

Figure 4. A proposed mechanism for poloxamer 407 gelation at raised temperatures. Poloxamer consists of PEG blocks (red) and polypropylene blocks (blue).

Poloxamer 407 and 188 have been used to form thermogelling systems to delivery econazole nitrate intravaginally [94]. Altering the ratio of pluronics allowed for modification of gelation temperature and rheological behaviour. It was found that particular blends of poloxamer 407 (15-20 %) and poloxamer 188 (10-20 %) gave steady econazole release over 8 h. The release profiles demonstrated non-Fickian diffusion, typical for poloxamers. Ibrahim et al [95] also used thermosensitive poloxamer 407 and 188 mixtures for metronidazole delivery. It was found that increasing the concentration of poloxamer 407 from 15 to 30 % led to a reduction in gelation temperature from >40 °C to 10 ± 1 °C. It was also found that mucoadhesive force increased with concentration and by the addition of poloxamer 188, which was attributed to specific interactions between F68 and the mucosa. This effect was also seen by Aka-Any-Grah et al [96]. A formulation of 20 % poloxamer 407 and 10 % poloxamer 188 was taken on for further evaluation, which had a gelation temperature of 28 °C. It was found that this formulation released 90 % of its metronidazole load over 12 hours in a more sustained manner than a commercial formulation, Tricho® gel. In a 4 week clinical trial, the pluronic gel showed a significant improvement in cure rate relative to the conventional vaginal gel group (80 versus 47 %, respectively, $p = 0.034$). It should be noted, however, that few details of the study are included within the publication, and that the sample size was small ($n = 20$). Aka-Any-Grah et al [96] also studied mixtures of poloxamer 407 and 188, and found that the incorporation of poloxamer 188 improved the gel's resistance to dilution in simulated vaginal fluid. The authors do demonstrate, however, that this improvement in elasticity is a result of the increase in overall pluronic content, and not a result of specific synergy polymers.

Chang et al [97] devised a thermogelling systems comprised of poloxamer 407, poloxamer 188, and polycarbophil for intravaginal administration of clotrimazole. Increasing polycarbophil or poloxamer 188 content improved mucoadhesion, but also increased the work needed to expel the formulations through a syringe. Controlled release was demonstrated over 8 h for formulations with poloxamer 407, poloxamer 188, and polycarbophil ratios of 15/15/0.2 and 15/20/0.2. Release rates were lower in the 15/20/0.2 formulation, which the authors suggest could be correlated with the increased viscoelastic modulus of that preparation hindering drug diffusion.

Thermogelling poloxamer 407 systems containing propolis and carbopol 934P have been studied by Pereira et al [98]. Propolis is a resin collected by bees, which has fungicidal activity. 15 % (w/w) poloxamer 407 and 0.25 % carbopol 349P (F15/0.25) or 20 % (w/w) poloxamer 407 and 0.15 % carbopol 349P (F20/0.15) released approximately 70 and 90 % of propolis over 24 h, in a controlled manner with no burst release, respectively.

Andrews et al [99] investigated complex gel formulations containing HEC, polyvinylpyrrolidone (PVP), Poloxamer 407 (PL), and either polycarbophil (PC) or poly(methylvinylether-co-maleic anhydride) as mucoadhesive agents. These so-called "rheologically-structured fluids" displayed several properties commensurate with an effective vaginal drug delivery vehicle. Some formulations underwent sol-gel transition at 15 °C, which would allow the preparation to flow through an applicator before gelation *in situ*. Furthermore, the gels were resistant to dilution in simulated vaginal fluid. The release of erioglaucine dye was controlled over the 24 h measurement window (figure 5).

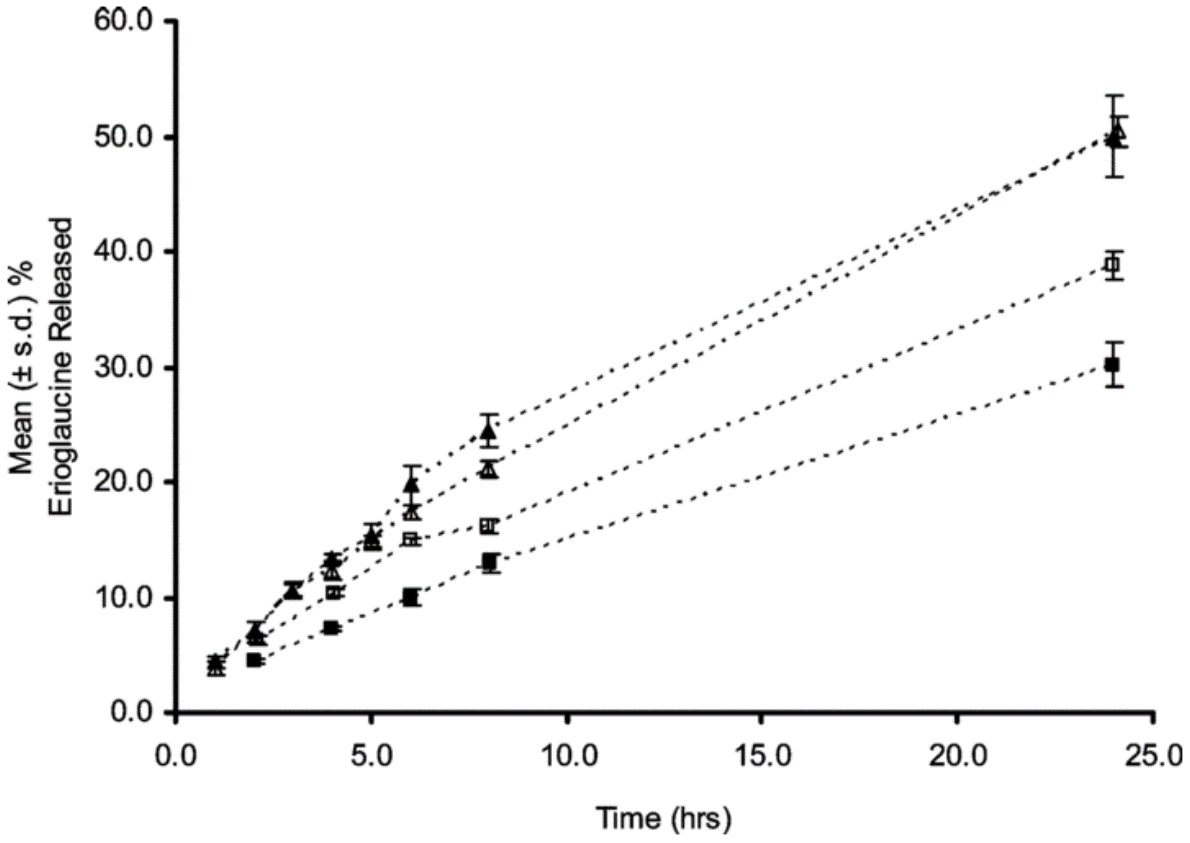

Figure 5. Erioglaucine release from gel blends containing poloxamer 407. (Filled square) HEC 5%/PC 3%/PL 10%/PVP 4% (w/w); (Filled triangle) HEC 5%/PC 3%/PVP 4%; (w/w); (open triangle) HEC 5%/Gantrez 3%/PL 10/PVP 4% (w/w); (open square) HEC 5%/GANT 3%/PVP 4% (w/w). Reused from Andrews et al [99] with permission from ACS. Direct link: http://pubs.acs.org/doi/abs/10.1021/bm9003332.

"Rheologically structured fluids" have also been investigated for the intravaginal delivery of recombinant HIV-1 clade-C trimeric gp140 envelope protein CN54gp140 for vaginal immunisation [100]. HEC at 3 and 5 % was mixed with polycarbophil, and poly(vinylpyrrolidone). Increasing HEC concentration from 3 to 5 % resulted in increased viscosity from 134 ± 9 Pa.s to 200 ± 5 Pa.s (at 1.14 Hz), but a reduction in mucoadhesion. Directly placing the gels into a phosphate buffered saline + Tween solution allowed for the *in vitro* release of the protein to be measured by ELISA. Over 24 h, 45.7 % of CN54gp140 was released from the 3% HEC "rheologically structured" gel, whilst 57.36 % was released from a HEC control gel. Another control, carbopol alone, released 98.17 % of its load over 20 minutes. This demonstrated that the complex "rheologically structured fluids" sustained the release of protein better than their constituent components, consistent with the greater overall polymer concentration. Once administered intravaginally, the optimised dosage forms were able to elicit immune response in rabbits.

Kim et al [101] used a thermogelling system comprised of pluronic derivatives designed to break down in the acidic environment of the vagina. Amphotericin B was incorporated into the gels within a hydroxypropyl--cyclodextrin inclusion complex. This led to gels which gave a pH-triggered release at the slightly acidic pH of the vagina (pH 5), delivering ~ 50 % of the incorporated amphotericin B over 4 h. The materials produced were found to be nontoxic to HEK293 cells and did not show inflammation or necrosis to murine vaginal tissue as determined by histology. The incorporation of clotrimazole-cyclodextrin inclusion complexes into gels containing poloxamer 407 has been studied by Bilensoy et al [102]. These gels allowed for controlled release of clotrimazole over 100 h, with no burst release.

The effect of k-carrageenan on the release rate of acyclovir from poloxamer 407 gels was studied by Liu et al [103]. It was reported that poloxamer 407 gels suffered from rapid erosion in vaginal fluid, leading to immediate drug release. It was found that the addition of carrageenan significantly decreased the release rates, slowed dissolution and hindered gel erosion in a concentration-dependent manner. Carbopol was then added to the formulations to improve mucoadhesion. In a rat study, it was found that the poloxamer, carrageenan, and carbopol complex gels retained acyclovir in the vagina up to a 12 h measurement point, whilst controls of poloxamer alone, poloxamer + carbopol, and poloxamer + carrageenan did not (Figure 6).

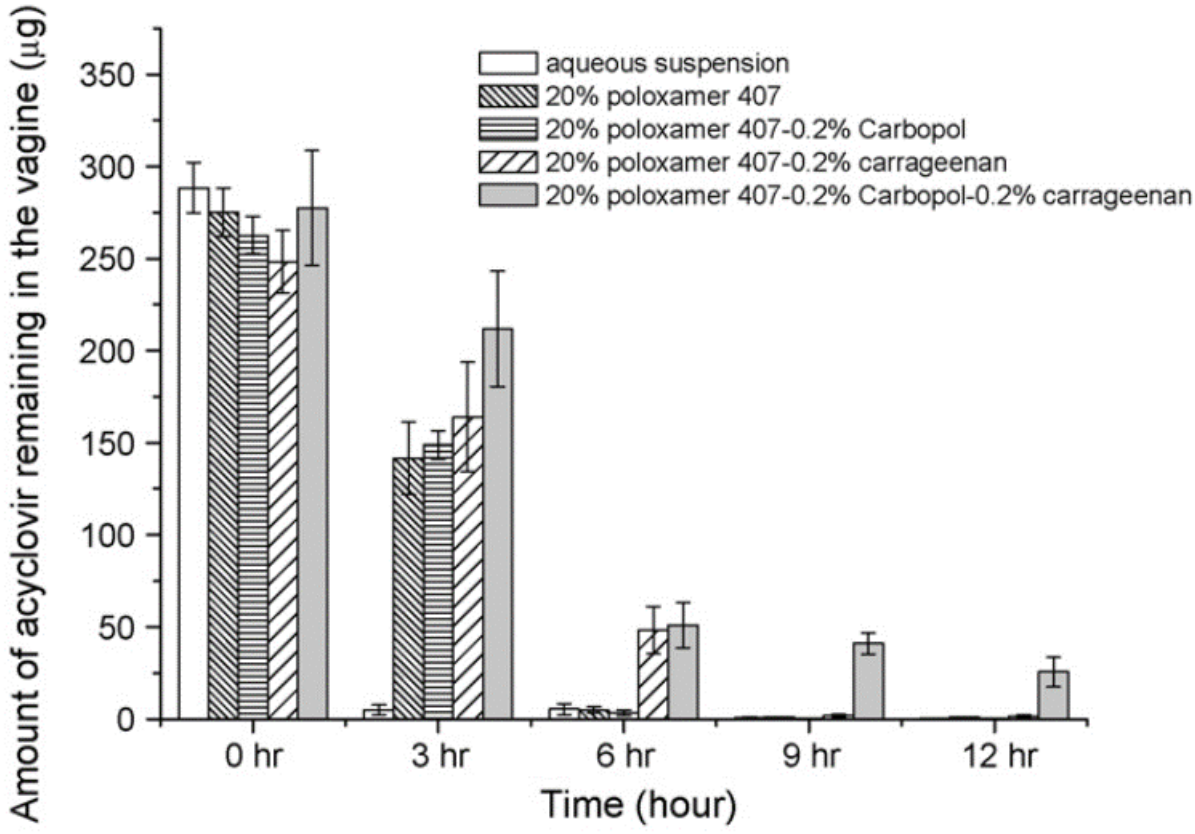

Figure 6. Blending of carbopol and carrageenan into poloxamer 407 formulations improves the retention of acyclovir in the vagina of rats [103]. Reused with permission from Elsevier.

Almomen et al [104] developed thermogelling glycol-chitosan hydrogels for intravaginal delivery of progesterone. Glycol-chitosan showed a concentration and pH dependent gelation temperature of between 15 and 30 °C. Incorporation of progesterone increased the gelation temperature of 5 % glycol-chitosan from 30.9±1.6 to 34.3±1.6 °C. Release of progesterone occurred over 4 h, in a biphasic manner, during which time the glycol-chitosan hydrogel eroded by approximately 50 %.

5.0 THE SENSORIAL PROPERTIES OF VAGINAL GELS

The process of administering a vaginal gel is less familiar for the patient than, for example, taking a tablet or suspension orally. Furthermore, after administration, gel formulations may be perceptible to the patient, or interfere with other activities, e.g. coitus, leading to an unwillingness to apply a product. Thus, the sensorial experience of using intravaginal products is of great importance in assuring patient adherence and compliance to the treatment. This has been particularly damaging in HIV pre-exposure prophylaxis trials using gel products. Mehendale et al [105] studied the sensorial attributes of an intravaginal tenofovir gel and found that whilst women liked the ease of application, messiness and leakage of the product were disliked. In addition, the microbicides were more accepted in women who used them in a coitally-dependent manner. Complaints of product leakage leading to poor patient adherence indicates that the effective retention of vaginal gels is important not just from the perspective of biological efficacy, but also that poor retention may reduce efficacy via a reduction in patient adherence. Mahan and co-workers [106] have attempted to link the rheology of commercial intravaginal products to their sensorial attributes, as measured using hand-feel by a sensory panel. Correlation could be made between rheological attributes and sensory perceptions of the product.

Morrow et al [107] evaluated couples sensory perceptions of HEC gels verses vaginal film during coitus. Both female and male partners were found to have greater sensorial experiences with gels than films. It has also been found that the volume of gel used affects user sensory perception, with larger volumes of an HEC perceived as more lubricative and messy than lower volumes [108]. Van den Berg et al [109] explored the patient acceptability of long-acting topical vaginal gels. All of the products studied were shear-thinning commercial gels. It was found that the women preferred gels with low viscosity for lubricative purposes, but for HIV/STI prevention a thicker gel would be preferred. Overall, the major concern of the study group was leakage of gel. It was also found that women responded positively to the idea of a gel that was initially thin, then thickened within a short time-frame. This supports the concept of *in situ* gelling products which increase in viscosity after application. These products include thermogelling polymer solutions which increase in viscosity upon warming, as well as thixotropic and pseudoplastic gels which reduce in viscosity during the application of shear.

6.0 BARRIERS TO THE APPROVAL OF NEW INTRAVAGINAL MEDICINES – QUALITY, SAFETY AND EFFICACY

The majority of published works focus on the understanding and optimisation of intravaginal gels, with little discussion of translation into a product. Herein is discussed some barriers to the approval of intravaginal medicines which must be carefully considered during product development. These are split into three key areas: quality, safety and efficacy (clinical trial). The regulatory information provided is taken largely from the United States Food and Drug Administration (FDA) and the USP. The FDA provides "guidance for industry" relevant to intravaginal drug delivery for specific applications, namely: vaginal microbicides for prevention of HIV infection [110]; vulvovaginal candidiasis (draft) [111]; bacterial vaginosis (draft) [112]; noncontraceptive estrogen drug products (draft) [113], estrogen and estrogen/progestin drug products (draft) [114] and vaginal contraceptive drugs [115]. There are no drug monographs relating to intravaginal gels available in the USP. The European Medicines Agency (EMA) provide some guidance for topical products, outlined in the relevant sections below.

6.1 ASSESSING THE QUALITY OF INTRAVAGINAL MEDICINES

The quality of a topical product must be thoroughly understood and characterised to satisfy regulatory bodies. The USP lists several product quality tests for topical products, some of these are "universal tests" which apply to all topicals, "specific Tests" to be considered as appropriate, and "specific tests

for topically applied semi-solid products". These USP tests may be used as a benchmark to assure quality of a product. Universal tests cover qualitative description of product, identification tests to establish drug compounds present, assay to determine stability, and assessment of impurities. Quality-related tests applicable to vaginal gels are outlined in table 2.

Table 2. USP tests for intravaginal gels

Test	Product	Comments
Minimum Fill	All creams, gels, lotions, ointments, pastes, powders, aerosols, and sprays that are packaged in containers	To ensure that the amount of material in the product conforms to that on the label.
Uniformity of dosage units	All topical and transdermal products	Required for topicals intended for systemic delivery, or where deviations in concentration may cause irritation or undesired systemic exposure, and which are packaged in single-unit containers.
Water content	All topical and transdermal products	
Microbial Limits	All topical and transdermal products	For nonsterile products microbial acceptance limits apply
Preservative content	All topical and transdermal products	Acceptance criteria must be established
Antioxidant content	All topical and transdermal products	
Sterility	All topical and transdermal products	Must be ensured for certain applications e.g. woundcare
pH	All topical and transdermal products	
Particle Size	All topical and transdermal products	For disperse systems. May include evidence of any instability
Crystal formation	All topical and transdermal products	Microscopic evaluation of crystal formation in product
Apparent viscosity	Topical Semi-solids	May be established based on capillary, rotational, or rolling ball methods. Product-specific acceptance criteria need to be defined.
Uniformity in containers	Topical Semi-solids	To ensure homogeneity of API content throughout container.

In 2015, the EMA released a concept paper to develop guidelines on quality and equivalence in topicals (EM/CHMP/QWP/5581852014) [116]. This paper outlines current issues with the quality of topical medicines, in particular suggesting that shelf-life should be based not only on stability, but also on *in vitro* performance to ensure equivalence of product during storage. The guidance also suggests the possibility of therapeutic equivalence waivers based on pharmaceutical equivalence of product. These guidelines are yet to be published and thus are not yet in place.

Transmucosal products, i.e. those delivering drugs across the mucosa, are likely to incorporate quantities of drug higher than is intended to be delivered to the patient to ensure flux through the mucosa. The FDA recommends quantification of residual drug remaining in the product after it has

delivered its dose [117], as this may affect its safety, efficacy, and quality. It is recommended that the transmucosal product contain as little excess drug as possible so that residual drug be minimised. This can be achieved through formulation design, the use of penetration enhancers, and incorporation of mucoadhesives, so that drug may pass through the mucosa as efficiently as possible.

6.2 ENSURING THE SAFETY OF POLYMERS FOR INTRAVAGINAL DRUG DELIVERY

The safety of medicinal products must be assured through a series of *in vitro* and *in vivo* tests prior to first use in humans, followed by clinical patient evaluation to demonstrate safety. There is a large literature base focussing on the development of novel materials for mucosal drug delivery. These novel materials may not be incorporated into intravaginal products without regulatory approval in their own right. When a new drug application is submitted, the presence of novel excipients in the medicine must be declared. The safety of these excipients must be supported by appropriate toxicological studies and the FDA provides guidance for industry on "Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients" [118]. This process adds a significant time, cost and risk burden for any product development pathway which makes the introduction of novel excipients into medicines challenging and unattractive to the pharmaceutical industry. The USP provides additional "<1074> excipient biological safety evaluation guidelines" which gives an overview of toxicological tests to consider for mucosal drug delivery, but highlights that for regulatory approval, the regulators' specific guidelines must be met. Guidance for industry is available from the FDA for "nonclinical" safety studies to precede use of medicines in humans, which aims to revise ICH harmonization of recommendations [119]. These guidelines cover a battery of nonclinical tests, such as acute toxicity, repeated dose toxicity, local tolerance, genotoxicity and carcinogenicity studies.

The EMA require local tolerance testing of medicines prior to evaluation in humans (Document: EMA/CHMP/SWP/2145/2000). This recommendation requires testing on the site of intended application, the vagina, but also those sites that might come into contact through accidental or unavoidable exposure, e.g. skin and the glans penis. The EMA require that extensive *in vitro* evaluation of the product's potential to cause harm be evaluated prior to *in vivo* studies. The EMA also require that the sensitising potential of intravaginal product be evaluated by e.g. guinea pig models.

The FDA has specific guidance for products intending to act as contraceptives or prevent the transmission of STDs [120]. In brief, the FDA recommends that these medicines undergo toxicity testing at three dose levels using controls which elicit frank toxicity at the high dose and no toxicity at the lowest dose. Pharmacokinetics are required, given the high permeability of the reproductive mucosal sites. In addition, it is advised that any product undergo irritation study in rabbits, genotoxicity assessment (e.g. AMES testing, *in vitro* mouse lymphoma tk assay, and mouse micronucleus test), reproductive toxicity study, and evaluation of carcinogenicity.

6.2.1 The transfer of drug from the vagina to other sites

Transfer of drug from vagina to the penis during coitus is possible. For instance, crinone, a progesterone gel to treat infertility, contains sorbic acid which may cause irritation or contact dermatitis and it is suggested that partners wear a condom during coitus due to potential for irritation of the penis [121]. Contraceptive products, such as spermicide gels, are frequently used as an alternative to barrier contraception, so exposure of the glans penis to drug is inevitable. Drug transfer during coitus has also been investigated for an intravaginal estradiol cream. It was found that men absorb vaginal estradiol during intercourse, whereas in women coitus reduced estradiol absorption [122]. The authors indicate that frequent exposure may lead to feminizing changes in men. There has

also been a clinical trial demonstrating coital transfer of progesterone, used to assist reproduction [123]. It was found that unprotected intercourse during usage of an intravaginal progesterone cream led to significantly reduced absorption in women, possibly affecting efficacy, and transfer of drug to the male partner's system, potentially causing an adverse reaction e.g. reduced libido. As these creams are used to assist reproduction, condom use is not an option to stop transfer. The transfer of levonorgestrel, a contraceptive progestogen, from an intravaginal carrageenan gel has also been investigated, however in this case there was no significantly reduced absorption, and levonorgestrel was only detected in 2 out of 12 male partners [124]. This suggests that the nature of the drug and/or dosage form may affect transfer during coitus. Vaginal use of sulfathiazole/sulfacetamide/sulfabenzamide cream was reported to be the cause of a partner's penile drug eruption following intercourse [125]. The male partner had a known sensitivity to trimethoprim/sulfamethoxazole.

In addition to drug transfer to a partner, administration of a drug intravaginally can lead to appearance of drug in other locations of the body. The transfer of drug from the vagina to the uterus, the "uterine first pass effect", occurs via transfer of drug from vaginal vein to the uterine arterial blood, which also allows for transfer to the urethra [126]. Additionally, drug transfer to the rectum is possible. A trial studying the pharmacokinetics of a CCR5 inhibitor in macaques found that there was significant appearance of drug in the rectal fluid of macaques following intravaginal administration, and *vice versa* [127]. This demonstrates that there is efficient transfer of drug from vagina to rectum. This is likely a result of the diffusion of drug across the connecting tissue, though it was also postulated that drug could be taken into the uterine artery and then distributed to the rectum via the blood vessels of the arteriovenous plexus.

Systemic absorption of drugs for the treatment of local disease is also possible. For instance, it was found that the antibiotic clindamycin had an absolute bioavailability of up to 13 % following intravaginal application of a clindamycin cream [128]. Another example is the systemic absorption of tioconazole following the use of pessaries to treat vaginal candidiasis [129]. This leads to unnecessary exposure of the system to the drug and potential for adverse drug reactions. There are also concerns for the use of some intravaginal medicines in pregnant women, where drug may be absorbed into the system and act on a developing fetus. There are suggestions, for instance, that intravaginal fluconazole medicines be avoided during pregnancy due to the potential for teratogenicity [130].

6.3 RECENT CLINICAL TRIALS OF INTRAVAGINAL MEDICINES

The majority of intravaginal formulations would follow the approval pathways for new drug products, receive marketing authorisation on those grounds, and thus require clinical trial. In some cases, however, intravaginal medicines may follow the processes associated with medical devices. For instance, vaginal moisturisers for the treatment of vaginal dryness are classified as Class IIa Medical Devices by the UK's Medicines and Healthcare products Regulatory Agency [131] and thus may be produced under responsibility of the manufacturer. For generic intravaginal medicines with systemic effect, bioequivalence studies may be used as a surrogate, but for local action therapeutic efficacy must still be demonstrated. This section will focus mainly on those medicines which have failed clinical trials, highlighting potential pitfalls, and will also discuss individual trials which have implication for formulation design or the future direction of intravaginal drug delivery.

There have been several clinical trials looking at the efficacy of vaginally administered antiretrovirals in the prevention of HIV transmission, with mixed results. One of the first topical intravaginal HIV PrEP agents was the non-ionic surfactant nonoxynol-9. This product failed to demonstrate efficacy at phase II/III clinical trial spread across Benin, Côte d'Ivoire, South Africa and Thailand. It was found that nonoxynol-9 use caused adverse toxic events which enhanced HIV infection [132]. The majority of

adverse events were related to vaginal mucosa irritation. It was subsequently discovered that nonoxynol-9 could promote HIV transmission through interleukin-1-mediated NF-kappaB activation, which lead to the immune cell recruitment, which provided host cells for HIV-1 [133]. More promising results have been found with tenofovir-containing medicines. Tenofovir is a reverse-transcriptase inhibitor, which has been formulated into a gel, with the intention of reducing HIV transmission during coitus. The CAPRISA 004 phase IIb clinical trial evaluated the efficacy and the safety of a 1 % tenofovir vaginal gel in the prevention of HIV infection in South African women [134]. CAPRISA 004 concluded that application of the gel before and after coitus reduced HIV infection by 39 %, and that women who used the gel more frequently had a 54 % reduction in HIV transmission. This promising trial was expanded in the FACTS001 phase III trial, conducted on 2059 South African women, which failed to prove efficacy. It was found that the gel was effective in women who used the gel in >72 % of sex acts, however only 20 % of women used it in this manner. This indicates that most women were unable to use the gel in a manner that rendered it effective, or simply chose not to adhere to the treatment regimen. It has been suggested that poor patient adherence to tenofovir gel medicines is a result of messiness and leakage of the medicine, which were found to be disliked features in a sensory study [105]. Thus, future medicines which reduce messiness and leakage are highly desirable

BufferGel (ReProtect Inc, USA) and 0.5% PRO2000 (Endo Pharmaceuticals Solutions Inc., USA), were sulphated cellulose products which intended to reduce HIV transmission by buffering and by inhibiting viral attachment, respectively. In clinical trials, neither product demonstrated efficacy at preventing HIV transmission [135]. An analysis of the users of 0.5% PRO2000 during an earlier phase I clinical trial found that 97.2 % of women liked the product, but that 70 % did not like the smell and indicated that they would prefer a product that would go unnoticed by men [136]. SAVVY vaginal gel (C31G) was a myristamine oxide and cetyl betaine-containing product for HIV PrEP developed by Cellegy Pharmaceuticals. The surfactants contained within the gel were intended to disrupt the membrane of the HIV pathogen [137]. Phase III clinical trials of SAVVY in Ghana and Nigeria were terminated midway due to a lack of evidence for efficacy [138,139]. Several factors may have contributed to the failure of the trial [139]. Low HIV incidence was found in the placebo group – it is suggested that those who wish to join trials are possibly inclined to safer behaviour. Patient adherence may be low, or over-reported. Condom-use during the trial confounded findings – the gel was reportedly used alone in only 9 % of coital acts. Carraguard is a carrageenan-based product intended for use in PrEP. It was found to be safe in phase I and II clinical trials and demonstrated antiviral activity in vitro. However, a phase III clinical trial was unable to show that Carraguard reduces the risk of male-to-female transmission of HIV. This was put down to poor adherence to the product (it was used in only 42.1 % of sexual acts), that the authors attribute to patients forgetting to use the medicines, or running out of supply. They also suggested that the control product, a methylcellulose gel, may have been imparting a protective effect. The overall challenges with adherence to topical HIV PrEP have been reviewed by Gengiah et al [140], who report that for vaginal gels, the biggest issue with adherence related to formulation is the “wetness and leakage” of vaginal gels. The authors also highlight issues with partners feeling vaginal rings during intercourse, highlighting advantages of gel-based medicines.

Vivagel® is a dendrimer formulation for the prevention of HIV and treatment of bacterial vaginosis developed by Starpharma (Australia). It was fast streamed by the FDA in 2006 for the prevention of HIV. Vivagel® has undergone a significant number of clinical trials, but is not yet approved for the prevention of HIV. It is, however, marketed in Australia in the form of a condom with Vivagel® lubricant, and has approval in the European Union for the treatment and relief of bacterial vaginosis [141]. In phase I clinical trials it was found that Vivagel® caused no serious adverse events, but that there was a significantly higher incidence of low-grade adverse events in one study [142]; another found evidence of mild irritation and inflammation [143].

The US-based Contraceptive Research and Development Program’s (CONRAD) “Quatro Study” (clinicaltrials.gov identifier: NCT02602366) aims to assess the acceptability and user preference of

four different vaginal microbicide products; a rapidly-disintegrating vaginal insert, intravaginal ring, film, and gel. The trial aimed to finish in April 2017, and results have not yet been published, but will offer an insight to patient preference between dosage forms.

In situ gelling metronidazole formulations based on poloxamer 407 and F68 have been investigated for treatment of bacterial vaginosis in a pilot study by Shabaan et al [144]. It was found that cure rates were significantly higher in the in situ gel group compared to a conventional gel (85 v 71 % at week one; 80 % v 47.4 % at week four). There was also a lower number of cases of vaginal discharge reported in the study.

Vaginal gels containing lidocaine have been investigated as a method of reducing the pain associated with intrauterine device insertion, but clinical trial failed due to lack of efficacy [145]. There did appear to be some anaesthetic effect, as the pain associated with placement of a tenaculum (a surgical forcep) was reduced in the lidocaine gel group.

Preterm births are associated with an early shortening of the cervix. Intravaginal progesterone gel therapy has been demonstrated to reduce the number of cervical shortening events, and mean cervix length, in a large clinical trial of women with a history of preterm birth [146]. This therapy has the potential to reduce numbers of prematurely born infants.

8.0 Conclusions

Vaginal drug delivery offers many opportunities for novel treatments of local disease, and for systemic drug delivery. It is, however, a difficult route of administration due to its sensitivity and the difficulty in retaining dosage forms. Gel formulations currently in use offer ease-to-use treatments for a variety of conditions, and recent advances in the area of pre-exposure HIV prophylaxis has increased interest in vaginal delivery. Users of vaginal gels often complain of messiness and leakage, which reduces their adherence to a treatment regimen, thus lowering efficacy. Sensory evaluation of vaginal gels is therefore crucial before clinical trial takes place, in order to ensure adherence to treatments. Efforts to improve the retention of vaginal medicines involves the incorporation of “mucoadhesive” excipients or the thickening of gels within the vagina, using pseudoplastic or thixotropic gel systems which shear-thin during application, or using thermogelling materials, which increase in viscosity after warming in the vagina. Synthetic materials have been created to enhance mucoadhesion and retention in the vagina, however these polymers have not yet been approved for use in humans and there is a greater need for researchers to consider the toxicity and irritancy of these products during their design. In particular, irritation of the vaginal mucosa has been a significant issue in PrEP research, enhancing HIV transmission.

References

[1] A.D. Woolfson, R.K. Malcolm, R. Gallagher, Drug delivery by the intravaginal route., Crit. Rev. Ther. Drug Carrier Syst. 17 (2000) 509–555. doi:10.1615/CritRevTherDrugCarrierSyst.v17.i5.30.

[2] R. Karl Malcolm, S.M. Fetherston, C.F. McCoy, P. Boyd, I. Major, Vaginal rings for delivery of HIV microbicides, Int. J. Womens. Health. 4 (2012). doi:10.2147/IJWH.S36282.

[3] R.K. Malcolm, K.L. Edwards, P. Kiser, J. Romano, T.J. Smith, Advances in microbicide vaginal rings, Antiviral Res. 88 (2010). doi:10.1016/j.antiviral.2010.09.003.

[4] O.J. D'Cruz, F.M. Uckun, Vaginal microbicides and their delivery platforms., Expert Opin. Drug Deliv. (2014) 1–18. doi:10.1517/17425247.2014.888055.

[5] L.M. Ensign, R. Cone, J. Hanes, Nanoparticle-based drug delivery to the vagina: A review, J. Control. Release. 190 (2014) 500–514. doi:10.1016/j.jconrel.2014.04.033.

[6] J. das Neves, R. Nunes, A. Machado, B. Sarmento, Polymer-based nanocarriers for vaginal drug delivery, Adv. Drug Deliv. Rev. 92 (2015) 53–70. doi:10.1016/j.addr.2014.12.004.

[7] C.M. Caramella, S. Rossi, F. Ferrari, M.C. Bonferoni, G. Sandri, Mucoadhesive and thermogelling systems for vaginal drug delivery, Adv. Drug Deliv. Rev. (2015). doi:10.1016/j.addr.2015.02.001.

[8] A. Hussain, F. Ahsan, The vagina as a route for systemic drug delivery, J. Control. Release. 103 (2005) 301–313. doi:10.1016/j.jconrel.2004.11.034.

[9] S.G. Antimisiaris, S. Mourtas, Recent advances on anti-HIV vaginal delivery systems development, Adv. Drug Deliv. Rev. 92 (2015) 123–145. doi:10.1016/j.addr.2015.03.015.

[10] M. Ghosh, Secreted Mucosal Antimicrobials in the Female Reproductive Tract that are Important to Consider for HIV Prevention, Am. J. Reprod. Immunol. 71 (2014) 575–588. doi:10.1111/aji.12250.

[11] K. Sharif, O. Olufowobi, The Structure, Chemistry and Physics of Human Cervical Mucus, in: J.A. Jordan, A. Singer (Eds.), The Cervix, Blackwell Publishing Ltd., 2009: pp. 157–168.

[12] D.L. Patton, S.S. Thwin, a Meier, T.M. Hooton, a E. Stapleton, D. a Eschenbach, Epithelial cell layer thickness and immune cell populations in the normal human vagina at different stages of the menstrual cycle., Am. J. Obstet. Gynecol. 183 (2000) 967–973. doi:10.1067/mob.2000.108857.

[13] A.K. Ildgruben, I.M. Sjöberg, M.L.K.C. Hammarström, Influence of hormonal contraceptives on the immune cells and thickness of human vaginal epithelium, Obstet. Gynecol. 102 (2003) 571–582. doi:10.1016/S0029-7844(03)00618-5.

[14] C.A. Squier, M.J. Mantz, P.M. Schlievert, C.C. Davis, Porcine vagina ex vivo as a model for studying permeability and pathogenesis in mucosa, J. Pharm. Sci. 97 (2008) 9–21. doi:10.1002/jps.21077.

[15] C.A. Squier, M.J. Mantz, P.M. Schlievert, C.C. Davis, Porcine vagina ex vivo as a model for studying permeability and pathogenesis in mucosa, J. Pharm. Sci. 97 (2008) 9–21. doi:10.1002/jps.21077.

[16] K.T. Barnhart, A. Izquierdo, E.S. Pretorius, D.M. Shera, M. Shabbout, A. Shaunik, Baseline dimensions of the human vagina, Hum. Reprod. 21 (2006) 1618–1622. doi:10.1093/humrep/del022.

[17] P.B. Pendergrass, M.W. Belovicz, C.A. Reeves, Surface area of the human vagina as measured from vinyl polysiloxane casts, Gynecol. Obstet. Invest. 55 (2003) 110–113. doi:10.1159/000070184.

[18] A.B. Sassi, K.D. McCullough, M.R. Cost, S.L. Hillier, L.C. Rohan, Permeability of tritiated water through human cervical and vaginal tissue, J. Pharm. Sci. 93 (4AD) 2009–2016.

[19] P. van der Bijl, I.O. Thompson, C. a Squier, Comparative permeability of human vaginal and buccal mucosa to water., Eur. J. Oral Sci. 105 (1997) 571–5. doi:10.1111/j.1600-0722.1997.tb00219.x.

[20] C.A. Lesch, C.A. Squier, A. Cruchley, D.M. Williams, P. Speight, The Permeability of Human

Oral Mucosa and Skin to Water, J. Dent. Res. 68 (1989) 1345–1349. doi:10.1177/00220345890680091101.

[21] D.H. Owen, D.F. Katz, A vaginal fluid simulant, Contraception. 59 (1999) 91–95. doi:10.1016/S0010-7824(99)00010-4.

[22] F.C. Chrétien, J. Berthou, Crystallographic investigation of the dried exudate of the major vestibular (Bartholin's) glands in women, Eur. J. Obstet. Gynecol. Reprod. Biol. 135 (2007) 116–122. doi:10.1016/j.ejogrb.2006.06.031.

[23] M. Silvina, J. Tomás, M.E. Nader-macías, Effect of a medium simulating vaginal fluid on the growth and expression of beneficial characteristics of potentially probiotic lactobacilli, Commun. Curr. Res. Ad Educ. Top. Trends Appl. Microbiol. (2007) 732–739.

[24] J.L. V Shaw, C.R. Smith, E.P. Diamandis, Proteomic analysis of human cervico-vaginal fluid, J. Proteome Res. 6 (2007) 2859–2865. doi:10.1021/pr0701658.

[25] A. Stone, C.J. Gamble, The quantity of vaginal fluid, Am. J. Obstet. Gynecol. 78 (1959) 279–281.

[26] C. Mitchell, K. Paul, K. Agnew, R. Gaussman, R.W. Coombs, J. Hitti, Estimating volume of cervicovaginal secretions in cervicovaginal lavage fluid collected for measurement of genital HIV-1 RNA levels in women, J. Clin. Microbiol. 49 (2011) 735–736. doi:10.1128/JCM.00991-10.

[27] M.J. Godley, Quantitation of vaginal discharge in healthy volunteers., Br. J. Obstet. Gynaecol. 92 (1985) 739–742.

[28] M.R.C. Marques, R. Loebenberg, M. Almukainzi, Simulated biological fluids with possible application in dissolution testing, Dissolution Technol. 18 (2011) 15–28. doi:10.1002/jps.23029.

[29] E.R. Boskey, R. a Cone, K.J. Whaley, T.R. Moench, Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source., Hum. Reprod. 16 (2001) 1809–1813. doi:10.1093/humrep/16.9.1809.

[30] J. Ravel, P. Gajer, Z. Abdo, G.M. Schneider, S.S.K. Koenig, S.L. McCulle, S. Karlebach, R. Gorle, J. Russell, C.O. Tacket, R.M. Brotman, C.C. Davis, K. Ault, L. Peralta, L.J. Forney, Vaginal microbiome of reproductive-age women., Proc. Natl. Acad. Sci. U. S. A. 108 Suppl (2011) 4680–4687. doi:10.1073/pnas.1002611107.

[31] C.A. Fox, S.J. Meldrum, B.W. Watson, Continuous measurement by radio-telemetry of vaginal pH during human coitus., J. Reprod. Fertil. 33 (1973) 69–75. doi:10.1530/jrf.0.0330069.

[32] S.S. Olmsted, J.L. Padgett, a I. Yudin, K.J. Whaley, T.R. Moench, R. a Cone, Diffusion of macromolecules and virus-like particles in human cervical mucus., Biophys. J. 81 (2001) 1930–1937. doi:10.1016/S0006-3495(01)75844-4.

[33] I. Carlstedt, H. Lindgren, J.K. Sheehan, U. Ulmsten, L. Wingerup, Isolation and characterization of human cervical-mucus glycoproteins., Biochem. J. 211 (1983) 13–22.

[34] N.A. Peppas, Y. Huang, Nanoscale technology of mucoadhesive interactions., Adv. Drug Deliv. Rev. 56 (2004) 1675–87. doi:10.1016/j.addr.2004.03.001.

[35] Y. Andersch-Björkman, K. a Thomsson, J.M. Holmén Larsson, E. Ekerhovd, G.C. Hansson, Large scale identification of proteins, mucins, and their O-glycosylation in the endocervical mucus during the menstrual cycle., Mol. Cell. Proteomics. 6 (2007) 708–716. doi:10.1074/mcp.M600439-MCP200.

[36] M. Elstein, Functions and physical properties of mucus in the female genital tract., Br. Med. Bull. 34 (1978) 83–88.

[37] M.T. Cook, V. V. Khutoryanskiy, Mucoadhesion and mucosa-mimetic materials—A mini-review, Int. J. Pharm. 495 (2015) 991–998. doi:10.1016/j.ijpharm.2015.09.064.

[38] V. V Khutoryanskiy, Advances in mucoadhesion and mucoadhesive polymers., Macromol. Biosci. 11 (2011) 748–64. doi:10.1002/mabi.201000388.

[39] A. Sosnik, J. das Neves, B. Sarmento, Mucoadhesive polymers in the design of nano-drug delivery systems for administration by non-parenteral routes: A review, Prog. Polym. Sci. 39 (2014) 2030–2075. doi:10.1016/j.progpolymsci.2014.07.010.

[40] J.D. Smart, The basics and underlying mechanisms of mucoadhesion., Adv. Drug Deliv. Rev. 57 (2005) 1556–1568. doi:10.1016/j.addr.2005.07.001.

[41] H.E. Friedl, S. Dünnhaupt, C. Waldner, A. Bernkop-Schnürch, Preactivated thiomers for vaginal drug delivery vehicles, Biomaterials. 34 (2013) 7811–7818. doi:10.1016/j.biomaterials.2013.06.021.

[42] J. Iqbal, G. Shahnaz, S. Dünnhaupt, C. Müller, F. Hintzen, A. Bernkop-Schnürch, Preactivated thiomers as mucoadhesive polymers for drug delivery., Biomaterials. 33 (2012) 1528–35. doi:10.1016/j.biomaterials.2011.10.021.

[43] M.T. Cook, S. a. Schmidt, E. Lee, W. Samprasit, P. Opanasopit, V. V. Khutoryanskiy, Synthesis of mucoadhesive thiol-bearing microgels from 2-(acetylthio)ethylacrylate and 2-hydroxyethylmethacrylate: novel drug delivery systems for chemotherapeutic agents to the bladder, J. Mater. Chem. B. (2015). doi:10.1039/C5TB00834D.

[44] E. Jabbari, N. Wisniewski, N.A. Peppas, Evidence of mucoadhesion by chain interpenetration at a poly (acrylic acid)/mucin interface using ATR-FTIR spectroscopy, J. Control. Release. 26 (1993) 99–108. doi:10.1016/0168-3659(93)90109-I.

[45] O. Reichman, J. Sobel, Desquamative inflammatory vaginitis, Bailliere's Best Pract. Res. Clin. Obstet. Gynaecol. (2014). doi:10.1016/j.bpobgyn.2014.07.003.

[46] L. Edwards, Dermatologic causes of vaginitis: A clinical review, Dermatol. Clin. 28 (2010) 727–735. doi:10.1016/j.det.2010.07.004.

[47] C. Schmitt, J.D. Sobel, C. Meriwether, Bacterial Vaginosis: Treatment with Clindamycin Cream Versus Oral Metronidazole, Obstet. Gynecol. 79 (2005) 1020–1023.

[48] P.L. Fidel, Immunity to Candida., Oral Dis. 8 Suppl 2 (2002) 69–75.

[49] J.R. Schwebke, D. Burgess, Trichomoniasis, Clin. Microbiol. Rev. 17 (2004) 794–803. doi:10.1128/CMR.17.4.794-803.2004.

[50] J.R. Schwebke, S.Y. Lensing, J. Sobel, Intravaginal metronidazole/miconazole for the treatment of vaginal trichomoniasis, Sex. Transm. Dis. 40 (2013) 710–714. doi:10.1097/01.olq.0000431069.38601.d5.

[51] Joint Formulary Committe, British National Formulary, BMJ Gr. Pharm. Press. 65 (2014). http://www.ncbi.nlm.nih.gov/pubmed/10981569.

[52] S. Sanchez, P.J. Garcia, K.K. Thomas, M. Catlin, K.K. Holmes, Intravaginal metronidazole gel versus metronidazole plus nystatin ovules for bacterial vaginosis: A randomized controlled trial, Am. J. Obstet. Gynecol. 191 (2004) 1898–1906. doi:10.1016/j.ajog.2004.06.089.

[53] V. Brache, L.J. Payán, A. Faundes, Current status of contraceptive vaginal rings, Contraception. 87 (2013) 264–272. doi:10.1016/j.contraception.2012.08.037.

[54] A.R. Thurman, M.R. Clark, J.A. Hurlburt, G.F. Doncel, Intravaginal rings as delivery systems for microbicides and multipurpose prevention technologies, Int. J. Womens. Health. 5 (2013) 695–708. doi:10.2147/IJWH.S34030.

[55] S. Garg, K.R. Tambwekar, K. Vermani, A. Garg, C.L. Kaul, L.J.D. Zaneveld, Compendium of Pharmaceutical Excipients for Vaginal Formulations, Pharm. Technol. (2001) 14–25.

[56] I. Lete, M.C. Cuesta, J.M. Marín, S. Guerra, Vaginal health in contraceptive vaginal ring users - A review., Eur. J. Contracept. Reprod. Health Care. 18 (2013) 234–41. doi:10.3109/13625187.2013.801954.

[57] M.M. Ramirez, Labor Induction: A Review of Current Methods, Obstet. Gynecol. Clin. North Am. 38 (2011) 215–225. doi:10.1016/j.ogc.2011.02.012.

[58] J.L. Tenore, Methods for cervical ripening and induction of labor, Am. Fam. Physician. 67 (2003) 2123–2128.

[59] J.D. Stewart, W.F. Rayburn, K.C. Farmer, E.M. Liles, A.H. Schipul, J.R. Stanley, Effectiveness of prostaglandin E2 intracervical gel (Prepidil), with immediate oxytocin, versus vaginal insert (Cervidil) for induction of labor, Am. J. Obstet. Gynecol. 179 (1998) 1175–1180. doi:10.1016/S0002-9378(98)70127-9.

[60] L.M. Ferguson, L.C. Rohan, The importance of the vaginal delivery route for antiretrovirals in HIV prevention, Ther. Deliv. 2 (2011) 1535–1550. doi:10.4155/tde.11.126.

[61] K. Shannon, J. Csete, Violence, Condom Negotiation, and HIV/STI Risk Among Sex Workers., JAMA. 304 (2010) 573–574. doi:10.1001/jama.2010.1090.

[62] J.M. Baeten, T. Palanee-Phillips, E.R. Brown, K. Schwartz, L.E. Soto-Torres, V. Govender, N.M. Mgodi, F. Matovu Kiweewa, G. Nair, F. Mhlanga, S. Siva, L.-G. Bekker, N. Jeenarain, Z. Gaffoor, F. Martinson, B. Makanani, A. Pather, L. Naidoo, M. Husnik, B.A. Richardson, U.M. Parikh, J.W. Mellors, M.A. Marzinke, C.W. Hendrix, A. van der Straten, G. Ramjee, Z.M. Chirenje, C. Nakabiito, T.E. Taha, J. Jones, A. Mayo, R. Scheckter, J. Berthiaume, E. Livant, C. Jacobson, P. Ndase, R. White, K. Patterson, D. Germuga, B. Galaska, K. Bunge, D. Singh, D.W. Szydlo, E.T. Montgomery, B.S. Mensch, K. Torjesen, C.I. Grossman, N. Chakhtoura, A. Nel, Z. Rosenberg, I. McGowan, S. Hillier, Use of a Vaginal Ring Containing Dapivirine for HIV-1 Prevention in Women, N. Engl. J. Med. (2016) 1–12. doi:10.1056/NEJMoa1506110.

[63] A. Nel, S. Kapiga, L. Bekker, D. Borremans, Z. Rosenberg, for the IPM 027/The Ring Study Research Center Teams, Safety and Efficacy of Dapivirine Vaginal Ring for HIV-1 Prevention in African Women, in: Conf. Retroviruses Opportunistic Infect., 2016: p. Abstract 110LB. http://www.croiconference.org/sessions/safety-and-efficacy-dapivirine-vaginal-ring-hiv-1-prevention-african-women.

[64] K.C. Anukam, E. Osazuwa, G.I. Osemene, F. Ehigiagbe, A.W. Bruce, G. Reid, Clinical study comparing probiotic Lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis, Microbes Infect. 8 (2006) 2772–2776. doi:10.1016/j.micinf.2006.08.008.

[65] W. Ya, C. Reifer, L.E. Miller, Efficacy of vaginal probiotic capsules for recurrent bacterial vaginosis: A double-blind, randomized, placebo-controlled study, Am. J. Obstet. Gynecol. 203 (2010). doi:10.1016/j.ajog.2010.05.023.

[66] P. Mastromarino, B. Vitali, L. Mosca, Bacterial vaginosis: a review on clinical trials with probiotics., New Microbiol. 36 (2013) 229–38. http://www.ncbi.nlm.nih.gov/pubmed/23912864.

[67] R. Barrons, D. Tassone, Use of Lactobacillus probiotics for bacterial genitourinary infections in

women: A review, Clin. Ther. 30 (2008) 453–468. doi:10.1016/j.clinthera.2008.03.013.

[68] A.C. Senok, H. Verstraelen, M. Temmerman, G.A. Botta, Probiotics for the treatment of bacterial vaginosis., Cochrane Database Syst. Rev. (2009) CD006289. doi:10.1002/14651858.CD006289.pub2.

[69] J.M. Marrazzo, R.L. Cook, H.C. Wiesenfeld, P.J. Murray, B. Busse, M. Krohn, S.L. Hillier, Women's satisfaction with an intravaginal Lactobacillus capsule for the treatment of bacterial vaginosis., J. Womens. Health (Larchmt). 15 (2006) 1053–1060. doi:10.1089/jwh.2006.15.1053.

[70] M.L. Stewart, I. Stewart, Vaginal applicator, USD320084 S, 1991.

[71] R.F. Omar, S. Trottier, G. Brousseau, C. Ouellet, A. Danylo, T. Ong, M.G. Bergeron, Universal Vaginal Applicator for the Uniform Distribution of Vaginal Gel and Cream Formulations: A Magnetic Resonance Imaging Study, J. Obstet. Gynaecol. Canada. 36 (2014) 42–50. doi:10.1016/S1701-2163(15)30682-4.

[72] R.F. Omar, S. Trottier, G. Brousseau, A. Lamarre, Alexandre Gagnon, M.G. Bergeron, Distribution of a vaginal gel (Invisible Condom®) before, during and after simulated sexual intercourse and its persistence when delivered by two different vaginal applicators: a magnetic resonance imaging study, Contraception. 77 (2008) 447–455. doi:10.1016/j.contraception.2008.01.015.

[73] F.J. Ahmad, M.A. Alam, Z.I. Khan, R.K. Khar, M. Ali, Development and in vitro evaluation of an acid buffering bioadhesive vaginal gel for mixed vaginal infections, Acta Pharm. 58 (2008) 407–419. doi:DOI 10.2478/v10007-008-0023-2.

[74] C. Pedersen, A. Slepenkin, S.B.E. Andersson, J.H. Fagerberg, C.A.S. Bergstr??, E.M. Peterson, Formulation of the microbicide inp0341 for in vivo protection against a vaginal challenge by chlamydia trachomatis, PLoS One. 9 (2014) 1–12. doi:10.1371/journal.pone.0110918.

[75] S. Gupta, I.P. Kaur, V. Prabha, Evaluation of antifertility effect of gel formulation containing sperm immobilizing factor: In vitro and in vivo studies, Eur. J. Pharm. Sci. 81 (2016) 67–74. doi:10.1016/j.ejps.2015.10.004.

[76] P.F. Kiser, A. Mahalingam, J. Fabian, E. Smith, F.R. Damian, J.J. Peters, D.F. Katz, H. Elgendy, M.R. Clark, D.R. Friend, Design of tenofovir-UC781 combination microbicide vaginal gels, J. Pharm. Sci. 101 (2012) 1852–1864. doi:10.1002/jps.23089.

[77] S. Chopra, S.K. Motwani, Z. Iqbal, S. Talegaonkar, F.J. Ahmad, R.K. Khar, Optimisation of polyherbal gels for vaginal drug delivery by Box-Behnken statistical design, Eur. J. Pharm. Biopharm. 67 (2007) 120–131. doi:10.1016/j.ejpb.2006.12.013.

[78] M.C. Bonferoni, P. Giunchedi, S. Scalia, S. Rossi, G. Sandri, C. Caramella, Chitosan gels for the vaginal delivery of lactic acid: relevance of formulation parameters to mucoadhesion and release mechanisms., AAPS PharmSciTech. 7 (2006) 104. doi:10.1208/pt0704104.

[79] Z.A. Senyiğit, S.Y. Karavana, B. Eraç, O. Gürsel, M.H. Limoncu, E. Baloğlu, Evaluation of chitosan based vaginal bioadhesive gel formulations for antifungal drugs., Acta Pharm. 64 (2014) 139–56. doi:10.2478/acph-2014-0013.

[80] F. Tuğcu-Demiröz, F. Acartürk, D. Erdoğan, Development of long-acting bioadhesive vaginal gels of oxybutynin: Formulation, in vitro and in vivo evaluations, Int. J. Pharm. 457 (2013) 25–39. doi:10.1016/j.ijpharm.2013.09.003.

[81] S. Svenson, R.K. Prud'homme, Multifunctional Nanoparticles for Drug Delivery Applications: Imaging, targeting, and delivery, Springer, New York, 2012.

[82] R.S. Navath, A.R. Menjoge, H. Dai, R. Romero, S. Kannan, R.M. Kannan, Injectable PAMAM dendrimer-PEG hydrogels for the treatment of genital infections: Formulation and in vitro and in vivo evaluation, Mol. Pharm. 8 (2011) 1209–1223. doi:10.1021/mp200027z.

[83] L. Perioli, V. Ambrogi, L. Venezia, C. Pagano, M. Ricci, C. Rossi, Chitosan and a modified chitosan as agents to improve performances of mucoadhesive vaginal gels, Colloids Surfaces B Biointerfaces. 66 (2008) 141–145. doi:10.1016/j.colsurfb.2008.06.005.

[84] H.E. Friedl, S. Dünnhaupt, C. Waldner, A. Bernkop-Schnürch, Preactivated thiomers for vaginal drug delivery vehicles., Biomaterials. 34 (2013) 7811–8. doi:10.1016/j.biomaterials.2013.06.021.

[85] E.E. Hassan, J.M. Gallo, A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength., Pharm. Res. 7 (1990) 491–495. doi:10.1023/A:1015812615635.

[86] E. Cevher, D. Sensoy, M.A.M. Taha, A. Araman, Effect of thiolated polymers to textural and mucoadhesive properties of vaginal gel formulations prepared with polycarbophil and chitosan., AAPS PharmSciTech. 9 (2008) 953–65. doi:10.1208/s12249-008-9132-y.

[87] Michael E Aulton, Aulton's Pharmaceutics, 2013. doi:10.1007/s13398-014-0173-7.2.

[88] Ž. Pavelić, N. Škalko-Basnet, R. Schubert, Liposomal gels for vaginal drug delivery, Int. J. Pharm. 219 (2001) 139–149. doi:10.1016/S0378-5173(01)00637-8.

[89] Ž. Pavelić, N. Škalko-Basnet, J. Filipović-Grčić, A. Martinac, I. Jalšenjak, Development and in vitro evaluation of a liposomal vaginal delivery system for acyclovir, J. Control. Release. 106 (2005) 34–43. doi:10.1016/j.jconrel.2005.03.032.

[90] D. Chen, K. Sun, H. Mu, M. Tang, R. Liang, A. Wang, S. Zhou, H. Sun, F. Zhao, J. Yao, W. Liu, pH and temperature dual-sensitive liposome gel based on novel cleavable mPEG-Hz-CHEMS polymeric vaginal delivery system, Int. J. Nanomedicine. 7 (2012) 2621–2630. doi:10.2147/IJN.S31757.

[91] a Chatterjee, B.B. Bhowmik, Y.S. Thakur, Formulation, In Vitro and In Vivo Pharmacokinetics of Anti-HIV Vaginal Bioadhesive Gel., J. Young Pharm. 3 (2011) 83–89. doi:10.4103/0975-1483.80290.

[92] A. Chatterjee, L. Kumar, B.B. Bhowmik, A. Gupta, Microparticulated anti-HIV vaginal gel: In vitro-in vivo drug release and vaginal irritation study., Pharm. Dev. Technol. 16 (2011) 466–473. doi:10.3109/10837450.2010.485318.

[93] L.A. Frank, G. Sandri, F. D'Autilia, R. V. Contri, M.C. Bonferoni, C. Caramella, A.G. Frank, A.R. Pohlmann, S.S. Guterres, Chitosan gel containing polymeric nanocapsules: A new formulation for vaginal drug delivery, Int. J. Nanomedicine. 9 (2014) 3151–3161. doi:10.2147/IJN.S62599.

[94] E. Baloglu, S.Y. Karavana, Z.A. Senyigit, S. Hilmioglu-Polat, D.Y. Metin, O. Zekioglu, T. Guneri, D.S. Jones, In-situ gel formulations of econazole nitrate: Preparation and in-vitro and in-vivo evaluation, J. Pharm. Pharmacol. 63 (2011) 1274–1282. doi:10.1111/j.2042-7158.2011.01315.x.

[95] el Ibrahim, S. Ismail, G. Fetih, O. Shaaban, K. Hassanein, N.H. Abdellah, Development and characterization of thermosensitive pluronic-based metronidazole in situ gelling formulations for vaginal application, Acta Pharm. 62 (2012) 59–70. doi:10.2478/v10007-012-0009-y.

[96] A. Aka-Any-Grah, K. Bouchemal, A. Koffi, F. Agnely, M. Zhang, M. Djabourov, G. Ponchel, Formulation of mucoadhesive vaginal hydrogels insensitive to dilution with vaginal fluids, Eur. J. Pharm. Biopharm. 76 (2010) 296–303. doi:10.1016/j.ejpb.2010.07.004.

[97] J. Yun Chang, Y.K. Oh, H. Soo Kong, E. Jung Kim, D. Deuk Jang, K. Taek Nam, C.K. Kim, Prolonged antifungal effects of clotrimazole-containing mucoadhesive thermosensitive gels on vaginitis, J. Control. Release. 82 (2002) 39–50. doi:10.1016/S0168-3659(02)00086-X.

[98] R.R. de Araújo Pereira, J.S. Ribeiro Godoy, T.I. Stivalet Svidzinski, M.L. Bruschi, Preparation and characterization of mucoadhesive thermoresponsive systems containing propolis for the treatment of vulvovaginal candidiasis, J. Pharm. Sci. 102 (2013) 1222–1234. doi:10.1002/jps.23451.

[99] G.P. Andrews, L. Donnelly, D.S. Jones, R.M. Curran, R.J. Morrow, A.D. Woolfson, R.K. Malcolm, Characterization of the rheological, mucoadhesive, and drug release properties of highly structured gel platforms for intravaginal drug delivery, Biomacromolecules. 10 (2009) 2427–2435. doi:10.1021/bm9003332.

[100] R.M. Curran, L. Donnelly, R.J. Morrow, C. Fraser, G. Andrews, M. Cranage, R.K. Malcolm, R.J. Shattock, A.D. Woolfson, Vaginal delivery of the recombinant HIV-1 clade-C trimeric gp140 envelope protein CN54gp140 within novel rheologically structured vehicles elicits specific immune responses, Vaccine. 27 (2009) 6791–6798. doi:10.1016/j.vaccine.2009.08.088.

[101] Y.T. Kim, B.K. Shin, V.K. Garripelli, J.K. Kim, E. Davaa, S. Jo, J.S. Park, A thermosensitive vaginal gel formulation with HP??CD for the pH-dependent release and solubilization of amphotericin B, Eur. J. Pharm. Sci. 41 (2010) 399–406. doi:10.1016/j.ejps.2010.07.009.

[102] E. Bilensoy, M.A. Rouf, I. Vural, A.A. Hincal, Thermosensitive vaginal gel formulation for the controlled release of clotrimazole via complexation to beta-cyclodextrin, J. Control. Release. 116 (2006) e107–e109.

[103] Y. Liu, Y. ying Zhu, G. Wei, W. yue Lu, Effect of carrageenan on poloxamer-based in situ gel for vaginal use: Improved in vitro and in vivo sustained-release properties, Eur. J. Pharm. Sci. 37 (2009) 306–312. doi:10.1016/j.ejps.2009.02.022.

[104] A. Almomen, S. Cho, C.H. Yang, Z. Li, E.A. Jarboe, C.M. Peterson, K.M. Huh, M.M. Jan??t-Amsbury, Thermosensitive Progesterone Hydrogel: A Safe and Effective New Formulation for Vaginal Application, Pharm. Res. (2015) 2266–2279. doi:10.1007/s11095-014-1616-8.

[105] S. Mehendale, S. Deshpande, R. Kohli, S. Tsui, E. Tolley, Acceptability of coitally-associated versus daily use of 1% tenofovir vaginal gel among women in Pune, India, Int. Health. 4 (2012) 63–69. doi:10.1016/j.inhe.2011.11.003.

[106] E.D. Mahan, T. Zaveri, G.R. Ziegler, J.E. Hayes, Relationships between perceptual attributes and rheology in over-the-counter vaginal products: A potential tool for microbicide development, PLoS One. 9 (2014) e105614. doi:10.1371/journal.pone.0105614.

[107] K.M. Morrow, R.K. Rosen, J.L. Fava, L.C. Rohan, E.M. Kojic, D.R. Friend, D.F. Katz, R.W. Buckheit, "'He Said, She Said.'" Exploring Couples' Sensory Perceptions and Experiences with Vaginal Gels & Film: Implications for Microbicide Development, AIDS Res. Hum. Retroviruses. 30 (2014) A86–A87.

[108] K.M. Morrow, R.K. Rosen, S.E. Vargas, D.F. Katz, J.L. Fava, E.M. Kojic, D.R. Friend, L.C. Rohan, A.S. Ham, R.W. Buckheit, More.? Less.? Just Right.? The Role of Perceived Volume in Gel and Film Perceptibility During Intercourse, and its Impact on Product Preference, AIDS Res Hum Retroviruses. 30 (2014) A145.

[109] J.J. Van Den Berg, R.K. Rosen, D.E. Bregman, L.A. Thompson, K.M. Jensen, P.F. Kiser, D.F. Katz, K. Buckheit, R.W. Buckheit, K.M. Morrow, "set it and forget it": Women's perceptions and opinions of long-acting topical vaginal gels, AIDS Behav. 18 (2014) 862–870. doi:10.1007/s10461-013-0652-4.

[110] FDA, Guidance for Industry Vaginal Microbicides : Development for the Prevention of HIV Infection Guidance for Industry Vaginal Microbicides : Development for the Prevention of HIV Infection, (2014).

[111] FDA, Vulvovaginal Candidiasis : Developing Drugs for Treatment Guidance for Industry Vulvovaginal Candidiasis : Developing Drugs for Treatment Guidance for Industry, (2016).

[112] FDA, Bacterial Vaginosis : Developing Drugs for Treatment Guidance for Industry Bacterial Vaginosis : Developing Drugs for Treatment Guidance for Industry, (2016).

[113] FDA, Guidance for Industry Noncontraceptive Estrogen Drug Guidance for Industry Noncontraceptive Estrogen Drug Products for the Treatment of Vasomotor Symptoms and Vulvar and Vaginal Atrophy Symptoms — Recommended Prescribing Information for Health Care Provid, (n.d.).

[114] FDA, Guidance for Industry Guidance for Industry Estrogen and Estrogen / Progestin Drug, (2003).

[115] FDA, Guidance for the Development of Vaginal Contraceptive Drugs, (1997).

[116] EMA Committee for Medicinal Products for Human use, Concept paper on the development of a guideline on quality and equivalence of topical products, EMA/CHMP/Q (2014).

[117] FDA, Guidance for Industry Residual Drug in Transdermal and Related Drug Delivery Systems Guidance for Industry Residual Drug in Transdermal and Related Drug Delivery, (2011).

[118] FDA, Guidance for Industry Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients, 2005.

[119] FDA, Guidance for Industry M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, 2010.

[120] FDA, Nonclinical Pharmacology/Toxicology Development of Topical Drugs Intended to Prevent the Transmission of Sexually Transmitted Diseases (STD) and/or for the Development of Drugs Intended to Act as Vaginal Contraceptives, (n.d.). https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm125951.htm (accessed June 2, 2017).

[121] MerckSerono, Crinone® 8% w/w Vaginal Gel - SPC, (n.d.).

[122] B. Hurst, A. Jones, M. Elliot, P. Marchburn, M. Matthews, Absorption of vaginal estrogen cream during sexual intercourse: a prospective, randomized, controlled trial., J. Reprod. Med. 53 (2008) 29–32.

[123] K.S. Merriam, K.A. Leake, M. Elliot, M.L. Matthews, R.S. Usadi, B.S. Hurst, Sexual absorption of vaginal progesterone: A randomized control trial, Int. J. Endocrinol. 2015 (2015). doi:10.1155/2015/685281.

[124] V. Brache, H. Croxatto, N. Kumar, R. Sitruk-Ware, L. Cochon, V. Schiappacasse, I. Sivin, C. Munoz, R. Maguire, A. Faundes, Effect of sexual intercourse on the absorption of levonorgestrel after vaginal administration of 0.75 mg in Carraguard® gel: a randomized, cross-over, pharmacokinetic study, Contraception. 79 (2009) 150–154. doi:10.1038/jid.2014.371.

[125] J. Zargooshi, H. Kavoussi, E. Rahmanian, H. Motaee, M. Kohzadi, S. Nourizad, Postcoital Penile Drug Eruption in a Co-Trimoxazole-Sensitive Patient Following Vaginal Use of Triple Sulfa Vaginal Cream by His Partner, J. Sex. Med. 9 (2012) 758–760.

[126] N. Einer-Jensen, R.H.F. Hunter, Counter-current transfer in reproductive biology,

Reproduction. 129 (2005) 9–18. doi:10.1530/rep.1.00278.

[127] R.K. Malcolm, D. Lowry, P. Boyd, L. Geer, R.S. Veazey, L. Goldman, P.J. Klasse, R.J. Shattock, J.P. Moore, Pharmacokinetics of a CCR5 inhibitor in rhesus macaques following vaginal, rectal and oral application, J. Antimicrob. Chemother. 69 (2014) 1325–1329. doi:10.1093/jac/dkt506.

[128] M.T. Borin, Systemic Absorption of Clindamycin Following Intra vaginal Application of Clindamycin Phosphate 1% Cream, J. Clin. Pharmacol. 30 (1990) 33–38. doi:10.1002/j.1552-4604.1990.tb03435.x.

[129] E.T. Houang, A.G. Lawrence, Systemic absorption and persistence of tioconazole in vaginal fluid after insertion of a single 300-mg tioconazole ovule, Antimicrob. Agents Chemother. 27 (1985) 964–965.

[130] C.T. King, P.D. Rogers, J.D. Cleary, S.W. Chapman, Antifungal therapy during pregnancy., Clin. Infect. Dis. 27 (1998) 1151–1160. http://www.ncbi.nlm.nih.gov/pubmed/9827262.

[131] D. Edwards, N. Panay, Treating vulvovaginal atrophy/genitourinary syndrome of menopause: how important is vaginal lubricant and moisturizer composition?, Climacteric. 19 (2016) 151–161. doi:10.3109/13697137.2015.1124259.

[132] L. Van Damme, G. Ramjee, M. Alary, B. Vuylsteke, V. Chandeying, H. Rees, P. Sirivongrangson, L. Mukenge-Tshibaka, V. Ettiègne-Traoré, C. Uaheowitchai, S.S. Abdool Karim, B. Mâsse, J. Perriëns, M. Laga, Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: A randomised controlled trial, Lancet. 360 (2002) 971–977. doi:10.1016/S0140-6736(02)11079-8.

[133] R.N. Fichorova, L.D. Tucker, D.J. Anderson, The Molecular Basis of Nonoxynol-9–Induced Vaginal Inflammation and Its Possible Relevance to Human Immunodeficiency Virus Type 1 Transmission, J. Infect. Dis. 184 (2001) 418–428. doi:10.1086/322047.

[134] K.Q. Abdool, S.S. Abdool Karim, J. a Frohlich, a C. Grobler, C. Baxter, L.E. Mansoor, a B. Kharsany, S. Sibeko, K.P. Mlisana, Z. Omar, T.N. Gengiah, S. Maarschalk, N. Arulappan, M. Mlotshwa, L. Morris, D. Taylor, T. Group., Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women., Science (80-.). 329 (2010) 1168–1174. doi:10.1126/science.1193748.Effectiveness.

[135] S.S. Abdool Karim, B.A. Richardson, G. Ramjee, I.F. Hoffman, Z.M. Chirenje, T. Taha, M. Kapina, L. Maslankowski, A. Coletti, A. Profy, T.R. Moench, E. Piwowar-Manning, B. Mâsse, S.L. Hillier, L. Soto-Torres, Safety and effectiveness of BufferGel and 0.5% PRO2000 gel for the prevention of HIV infection in women, AIDS. 25 (2011) 957–966. doi:10.1097/QAD.0b013e32834541d9.

[136] N. Joglekar, S. Joshi, M. Kakde, G. Fang, M. Cianciola, S. Reynolds, S. Mehendale, THE HIV PREVENTION TRIAL NETWORK (H, Acceptability of PRO2000 Vaginal Gel among HIV un-infected Women in Pune, India, AIDS Care. 19 (2007) 817–821. doi:10.1080/09540120601133576.

[137] T.N. Gengiah, A. Moosa, A. Naidoo, L.E. Mansoor, Adherence challenges with drugs for pre-exposure prophylaxis to prevent HIV infection, Int. J. Clin. Pharm. 36 (2014) 70–85. doi:10.1007/s11096-013-9861-1 [doi].

[138] L. Peterson, K. Nanda, B.K. Opoku, W.K. Ampofo, M. Owusu-Amoaka, A.Y. Boakye, W. Rountree, A. Troxler, R. Dominik, R. Roddy, L. Dorflinger, SAVVY® (C31G) gel for prevention of HIV infection in women: A phase 3, double-blind, randomized, placebo-controlled trial in Ghana, PLoS One. 2 (2007). doi:10.1371/journal.pone.0001312.

[139] P.J. Feldblum, A. Adeiga, R. Bakare, S. Wevill, A. Lendvay, F. Obadaki, M.O. Olayemi, L. Wang, K. Nanda, W. Roundtree, SAVVY vaginal gel (C31G) for prevention of HIV infection: A randomized controlled trial in Nigeria, PLoS One. 3 (2008). doi:10.1371/journal.pone.0001474.

[140] T.N. Gengiah, A. Moosa, A. Naidoo, L.E. Mansoor, Adherence challenges with drugs for pre-exposure prophylaxis to prevent HIV infection, Int. J. Clin. Pharm. 36 (2014) 70–85. doi:10.1007/s11096-013-9861-1 [doi].

[141] Starpharma, VivaGel Availability, (n.d.). http://www.starpharma.com/vivagel/vivagel_availability (accessed June 5, 2017).

[142] A.-B. Moscicki, R. Kaul, M. Yifei, M.E. Scott, I.I. Daud, E.A. Bukusi, S. Shiboski, A. Rebbapragada, S. Huibner, C.R. Cohen, C. Author, Measurement of mucosal biomarkers in a phase 1 trial of intravaginal 3% SPL 7013 gel (VivaGel ®) to assess expanded safety, J Acquir Immune Defic Syndr J Acquir Immune Defic Syndr. Febr. 1 (2012) 134–140. doi:10.1097/QAI.0b013e31823f2aeb.

[143] C.R. Cohen, J. Brown, A.B. Moscicki, E.A. Bukusi, J.R.A. Paull, C.F. Price, S. Shiboski, A phase I randomized placebo controlled trial of the safety of 3% SPL7013 gel (VivaGel??) in healthy young women administered twice daily for 14 days, PLoS One. 6 (2011). doi:10.1371/journal.pone.0016258.

[144] O.M. Shaaban, G.N. Fetih, N.H. Abdellah, S. Ismail, M. a. Ibrahim, E.S. a Ibrahim, Pilot randomized trial for treatment of bacterial vaginosis using in situ forming metronidazole vaginal gel, J. Obstet. Gynaecol. Res. 37 (2011) 874–881. doi:10.1111/j.1447-0756.2010.01457.x.

[145] R.B. Rapkin, S.L. Achilles, E.B. Schwarz, L. Meyn, M. Cremer, C.M. Boraas, B.A. Chen, Self-Administered Lidocaine Gel for Intrauterine Device Insertion in Nulliparous Women, Obstet. Gynecol. 128 (2016) 621–628. doi:10.1097/AOG.0000000000001596.

[146] J.M. O'Brien, E.A. Defranco, C.D. Adair, D.F. Lewis, D.R. Hall, H. How, M. Bsharat, G.W. Creasy, G. Progesterone Vaginal Gel Study, Effect of progesterone on cervical shortening in women at risk for preterm birth: secondary analysis from a multinational, randomized, double-blind, placebo-controlled trial, Ultrasound Obs. Gynecol. 34 (2009) 653–659. doi:10.1002/uog.733810.1002/uog.7338.