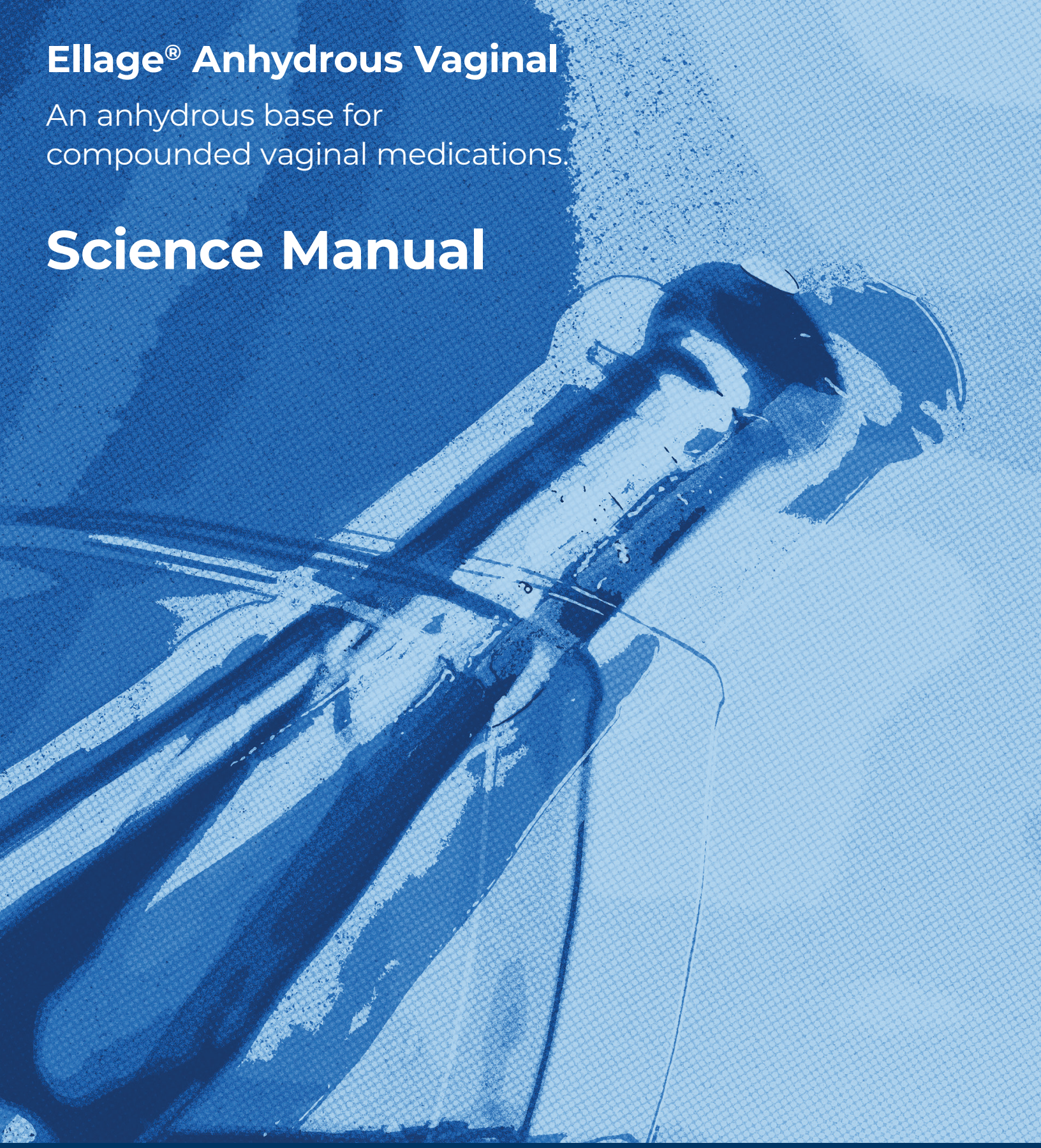
Ellage® Anhydrous Vaginal

An anhydrous base for
compounded vaginal medications.

Science Manual



PCCA® SCIENCE

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Topical/Vaginal Treatment of Sexual Function Disorders

SUMMARY: Two female patients were diagnosed primarily with low libido and were prescribed sildenafil 2% combined with arginine hydrochloride (HCl) in PCCA Ellage® Anhydrous Vaginal (PCCA Formula 14338), to be applied once a day for approximately 3 months. Both patients were satisfied with the treatment outcome and reported no adverse events. The biggest improvements attributed to the compounded topical/vaginal treatment were in the desire and arousal sexual domains, followed by the satisfaction, lubrication and pain domains.

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Introduction:

It is estimated that sexual function disorders affect about 43% of women, impacting significantly on their interpersonal functioning and overall quality of life[1]. Over recent years, there has been an increase in the clinical and academic interest in Female Sexual Dysfunction (FSD), which is commonly divided into four key domains: sexual pain, low desire, low arousal, and orgasmic dysfunction. Sexual pain is a common complaint in women of all ages and may include pain at the vulva, deep pain with penetration, or tightening of the pelvic musculature. Women with low desire will experience an absence or reduction in sexual fantasies and desire for sexual activity that causes distress. Sexual desire is fluid throughout a lifetime; however, at various points in one's life, low sexual desire may cause significant distress. Low arousal may manifest as a decrease in vaginal lubrication and a decrease in genital warmth related to blood flow. Orgasmic dysfunction requires distinguishing between completely absent orgasms and delayed or less intense orgasms[2]. The purpose of this case series is to discuss the clinical efficacy of a compounded topical/vaginal treatment of two women diagnosed with FSD.

Case Reports:

Two female patients, aged 49 and 58 years old, were diagnosed primarily with low libido and were prescribed sildenafil 2% combined with arginine hydrochloride (HCl) in PCCA Ellage® Anhydrous Vaginal (Figure 1). In both cases, the compounded topical/vaginal treatment was applied once a day, in the evening, over a period of approximately 3 months. The corresponding PCCA Formula 14338 is displayed in Table 1.

Figure 1. *PCCA Ellage® Anhydrous Vaginal.*

Rx	
Sildenafil Citrate USP	2.8 g
Arginine HCl USP	5 g
Glycerin USP (Natural)	5 g
Base, PCCA Ellage® Anhydrous Vaginal	87.2 g

Table 1. *Sildenafil 2%, Arginine HCl Topical/Vaginal (Ellage® Anhydrous).*

Sildenafil citrate is a type-5 phosphodiesterase (PDE_5) inhibitor which is commonly indicated in low libido and may be prescribed either alone or combined with arginine HCl[2-4]. Ellage is a mucoadhesive, anhydrous and self-emulsifying vaginal base, which is expected to adhere to the vaginal mucosa for a long period of time, despite the variable secretions of vaginal fluid, due to its high retention potential demonstrated *in vitro*[5,6].

Methodology:

The Female Sexual Function Index (FSFI) was the research instrument used in this case series to evaluate the clinical efficacy of the compounded topical/vaginal treatment. This multidimensional, self-reported instrument is comprised of 19 items which evaluate six domains of the female sexual function (desire, subjective arousal, lubrication, orgasm, satisfaction, and pain). Individual domain scores and a full scale (overall) score are derived from the computational formulas by Rosen *et al.* (2000). Scores are rated from 0-5; a domain score of zero indicates that the subject reported having no sexual activity[7].

The patients were requested to complete the FSFI before and after the compounded topical/vaginal treatment. Informed consent was provided by both patients.

Topical/Vaginal Treatment of Sexual Function Disorders

Results and Discussion:

The two female patients were satisfied with the treatment outcome and reported no adverse events. The FSFI was completed before treatment and approximately 3 months after treatment. When compared to baseline, there was an improvement in almost all domains of the female sexual function (desire, subjective arousal, lubrication, orgasm, satisfaction, and pain). Orgasm was the only domain which remained unchanged throughout treatment but this a complex domain that requires a multidimensional approach to treatment.

The biggest improvements attributed to the compounded topical/vaginal treatment were in the desire and arousal domains. Satisfaction improved equally but moderately in both patients; this is also a complex domain, less tangible than all other domains of the female sexual function. Lubrification, on the other hand, showed higher improvements in one patient but both reported increased lubrification. With regards to the pain domain, there was a remarkable improvement in one patient who suffered from deep pain with penetration. The patient commented the following:

"I need more (compounded topical/vaginal treatment) because it really did help with the pain."

The other patient did not suffer from pain and thus the scores remained unchanged.

The overall improvements in the female sexual function of both patients following the treatment with Sildenafil 2%, Arginine HCl Topical/Vaginal (Ellage Anhydrous) suggest that this compounded medication is a promising treatment option for female patients with sexual function disorders, in particular low libido. The vaginal route of administration was chosen because the vaginal mucosa offers a large surface area with a rich blood supply, making it an ideal site for the delivery of medication. Also, it is well documented that sildenafil citrate and arginine HCl are effective in enhancing sexual desire and arousal[2-4]. When incorporated in PCCA Ellage Anhydrous Vaginal, the contact time between the active pharmaceutical ingredients and the vaginal mucosa is likely to be extended because of the mucoadhesive properties of the compounding base[5,6]. Therefore, Ellage was the compounding base chosen to effectively deliver sildenafil citrate and arginine HCl.

Figure 2. *Representation of sexual function disorders (adapted from VGstockstudio/Shutterstock.com).*

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Technical Reports

Effect of Ellage™ and Other PCCA Proprietary Bases on the pH of Vaginal Fluid

SUMMARY: The mildly acidic vaginal pH is the result of physiological processes and the microbiology flora. Ellage Anhydrous Vaginal, VersaBase® Cream and MucoLox™/VersaBase Gel (50:50) are unlikely to change the vaginal pH, which is essential for normal mucosal function and protection against infection.

Introduction:

The pH (potential of hydrogen) is a quantitative measure of the hydrogen ion concentration [H^+] in a solution and it indicates if the solution is acid or alkaline. Mathematically, the pH corresponds to the negative of the base 10 logarithm of the H^+ molar concentration and is represented by the following formula: pH = -log10[H^+].

The pH scale, also called the acid-base scale, ranges from 0 to 14 and it inversely indicates the [H+]. As such, the higher the hydrogen ion concentration, the lower the pH. A solution with a pH less than 7 is considered acidic; a solution with a pH greater than 7 is considered alkaline, or basic; a solution with a pH of 7 is considered neutral, as displayed in Figure 1. The pH is commonly measured by a pH meter or, less accurately, by paper test strips [1].

The human body compartments and biological fluids have characteristic pH ranges that are very important to maintain a healthy balance of acidity and alkalinity. For instance, the normal physiological pH of the blood is 7.3-7.5 whereas the pH of the vagina is moderately acidic ≈4.5. Any disruptions of these acid-base balances can lead to the development of a variety of diseases [2]. The purpose of this *in vitro* study is to investigate the effect of the PCCA proprietary bases Ellage Anhydrous Vaginal, VersaBase Cream and MucoLox/VersaBase Gel (50:50) on the pH of vaginal fluid, in comparison to an over-the-counter (OTC) long-lasting vaginal moisturizer.

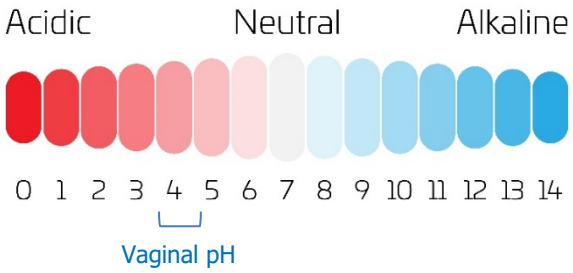

Figure 1. *The pH scale, adapted to indicate vaginal pH; stock illustration ID: 1215457015 (adapted from Pyty /Shutterstock.com).*

Methodology:

A Vaginal Fluid Simulant (VFS) was prepared in the PCCA R&D Lab to model the fluid produced in the human vagina by healthy, nonpregnant premenopausal women. The composition of the fluid medium, displayed in Table 1, was based on the research by Owen and Katz (1999) who gave particular importance to the pH and osmolarity of the VFS [3].

About 50 mL of the VFS were placed in a water bath until 37°C. The pH of the VFS was measured using a HORIBA compact pH meter (LAQUAtwin-pH-22) and an average of 4 readings was considered baseline. A small volume of 0.5 mL of VFS was removed and added to a beaker. A total of 5 g of Ellage Anhydrous Vaginal was added to the beaker and mixed gently. The pH of the resulting solution was measured in duplicate. Again 0.5 mL of VFS was removed and added to the same beaker, followed by another set of pH measurements. This procedure was repeated for the following volumes of VFS: 4x1 mL and 2x2.5 mL, up to a total of 10 mL of VFS. This *in vitro* study was repeated for 5 g of VersaBase Cream, 5 g of MucoLox/VersaBase Gel (50:50) and 5 g of the OTC vaginal moisturizer.

Ingredient	*Quantity*
NaCl	3.51 g
KOH	1.4 g
$Ca(OH)_2$	0.222 g
BSA	0.018 g
Lactic acid	2.0 g
Acetic acid	1.0 g
Glycerol	0.16 g
Urea	0.4 g
Glucose monohydrate	5.0 g
HCl	qs pH 4.2-4.5
H_2O	qs 1,000 mL

Table 1. *Composition of the Vaginal Fluid Simulant prepared in the PCCA R&D Lab.*

Effect of Ellage™ and Other PCCA Proprietary Bases on the pH of Vaginal Fluid

Results and Discussion:

The pH ranges obtained in this study for the VFS and the PCCA proprietary bases were as follows: Ellage Anhydrous Vaginal [pH 4.51-4.645]; VersaBase Cream [4.535-4.935]; and MucoLox/VersaBase Gel (50:50) [4.465-4.68] (Figures 2-4).

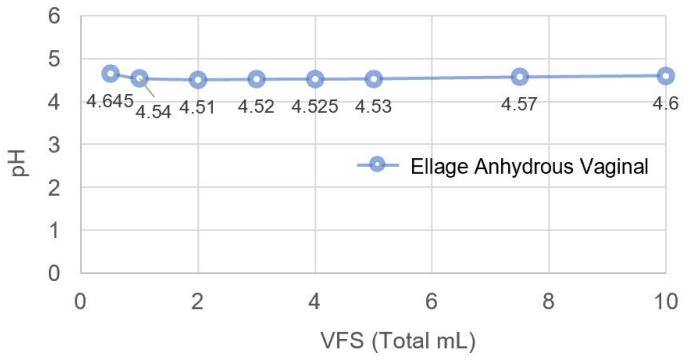

Figure 2. *Effect of Ellage Anhydrous Vaginal (5 g) on increasing volumes of VFS (0.5 – 10 mL).*

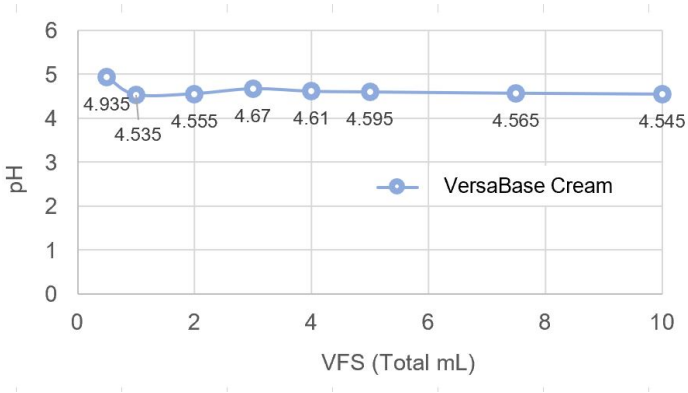

Figure 3. *Effect of VersaBase Cream (5 g) on increasing volumes of VFS.*

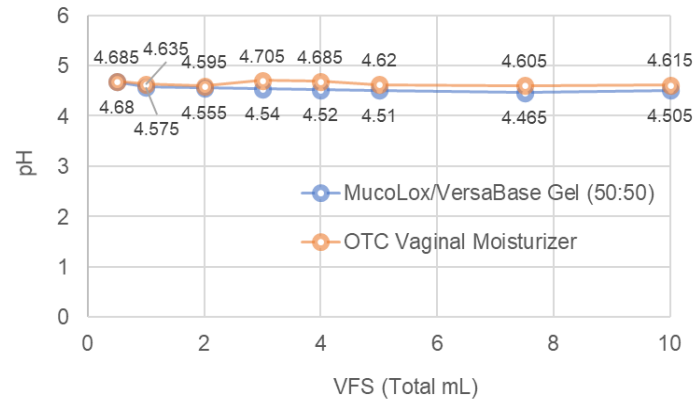

Figure 4. *Effect of MucoLox/VersaBase Gel (50:50) and the OTC vaginal moisturizer (5 g) on increasing volumes of VFS.*

Considering that the baseline pH of the VFS was 4.54, the PCCA proprietary bases had no significant effects on the pH of the fluid medium. In comparison, the OTC long-lasting vaginal moisturizer had also no significant effects on the VFS pH (Figures 2-4).

Conclusions:

The pH of the vagina is the result of physiological processes and the microbiology flora. It is important that the vaginal pH is kept mildly acidic ≈4.5 for normal mucosal function and protection against infection [2].

Any significant changes of vaginal pH are of clinical importance. For instance, a change to an elevated vaginal pH indicates an alteration of the microbial ecosystem which may result in bacterial vaginosis. In pregnant women, this elevated pH may indicate risk factors for preterm birth [2].

For these reasons, it is essential that feminine hygiene products and topical medications do not alter the vaginal pH when applied to the vaginal mucosa. This *in vitro* study has demonstrated that the PCCA proprietary bases Ellage Anhydrous Vaginal, VersaBase Cream and MucoLox/VersaBase Gel (50:50) are unlikely to contribute to any changes of the vaginal pH. This finding is key for compounding pharmacists to ensure the quality and safety of their vaginal formulations.

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The Leakage Test: Evaluation of the Leakage Potential of PCCA Ellage™

SUMMARY: Vaginal drug delivery offers many advantages but conventional vaginal dosage forms have the potential to leak rapidly due to the vaginal fluid that is continuously released. The leakage test has shown that PCCA Ellage Anhydrous Vaginal, a mucoadhesive base designed specifically to optimize drug delivery in the vaginal mucosa, and PCCA formulas 13845 and 13834 have high retention potential *in vitro* – superior to the OTC long-lasting vaginal moisturizer.

Introduction:

The vaginal mucosa offers a large surface area and rich blood supply, making it a promising site for delivery of medication in the treatment of several conditions (e.g., vaginitis, infections) and also in hormone replacement therapy. Vaginal drug delivery, however, faces a multitude of challenges; in particular, the leakage potential of drugs due to the vaginal fluid that is continuously released [1]. Conventional vaginal dosage forms such as creams, gels, and foams have a limited residence time (time at the site of action) and efforts are made to prolong the contact time between the medication and the mucosal tissue. Also, conventional dosage forms are commonly runny or messy, especially the vaginal gels [2]. PCCA Ellage Anhydrous Vaginal is a mucoadhesive base designed specifically to optimize drug delivery in the vaginal mucosa. Compounded medicines prepared with PCCA Ellage are likely to remain at the site of action for a longer period of time, without leakage or messiness, potentially improving patient acceptance and compliance.

The aim of this study was to test *in vitro* the leakage potential of PCCA Ellage, alone and with common active pharmaceutical ingredients, in comparison to an over-the-counter (OTC) vaginal moisturizer of reference that claims to be long-lasting (up to 3 days). Upon vaginal administration, gels and creams become diluted with vaginal fluids which may lead to changes in their rheological and mucoadhesive properties, with increased leakage and runny discomfort. In order to simulate *in vivo* conditions and account for any loss of beneficial physical properties, the leakage test was also performed on the corresponding diluted formulas for PCCA Ellage; Estriol 0.1% and Testosterone 0.1% in Ellage (PCCA formula 13845); Amitriptyline 2% and Baclofen 2% in Ellage (PCCA formula 13834); and the OTC long-lasting vaginal moisturizer.

Methodology:

The leakage test was conducted at the PCCA R&D Lab in accordance to an adapted method of the *in vitro* leakage potential test by Andrade *et al.* [3]. Initially, a Vaginal Fluid Simulant (VFS) was prepared to model the fluid produced in the human vagina by healthy, nonpregnant premenopausal women. The composition of the fluid medium was based on the research by Owen and Katz and includes the following ingredients: NaCl 3.51 g, KOH 1.4 g, $Ca(OH)_2$ 0.222 g, BSA 0.018 g, lactic acid 2.0 g, acetic acid 1.0 g, glycerol 0.16 g, urea 0.4 g, glucose monohydrate 5.0 g, HCl qs pH 4.2-4.5 and H_2O qs 1,000 mL [4].

A total of 4 test formulas were prepared, as follows: PCCA Ellage; Estriol 0.1% and Testosterone 0.1% in Ellage; Amitriptyline 2% and Baclofen 2% in Ellage; and the OTC long-lasting vaginal moisturizer. A diluted version of these formulas was also prepared by adding 2 mL of each formula to 0.75 mL of VFS. As a result, a total of 8 formulas (diluted and undiluted) were prepared for the leakage test.

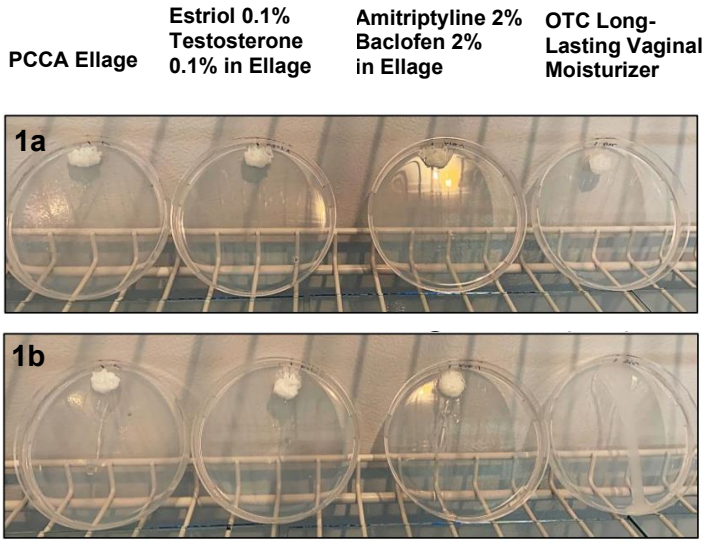
An aliquot of 25 mL of agar solution – 1.5% of agarose (w/v) in VFS – was poured into a Petri dish (100 mm) and the resulting agar plate was placed at room temperature for solidifying. Each formula was tested in triplicate so this procedure was repeated for a total of 24 Petri dishes. Before the experiment, the agar plates were placed inside an incubator (VWR; model 2020) at 37°C, in a vertical position and at an angle of 60°.

Using a syringe, an aliquot of 0.5 mL of each test formula was deposited onto the top of the agar plate and the time taken for the test formulas to reach the bottom of the agar plate was recorded, up to a maximum of 5 minutes. The running distance of the test formulas was measured along the agar plate.

The Leakage Test: Evaluation of the Leakage Potential of PCCA Ellage™

Results and Discussion:

The 4 test formulas were divided in two groups: diluted and undiluted, with 12 agar plates in each group. There was no change for the undiluted test formulas as these remained on the top of the agar plates, where they were originally placed, throughout the study period of 5 minutes (Figure 1a, top). This fact demonstrates that PCCA Ellage, PCCA formulas 13845 and 13834, as well as the OTC long-lasting vaginal moisturizer, have high retention potential to the warm and moisturized surface containing VFS.



Figures 1a and 1b. *The leakage test (randomly selected agar plates) for PCCA Ellage, PCCA formulas 13845 and 13834, and the OTC long-lasting vaginal moisturizer: undiluted formulas (1a) and diluted formulas (1b).*

When the test formulas were diluted with the VFS, to simulate *in vivo* conditions where vaginal dosage forms become diluted with vaginal fluids, it was observed that PCCA Ellage and the corresponding PCCA formulas still remained on the top of the agar plates (Figure 1b, bottom). The average running speed of these formulas on the agar plates was close to zero, as shown in Figure 2, which reinforces the unique high retention potential of PCCA Ellage. On the contrary, the OTC long-lasting vaginal moisturizer ran down the agar plate, travelling across 10 cm, and reached the bottom of the plate in less than 10 seconds (average running speed of 14.44 mm/s, Figure 2).

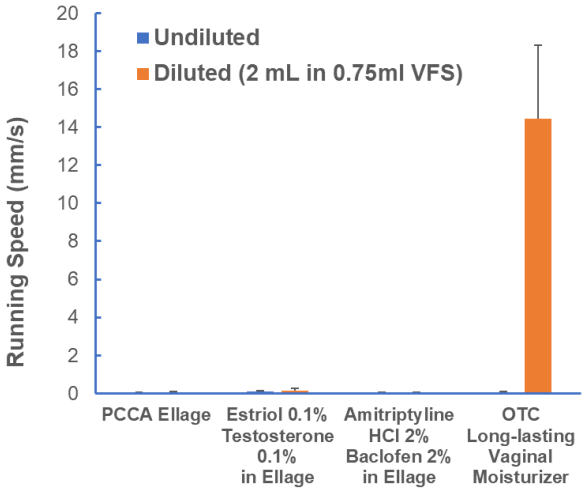


Figure 2. *The leakage test: average running speed for PCCA Ellage, PCCA formulas 13845 and 13834, and the OTC long-lasting vaginal moisturizer (diluted and undiluted formulas).*

Conclusions:

The leakage test has shown that PCCA Ellage Anhydrous Vaginal, and its corresponding PCCA formulas, have high retention potential *in vitro* – superior to the OTC long-lasting vaginal moisturizer. As such, PCCA Ellage is expected to adhere *in vivo* to the vaginal mucosa for a long period of time, despite the regular secretions of vaginal fluid, without leakage or messiness.

References:

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Evaluation of the Irritation Potential of PCCA Ellage™ Anhydrous Vaginal *Part 1: Hen's Egg Test-Chorioallantoic Membrane Assay*

SUMMARY: The evaluation of the irritation potential by the HET-CAM assay is part of the product safety assessment for PCCA Ellage™ Anhydrous Vaginal. A preliminary study was conducted at PCCA R&D and another study was outsourced to a specialized company. Both studies have shown that PCCA Ellage has no ocular irritation potential (IS<5). This finding strongly suggests that PCCA Ellage is also no irritant to the vaginal mucosal membrane and it is thus expected to be clinically safe *in vivo*.

At PCCA R&D, we respect animal welfare and we do not test our products on animals. Instead, we are proud to collaborate with institutions that provide alternatives to the use of animals for scientific purposes.

Introduction:

The evaluation of the irritation potential is part of the product safety assessment for PCCA Ellage Anhydrous Vaginal. There is evidence in the literature to suggest testing vaginal irritation with the Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) assay, an *in vitro* alternative to the international standard Draize rabbit *in vivo* ocular irritation test. The irritation potentials for the eye and the vaginal mucosa are similar and, as such, any skin or eye irritant substance shall be directly labelled as a potential vaginal irritant [1,2].

The HET-CAM assay is a rapid, sensitive and inexpensive toxicity test that has been widely used to evaluate the potential ocular irritation of substances by measuring the ability to induce toxicity in the CAM of a chicken egg [1]. The HET-CAM test method recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is applicable to nonregulatory, validation or optimization of preclinical studies (NIH Publication No. 10-7553 – 2010).

The majority of vaginal products are intended to be self-administered and, as such, it is desirable that these products offer maximum comfort at the time of application and during the time of use [1]. PCCA Ellage is a mucoadhesive vaginal base that was developed to remain at the site of application. It is therefore very important to evaluate the irritation potential of this base in order to ensure the safety of the corresponding compounded medicines.

Aim & Methodology:

The aim of this study was to test the irritation potential of the vaginal base PCCA Ellage in comparison to positive and negative controls.

A preliminary study was conducted at PCCA R&D (Figures 1a and 1b), which was followed by the experiment V20-4095 at Consumer Product Testing Company, Inc. (CPT^{SM}) (Fairfield, NJ). The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) HET-CAM Recommended Test Method (NIH Publication No. 10-7553 – 2010) was the protocol followed at PCCA R&D with the negative and positive test controls, 0.9% NaCl and 0.1N NaOH, respectively. A modification of the HET-CAM Luepke and Kemper (1986) was the protocol followed by the outsourced facility [3] with popular eye cosmetics as negative test controls: Nivea Visage Liposome Eye Contour Gel and Pond's Revitalizing Eye Gel with Vitamin E. There are variable scoring schemes for the HET-CAM assay. The Irritation Scores (IS) adopted classifies the test products as no irritants for IS between 0 and 4.9; and as irritants for IS greater than 5 [4,5].

Figure 1. *Exposing the chorioallantoic membranes by the PCCA R&D team.*

Evaluation of the Irritation Potential of PCCA Ellage™ Anhydrous Vaginal *Part 1: Hen's Egg Test-Chorioallantoic Membrane Assay*

Results and Discussion:

The preliminary study at PCCA R&D has demonstrated that both PCCA Ellage and the 0.9% NaCl have no ocular irritation potentials (IS=0). Contrarily, the 0.1N NaOH is strongly irritative (IS=17) as shown by the lysis (vessels disintegration), hemorrhage (vessels bleeding) and coagulation (blood clotting) displayed in Figure 2. This experiment was extended for a total of 20 minutes and PCCA Ellage still presented no irritation potential.

The outsourced study by CPT[SM] yielded comparable results for PCCA Ellage (lot number 0527009), with an IS of 2.50 which is considered non-irritant by Gilleron *et al.* (IS=0-4.9) [4,5]. The negative controls were also classified as non-irritants with IS of 3.0 and 2.0 for the Nivea Visage Liposome Eye Contour Gel and the Pond's Revitalizing Eye Gel with Vitamin E, respectively. All irritation scores correspond to an average of 4 eggs tested per product. PCCA Ellage and the controls were all diluted to 50% in this experiment because previous studies have shown that the CAM of the hen's egg is more sensitive to liquid irritants than is the rabbit eye [6].

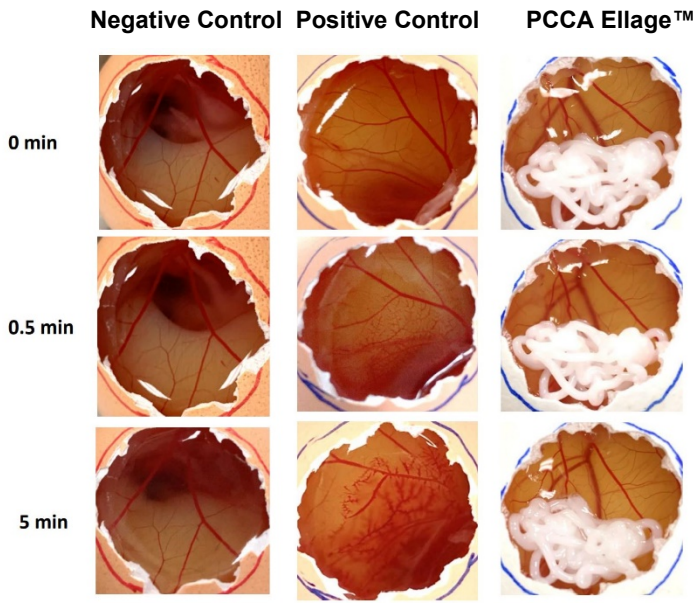

Figure 2. *Test eggs exposed to PCCA Ellage and controls (0.9% NaCl and 0.1N NaOH) for a contact time of 5 minutes.*

Conclusions:

The HET-CAM assay is perceived as an ideal *in vitro* test to evaluate the ocular irritation potential (topical toxicity) of substances. When transposed and applied to the vaginal irritation potential, this assay widens the preclinical safety assessment portfolio of vaginal products [1].

These preliminary and outsourced HET-CAM assays have shown that PCCA Ellage has no ocular irritation potential (IS<5). This finding strongly suggests that PCCA Ellage is also a non-irritant to the vaginal mucosal membrane and is thus expected to be clinically safe *in vivo*.

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Evaluation of the Irritation Potential of PCCA Ellage™ Anhydrous Vaginal *Part 2: Safety and Toxicological Profile by the MTT Assay*

SUMMARY: The evaluation of the safety and toxicological profile by the MTT assay is part of the safety assessment for PCCA Ellage™ Anhydrous Vaginal. This study builds on the HET-CAM assay (Part 1) by demonstrating that the toxic exposure time (ET_{50}) of PCCA Ellage is superior to 24 hours. It is concluded that PCCA Ellage has a good safety and toxicological profile on the vaginal mucosa, comparable to two reference OTC vaginal lubricants, and it is thus expected to be clinically safe *in vivo*.

Introduction:

The vaginal mucosa is a promising site for delivery of medication in the treatment of several conditions (e.g., vaginitis, infections) and also in hormone replacement therapy. Vaginal drug delivery, however, faces a multitude of challenges; in particular, the leakage potential of drugs due to the vaginal fluid that is continuously released. Mucoadhesive vaginal dosage forms are preferred over conventional gels and creams in order to prolong the contact time between the medication and the mucosal tissue, thus avoiding leakage and messiness. Considering the increased residence time, it is important to ensure that these mucoadhesive dosage forms are non-toxic and non-irritating to the vaginal mucosa [1].

The aim of this study was to evaluate the safety and toxicological profile of PCCA Ellage™ Anhydrous Vaginal, a mucoadhesive base designed specifically to remain in the vaginal mucosa for a longer period of time, in comparison to a positive control, the spermicide Gynol II, and two reference over-the-counter (OTC) vaginal lubricants (OTCs I&II). A 3-dimensional (3D) model of the human vaginal mucosa was used for the *in vitro* testing (Figure 1).

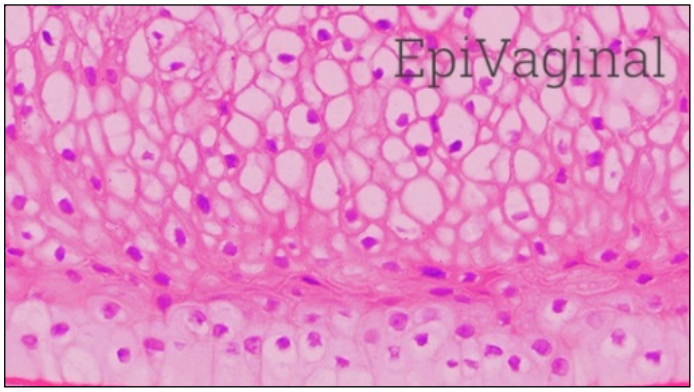


Figure 1. *Illustration of the EpiVaginal™ tissue model (adapted from MatTek Life Sciences, 2020)* [2].

Methodology:

The EpiVaginal™ (VEC-100) by MatTek Life Sciences (Ashland, MA) is a highly differentiated tissue that closely parallels the *in vivo* vaginal epithelial tissue. It is therefore an ideal *in vitro* research tool for safety and toxicological testing of feminine products [2].

The VEC-100 cells were maintained in the supplied culture media and stored in accordance to the manufacturer's protocol until the initiation of the study. Following preparation of the cells, the EpiVaginal tissues were treated in duplicate with 100 µL of the test products (PCCA Ellage, OTCs I&II, and the control) for 1, 4, 16 and 24 hours. A set of EpiVaginal tissues remained untreated (in duplicate) to serve as a negative control. Following the exposure period, the dosing solutions were removed and the cells were analyzed for cell viability by the MTT Effective Time 50 (ET_{50}) assay.

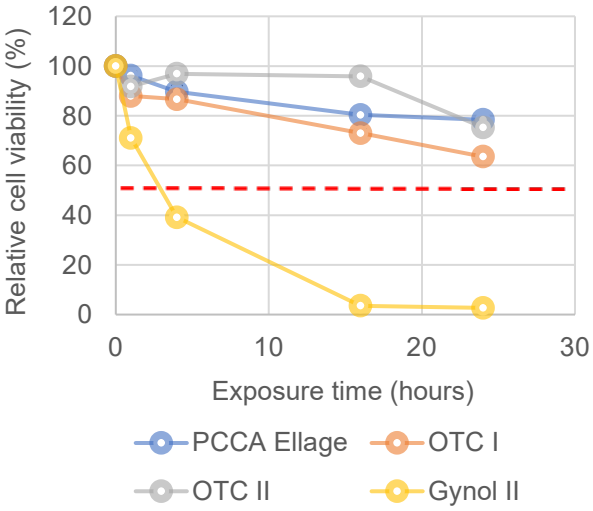
The MTT ET_{50} assay consists of measuring the reduction of MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide) by the cells. Succinate dehydrogenase enzymes within the mitochondria of viable cells have the ability to reduce soluble yellow tetrazonium salt of MTT to an insoluble purple formazan derivative. MTT is therefore an indicator of cell viability as the tissues are evaluated for their ability to reduce soluble-MTT (yellow) to formazan-MTT (purple) [3].

The MTT solution was prepared in the provided medium and added to the basal side of each tissue, followed by an incubation period of the tissues for 3 hours at 37°C. The purple formazan product was then extracted using the provided extractant, which was previously applied to both the apical and basal side of the tissues. Sample absorbance was read at 570 nm and reference absorbance at 650 nm with CLARIOstar – BMG Labtech Plate reader.

Evaluation of the Irritation Potential of PCCA Ellage™ Anhydrous Vaginal *Part 2: Safety and Toxicological Profile by the MTT Assay*

Table 1, Figure 2. *Safety and toxicological profiles of PCCA Ellage, OTCs I&II and Gynol II for up to 24 hours.*

Time (hrs)	Relative cell viability (% mean ± SD)			
	PCCA Ellage	OTC I	OTC II	Gynol II
0	100.00±3.17	100.00±3.17	100.01±3.17	100.00±3.17
1	96.13±2.01	87.95±1.03	91.81±1.60	84.75±3.21
4	89.71±0.21	86.63±9.81	96.89±1.04	39.16±3.05
16	80.35±2.33	72.99±1.55	95.87±9.82	3.54±0.10
24	78.36±0.19	63.52±1.63	75.3±17.94	2.69±0.17



Results and Discussion:

The viability of the vaginal cells following exposure to the test products is represented by the absorbance of the respective extracts and expressed in percentage relative to the negative control. The greater the absorbency of the extracts, the greater the amount of MTT reduced by succinate dehydrogenase and, as a result, the higher the percent relative cell viability within the tissue.

At the start of the study (t=0 hours), the relative viability of the cells was 100% for all the tissues. Following 16 hours, the viability of the cells exposed to the positive control was less than 5%, which means that the vaginal tissue was no longer functional and thus confirms the toxicity of Gynol II. On the contrary, the viability of the cells exposed to PCCA Ellage was 78% following 24 hours, as shown in Table 1, Figure 2.

The toxic exposure time (ET_{50}) is the time when cell viability is reduced to 50%. The ET_{50} is represented by a red dashed line in Figure 2. According to the results obtained, the ET_{50} of the positive control is approximately 3 hours, as opposed to the ET_{50} of PCCA Ellage and the OTCs I&II which are superior to 24 hours (Table 1, Figure 2). This study demonstrates that PCCA Ellage has a good safety and toxicological profile on the vaginal mucosa, comparable to the reference OTC vaginal lubricants.

Conclusions:

The safety of vaginal compounded medicines is very important since damage to the vaginal mucosa weakens the natural defenses and increases the risk of infections such as HIV and herpes simplex [4].

The safety assessment for PCCA Ellage Anhydrous Vaginal was evaluated *in vitro* by the HET-CAM (Part 1) and the MTT (Part 2) assays. Both assessments have shown that PCCA Ellage is likely to remain at the site of action for a prolonged period of time without causing damage (irritancy/toxicity) to the vaginal tissue. As such, PCCA Ellage is expected to be clinically safe *in vivo*.

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In Vitro Drug Release of *Estriol 0.1% and Testosterone 0.1%* from VersaBase® Cream and PCCA Ellage™ Anhydrous Vaginal

SUMMARY: The *in vitro* drug release, a product performance test for topical drug products, is mainly used during the research and development phase of a new product to ensure its performance and comparability to a product of reference. This test has demonstrated that estriol 0.1% and testosterone 0.1% have comparable release profiles when incorporated in the well-established PCCA VersaBase Cream *versus* the newly-developed PCCA Ellage Anhydrous Vaginal.

Introduction:

Semisolid dosage forms, such as creams and gels, may be considered extended-release preparations and their drug release depends largely on the formulation. During the research and development phase, it is important to test the *in vitro* drug release of the new product to ensure its performance and comparability to a product of reference. This test is not intended though to predict *in vivo* performance, as opposed to the skin percutaneous absorption studies, since the primary factor that impacts bioavailability and clinical performance is skin permeation. However, this test can detect *in vitro* changes, as a result of formulation differences, that may correspond to altered *in vivo* performance of the dosage form. For this reason, its main purpose and use is comparison testing in which any difference in delivery rate is undesirable [1]. This test is required by the FDA to determine the acceptability of minor process and/or formulation changes in commercially-approved semisolid dosage forms [2]. The Unites States Pharmacopoeia (USP) recognizes different apparatus for the *in vitro* drug release test in the monograph <1724> Semisolid Drug Products – Performance Tests [1].

The aim of this study was to evaluate and compare the *in vitro* drug release of the following PCCA formulas:

- Estriol 0.1% and Testosterone 0.1% in PCCA VersaBase® Cream (PCCA Formula 13931)
- Estriol 0.1% and Testosterone 0.1% in PCCA Ellage™ Anhydrous Vaginal (PCCA Formula 13845)

VersaBase Cream is a well-established and referenced compounding base for topical and vaginal hormone replacement therapy, whereas Ellage is a newly-developed anhydrous vaginal base with superior mucoadhesive and self-emulsifying properties.

Methodology:

The *in vitro* drug release test was evaluated using the Franz Diffusion System (surface area 1.77 cm^2) for a group of 6 diffusion cells, which were mounted in a diffusion apparatus including Vaginal Fluid Simulant (VFS) as the receptor medium. The study methodology was adapted from the USP monograph <1724> Semisolid Drug Products – Performance Tests [1].

Initially, the VFS was prepared to model the fluid produced in the human vagina by healthy, nonpregnant premenopausal women. The composition of the fluid medium was based on the research by Owen and Katz and includes the following ingredients: NaCl 3.51 g, KOH 1.4 g, $Ca(OH)_2$ 0.222 g, BSA 0.018 g, lactic acid 2.0 g, acetic acid 1.0 g, glycerol 0.16 g, urea 0.4 g, glucose monohydrate 5.0 g, HCl qs pH 4.2-4.5 and H_2O qs 1,000 mL [3]. The VFS was degassed by filtering through a 0.2 µm membrane and maintaining a vacuum for 2 min; it was then warmed in a water bath at 37°C. The dialysis membranes (cut off 12-14 kD) were soaked in water overnight, followed by VFS for 30 min at 37°C. The dialysis membranes were then mounted on the Franz diffusion cells (no bubbles) and applied 100 µL of VFS, prior to dosing 200 mg of the test samples (PCCA Formulas 13845 and 13931). The receptor medium solution was stirred magnetically at approximately ~600 RPM with the water jacket temperature controlled to maintain at 37 ± 1.00°C. The receptor medium samples were collected at 1, 2, 3, 4, 5 and 6 hours by stopping the stirrer, withdrawing 1 mL of sample, and replacing the same volume with VFS. All receptor medium samples were filtered with a PVDF membrane prior to quantification of estriol / testosterone by the analytical method Ultra High Performance Liquid Chromatography (UPLC) with Ultraviolet Photodiode Array (PDA).

In Vitro Drug Release of *Estriol 0.1% and Testosterone 0.1%* from VersaBase® Cream and PCCA Ellage™ Anhydrous Vaginal

It consisted of a reverse phase, gradient chromatographic method with two different mobile phases: deionized water (A) and acetonitrile (B). The chromatographic column used was an Acquity UPLC BEH C_{18} (1.7 µM) 2.1 mm x 100 mm. The injection volume was 10 µL and the flow rate was 0.5 mL/min with a run time of 5 minutes. The column temperature was maintained at 50°C and the sample tray at 6°C. The ultraviolet PDA detector was set to an acquisition wavelength of 190–400 nm, with a detection wavelength of 245 nm for testosterone and 280 nm for estriol.

Results and Discussion:

Estriol 0.1% exhibited a similar *in vitro* release profile from both Ellage and VersaBase Cream throughout the study period of 6 hours. The amount released from Ellage was higher at all time points in comparison to VersaBase Cream. By the end of the study, a total of 30.4 µg/cm^2 (27%) and 13.1 µg/cm^2 (11.5%) of estriol had been released from Ellage and VersaBase Cream, respectively (Figure 1).

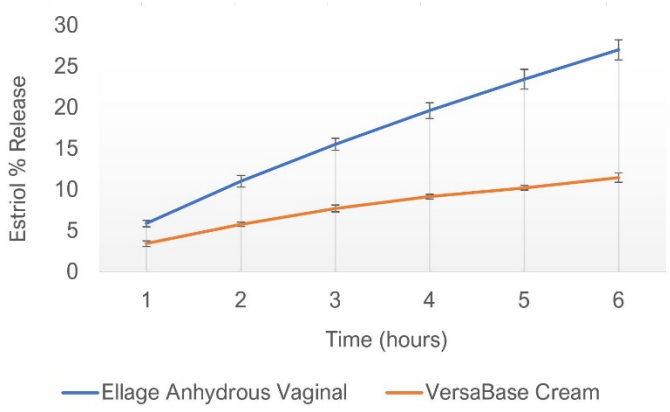

Figure 1. *In vitro percentage release of estriol from PCCA proprietary bases for 6 hours.*

Likewise, testosterone 0.1% exhibited a similar *in vitro* release profile from both Ellage and VersaBase Cream throughout the study period of 6 hours. However, the amount released from VersaBase Cream was higher at all time points in comparison to Ellage (Figure 2). By the end of the study, a total of 13.6 µg/cm2 (11.2%) and 7 µg/cm2 (6%) of estriol had been released from VersaBase Cream and Ellage, respectively (Figure 2).

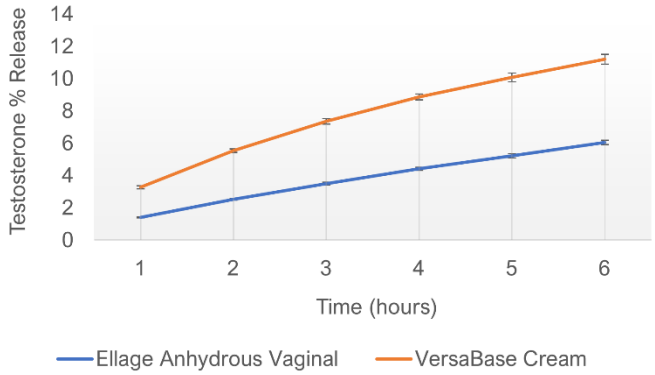

Figure 2. *In vitro percentage release of testosterone from PCCA proprietary bases for 6 hours.*

This comparative study was not designed to evaluate any statistical differences between the two PCCA proprietary bases. Instead, it is able to provide qualitative insights on the drug release performance of the bases.

Conclusions:

The *in vitro* drug release is a product performance test for topical drug products mainly used during the product research and development phase. According to the USP this test is not a measure of bioavailability but instead a demonstration of product comparability or compliance with FDA guidelines [1,2]. In the present study, the *in vitro* drug release test has demonstrated that estriol 0.1% and testosterone 0.1% have comparable release profiles when incorporated in the well-established PCCA VersaBase Cream *versus* the newly-developed PCCA Ellage Anhydrous Vaginal.

References:

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In Vitro Drug Release of *Amitriptyline 2% and Baclofen 2%* from VersaBase® Cream and PCCA Ellage™ Anhydrous Vaginal

SUMMARY: The *in vitro* drug release, a product performance test for topical drug products, is mainly used during the research and development phase of a new product to ensure its performance and comparability to a product of reference. This test has demonstrated that amitriptyline 2% and baclofen 2% have comparable release profiles when incorporated in the well-established PCCA VersaBase Cream *versus* the newly-developed PCCA Ellage Anhydrous Vaginal.

Introduction:

Semisolid dosage forms, such as creams and gels, may be considered extended-release preparations and their drug release depends largely on the formulation. During the research and development phase, it is important to test the *in vitro* drug release of the new product to ensure its performance and comparability to a product of reference. This test is not intended though to predict *in vivo* performance, as opposed to the skin percutaneous absorption studies, since the primary factor that impacts bioavailability and clinical performance is skin permeation. However, this test can detect *in vitro* changes, as a result of formulation differences, that may correspond to altered *in vivo* performance of the dosage form. For this reason, its main purpose and use is comparison testing in which any difference in delivery rate is undesirable [1]. This test is required by the FDA to determine the acceptability of minor process and/or formulation changes in commercially approved semisolid dosage forms [2]. The Unites States Pharmacopoeia (USP) recognizes different apparatus for the *in vitro* drug release test in the monograph <1724> Semisolid Drug Products – Performance Tests [1].

The aim of this study was to evaluate and compare the *in vitro* drug release of the following PCCA formulas:

- Amitriptyline 2% and Baclofen 2% in PCCA VersaBase® Cream (PCCA Formula 6991)
- Amitriptyline 2% and Baclofen 2% in PCCA Ellage™ Anhydrous Vaginal (PCCA Formula 13834)

VersaBase Cream is a well-established and referenced compounding base for topical and vaginal hormone replacement therapy, whereas PCCA Ellage is a newly-developed anhydrous vaginal base with superior mucoadhesive and self-emulsifying properties.

Methodology:

The *in vitro* drug release test was evaluated using the Franz Diffusion System (surface area 1.77 cm^2) for a group of 6 diffusion cells, which were mounted in a diffusion apparatus including Vaginal Fluid Simulant (VFS) as the receptor medium. The study methodology was adapted from the USP monograph <1724> Semisolid Drug Products – Performance Tests [1].

Initially, the VFS was prepared to model the fluid produced in the human vagina by healthy, nonpregnant premenopausal women. The composition of the fluid medium was based on the research by Owen and Katz and includes the following ingredients: NaCl 3.51 g, KOH 1.4 g, $Ca(OH)_2$ 0.222 g, BSA 0.018 g, lactic acid 2.0 g, acetic acid 1.0 g, glycerol 0.16 g, urea 0.4 g, glucose monohydrate 5.0 g, HCl qs pH 4.2-4.5 and H_2O qs 1,000 mL [3]. The VFS was degassed by filtering through a 0.2 µm membrane and maintaining a vacuum for 2 min; it was then warmed in a water bath at 37°C. The dialysis membranes (cut off 12-14 kD) were soaked in water overnight, followed by VFS for 30 min at 37°C. The dialysis membranes were then mounted on the Franz diffusion cells (no bubbles) and applied 100 µL of VFS, prior to dosing 200 mg of the test samples (PCCA Formulas 6991 and 13834). The receptor medium solution was stirred magnetically at approximately ~600 RPM with the water jacket temperature controlled to maintain at 37 ± 1.00°C. The receptor medium samples were collected at 1, 2, 3, 4, 5 and 6 hours by stopping the stirrer, withdrawing 1 mL of sample, and replacing the same volume with VFS. All receptor medium samples were filtered with a PVDF membrane prior to quantification of amitriptyline and baclofen by the analytical method Ultra High Performance Liquid Chromatography (UPLC) with Ultraviolet Photodiode Array (PDA).

In Vitro Drug Release of *Amitriptyline 2% and Baclofen 2%* from VersaBase® Cream and PCCA Ellage™ Anhydrous Vaginal

It consisted of a reverse phase, gradient chromatographic method with two different mobile phases: 0.1% formic acid in deionized water (A) and 0.1% formic acid in acetonitrile (B). The chromatographic column used was an Acquity UPLC BEH C_{18} (1.7 µM) 2.1 mm x 100 mm. The injection volume was 1 µL and the flow rate was 0.6 mL/min with a run time of 5.5 minutes. The column temperature was maintained at 65°C and the sample tray at 6°C. The ultraviolet PDA detector was set to an acquisition wavelength of 190–400 nm, with a detection wavelength of 240 nm for amitriptyline and 220 nm for baclofen.

Results and Discussion:

Amitriptyline 2% exhibited a similar *in vitro* release profile from both Ellage and VersaBase Cream throughout the study period of 6 hours. The amount released from VersaBase Cream was slightly higher at all time points in comparison to Ellage. By the end of the study, a total of 1,589.1 µg/cm^2 (68.3%) and 1,429.2 µg/cm^2 (63.4%) of amitriptyline had been released from VersaBase Cream and Ellage, respectively (Figure 1).

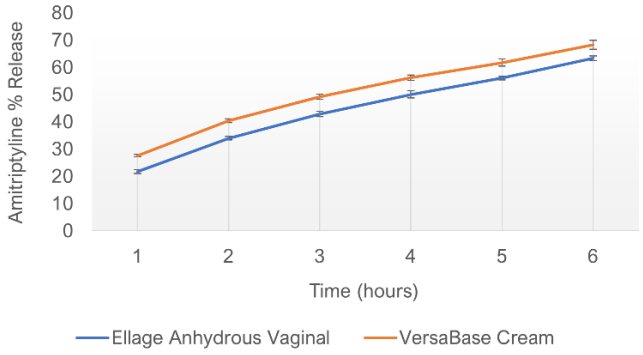

Figure 1. *In vitro percentage release of amitriptyline from PCCA proprietary bases for 6 hours.*

Likewise, baclofen 2% exhibited a similar *in vitro* release profile from both Ellage and VersaBase Cream throughout the study period of 6 hours. In this study. the amount released from Ellage was higher at all time points in comparison to VersaBase Cream. By the end of the study, a total of 1,962.3 µg/cm^2 (86%) and 1,482.6 µg/cm^2 (64.1%) of baclofen had been released from Ellage and VersaBase Cream, respectively (Figure 2).

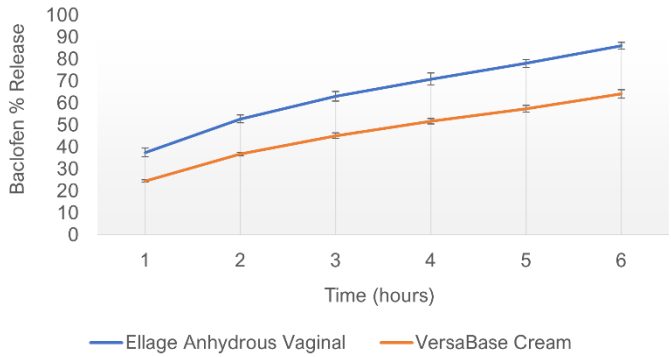

Figure 2. *In vitro percentage release of baclofen from PCCA proprietary bases for 6 hours.*

This comparative study was not designed to evaluate any statistical differences between the two PCCA proprietary bases. Instead, it is able to provide qualitative insights on the drug release performance of the bases.

Conclusions:

The *in vitro* drug release is a product performance test for topical drug products mainly used during the product research and development phase. According to the USP this test is not a measure of bioavailability but instead a demonstration of product comparability or compliance with FDA guidelines [1,2]. In the present study, the *in vitro* drug release test has demonstrated that amitriptyline 2% and baclofen 2% have comparable release profiles when incorporated in the well-established PCCA VersaBase Cream *versus* the newly-developed PCCA Ellage Anhydrous Vaginal.

References:

1. The United States Pharmacopeial Convention (2014) 'General Information / <1724> Semisolid Drug Products – Performance Tests. *USP 37 -NF 32*. Rockville: USP, p. 1273-84.

2. FDA (1997) 'Guidance Document / SUPAC-SS: Nonsterile Semisolid Dosage Forms; Scale-Up and Post-Approval Changes: Chemistry, Manufacturing and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation'. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/supac-ss-nonsterile-semisolid-dosage-forms-scale-and-post-approval-changes-chemistry-manufacturing.

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Evaluation of the Mucoadhesive Properties of PCCA Ellage™ *Part 1:* Ex Vivo *Bioadhesion Testing Using a Texture Analyzer*

SUMMARY: The bioadhesion testing on porcine vaginal tissues evaluates the ability of the test formulations to adhere to *ex vivo* biological surfaces. A comparative study was conducted by testing simultaneously the new PCCA Ellage *versus* MucoLox™/VersaBase® Gel (50:50) and an OTC long-lasting vaginal moisturizer. The three test products exhibited similar bioadhesion (differences not statistically significant), as opposed to the negative control. It is concluded that PCCA Ellage Anhydrous Vaginal has a good bioadhesion profile.

Introduction:

The vaginal mucosa offers a large surface area and rich blood supply making it a promising site for delivery of medication but it faces a multitude of challenges; in particular, the leakage potential of drugs due to the vaginal fluid that is continuously released [1]. Mucoadhesion is widely recognized as an important property in vaginal drug delivery and there are various analytical methods for the *in vitro / ex vivo* evaluation of the mucoadhesive properties of vaginal semisolid formulations. Bioadhesion, in particular, represents the ability of a formulation to adhere to a biological surface (e.g., *ex vivo* vaginal epithelium) and it is measured by the tensile force or work of adhesion using commercially available texture analysers, as the example shown in Figure 1 [2-3].

The aim of this study was to evaluate the bioadhesion properties of PCCA Ellage using porcine vaginal tissues. A comparative study was conducted by testing simultaneously the well-established combination MucoLox/VersaBase Gel (50:50) and the over-the-counter (OTC) vaginal moisturizer of reference that claims to be long-lasting (up to 3 days).

Figure 1. *CTX Texture Analyzer (Ametek Brookfield, USA) equipped with a load cell of 1.5 Kg.*

Methodology:

Frozen *ex vivo* porcine vaginal tissues were obtained from BioIVT (Westbury, NY) and reserved in an airtight bag at -20ºC. A Vaginal Fluid Simulant (VFS) was prepared to model the fluid produced in the human vagina by healthy, nonpregnant premenopausal women [4]. Prior to the experiment, the vaginal tissues were thawed at room temperature and cut to excise the epithelium surface. Using a biopsy scalpel punch, the virginal epithelia was further cut into a surface area of approximately 10-mm diameter.

The bioadhesion testing was evaluated using the CTX Texture Analyzer (Ametek Brookfield, USA) equipped with a load cell of 1.5 Kg (Figure 1). The three test products [PCCA Ellage, MucoLox/VersaBase Gel (50:50) and the OTC vaginal moisturizer] were premixed with the VFS. For each experiment, a sample of 0.05 mL of test product in VFS was added to the cellulose acetate membrane, which was fixed to the texture analyzer using a mucoadhesion rig. A total of 6 replicates were carried out per test product. A negative control was used by adding 0.05 mL of VFS to the cellulose acetate membrane. All experiments were conducted at the body temperature of 37ºC.

The porcine epithelia was attached to the cylinder probe of the texture analyzer using cyanoacrylate glue. For each experiment, the cylinder probe was lowered at a speed of 2.5 mm/min using a trigger force of 1 g, followed by a force of 60 g to bring the epithelia into contact with the test product. The contact/hold time between the porcine epithelia and the test product was 3 min. The cylinder probe was then lifted at a speed of 2.5 mm/min to separate the epithelia from the test product. The work of adhesion (g·mm) was calculated as the area under the force *versus* the displacement curve.

Evaluation of the Mucoadhesive Properties of PCCA Ellage™ *Part 1:* Ex Vivo *Bioadhesion Testing Using a Texture Analyzer*

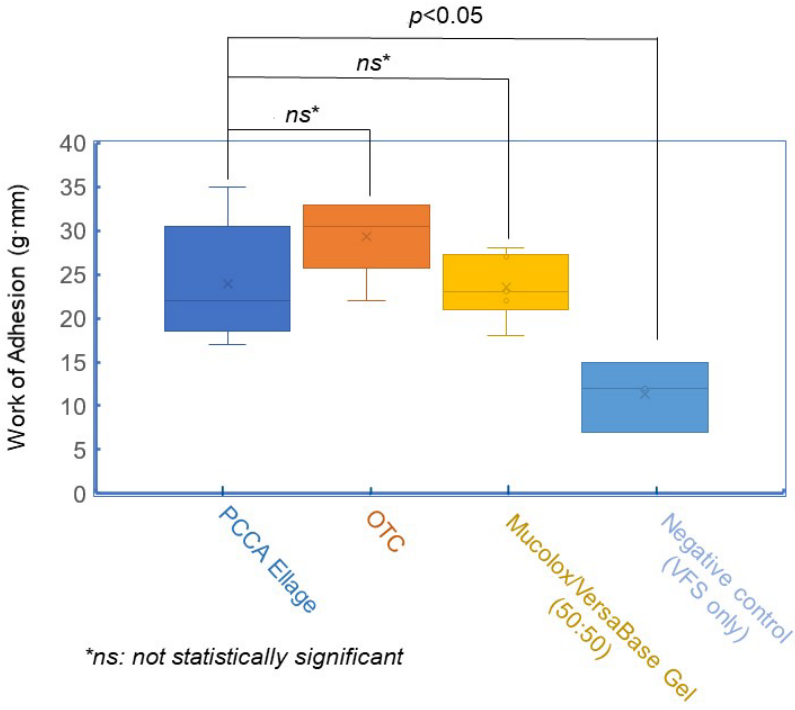

Figure 2. *Box plot: work of adhesion for the three test products and the negative control:*

Each box represents the values between the first and the third quartile.

The whiskers represent the distances between the minimum and maximum work of adhesion obtained for the 6 replicates.

The median is shown as a line and the calculated average is shown as a cross within each box.

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Results & Discussion:

The average work of adhesion obtained for each test product (6 replicates) is very similar, as follows: PCCA Ellage 24 ± 6.78 g·mm; MucoLox/VersaBase Gel (50:50) 24 ± 3.62 g·mm; and OTC 29 ± 4.23 g·mm. These differences are not statistically significant which demonstrate that PCCA Ellage has a good bioadhesion profile, similar to the well-established combination MucoLox/VersaBase Gel (50:50) and the OTC long-lasting vaginal moisturizer. The standard deviations obtained are likely due to variations of the excised epithelium surface.

In contrast, the work of adhesion for the negative control is much lower (11 ± 4.04 g·mm) and it is statistically significant (p<0.05), which shows that the VFS alone has minimum bioadhesion. Despite its limitations, this analytical method is a valuable, reliable and reproducible tool to determine the mucoadhesive properties of vaginal semisolid formulations.

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Evaluation of the Mucoadhesive Properties of PCCA Ellage™ *Part 2:* Ex Vivo *Testing on Animal Vaginal Tissues*

SUMMARY: The *ex vivo* testing on animal vaginal tissues, together with the bioadhesion testing (PCCA document 99836), evaluate the mucoadhesive properties of PCCA Ellage. A comparative study was conducted by testing simultaneously the well-established combination MucoLox™/VersaBase® Gel (50:50) and a reference OTC long-lasting vaginal moisturizer. It was concluded that PCCA Ellage, as well as MucoLox/VersaBase Gel (50:50), exhibit prolonged mucoadhesion to *ex vivo* animal vaginal tissues.

Introduction:

The vaginal mucosa offers a large surface area and rich blood supply making it a promising site for delivery of medication but it faces a multitude of challenges; in particular, the leakage potential of drugs due to the vaginal fluid that is continuously released [1].

A series of studies were conducted to evaluate the mucoadhesive properties of PCCA Ellage Anhydrous Vaginal. The *Part 1: Ex Vivo Bioadhesion Testing Using a Texture Analyzer* (PCCA document 99836) has demonstrated that PCCA Ellage has a good bioadhesion profile. On the other hand, the leakage test (PCCA document 99816) has shown that PCCA Ellage, and its corresponding PCCA formulas (13834 and 13845), have high retention potential *in vitro* (agar plates at 37°C).

The aim of this study was to build on the existing results by evaluating the mucoadhesive properties of PCCA Ellage using *ex vivo* animal vaginal tissues. A comparative study was conducted by testing simultaneously the well-established combination MucoLox/VersaBase Gel (50:50) and the over-the-counter (OTC) vaginal moisturizer of reference that claims to be long-lasting (up to 3 days).

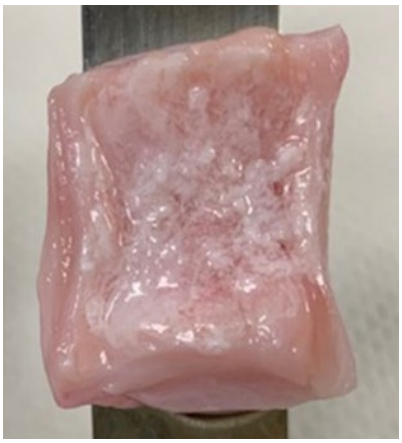

Figure 1. *Ex vivo animal vaginal tissue exposed to PCCA Ellage (t= 0 min).*

Methodology:

Frozen pig vaginal tract (BioIVT, Westbury) was cut into small pieces with a square mucosal surface area of approximately 2x2 cm. The *ex vivo* vaginal tissues were washed with Hanks' Balanced Salt Solution (HBSS) and equilibrated at 37°C in HBSS prior to the experiment.

A Vaginal Fluid Simulant (VFS) was prepared to model the fluid produced in the human vagina by healthy, nonpregnant premenopausal women [2]. The *ex vivo* vaginal tissues were fixed on stainless steel using cyanoacrylate glue and were washed with the VFS. The fluorescent dye fluorescein sodium (Sigma-Aldrich, F6377) was dissolved in ethanol and a trace amount was added to the three products: PCCA Ellage, MucoLox/VersaBase Gel (50:50), and the OTC long-lasting vaginal moisturizer. Each fluorescently labeled product was directly applied on the top of the *ex vivo* vaginal tissues (50 µL/cm^2) and it was spread evenly using a pellet pestle. A control test was prepared by applying PCCA Ellage (without the fluorescent dye) to the *ex vivo* vaginal tissues (50 µL/cm^2), as displayed in Figure 1.

The fluorescently labelled and control tissues were rinsed intermittently in VFS by dipping the stainless steel up and down, three consecutive times, at the following pre-determined time points: 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 40 min, 50 min, 60 min, 70 min, 80 min and 90 min. The labelled tissues were observed under UV light at 390 nm and images were acquired at the same lighting and angle using an iPhone camera.

This test was conducted at the PCCA R&D Laboratory in accordance to the *in vitro* studies by Song *et al.* and Cazorla-Luna *et al.* [3,4].

Evaluation of the Mucoadhesive Properties of PCCA Ellage™ *Part 2:* Ex Vivo *Testing on Animal Vaginal Tissues*

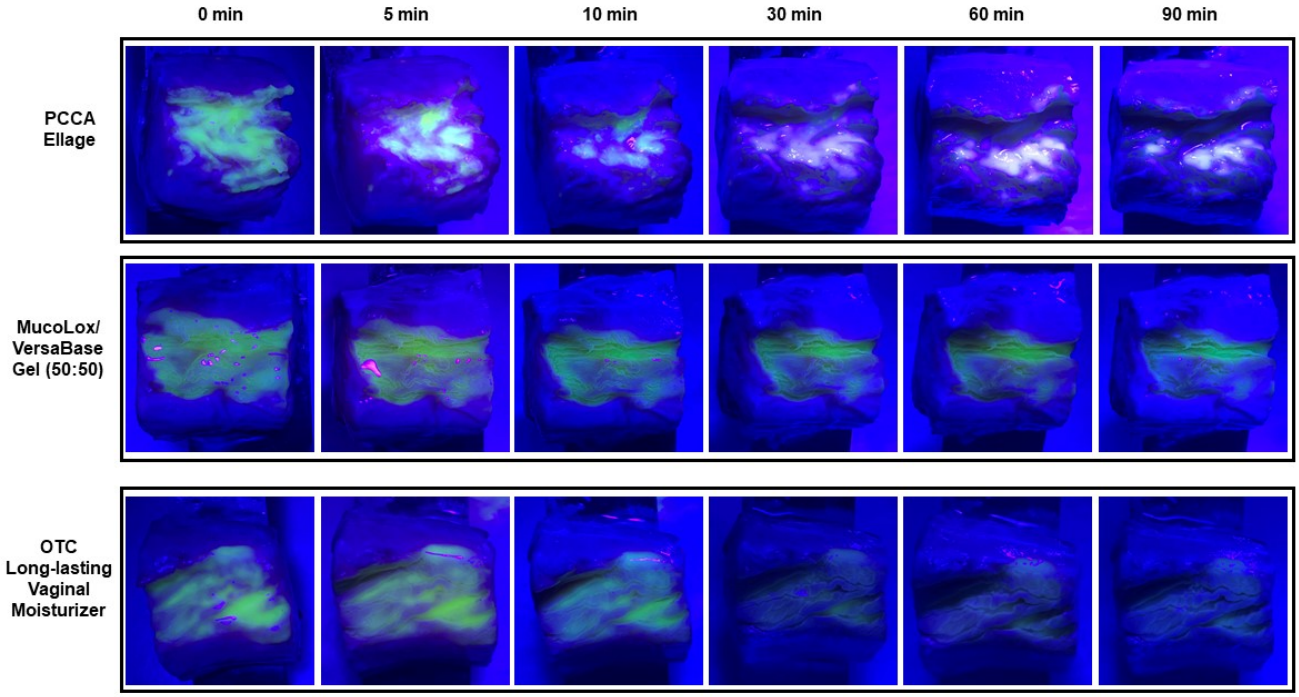

Figure 2. *Ex vivo animal vaginal tissues exposed to 3 products (PCCA Ellage, MucoLox/VersaBase Gel (50:50) and an OTC Long-lasting Vaginal Moisturizer), dyed with fluorescein (green color) and analysed for a study period of 90 minutes.*

Results & Discussion:

The testing products MucoLox/VersaBase Gel (50:50) and OTC are both clear, as opposed to PCCA Ellage which is white color. For this reason, the fluorescent dye was needed to visualize the mucoadhesion properties of the clear testing products. As shown in Figure 2, the MucoLox/VersaBase Gel (50:50) exhibited longer mucoadhesion at all time points in comparison to the OTC, which showed almost no green color by the end of the study.

The fluorescent dye was added to the three testing products in order to expose the *ex vivo* tissues to the same experimental conditions. However, since PCCA Ellage is an anhydrous vaginal base and the fluorescein sodium is water soluble, it was to be expected that the green color would fade with the intermitted rinsing in VFS. However, the white color shown in the control test (Figure 1) was clearly visible at all time points in Figure 2, which demonstrates that PCCA Ellage exhibits prolonged mucoadhesion to *ex vivo* vaginal tissues.

References:

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Evaluation of the Self-Emulsifying Properties of PCCA Ellage™ *Part 1: Microscopic Evaluation*

SUMMARY: The self-emulsifying properties of PCCA Ellage were evaluated under the microscope and compared to VersaBase® Cream and PCCA Plasticized™. Upon mixture with VFS and incubation at 37°C, PCCA Ellage created a spontaneous emulsion and the resulting droplets decreased in size with prolonged incubation. On the contrary, there were no changes in the properties for VersaBase Cream and PCCA Plasticized. PCCA Ellage exhibited its self-emulsifying properties even in the presence of small amounts of VFS, a very important finding taking into account the variable amounts of vaginal fluids in women.

Introduction & Methodology:

PCCA Ellage Anhydrous Vaginal is an innovative proprietary base with self-emulsifying properties that creates a spontaneous emulsion when it comes into contact with water from vaginal fluids. This emulsion releases the active ingredients from the base to the mucosa. Once the active ingredients are released, the emulsifier system in the base also holds the drugs to the surface and increases the contact time. This novel and promising technology, named Self-Emulsifying Drug Delivery Systems (SEDDS), has the potential to maximize drug solubility and bioavailability [1-2].

Initially, two laboratory tests were performed at PCCA R&D to evaluate the SEDDS, in particular the formation of droplets, for PCCA Ellage in comparison with other test products. Initially, PCCA Ellage, VersaBase Cream and PCCA Plasticized were mixed gently with Vaginal Fluid Simulant (VFS) in a 1:2 ratio (w/v). Following 5 min of incubation at 37°C, a sample of each vial was observed under a light microscope with a total magnification of 100x. Afterwards, 10 µL of VFS at 37°C were placed on glass slides and added were equal volumes of the same test products (1:1 ratio). The mixtures were spread with a pipette tip, incubated at 37°C for 1 min and 5 min, and protected with a cover slip for microscopic evaluation at a magnification of 100x (10x objective lens and 10x ocular lens).

Afterwards, a third laboratory test was performed at PCCA R&D to evaluate the formation of droplets with increasing volumes of VFS, as the research by Rohrer *et al*. [3]. PCCA Ellage was mixed gently with VFS (90:10, 75:25 and 50:50 ratios) and, following 5 min of incubation at 37°C, a sample of each mixture was observed under a light microscope with a total magnification of 10x.

Results & Discussion:

PCCA Plasticized, as expected, did not mix with the VFS because it is an anhydrous gel. VersaBase, on the other hand, is an emulsifying cream and, as such, it made a homogeneous emulsion upon gentle mixing with VFS. PCCA Ellage showed a similar behavior to VersaBase Cream, as shown in Figure 1.

Under the microscope, it was observed that the original PCCA Ellage and PCCA Plasticized do not have any droplets, as expected, whereas VersaBase Cream contains a large number of droplets sized around or under 10 µm. Following mixture with VFS and incubation (1-5 min), there were no significant changes for VersaBase Cream. Likewise, there were no significant changes for PCCA Plasticized as the oil and water phases were kept separated, as shown in Figure 2. PCCA Ellage, on the contrary, developed droplets when mixed with the VFS and these droplets decreased in size from 1 min to 5 min of incubation at 37°C. This phenomenon is the process of self-emulsifying which is a unique property of PCCA Ellage. This process took place also when the active pharmaceutical ingredients (APIs) amitriptyline 2% and baclofen 2% were incorporated into PCCA Ellage (Figure 2).

The self-emulsifying properties for PCCA Ellage are observed even in the presence of small amounts of VFS. As shown in Figure 3, droplets were developed in all three mixtures (90:10, 75:25 and 50:50 ratios for PCCA Ellage:VFS). This property is very important in the clinical practice taking into account the variable amounts of vaginal fluids in women. When these fluids are low, depending on the menstrual cycle or in the presence of vaginal dryness, PCCA Ellage is expected to exhibit its self-emulsifying properties *in vivo* upon application to the vaginal mucosa.

Evaluation of the Self-Emulsifying Properties of PCCA Ellage™ *Part 1: Microscopic Evaluation*

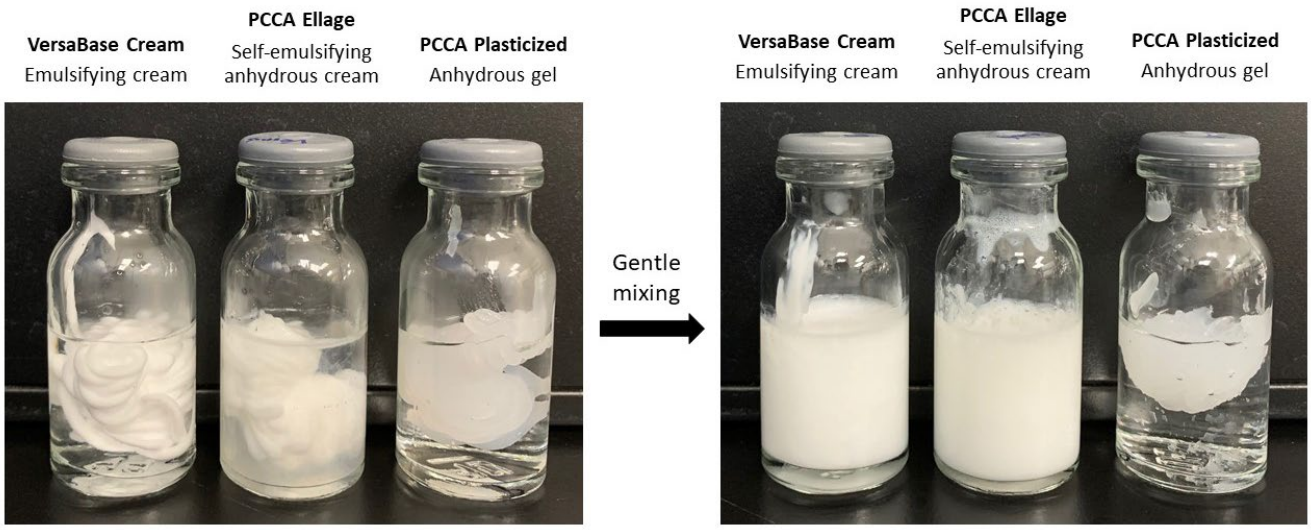

Figure 1. *Mixture of PCCA proprietary bases with VFS (1:2 ratio).*

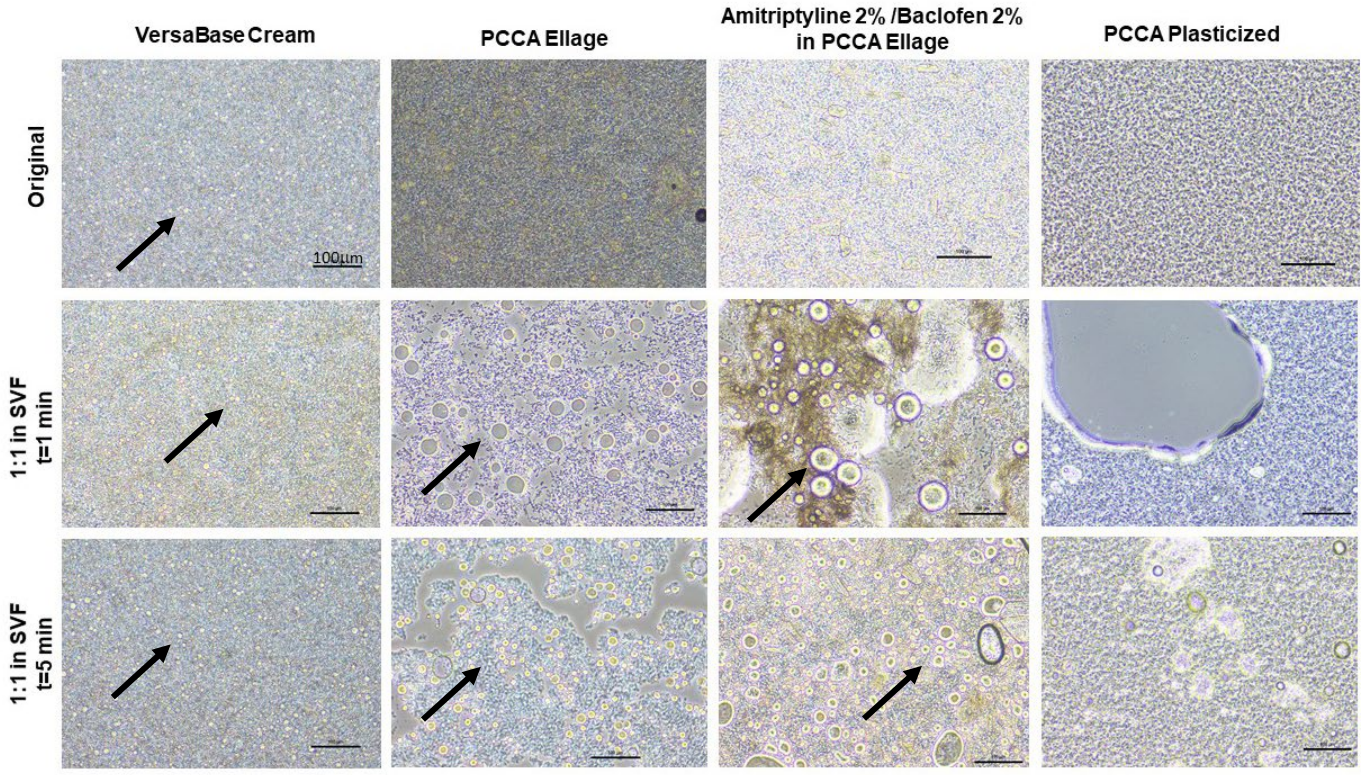

Figure 2. *Microscopic evaluation of droplet size formation (100x) for VersaBase Cream, PCCA Plasticized and PCCA Ellage, with and without APIs (selected droplets highlighted with arrows).*

Evaluation of the Self-Emulsifying Properties of PCCA Ellage™
Part 1: Microscopic Evaluation

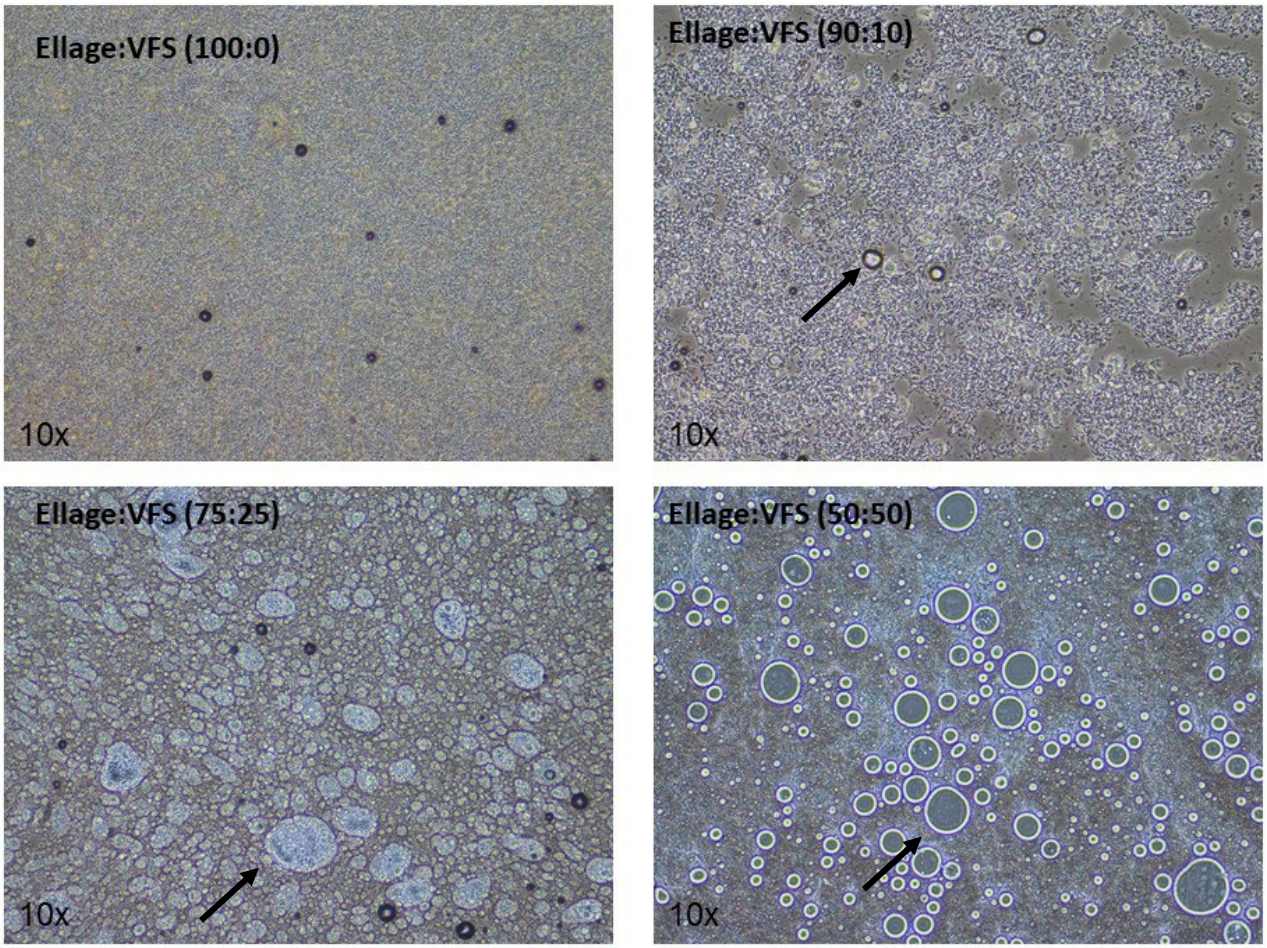

Figure 3. *Microscopic evaluation of droplet size formation (10x) for PCCA Ellage with increasing volumes of VFS (selected droplets highlighted with arrows).*

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[1] Vasconcelos, T., Marques, S. and Sarmento, B. (2018) 'Measuring the emulsification dynamics and stability of self-emulsifying drug delivery systems'. *European Journal of Pharmaceutics and Biopharmaceutics*, 123 (1-8). doi: 10.1016/j.ejpb.2017.11.003.

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Evaluation of the Self-Emulsifying Properties of PCCA Ellage™ *Part 2: Fluorescence Microscopy*

SUMMARY: The distribution pattern of lipophilic and hydrophilic substances in PCCA Ellage, with and without VFS, was evaluated by fluorescence microscopy (white and green fluorescent lights). Curcumin 1% (lipophilic) and fluorescein sodium 0.02% (hydrophilic) distributed evenly in PCCA Ellage alone but exhibited opposite behaviors when PCCA Ellage was gently mixed with VFS.

Introduction & Methodology:

PCCA Ellage Anhydrous Vaginal (Figure 1) is an innovative proprietary base with self-emulsifying properties that creates a spontaneous emulsion when it comes in contact with water from vaginal fluids. This emulsion releases the active ingredients from the base to the mucosa. Once the active ingredients are released, the emulsifier system in the base also holds the drugs to the surface and increases the contact time. This novel and promising technology, named Self-Emulsifying Drug Delivery Systems (SEDDS), has the potential to maximize drug solubility and bioavailability [1]. The purpose of this study is to explore further the self-emulsifying properties of PCCA Ellage by evaluating the distribution pattern of substances with different solubilities. A fluorescence microscopy test was performed at PCCA R&D using hydrophilic and lipophilic substances incorporated in PCCA Ellage. Fluorescein sodium (Sigma-Aldrich, #F6377) was the hydrophilic substance used (1 mg/mL water solubility) whereas curcumin (PCCA #C30-3497) was the lipophilic substance used (insoluble in water) [2]. These substances were selected for their fluorescence properties and represent both hydrophilic and lipophilic active pharmaceutical ingredients (APIs) that will be used in clinical practice.

Figure 1. *Photograph of PCCA Ellage 500 g container with TopiClick and applicator.*

The test formulations were prepared by adding fluorescein sodium 0.02%, or curcumin 1%, with glycerin 5% to PCCA Ellage. The formulations were mixed using the EMP at a setting of 5 for 2 min. A sample from each was saved for fluorescence microscopy. The test formulations were then gently mixed with a Vaginal Fluid Simulant (VFS) – 1:2 ratio (w/v) – at 37°C. Within 5 min from extemporaneous preparation, samples from both test formulations, with and without VFS, were observed under the microscope with white light and also green fluorescent light. Photographs were taken using a Nikon Eclipse TS100 inverted phase microscope coupled with the NIS-Elements imaging software.

Results & Discussion:

The hydrophilic and lipophilic substances distributed evenly in PCCA Ellage when mixed for 2 min in the EMP, as shown by the homogeneous fluorescence under green fluorescent light (Figure 2). Upon mixing with VFS at 37°C, PCCA Ellage exhibited its unique self-emulsifying properties by creating an emulsion, i.e., dispersed lipophilic droplets in an aqueous continuous phase. As expected, the hydrophilic and lipophilic substances displayed opposite behaviors within the self-emulsifying cream, as shown in Figure 3. The insoluble curcumin was encapsulated inside the droplets whereas the soluble fluorescein sodium remained in the aqueous phase, outside the droplets (examples highlighted with arrows for both white and green fluorescent lights). In clinical practice, lipophilic APIs are thus expected to exhibit a slow drug release from the PCCA Ellage Anhydrous Vaginal due to the encapsulation whereas hydrophilic APIs are expected to exhibit a fast drug release.

Evaluation of the Self-Emulsifying Properties of PCCA Ellage™ *Part 2: Fluorescence Microscopy*

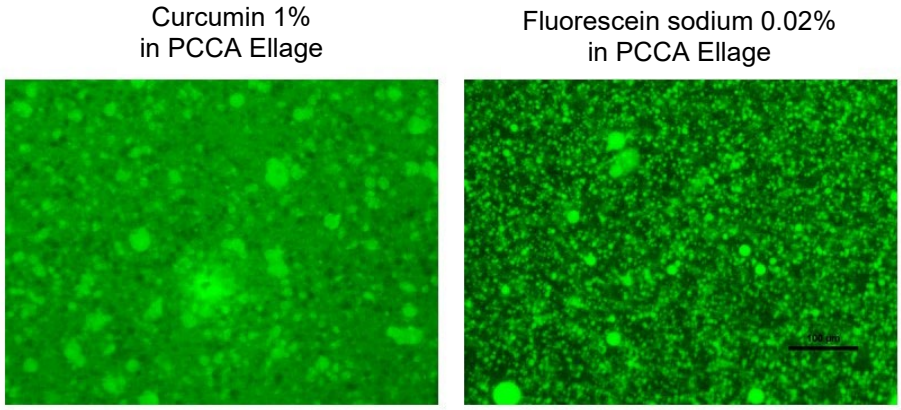

Figure 2. *Fluorescence microscopy (green fluorescent light) for curcumin 1% and fluorescein sodium 0.02% in PCCA Ellage.*

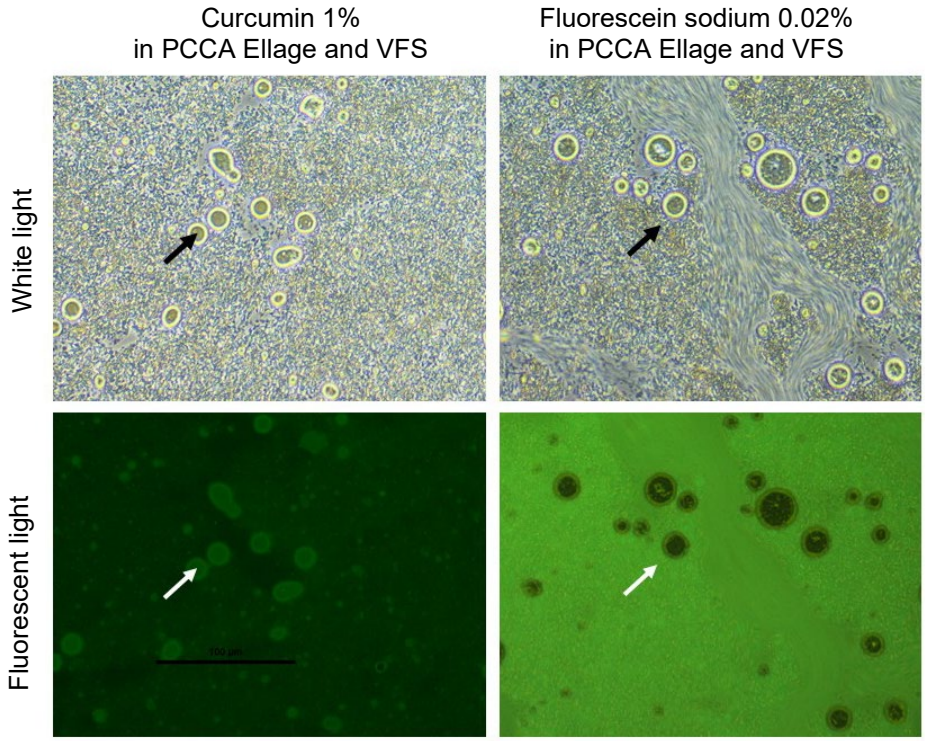

Figure 3. *Fluorescence microscopy (white and green fluorescent lights) for curcumin 1% and fluorescein sodium 0.02% in PCCA Ellage with VFS. Arrows highlight the interior of selected droplets for curcumin and the exterior of selected droplets for the fluorescein sodium, where the lipophilic and hydrophilic substances are respectively located.*

[1] Vasconcelos, T., Marques, S. and Sarmento, B. (2018) 'Measuring the emulsification dynamics and stability of self-emulsifying drug delivery systems'. *European Journal of Pharmaceutics and Biopharmaceutics*, 123 (1-8). doi:10.1016/j.ejpb.2017.11.003.

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Scientific Posters

POSTER

Development of a mucoadhesive, anhydrous and self-emulsifying vaginal base for compounded medications

12th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology (PBP) 2021

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pccarx.com/pdf_files/100145_Poster_Ellage-DevVagBase.pdf

Author(s): Daniel Banov, Maria Carvalho, Guiyun Song, Yi Liu, Christine Vu, Kendice Ip, and Ashley Shan

Introduction: The vaginal mucosa offers a large surface area and rich blood supply, making it a promising site for delivery of medication in the treatment of several conditions and also in hormone replacement therapy. However, vaginal drug delivery faces multiple challenges; in particular, the leakage potential of drugs due to the vaginal fluid that is continuously released [1]. Conventional dosage forms such as creams, gels, and foams have limited contact time with the vaginal mucosa and are commonly runny or messy, especially the vaginal gels [2]. Vaginal compounded medications offer an alternative treatment option that is customized to meet the individual needs of each and every woman. The purpose of our research was to develop a mucoadhesive, anhydrous and self-emulsifying base for the preparation of vaginal compounded medications. Mucoadhesive to increase the contact time between the medication and the vaginal mucosa, potentially improving patient acceptance and compliance. Self-emulsifying to create a spontaneous emulsion when it comes into contact with water from vaginal fluids. This emulsion then releases the active pharmaceutical ingredients (APIs) from the base to the vaginal mucosa. Once the APIs are released, the emulsifier system in the base also holds the drugs to the surface and increases the contact time. This novel and promising technology, named Self-Emulsifying Drug Delivery Systems (SEDDS), has the potential to maximize drug solubility and bioavailability.

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Journal Articles

Vulvovaginal Symptoms in a Postmenopausal Woman: A Case Study

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Abstract

A postmenopausal female patient was suffering from vulvovaginal symptoms such as dryness and irritation, which were affecting her relationship with her partner and her overall quality of life. The patient was instructed to apply an estriol 0.1% vaginal ointment (PCCA Ellage Anhydrous Vaginal) for a duration of three months. The safety and efficacy of the compounded treatment were evaluated using an online data collection form, which included the validated Vulvovaginal Symptom Questionnaire. Post-treatment results show that the vulvovaginal symptoms were no longer bothersome, and that the patient's relationship was no longer affected. There were no reports of undesirable effects as a result of the compounded treatment. This case study reinforces the benefits and convenience of using topical hormone replacement therapy in postmenopausal women.

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Compounding pharmacists around the world specialize in customizing medications to meet patient specific needs. Although compounded medications make up for only a small subset of the prescriptions filled, the art and science of compounding is relevant and necessary in almost every medical specialty. In particular, the area of women's health has always been a medical specialty that has lent itself to compounding due to the increase in popularity over the years for customized hormone replacement therapy (HRT). In addition to HRT, customization of prescriptions for vulvovaginal symptoms such as dryness, infectious disease, anorgasmia, and lichen sclerosus, among others, has also increased over the years. Practitioners have been able to use compounded medications for these conditions utilizing specific drugs and strengths of choice based off clinical data from the medical literature, as well as evidenced-based data. Out of the many potential vulvovaginal symptoms that patients may experience, vaginal dryness as a result of tissue atrophy is one of the most common presentations of the estrogen-deficient patient. One study estimates that one year after menopause the prevalence of vaginal dryness in patients is approximately 62% to 67%.[1] In another study, it was stated that "The impact of vaginal dryness on interpersonal relationships, quality of life, daily activities, and sexual function can be significant, but is frequently underestimated."[2] Estriol, a natural metabolite of estradiol, has been shown to be effective as a treatment option for vulvovaginal atrophy even when used at low doses.[3]

When a prescription is compounded for a topical or intra-vaginal medication, many factors related to formulation development must be considered by the compounding pharmacist. For instance, the formulation's:

- pH,
- viscosity,
- tissue adherence,
- drug delivery,
- risk of irritability, and
- compatibility with the active pharmaceutical ingredients (APIs).

All of these play a role in the safety and efficacy of the final compounded medication, in addition to patient acceptance and compliance. Most of these properties are dependent on the base (vehicle) that is chosen as the delivery system for the APIs. The beyond-use date (BUD) of the final compounded medication is also affected by the choice of the base. For instance, bases that contain water, or present a water activity equal or great than 0.06, will typically be given a much shorter BUD than their anhydrous counterparts due to the increased risks of pH changes and oxidation of the APIs that can occur in the presence of water.

PCCA Ellage Anhydrous Vaginal (Figure 1) is a proprietary compounding base developed specifically for vaginal applications. It is mucoadhesive, anhydrous, and self-emulsifying, and it has been shown to have a low potential for irritation.[4,5] PCCA Ellage is an ideal base for the intravaginal administration of hormones, anti-infectives, and other APIs or substances indicated in the treatment of vulvovaginal symptoms.

Case Report

A 66-year-old postmenopausal female patient was diagnosed with vaginal dryness and irritation. Additional vulvovaginal symptoms included pain and bleeding on intercourse. The physician prescribed estriol 0.1% vaginal ointment in PCCA Ellage, to be applied daily for two weeks, then every other day for two months, followed by twice weekly. The formula and method of preparation for the compounded vaginal ointment are detailed in the formula and method of preparation for estriol 0.1% vaginal ointment included in this article.

An online data collection form was developed on Microsoft Forms, a web platform that uses built-in analytics to evaluate responses and exports results to Microsoft Excel for additional data analysis. Initial questions addressed the patient's condition and treatment. For instance, "Please describe your condition to be treated with the compounded medication(s)." and "Which compounded medication(s) did you use to treat your condition?".

FIGURE 1.

BASE, PCCA ELLAGE ANHYDROUS VAGINAL WITH OPEN LID, TOPICLICK DEVICE, AND VAGINAL APPLICATOR.

Photo courtesy of the Professional Compounding Centers of America, Houston, Texas.

PATIENT'S TESTIMONIAL

"Wonderful result! The bleeding and dryness went away and I noticed that the vulva tissues were much more flexible and healthy feeling."

The Vulvovaginal Symptom Questionnaire (VSQ) was included in the online data collection form to measure the efficacy of the compounded vaginal ointment. The VSQ is a validated research instrument developed by Erekson et al[6] to measure specifically vulvovaginal symptoms and disease-specific quality-of-life in postmenopausal women. This questionnaire includes 21 items grouped in four distinct areas:

1. **Symptoms** (7 questions),
2. **Emotions** (4 questions),
3. **Life-impact** (5 questions), and
4. **Sexual impact** (5 questions, if applicable).

Responses to these questions are dichotomous (Yes/No) and self-explanatory (e.g., Have you been bothered by your vulva being dry?)[6] Scoring is determined by a change in a "Yes/No" answer before treatment to a "No/Yes" answer post-treatment.

A specific question regarding undesirable effects was also included the online data collection form to evaluate the safety of the compounded estriol vaginal ointment, as follows: "Did you experience any undesirable effects as a result of the compounded medication(s)?".

The patient was instructed to complete the online data collection form twice: 1) before treatment and 2) three months post-treatment to measure the safety and efficacy of the compounded estriol vaginal ointment.

Postmenopausal women commonly suffer from chronic vaginal atrophy, which is characterized by thinning, drying, and inflammation of the vaginal walls due to estrogen deficiency. Vaginally administered estriol induces normalization of the vaginal epithelium and thus helps to restore the normal microflora and a physiological pH in the vagina.

Results and Discussion

According to the VSQ prior to treatment, the patient was bothered by the following vulvovaginal symptoms: pain, irritation, bleeding, and dryness. These symptoms affected the patient's interaction with others, her desire to be with other people, and her sexual activity. The patient reported that she had recently initiated a relationship with a previous partner, following several years of abstinence, and her condition was having a profound impact on them. There were no complaints regarding vulvovaginal itching, burning, or stinging.

The patient applied the compounded estriol vaginal ointment as instructed for a duration of three months and noticed remarkable improvements on her vulvovaginal symptoms. The patient's testimonial was as follows: *Wonderful result! The bleeding and dryness went away and I noticed that the vulva tissues were much more flexible and healthy feeling.* According to the VSQ after treatment, the vulvovaginal pain, irritation, bleeding, and dryness were no longer bothersome. Likewise, the patient's interaction with others and her sexual activity were no longer affected. The patient's response to all these questions changed from a "Yes" to a "No".

When estriol is incorporated in PCCA Ellage, it is expected that the vaginal ointment adheres to the mucosa for an extended period of time, despite the regular secretions of vaginal fluid, due to the mucoadhesive properties of the base. There were no reports of undesirable effects as a result of the compounded

Rx

ESTRIOL 0.1% VAGINAL OINTMENT

For 100 g

Estriol USP Micronized (PCCA; E3)	0.1 g
Glycerin USP (Natural)	5 g
Base, PCCA Ellage Anhydrous Vaginal	94.9 g

Note: An alternative to step 3 in the method of preparation is to mix all ingredients for 2 minutes in an electronic mortar and pestle with a medium setting.

METHOD OF PREPARATION

1. Calculate the required quantity of each ingredient for the total amount to be prepared.
2. Weigh and/or measure each ingredient accurately.
3. Mix the estriol with the Glycerin USP, using a mortar and pestle to make a smooth paste.
4. Bring to final weight with Ellage Anhydrous Vaginal and mix until uniform.
5. Store in an air-tight, light-resistant container.

estriol vaginal ointment, which is consistent with the expected safety profile of the estriol vaginal ointment in PCCA Ellage.

Conclusion

Postmenopausal women commonly suffer from chronic vaginal atrophy, which is characterized by thinning, drying, and inflammation of the vaginal walls due to estrogen deficiency.[7] Vaginally administered estriol induces normalization of the vaginal epithelium and thus helps to restore the normal microflora and a physiological pH in the vagina.[8] Estriol therapy is described as a safe and effective therapy to alleviate the signs and symptoms of vaginal atrophy.[9,10] Pharmaceutical compounding allows estriol vaginal therapy to be customized to meet the patient's variable hormone needs. When estriol is incorporated in PCCA Ellage, it is expected that the vaginal ointment adheres to the mucosa for an extended period of time, despite the regular secretions of vaginal fluid, due to the mucoadhesive properties of the base. This case study reinforces the benefits and convenience of using topical hormone replacement therapy in postmenopausal women.

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Comprehensive Evaluation of a Mucoadhesive Self-Emulsifying Anhydrous Base for Vaginal Drug Delivery

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Abstract

Background/Objectives: Compounded vaginal creams are widely used for conditions such as hormone replacement therapy, vaginal dryness, low libido, vaginal infections, etc. Recent research highlights the potential of using anhydrous bases to extend shelf life, particularly when combined with self-emulsifying and mucoadhesive properties that improve mucosal retention and enhance drug bioavailability. This study provides in vitro and ex vivo evaluation of an anhydrous vaginal base. **Methods**: Key quality indicators such as irritation potential, leakage potential, pH compatibility, mucoadhesion, and self-emulsification were assessed using the chorioallantoic membrane Hen's Egg Test, MTT assay, texture analysis, and fluorescence microscopy. **Results**: The anhydrous vaginal base demonstrated high cell viability (>78%) and non-irritant potential (IS = 2.5) in in vitro assays. It maintained physiological vaginal pH (4.56 $\pm$ 0.05), showed strong mucoadhesive properties comparable to commercial products, and exhibited minimal leakage. Ex vivo studies confirmed its prolonged retention on vaginal tissues. The anhydrous vaginal base formed stable emulsions upon contact with vaginal fluid simulant, effectively distributing both lipophilic and hydrophilic compounds. **Conclusions**: Compared to water-containing bases, an anhydrous vaginal base shows advantages: longer retention time and lower leakage; adaptability to varying vaginal fluid levels; and efficient dispersion of both hydrophilic and lipophilic active pharmaceutical ingredients. These features support its potential use in compounded vaginal products, minimizing stability risks and enhancing patient compliance and therapeutic outcomes.

Keywords: vaginal drug delivery; mucoadhesive properties; self-emulsifying drug delivery systems; compounded formulations; bioadhesion testing

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1. Introduction

Pharmaceutical compounding offers a flexible approach to treating various gynecological conditions and supporting women's health by allowing healthcare providers to tailor formulations to meet specific patient needs. The ability to customize oral, topical, injectable, rectal, and vaginal dosage forms, their concentration, and combinations of active pharmaceutical ingredients (APIs) is particularly important [1]. According to current trends in pharmacy compounding for women's health [2], the most common indications include

hormone replacement therapy [3], vaginal dryness [4], low libido [5], vulvodynia [6], and vulvovaginal infections [7]. Vaginal dosage forms, including creams, play a traditional role in the treatment of gynecological diseases due to their ability to deliver APIs directly to the site of action [8]. This targeted delivery is especially important in the treatment of conditions where localized treatment can offer significant therapeutic benefits [9]. Clinical indications for prescribing compounded vaginal creams include pruritus/itching, lichen sclerosis, desquamative inflammatory vaginitis [10], decreased sex drive, radiation therapy inflammation, vaginal atrophy, vaginal and pelvic pain, candidiasis, human papillomavirus [11], radiation-induced vaginal stenosis, and dysbiosis of vaginal microbiome [12], among others.

The selection of APIs for vaginal delivery depends on the patient's therapeutic needs and the absence of commercially available formulations. Compounding pharmacists face the complex task of combining different APIs and excipients while ensuring compatibility [13,14] and the physical and chemical stability of vaginal creams [15,16]. To ensure the best possible treatment outcomes, they also need to meet efficacy and safety standards. The composition of compounded vaginal creams often includes APIs from various pharmacological groups [4,6,17–19]: hormones (estriol, testosterone, estradiol, progesterone, dehydroepiandrosterone, and hydrocortisone); anesthetics (ketamine hydrochloride, lidocaine hydrochloride, and tetracaine); corticosteroids (triamcinolone acetonide and clobetasol propionate); antihistamines (ketotifen); immunosuppressants (tacrolimus); natural soothing agent (aloe vera); antimicrobials and antifungals (mupirocin, nystatin, clotrimazole, clindamycin, and boric acid); chelating agents (edetate disodium); muscle relaxants (baclofen and diazepam); anticonvulsants (gabapentin and phenytoin); bronchodilators/phosphodiesterase inhibitors (theophylline); phosphodiesterase inhibitors (sildenafil); nonessential amino acid (arginine hydrochloride); calcium channel blockers/vasodilators (nifedipine); antidepressants/analgesics for neuropathic pain (amitriptyline hydrochloride); opioid receptor antagonist (naltrexone hydrochloride).

In Vitro and Ex Vivo Evaluation of Key Performance Parameters of Vaginal Semisolid Pharmaceutical Formulations

Promising areas for the development of compounded vaginal creams include the creation of non-irritating anhydrous bases with self-emulsifying and mucoadhesive properties. Such formulations can enhance bioavailability and minimize irritation, ensuring patient compliance. Properties of vaginal formulations are usually evaluated with a range of methods, including technological, in vitro, ex vivo, and in vivo techniques [20,21].

Non-animal in vitro methods encompass cell-based assays (human-derived vaginal epithelial and dendritic cells) and are frequently utilized to evaluate the potential for irritation and cytotoxicity (the MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) and the neutral red uptake (NRU) assay) [22,23].

The Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) assay is an in vitro alternative to the traditional in vivo Draize rabbit eye irritation test and assesses the level of hemorrhage, lysis, and coagulation, indicating the potential for irritation of the test product [20]. Incubated Hen's Egg Tests do not conflict with animal protection laws [24] and are recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) [24,25].

Premature leakage, driven by human vaginal fluid (HVF) clearance, significantly reduces the residence time of the drug on the mucosal surface, ultimately leading to subtherapeutic outcomes [26]. It is therefore the task of contemporary developments to improve the coating on the vaginal mucosa, enhance drug penetration, and slow leakage [27]. To address this issue, increasing mucoadhesion in vaginal formulations is a key strategy [28,29]. In vitro leakage testing evaluates the ability of undiluted and HVF-diluted

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formulations to remain on an inclined surface, thus assessing their potential in vivo retention. Bioadhesion, specifically, refers to the ability of a formulation to adhere to biological tissues, and it is commonly measured using texture analyzers by determining the tensile force or work of adhesion [30–33].

In normal conditions, the vagina has a unique microbiota and is covered by a thin layer of HVF, which maintains the internal physical and chemical environment and are indicators of a woman's health [19]. HFV pH is typically mildly acidic and crucial for the proper function of the vaginal mucosa and protection against infections [19,34,35]. Therefore, an evaluation of the effect of vaginal drug formulations on the pH of vaginal fluid is conducted, typically using a vaginal fluid simulant (VFS) [36,37].

Vaginal dosage forms with self-emulsifying properties improve drug solubility and bioavailability by spontaneously forming emulsions upon contact with HVF [38,39]. This mechanism enhances spreadability and penetration into target areas of the female reproductive tract [40]. Light and fluorescence microscopy are used to assess particle size and API distribution in the resulting emulsion [41].

The aim of this article is to provide a comprehensive in vitro and ex vivo evaluation of the key performance characteristics of an anhydrous vaginal base, focusing on its mucoadhesion, self-emulsification, and irritation potential.

2. Results

2.1. Human Vaginal–Ectocervical Tissue Viability MTT Assay

At the initial time point (exposure time = 0), all samples showed 100% cell viability. As exposure time increased, as expected, a significant decrease in cell viability was observed for the positive control (Gynol II), with cell viability dropping to less than 5% after 16 h. By 24 h, cell viability of the positive control was further reduced to 2.69%, indicating high toxicity. All other samples did not reach the toxic exposure time (ET_{50}) threshold within a day. After 24 h, the Ellage® Anhydrous Vaginal (ELAV) base maintained 78.36% cell viability, while Over-The-Counter (OTC) I and II vaginal lubricants maintained 63.52% and 75.3%, respectively. Thus, the ET_{50} for Gynol II was approximately 3 h, whereas for the ELAV base and both OTC lubricants, the ET_{50} was greater than 24 h. The results of relative cell viability are shown in Figure 1 and in Table S1.

2.2. Hen's Egg Test-Chorioallantoic Membrane Assay

In the preliminary study, both the ELAV base and 0.9% NaCl showed no irritation potential, with an irritation score (IS) of 0.00. In contrast, 0.1 N NaOH was highly irritative, with an IS of 17.00, as evidenced by lysis, hemorrhage, and coagulation (Figure 2). The CAM for test formulation was visually examined both around the edges of the sample and after gentle removal of the formulation to ensure clear observation of vascular endpoints. These findings were consistent even when the experiment was extended for 20 min, in which the ELAV base continued to show no irritation. In the outsourced study at CPT℠, similar results were obtained. The ELAV base demonstrated an IS of 2.50, which falls within the non-irritant range (IS 0–4.9) [42]. The cosmetic gels used as negative controls also exhibited low irritation, with IS values of 3.0 (Nivea Visage) and 2.0 (Pond's Eye Gel).

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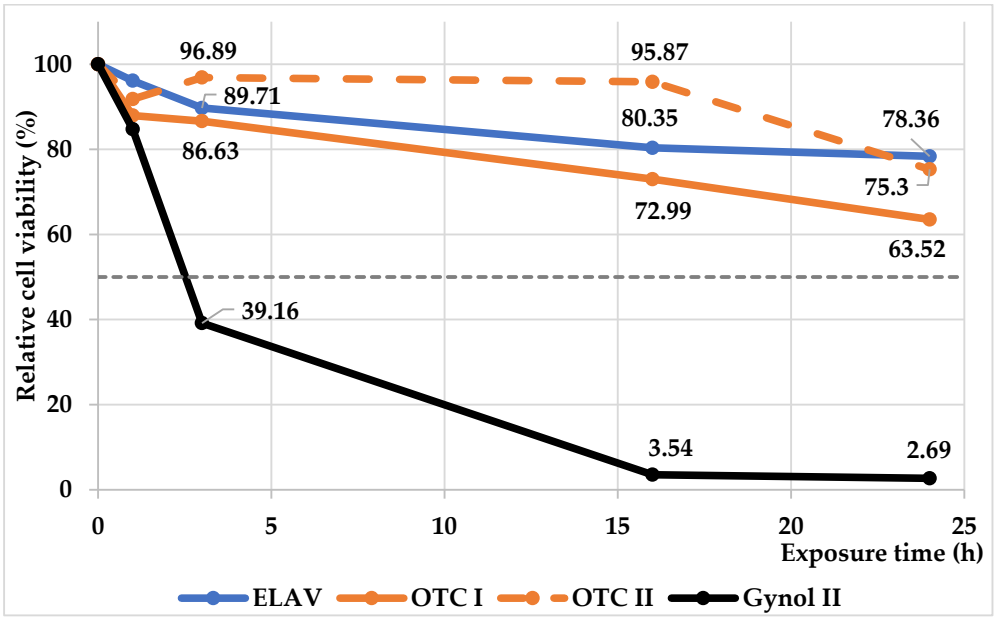

Figure 1. Cell viability of the EpiVaginal™ human vaginal–ectocervical tissue model following exposure to ELAV base, OTC vaginal lubricants (OTC I and OTC II), and Gynol II spermicide over time. The blue solid line represents ELAV base; the black solid line represents Gynol II; the orange solid and dashed lines represent OTC I and OTC II lubricants, respectively. ET_{50} values were determined as the exposure time required to reduce tissue viability to 50%.

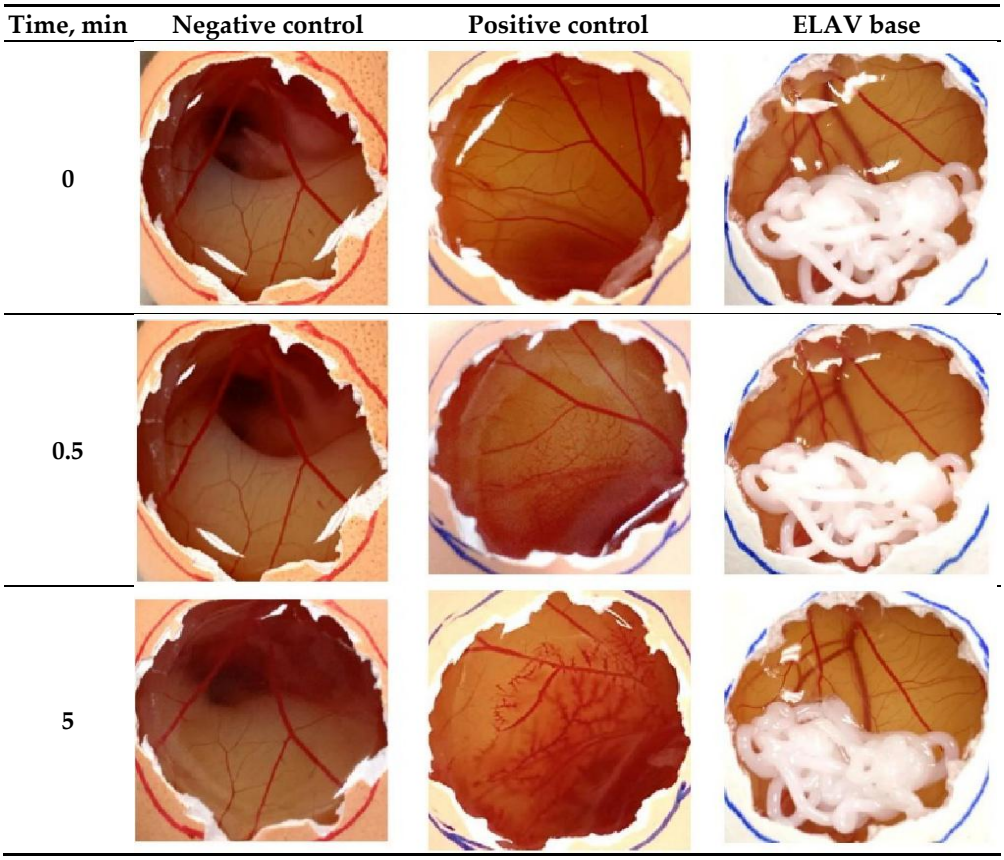

Figure 2. HET-CAM assay evaluating the irritation potential of ELAV base compared with 0.9% NaCl (negative control), and 0.1 N NaOH (positive control) during 5 min. The CAM was visually examined around the edges of the applied material and after gentle removal of the non-transparent formulation to ensure clear observation of vascular endpoints. No irritation reactions were observed for the ELAV base or the negative control.

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2.3. Effect of the Base on the pH of HVF

The pH measurements obtained showed minimal deviation from the baseline of 4.54 for all compounded bases. All tested bases (ELAV, VersaBase® Cream Compounding Base (VBC), MucoLox™ (ML)/VersaBase Gel (VBG) (50:50), OTC moisturizer) were found to maintain the mildly acidic environment of the VFS, comparable to the baseline (Table S2, Figure 3). For ELAV, the pH ranged from 4.51 to 4.65 and remained within the VFS pH range (4.54 ± 0.06) from the addition of 1 mL up to at least 8 mL. VBC showed a pH range of 4.54 to 4.94, with a higher initial pH value. ML/VBG (50:50) showed slight variations in pH values between 4.47 and 4.68. The OTC vaginal moisturizer also showed no significant effect on pH, with a range of 4.60 to 4.71.

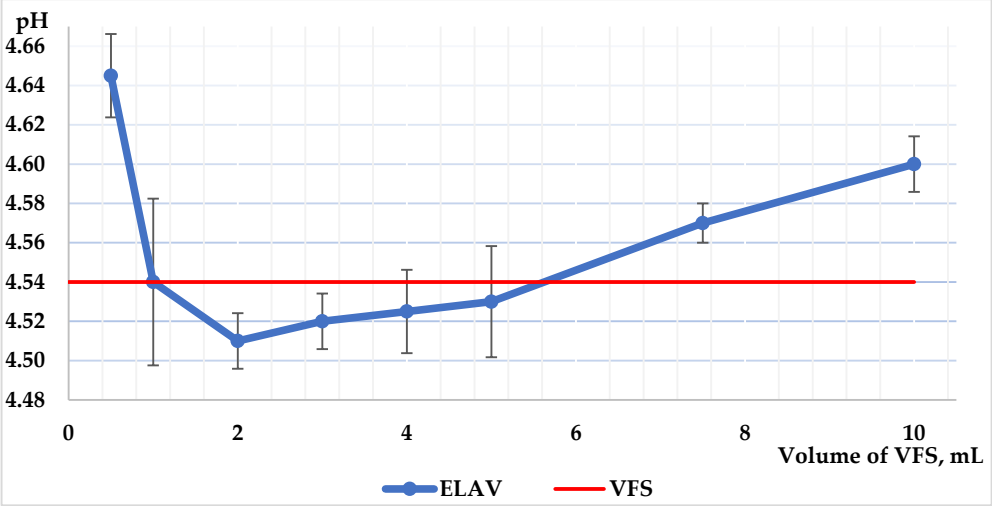

Figure 3. Effect of the ELAV base on the pH of VFS. The blue line represents the measured pH of the ELAV–VFS mixture, while the red line indicates the baseline pH range of VFS. Data represent mean values (n = 3).

2.4. Evaluation of the Mucoadhesive Properties

Ex vivo bioadhesion testing using a texture analyzer. The results showed that all three formulations demonstrated similar bioadhesion profiles, with the following average work-of-adhesion values (mean ± SD): ELAV base: 24 ± 6.78 g·mm, ML/VBG (50:50): 24 ± 3.62 g·mm, and the OTC vaginal moisturizer: 29 ± 4.23 g·mm (Figure 4). The differences between these results were not statistically significant, indicating that the ELAV base had a bioadhesion profile comparable to both ML/VBG (50:50) and the OTC vaginal moisturizer. The negative control (VFS alone) exhibited a significantly lower work of adhesion (11 ± 4.04 g·mm).

Ex vivo testing on porcine vaginal tissues. The ML/VBG (50:50) showed strong mucoadhesion throughout the 90 min study period, with only minor fading of fluorescence (Figure 5). In contrast, the OTC vaginal moisturizer showed significantly reduced fluorescence at the end of this study, suggesting a lower retention potential. Sodium fluorescein dye was effective in visualizing the mucoadhesive properties of both ML/VBG (50:50) and the OTC vaginal moisturizer, as they are both transparent formulations. The fluorescent dye was added to all three test products to ensure that the ex vivo tissues were exposed to identical experimental conditions, despite the ELAV base being white in color and not requiring the dye for visualization (Figure S1). As the ELAV base is an anhydrous formulation and fluorescein sodium is a water-soluble compound, it was expected that the dye would be washed away by VFS, causing the fluorescence to fade. Nevertheless, the white color of the ELAV base remained clearly visible at all time points for both the test and control samples, as confirmed by the images in Figure 5.

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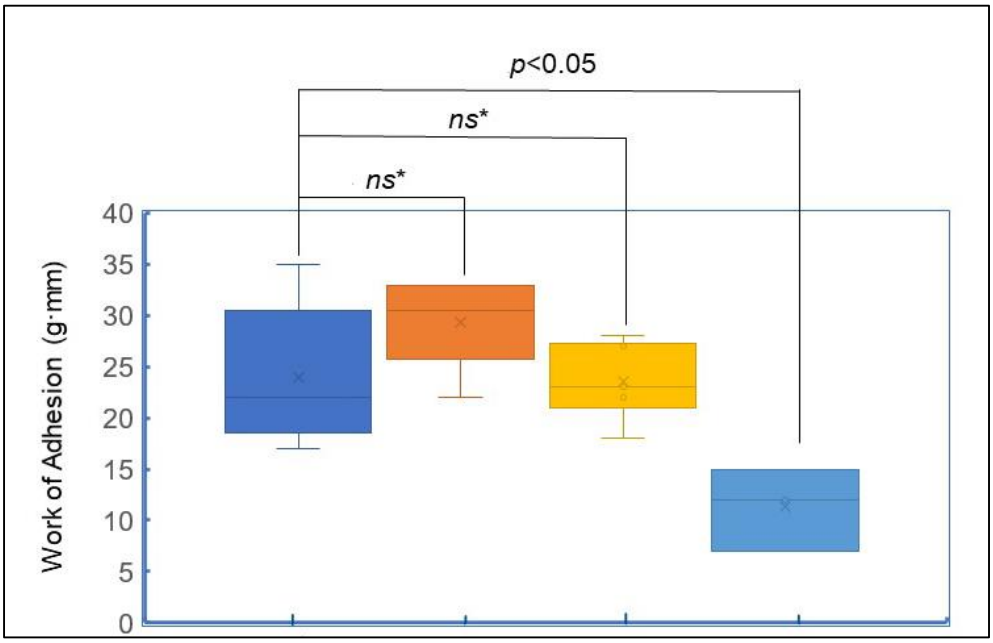

Figure 4. Work of adhesion determined by ex vivo mucoadhesion testing using a texture analyzer (n = 6). Box plots show the bioadhesive performance of the ELAV base (blue), ML/VBG (50:50) (orange), OTC vaginal moisturizer (yellow), and the negative control VFS (light blue). Each box represents the interquartile range (IQR), with whiskers indicating the minimum and maximum values. The horizontal line within each box represents the median, and the cross indicates the mean value. Statistical comparison between the tested formulations showed no significant differences (*ns**, not statistically significant).

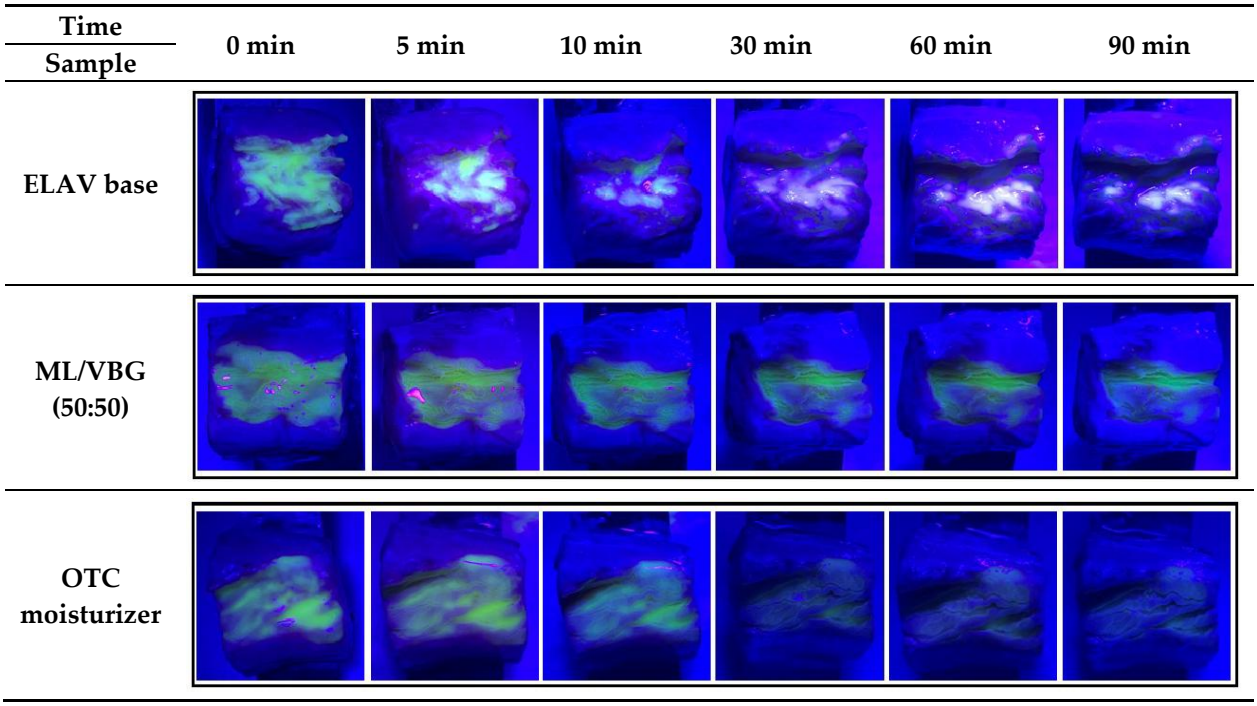

Figure 5. Ex vivo evaluation of mucoadhesion on porcine vaginal tissue over 90 min. Test formulations—ELAV base, ML/VBG (50:50), and an OTC vaginal moisturizer—were applied to the epithelial surface of porcine vaginal tissue and rinsed intermittently with VFS to mimic physiological conditions. Fluorescein sodium was incorporated into the formulations to visualize retention on the tissue surface under UV illumination. Images were captured at the indicated time points (0–90 min). Rows correspond to the tested formulations, while columns represent the observation time points. Persistent fluorescence indicates stronger retention of the formulation on the tissue surface.

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2.5. Evaluation of the Leakage Potential

The undiluted samples exhibited no significant change in migration distance and demonstrated no movement on the agar plates, maintaining their position for the duration of the test (Figure 6A). The running speed of all samples did not exceed 0.3 mm/s, indicating high retention and minimal leakage. The diluted samples of ELAV and its formulated products also demonstrated minimal movement, with an insignificant running speed of less than 0.4 mm/s. In contrast, the OTC vaginal moisturizer exhibited a notable degree of leakage, with a displacement of 10 cm within 10 s, indicating considerable leakage potential and an average running speed of 14.44 ± 1.89 mm/s (Figure 6B).

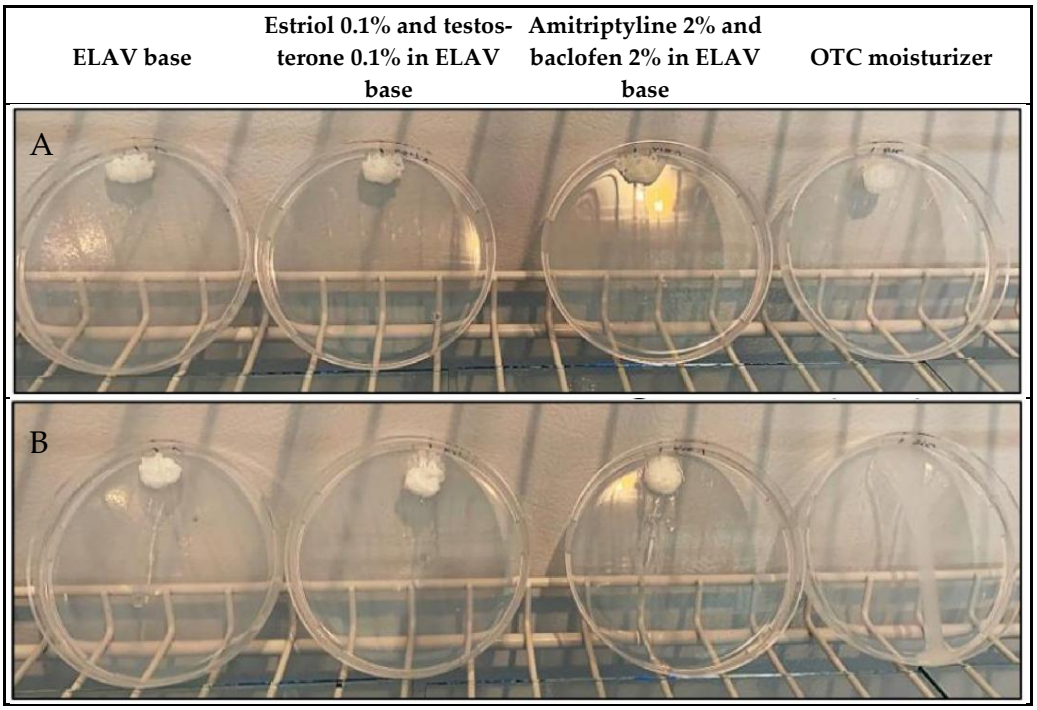

Figure 6. In vitro leakage test performed on agar plates inclined at 60°. Test samples included ELAV base, estriol 0.1%, and testosterone 0.1% in ELAV base; amitriptyline 2% and baclofen 2% in ELAV base; and an OTC long-acting vaginal moisturizer. (**A**) Undiluted formulations. (**B**) Formulations diluted with VFS. Images illustrate the migration behavior and leakage potential of the tested samples.

2.6. Self-Emulsifying Properties

Even the naked-eye evaluation showed that the ELAV base has distinct self-emulsification properties upon gentle mixing with VFS, which distinguishes it from VBC and the Plasticized Compounding Base (PCB) (Figure S2). After mixing with VFS and incubation at physiological temperature (37 °C), ELAV spontaneously formed an emulsion, the droplet size of which gradually decreased over time. This self-emulsification phenomenon was not observed in either VBC, which maintained a stable droplet size, or PCB, which did not emulsify due to its anhydrous gel form.

However, the ELAV and VBC emulsions behaved differently under microscopic observation for 5 min after VFS addition. A conventional emulsifying base, VBC, formed a homogenous emulsion with VFS, with droplet sizes of approximately 10 µm in diameter, and it remained stable. The ELAV base not only formed droplets but also displayed a reduction in droplet size over a period of 1 to 5 min (Figure S3). PCB remained phase-separated and did not form any droplets, indicating no emulsifying behavior. When APIs (amitriptyline 2% and baclofen 2%) were incorporated into the ELAV base, similar self-emulsifying behavior was observed, with droplet formation and size reduction proceeding as observed in the API-free formulation.

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The self-emulsifying behavior of the ELAV base was evident even when diluted with varying proportions of VFS (9:1, 3:1, and 1:1 ratios to VFS). Droplet formation was observed in all three ratios, underscoring the robustness of its emulsifying mechanism across a range of dilution levels (Figure S4).

The fluorescence microscopy results showed effective distribution of both lipophilic and hydrophilic substances in the ELAV base. When fluorescein sodium and curcumin were incorporated into ELAV and mixed for 2 min, each was evenly distributed and showed uniform fluorescence. After gently mixing ELAV with VFS in a 1:2 (*w*/*v*) ratio and incubating at 37 °C, the self-emulsification process was visually confirmed. Under UV light, it was clearly visible that curcumin (lipophilic) was encapsulated within the lipid droplets of the emulsion while fluorescein sodium (hydrophilic) remained in the surrounding aqueous phase. This separation within the emulsion, as shown in Figure 7 (green fluorescent light) and Figure S5 (white and green fluorescent light), highlights the efficiency of the ELAV self-emulsification process.

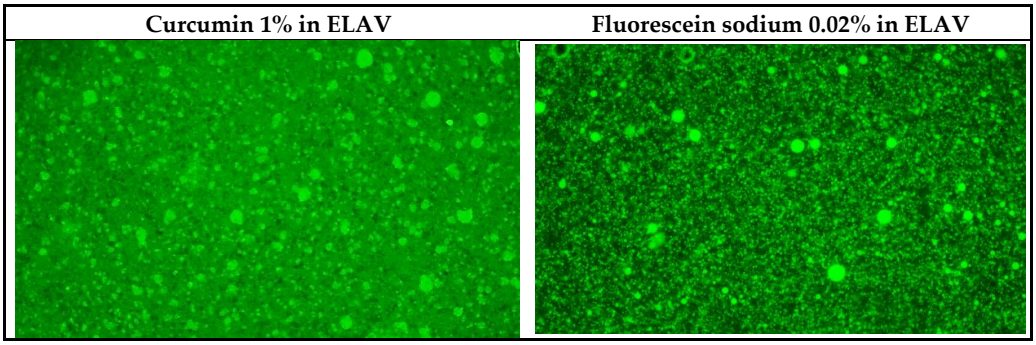

Figure 7. Fluorescence microscopy of ELAV base containing curcumin (lipophilic) and fluorescein sodium (hydrophilic) under green fluorescent light. The images illustrate the distribution of lipophilic and hydrophilic substances within the emulsion formed after mixing the ELAV base with VFS (100×).

3. Discussion

3.1. Cytotoxicity and Irritation Characteristics

The MTT assay results demonstrate that the ELAV base and OTC I and II lubricants have a favorable toxicological profile, with a significant level of cell viability after prolonged exposure compared to the positive control. The obtained results are consistent with the approval requirements for OTC lubricants and confirm their non-toxicity to human vaginal–ectocervical (VEC) tissues. The ET_{50} values indicate that the ELAV base is non-toxic (comparable to well-characterized and approved OTC lubricants) and is expected to be safely applied to the vaginal mucosa without causing cellular damage, making it suitable for vaginal applications.

The results of both HET-CAM assays show that the ELAV base has no significant irritation potential, as demonstrated by an IS of 0 and 2.50. These scores fall well below the irritation threshold (IS > 5). The obtained data correlate with the IS of industrially manufactured creams and also align with the results from the tissue viability MTT assay.

3.2. pH Compatibility

The results indicate that none of the bases, including ELAV, VBC, ML/VBG (50:50), or the OTC moisturizer, significantly altered the pH of the VFS. The initial pH results for VBC correlate with the values in the study of the physical and chemical stability of 0.25 mg/g estriol and 10 mg/g vaginal creams [16], as well as the pH values of ML and VBG [43,44] and the pH limits for the OTC moisturizer stated by the manufacturer. Given that the ELAV base does not significantly alter VFS's pH, it appears suitable for formulating vaginal

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preparations without compromising the physiological environment, making it a promising option for compounders delivering APIs.

3.3. Mucoadhesive Characteristics

During ex vivo bioadhesion testing using a texture analyzer, all experimental parameters were standardized, including contact force, contact time, sample temperature, and withdrawal speed, except for the biological variability in the tissue sample area. The standard deviations observed in the results likely stem from biological variations in the excised porcine epithelial tissues, which is a common limitation in ex vivo studies [45]. Despite this, the method proved to be a reliable and reproducible tool for evaluating mucoadhesive properties. The observed work-of-adhesion values suggest that all three samples can adhere effectively to the vaginal mucosa, potentially extending the residence time of the formulations. The bioadhesion testing results indicate that the ELAV base exhibits a strong mucoadhesive profile, comparable to the well-established combination of ML/VBG (50:50) and a marketed OTC vaginal moisturizer.

Ex vivo testing of mucoadhesive properties on porcine vaginal tissues shows that the ELAV base and ML/VBG (50:50) have similarly strong mucoadhesive profiles. The observed mucoadhesive properties of the ELAV base further support its potential suitability for use in extended-release vaginal formulations.

The results of bioadhesion tests using a texture analyzer correlate well with the ex vivo tests on porcine vaginal tissue and show that the ELAV base has a strong mucoadhesive profile, comparable to the well-established combination of ML/VBG (50:50). Despite the use of porcine tissue as a model for human vaginal mucosa, which may not fully replicate human physiological conditions, this study provides valuable insights into the mucoadhesive performance of the tested formulations.

The results of the in vitro leakage test illustrate the distinction between the properties of the ELAV base, its compounded formulations, and the OTC vaginal moisturizer. While traditional vaginal formulations, such as the OTC moisturizer included in our test, gels [46], and creams [47], often become diluted upon contact with vaginal fluids, which can result in increased leakage, the ELAV base demonstrated the ability to maintain its position on the simulated vaginal mucosa even after dilution. This finding suggests that the ELAV base may provide a longer residence time.

3.4. Self-Emulsification Characteristics

The self-emulsifying properties of the ELAV base demonstrated adaptability, allowing it to form emulsions effectively even with small volumes of VFS. This characteristic underscores its potential in enhancing drug delivery within the vaginal environment, which naturally experiences fluctuations in fluid levels (reduced vaginal fluid production, postmenopausal syndrome, etc.). The ability of the ELAV base to self-emulsify with limited fluid suggests that it may facilitate improved drug dispersion and adhesion to the mucosal surface, which are key factors in prolonged retention and therapeutic efficacy. The observed reduction in droplet size over time is an indicator of a robust emulsification process that can further enhance the bioavailability of encapsulated APIs. This dynamic adjustment in droplet formation allows the ELAV base to potentially deliver a more uniform and effective distribution of APIs, which may overcome limitations in spreadability or release as seen in traditional bases. The self-emulsifying properties of ELAV, as demonstrated in this fluorescence microscopy study, suggest that this base can adapt to varying vaginal fluid volumes and effectively encapsulate both hydrophilic and lipophilic APIs. The encapsulation of curcumin in the lipid droplets and the distribution of fluorescein sodium in the aqueous phase suggest that the ELAV base has the potential to release lipophilic APIs gradually

while allowing for a faster release of hydrophilic compounds. This dual-release mechanism may offer more tailored and efficient treatment options, depending on the solubility and pharmacokinetics of the incorporated APIs.

4. Materials and Methods

4.1. Materials

This study was designed to comprehensively evaluate properties of the anhydrous vaginal base (trade name: Ellage® Anhydrous Vaginal (PCCA, Houston, TX, USA)), hereinafter referred to as the ELAV base. ELAV is an off-white, shiny, smooth cream composed of Medium-Chain Triglycerides NF, Hard Fat NF, Polyoxyl Stearate NF, Glyceryl Monostearate NF, Poloxamer 407 NF, Glyceryl Ricinoleate, Polyoxyl 20, and Cetostearyl Ether NF. ELAV contains a self-emulsifying drug delivery system that creates a micro-emulsion when it comes in contact with water in vaginal fluid. This emulsion releases the APIs from the base to the mucosa. Once the APIs are released, the emulsifier system in the base also holds the drugs at the surface and increases the contact time. For comparisons of various methods, the following were used: a water-containing vaginal base (trade name: VersaBase® Cream (PCCA, Houston, TX, USA)), hereinafter referred to as VBC; a compounding base for mucous membranes (trade name: MucoLox™ (PCCA, Houston, TX, USA)), hereinafter referred to as ML; and a topical, vaginal, and rectal gel base (trade name: VersaBase Gel), hereinafter referred to as VBG, as well as other OTC vaginal lubricants, moisturizers, and a Plasticized Compounding Base (trade name: PCCA Plasticized™ (PCCA, Houston, TX, USA)), hereinafter referred to as PCB. The vaginal bases, APIs, and compounded medications were prepared and provided by PCCA (Professional Compounding Centers of America, Houston, TX, USA): Ellage® Anhydrous Vaginal (Lot 0527009), VersaBase® Cream (Lot 9156966), PCCA Plasticized™ (Lot 8559865), OTC I vaginal lubricant (Replens™ Long-Lasting Vaginal Moisturizer (Church & Dwight Co., Ewing Township, NJ, USA)), OTC II vaginal lubricant (Replens™ Moisture Restore External Comfort Gel (Church & Dwight Co., Ewing Township, NJ, USA)), MucoLox™ (Lot 8917769), VersaBase Gel (Lot 8581476), and Gynol II Spermicide (Caldwell Consumer Health LLC, Madison, NJ, USA). The compounded medications assessed in this study were as follows: estriol 0.1% and testosterone 0.1% in ELAV; estriol 0.1% and testosterone 0.1% in VBC; amitriptyline 2% and baclofen 2% in ELAV; amitriptyline 2% and baclofen 2% in VBC.

4.2. Experimental Design

The development of the vaginal vehicle was guided by critical target attributes—anhydrous composition, non-irritative nature, mucoadhesive properties, and self-emulsifying behavior. These characteristics were systematically assessed through a combination of in vitro and ex vivo methods aimed at evaluating biocompatibility and functional performance. The overall experimental strategy is summarized in Figure S6.

4.3. Human Vaginal–Ectocervical Tissue Viability MTT Assay

This study employed the EpiVaginal™ VEC tissue model (MatTek Life Sciences, Ashland, MA, USA). Four test products were evaluated: ELAV compounding base, OTC I and OTC II vaginal lubricants, and Gynol II Spermicide. The VEC-100 cells were maintained in culture media in accordance with the manufacturer's instructions until they were prepared for testing [22]. A volume of 100 µL of each product was applied to the tissue model in duplicate for 1, 4, 16, and 24 h, with an untreated set of tissues serving as a negative control and following the designated exposure periods. Following the exposure period, the test solutions were removed, and the 300 µL MTT solution (1 mg/mL) was applied to the basal side of the tissues. After a 3 h incubation period at 37 °C, the purple formazan product was

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extracted with extractant solution, and the optical density (OD) was measured at 570 nm with a reference wavelength of 650 nm using a CLARIOstar plate reader (BMG LABTECH GmbH, Ortenberg, Germany). The relative cell viability of each test product was calculated as a percentage in comparison to the negative control: % viability = OD (treated tissue)/OD (untreated tissue) × 100 [23].

4.4. Hen's Egg Test-Chorioallantoic Membrane Assay

The irritation potential of the ELAV compounding base was evaluated in comparison to positive (0.1 N sodium hydroxide solution) and negative (0.9% sodium chloride solution) controls, following the ICCVAM HET-CAM recommended test method [25]. Fertilized hen eggs were incubated at 37 °C for 9 days to allow the development of the CAM. After the incubation period, a small window was carefully created in the eggshell with the help of a scalpel and tweezers to expose the CAM for testing. For each test, 0.5 mL of the respective formulation (ELAV base and controls) was applied. The membrane was observed for an initial 5 min period, and observations were extended up to 20 min to detect any delayed reactions. Each experiment was conducted in triplicate. Data collection involved recording the occurrence and intensity of lysis, hemorrhage, and coagulation, as well as the time required for each reaction. Photographic documentation was undertaken at the outset of the experiment (0 min), after 0.5 min, and at the conclusion of the assays (5 min). The irritation score (IS) was calculated based on the presence and intensity of lysis (vessel disintegration), hemorrhage (vessel bleeding), and coagulation (blood clotting). The products were classified as non-irritants with IS 0–4.9 or irritants with IS $\geq$ 5 [42].

4.5. Effect of the Base on the pH of HVF

The evaluation of the base's effect on the pH of HVF was conducted in vitro using VFS following the methodology described below. Approximately 50 mL of VFS was heated to 37 °C, and the initial pH of the fluid was measured using a compact pH meter LAQUAtwin-pH-22 (Horiba Advanced Techno Co., Ltd., Kyoto, Japan). To 5.0 g of the ELAV base, VFS was added incrementally and mixed thoroughly. The VFS was added to the base in the following sequence to reach a final total volume of 10 mL, with pH measurements taken after each addition: 0.5 mL (2 times), 1 mL (4 times), and 2.5 mL (2 times). This procedure was repeated for 5.0 g of VBC, ML/VBG (50:50), and the OTC vaginal moisturizer. The VFS was prepared based on the composition proposed by Owen and Katz (1999) [36], and 1.0 L of this fluid contained the following: sodium chloride, 3.51 g; potassium hydroxide, 1.40 g; calcium hydroxide, 0.222 g; bovine serum albumin, 0.018 g; lactic acid, 2.00 g; acetic acid, 1.00 g; glycerol, 0.16 g; urea, 0.4 g; glucose, 5.0 g.

4.6. Evaluation of the Mucoadhesive Properties

4.6.1. Ex Vivo Bioadhesion Testing Using a Texture Analyzer

In order to evaluate the mucoadhesive properties of the ELAV compounding base, the well-established combination ML/VBG (50:50) and an OTC vaginal moisturizer, which claims to provide long-lasting effects for up to 3 days, were selected as references for a comparative study.

Experimental Procedure: Frozen porcine vaginal tissues (BioIVT, Westbury, NY, USA) (Lot #: PIG15407–PIG15409) were thawed at room temperature, and the epithelial surface was excised and cut into circular samples of approximately 10 mm in diameter using a biopsy scalpel punch. The bioadhesive properties of the ELAV base, ML/VBG (50:50), and the OTC vaginal moisturizer were evaluated by premixing each formulation with VFS in a ratio of 3:1 (*v*/*v*) [36]. Then, 0.05 mL of each test sample in VFS was applied to a cellulose acetate membrane, which was fixed to the CTX Texture Analyzer (Ametek Brookfield, Middleborough, MA, USA) using a mucoadhesion rig.

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The porcine epithelial tissue was attached to the cylinder probe of the texture analyzer using cyanoacrylate glue. The probe was lowered at a speed of 2.5 mm/min until it contacted the test product, applying a trigger force of 1 g, followed by an applied force of 60 g. After a 3 min hold time, the probe was raised at a speed of 2.5 mm/min, separating the epithelium from the test product. A total of six replicates were conducted for each formulation, along with a negative control (VFS alone). The experiments were performed at a constant temperature of 37 °C to simulate body conditions.

The work of adhesion (g·mm), representing the mucoadhesive force, was calculated as the area under the force versus displacement curve.

4.6.2. Ex Vivo Testing on Porcine Vaginal Tissues

This test was conducted in accordance with the in vitro studies by Song et al. [48] and Cazorla-Luna et al. [49]. Frozen porcine vaginal tissues (BioIVT, Westbury, NY, USA) were used for an ex vivo study. The tissues were thawed at room temperature, cut into 2 × 2 cm pieces, and washed with Hanks' balanced salt solution. Prior to the experiment, the tissues were equilibrated at 37 °C. Afterwards, the ex vivo vaginal tissues were fixed on stainless steel using cyanoacrylate glue and washed with the VFS [42].

Fluorescein sodium (Sigma-Aldrich, Burlington, MA, USA, F6377) (Lot SLBL5470V), a fluorescent dye, was dissolved in ethanol and added to the ELAV base, ML/VBG (50:50), and the OTC vaginal moisturizer to allow visualization of the mucoadhesion properties. Control groups of vaginal formulations without the fluorescent dye were also included. The test products were applied (50 µL/cm^2) to the surface of the porcine vaginal tissues and evenly distributed using a pellet pestle. The test tissues were rinsed intermittently in VFS by immersing the plates at predetermined time points (5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, and 90 min) to simulate vaginal conditions. Fluorescence was observed under UV light at 390 nm and captured using a smartphone camera (iPhone XII, Apple Inc., Cupertino, CA, USA).

4.7. Evaluation of the Leakage Potential

An adapted in vitro leakage test [27] was used, including VFS to mimic HVF with Petri dishes coated with agarose. Four samples were collected for analysis: ELAV base, estriol 0.1% and testosterone 0.1% in the ELAV base, amitriptyline 2% and baclofen 2% in the ELAV base, and an OTC long-acting vaginal moisturizer. Each sample was tested in both diluted and undiluted forms. Dilution was achieved by adding 0.75 mL of VFS to 2 mL of base or formulation, and mixing was carried out. To simulate a moist, vertical surface similar to vaginal mucosa, Petri dishes (100 mm) were prepared by filling each dish with 25 mL of a 1.5% (*w*/*v*) agarose solution in VFS, which was allowed to solidify at room temperature. Prior to the experiment, the agar plates were placed in an incubator Model 2020 (VWR™ International, Cornelius, OR, USA) at 37 °C for 1 h, positioned at a 60° angle. A 0.5 mL aliquot of each sample was applied in triplicate to the top of agar plates using a syringe. The migration distance of the test formulations along the agar plate was measured for 5 min.

4.8. Self-Emulsifying Properties

Light microscopy was conducted to observe the emulsifying dynamics of the ELAV base in comparison with PCB and VBC. The ELAV base, VBC, and PCB were first gently mixed with VFS in a 1:2 (*w*/*v*) ratio and incubated at 37 °C for 5 min (sample preparation). To assess droplet formation and size, each sample was observed under a light microscope. Then, 10 µL of VFS and 10 µL of the sample were placed on glass slides (1:1 ratio). The mixtures were placed with a pipette tip, incubated at 37 °C for 5 min, and covered with a coverslip for microscopic examination at 100× magnification (using a 10× objective

and a 10× ocular lens). To evaluate the self-emulsifying properties of the ELAV base when containing APIs, amitriptyline 2% and baclofen 2% were incorporated into the ELAV base. To assess droplet formation under different conditions, the ELAV base was also mixed with VFS at ratios of 9:1, 3:1, and 1:1 and examined under a light microscope at 10× magnification.

Fluorescence microscopy was used to evaluate the distribution patterns of hydrophilic fluorescein sodium (Sigma-Aldrich, #F6377) and lipophilic curcumin (PCCA, #C30-3497) substances within the formulations and to compare them with the performance of the reference bases. Test formulations were prepared by adding 0.02% fluorescein sodium and 1% curcumin into the ELAV base, together with 5% glycerol as a cosolvent (sample preparation). The formulations were mixed for 2 min using an electronic mortar and pestle (EMP) mixer, GAKO UNGUATOR (GAKO International GmbH, Munich, Germany), which was set to a speed of 5 to achieve a homogeneous distribution. Then, a portion of each sample with fluorescein or curcumin was mixed with VFS in a 1:2 (w/v) ratio and incubated at 37 °C to simulate vaginal conditions. Within 5 min after preparation, samples of the test formulations, with and without VFS, were observed under a combination of white and green fluorescent light. Fluorescence microscopy tests were performed using a Nikon Eclipse TS100 inverted phase microscope equipped with NIS-Elements imaging software (version 5.02, Nikon, Tokyo, Japan) and a Lumencor® MIRA Light Engine (4-NII-FA) (Lumencor, Inc., Beaverton, OR, USA). A filter set with excitation and emission spectra of 460–490 nm and 500–560 nm was used for fluorescence excitation, allowing detailed visualization of the self-emulsifying properties of the samples at 10× magnification.

5. Conclusions

This study confirms that the novel anhydrous ELAV base successfully embodies the key design properties essential for vaginal semisolid formulation: anhydrous composition, non-irritative nature, strong mucoadhesion, and effective self-emulsifying behavior. The ELAV base demonstrated a strong biocompatibility profile, showing no cytotoxicity or irritation in standardized assays (MTT and HET-CAM), and it maintained minimal impact on the physiological pH of VFS. Its strong mucoadhesive properties, confirmed through ex vivo testing and texture analysis, suggest enhanced retention time on vaginal mucosa, which is essential for prolonged therapeutic action. ELAV's self-emulsifying capability allows efficient dispersion of both lipophilic and hydrophilic APIs even in low-fluid environments. Compared to water-containing bases such as VBC and ML/VBG (50:50), ELAV offers several functional properties that may be beneficial under specific formulation conditions: longer retention time and lower leakage potential; the ability to adapt to varying vaginal fluid levels; and efficient dispersion of both hydrophilic and lipophilic APIs. Moreover, its anhydrous nature is expected to minimize the risk of API hydrolytic degradation, and the stability of promising formulations is planned for further investigation. ELAV thus emerges as an option for compounded vaginal formulations that demand these specific functional characteristics.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ph19040585/s1. Figure S1: Ex vivo porcine vaginal tissue immediately after exposure to ELAV base (t = 0 min). The image shows the initial state of the tissue prior to incubation or rinsing with vaginal fluid simulant (VFS). Figure S2: The result of mixing ELAV (1), VBC (2) and PCB (3) bases with vaginal fluid simulant (VFS) in a 1:2 (w/v). The images demonstrate differences in self-emulsifying behavior among the tested bases. Figure S3: Light microscopy (100×) showing droplet formation in VBC, PCB and ELAV bases before and after mixing with vaginal fluid simulant (VFS). Images illustrate droplet formation in ELAV base with and without incorporated APIs (amitriptyline 2% and baclofen 2%). Figure S4: Light microscopy (10×) showing

droplet formation in the ELAV base after mixing with different proportions of vaginal fluid simulant (VFS) (9:1, 3:1, and 1:1 ratios). The images illustrate the effect of increasing aqueous phase on the self-emulsification process. Figure S5: Fluorescence microscopy of ELAV base mixed with vaginal fluid simulant (VFS) containing curcumin (lipophilic) and fluorescein sodium (hydrophilic). Images obtained under white and green fluorescent light illustrate the localization of lipophilic curcumin within emulsion droplets and hydrophilic fluorescein sodium in the surrounding aqueous phase. Arrows indicate the distinct distribution of both substances. Figure S6: Experimental design for the evaluation of the key performance characteristics of the anhydrous vaginal base. Table S1: Relative cell viability for ELAV base, OTC I, OTC II, and Gynol II over time. Table S2: Effect of various vaginal bases on the pH of vaginal fluid simulant (n = 3).

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Abbreviations

The following abbreviations are used in this manuscript:

API	Active Pharmaceutical Ingredient;
CAM	Chorioallantoic Membrane;
ELAV	Ellage® Anhydrous Vaginal Base;
ET50	Toxic Exposure Time;
HET	Hen's Egg Test;
HVF	Human Vaginal Fluid;
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods;
IS	Irritation Score;
ML	MucoLox™ Compounding Base;
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide;
OD	Optical Density;
OTC	Over the Counter;
PCB	Plasticized™ Compounding Base;
PCCA	Professional Compounding Centers of America;
VBC	VersaBase® Cream Compounding Base;
VBG	VersaBase Gel Vaginal and Rectal Gel Base;
VEC	Vaginal–Ectocervical;
VFS	Vaginal Fluid Simulant.

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