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Development of a mucoadhesive delivery system for control release of doxepin with application in vaginal pain relief associated with gynecological surgery

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ABSTRACT

The main purpose of this study was to develop a semisolid mucoadhesive formulation for the non-invasive vaginal administration of doxepin (DOX) for relief of pain derived from the scarring process after surgery. An orafix® platform loading DOX was tested for adequate stability, rheology and vaginal mucoadhesion capacity. The formulation exhibited appropriate pH and was microbiologically stable. The rheological studies confirmed its pseudoplastic and thixotropic nature with prevalence of the elastic behavior component over the viscous one. Appropriate syringeability and spreadability results were also confirmed. Different experiments showed adequate mucoadhesion capacity even in the presence of simulated vaginal fluid. Finally, DOX release, permeation and retention in vaginal mucosa studies were also accomplished with promising results. DOX release kinetics followed the modified Higuchi model and the permeation studies did not render such high values as to suggest potential systemic absorption which could lead to undesirable systemic side effects. Therefore, we can hypostatize that the proposed formulation may assist to fill in the therapeutic gap regarding pure pain relief at local level in vagina.

1. Introduction

The vagina is an expandable, longitudinally S-shaped, fibromuscular tube. It extends from the cervix of the uterus to the vestibule with dimensions of approximately 7–10 cm in length and 2–5 cm in diameter. Although commonly known as a mucosa, it does not harbor secretory glands; it does not secret fluid *per se*. However, its epithelium surface is covered by a thin film of fluid mainly originated by transudation and cervical glands (Vanić and Škalko-Basnet, 2013).

The vagina is a non-invasive delivery route in women for drugs formulations with systemic and local effect. Seeking a local effect, it has traditionally been used for the delivery of drugs such as prostaglandins, antibiotics, antifungals, steroids, antivirals, antiprotozoal, antichlamydial and spermicidal agents (Vermani et al., 2002). Many different vaginal delivery systems have been used over the years. However, conventional dosage forms are associated with poor retention time, leakage and messiness, causing discomfort to patients and leading to poor patient compliance and loss of therapeutic efficacy (Rençber et al., 2017). Nevertheless, semisolid formulations have been the preferred ones (Palmeira-de-Oliveira et al., 2015). An ideal vaginal delivery system should present minimal leakage, long residence on the tissue and it should uniformly distribute throughout the entire vaginal cavity (Ochoa Andrade et al., 2014). In order to improve the residence time of vaginal semisolids, the incorporation of mucoadhesive polymers to the formulations is a widely extended practice.

As regards drug administration in mucosa, researchers may be tempted to extrapolate results regarding the oral route to vaginal administration, it must be taken into account that drug delivery is tissue specific and should be studied for each specific drug and route of administration (Sanz et al., 2015a). However, considering the significant structural similarities between the oral and vaginal mucosa (Thompson et al., 2001), it was considered appropriate to study the potential use of a typical oral mucoadhesive excipient for its application in vaginal mucosa. It was also taken into account that human vaginal mucosa is a good permeability model for human buccal mucosa (Van Eyk and Van Der Bijl, 2004). Thus, it was expected that an oral formulation may also

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render satisfactory results at vaginal level. Orafix®, a fat white vehicle for drug administration designed for oral application in buccal mucosa was selected as the main excipient to elaborate the proposed formulation. Orafix® is a fat, white, odorless, uniform, and slightly grainy to the touch cream.

Doxepin (DOX) is a tricyclic antidepressant which has proven analgesic and anesthetic effect with topical application onto buccal mucosa. It is believed that its therapeutic local effect is caused by the blockade of sodium channels in cutaneous nociceptors (Leenstra et al., 2014).

Some anesthetic and anti-inflammatory topical drugs for local effect at vaginal level are commercially available. However, the field of local vaginal analgesia is not properly covered nowadays. Any surgery is a trauma to the body and the healing process involves the formation of scars. The scarring may cause pain through neuropathic pain mechanisms. Unfortunately, the treatment of scar pain in gynecology has not received much attention. Current treatment may include several injections of local anesthetics, the application of an anesthetic ointment containing lidocaine and the administration of oral tricyclic antidepressant and antiepileptic drugs (Steege and Zolnoun, 2009).

This work was undertaken with the main objective of designing a suitable mucoadhesive semisolid dosage form for DOX vaginal delivery at local level. In order to do so, rheological and bioadhesive properties of the formulation were tested, as well as its physical and microbiological stability. *In vitro* and *ex vivo* studies were also performed to assess its potential drug release and mucosal permeation, including its mucosal drug retention capacity.

2. Materials and methods

2.1. Materials

Doxepin hydrochloride and menthol were obtained from Sigma-Aldrich (Madrid, Spain). Diethylene glycol monoethyl ether (Transcutol®) was a gift from Gattefossé (Saint-Priest Cedex, France). Orafix® was purchased from Fagron Ibérica (Barcelona, Spain). Double-distilled water was obtained through a Station 9000 purification unit. All other chemical and reagents were of analytical grade.

2.2. Tissue samples

Vaginal porcine mucosa was surgically removed immediately after the female pigs (three- to four-month-old) were sacrificed using an overdose of sodium thiopental anesthesia at the Animal Facility at Bellvitge Campus of Barcelona University (Barcelona, Spain). Mucosa was placed in Hank's balanced salt solution (HBSS) and refrigerated until its use no later than 24 h after extraction. Tissues that were not utilized were cryopreserved for further studies (Amores et al., 2014). This protocol was approved by the Animal Experimentation Ethical Committee of the University of Barcelona, Spain (CEEA-UB).

Vaginal mucosa integrity was studied by the measurement of the vaginal transmucosal water loss (VTMWL). VTMWL corresponds to the amount of water that passes from the interior of the body, through the outer layer of the vaginal mucosa, to the surrounding atmosphere via diffusion and evaporation processes and it is given in grams per square meter and hour (Amores et al., 2014). Each reading was performed at room temperature, in triplicate, using a DermaLab® module (Cortex Technology, Hadsund, Denmark). The metering device was placed perpendicular to the tissue, reaching a stable VTMWL result in 60 s approximately. VTMWL was initially measured *in vivo* in six anesthetized female pigs obtaining a maximum value of 37 g/h m^2 which was set as the maximum acceptable limit in the vaginal tissue to be used for permeation studies (median: 28.4 g/h m^2; min - max: 19.5–37.2 g/h m^2).

Goat tanned leather was used for the mucoadhesive experiments as it was supplied from a local commerce.

2.3. Mucoadhesive formulation

The proposed formulation was prepared by mixing up the preweighed ingredients in increasing amounts. Orafix® was selected as the bioadhesive platform to elaborate the semisolid formulation. Menthol (5%, w/w) and transcutol® (10%, w/w) were selected as DOX permeation enhancers according to previous studies performed on a different mucosa type (Sanz et al., 2017). DOX (5%, w/w) was first mixed with menthol and transcutol® and then, as a whole, combined with Orafix® in a mortar with a pestle. The resultant semisolid product was further mixed and homogenized using an Ultra-Turrax® T10 basic (IKA, Staufen, Germany).

2.4. Preparation of simulated vaginal fluid

A modified simulated vaginal fluid (SVF) was prepared to mimic the vaginal environment when needed for the mucoadhesive studies. SVF was adapted by adding 1.5% (w/v) mucin to that proposed by Owen and Katz (1999). This modification conferred higher viscosity and a characteristic mucin odor which better mimic the viscoelastic and mucoadhesive characteristics of natural vaginal secretion (das Neves et al., 2012; Vermani et al., 2002).

The preparation of SVF consisted in mixing glucose (0.5%, w/w), sodium chloride (0.351%, w/w), lactic acid (0.2%, w/w), potassium hydroxide (0.14%, w/w), acetic acid (0.1%, w/w), urea (0.04%, w/w), calcium hydroxide (0.0222%, w/w), bovine serum albumin (0.018%, w/w), glycerol (0.016%, w/w) and water. 1.5% mucin (w/v) was added to the solution and mixed in Ultra-Turrax®. A pH value of 4.2 was obtained by the addition of HCl 0.1N.

2.5. Rheological characterization

2.5.1. Rheology

Rheological parameters of the formula were studied using a Haake Rheostress 1 rheometer (Termo Fisher Scientific, Karlsurhe, Germany). The equipment was connected to a thermostatic circulator Thermo Haake Phoneix II + Haake C25P to assure constant temperature during the experiments. The tests were executed with a Haake Reowin® Job Manager v. 3.3 software (Thermo Electron Corporation, Karlsuhe, Germany) and data was analyzed with a Haake Reowin® Data Manager v. 3.3 software (Thermo Electron Corporation, Karlsuhe, Germany). Two different kinds of measurements were performed 24 h after preparation of the formulation: rotational measurements and oscillatory tests. All tests were performed by triplicate at 25 ± 0.2 °C.

Rotational tests were addressed with a plate-plate geometry (1 mm gap) using a fixed lower plate and a mobile upper plate (Haake PP60 Ti, 60 mm diameter). The shear stress (τ) was measured as a function of the shear rate (γ) and viscosity curves ($\eta = f(\gamma)$) and flow curves ($\tau = f(\gamma)$) were recorded. The shear rate ramp program included 3 min ramp-up period ($0 \rightarrow 100\ s^{-1}$, 1 min constant shear rate period ($100\ s^{-1}$), and finally, 3 min ramp-down period ($100 \rightarrow 0\ s^{-1}$). Steady-state viscosity (η, Pa·s) was determined from the constant share section at $100\ s^{-1}$. The apparent thixotropy (Pa/s) for the formulation was assessed by estimating the area of the hysteresis loop formed by the flow curves.

Oscillatory (dynamic) measurements were also performed with parallel plate-plate geometry (Haake PP60 Ti, 60 mm diameter, 0.5 mm gap separation between plates). An oscillatory stress sweep test was first carried out at a constant frequency of $1\ s^{-1}$ with an increasing shear stress (1→200 Pa) to study the linear viscoelastic region (LVR) of the sample. Once the LVR was set, a frequency sweep test was performed modifying the frequency range within 0.1-10 s^{-1} at a constant shear rate within the observed LVR. The study of the storage modulus (G'), loss modulus (G''), phase angle (δ) and complex viscosity (η^*) were used to characterize the sample.

2.5.2. Extensibility (spreadability)

The extensibility test was assessed based on the method previously described by Campaña-Seoane et al. (2014). Briefly, 1 g of sample was placed between 2 glass slides of 5 cm^2, as centered as possible. Force was generated onto the upper plate by adding known weights, so the sample was compressed to uniform thickness (known weights: 20, 50, 100, 200 and 300 g). After 60 s, the weights were removed and the area of the sample was recorded. The sample was tested in triplicate for each weight at room temperature.

2.5.3. Syringeability (extrusion)

Syringeability was measured with a TA.XT plus texture analyzer (Stable Micro Systems, Ltd., Surrey, England) as the necessary force required to expel the formulation from a syringe. The formulation was carefully loaded into 1 mL syringes to a preselected height (2 cm) avoiding the formation of air bubbles. The syringe was vertically placed on a support and its plunger was put in contact with the punch of the texture analyzer. The punch was moved downwards for at a 2.0 mm/s rate. The applied force was recorded as a function of the distance, thus, determining the work required to expel the formulation from the syringe. The experiment was performed 7 times at room temperature.

2.6. Mucoadhesive characterization

2.6.1. Rotating cylinder method

The method for the shear stress test was based on the rotational cylinder method using a dissolution apparatus as described by Nowak et al. (2015). Instead of attaching the mucosa to a stainless steel cylinder inside a vessel containing fluid, a paddle was used (USP dissolution test apparatus 2 - paddle apparatus -). Freshly excised vaginal mucosa was carefully clamped to each paddle as flat as possible. Vessels were filled with phosphate buffer saline (PBS) pH 4.5 to mimic the conditions of the vagina environment. Temperature was kept at 37 °C and paddles rotated at 100 rpm. Approximately 0.5 g of the test formulation was applied to each mucosa prior to its immersion in the PBS. For half of the replicates, SVF was added to the mucosa prior to the application of the semisolid sample. Thus, half of the replicates presented the sample in direct contact with the mucosa and, for the other half, the sample was applied to a mucosa covered with a SVF layer .Tests were performed in triplicate and the residence time under stirring was assessed. Fig. 1 illustrates how the test was performed.

2.6.2. Gravimetric method

The retention time of the formulation was studied according to the method reported by Vermani et al. (2002). Intact tubular vaginas soaked in HBSS were vertically suspended with the help of few stands. Vagina was previously covered with a wet cotton pad moistened with HBSS and surrounded by aluminum foil to keep the mucosa moist. An analytical balance was placed below the suspended tissues to weight the falling cream. The test was performed in triplicate, inserting SVF to one of the two vaginas prior to the test formulation each time. Samples (4 mL) were applied with a 12 mL syringe to each vagina and the amount of cream falling down was recorded for 6 h. The whole experimental equipment is illustrated in Fig. 2.

2.6.3. Texture analyzer

A texture analyzer TA-XT Plus (Stable Micro Systems, Ltd., Surrey, England) was chosen for the more complex tests to study the bioadhesivity properties of the formulation by the measurement of the maximum force (detachment force, F_{dt}) and the work needed to separate the semisolid formulation from the vaginal mucosa (work of adhesion, W_{ad}). For these types of studies, vaginal tissues obtained from several animal species have been worldwide used as the substrate (das Neves et al., 2008). However, goat tanner leather was finally selected for the experiments as it is a good model for bioadhesion determination and it has proven similar results when compared to cow vagina (Campaña-Seoane et al., 2014). Briefly, goat tanned leather was cut into small discs (2 cm diameter) and stuck to the upper and lower supports with cyanoacrylate adhesive. 0.2 mL semisolid formulation samples were placed on the lower leather disc. Then, a compression/extension stage at a constant rate of 1 mm/s was applied, maintaining a contact force between the substrate and the semisolid of 0.5 N for 300 s. Results were plotted as force *versus* elongation providing the maximum force required to detach the formulation from the substrate and the area under the curve or mucodhesion work.

Finally, the potential effect of vaginal fluid on the mucoadhesive properties was also studied with the same device and goat tanner leather (2 cm diameter). One leather disc was fixed to the lower face of the probe and the other one was stuck on the center of a Petri dish previously fixed to the lower support by a clamp. Samples (0.2 mL) were placed on the lower support and the system was equilibrated for 1 min at 30 °C. 25 mL of SVF at 37 °C were added to the Petri dish and the upper support was lowered at a constant rate of 1 mm/s until contact between the formulation and the leather surface was achieved. Then, intimate contact between the substrate and the sample was assured by applying a 0.5 N force for 60 s. Next, the upper probe was brought into its initial position at a speed of 1 mm/s and the force *versus* elongation curve was recorded. After 1 min, the extension/compression cycle was

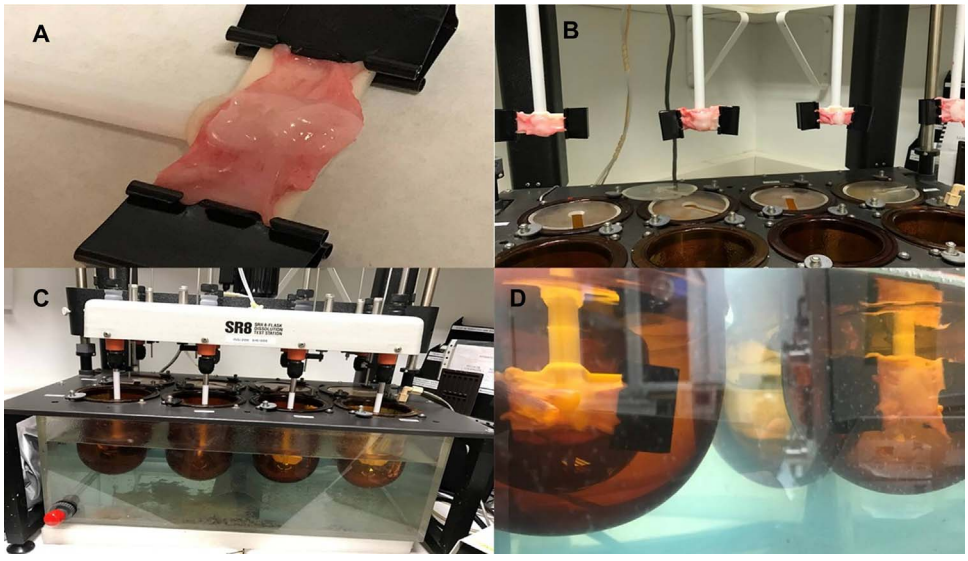

Fig. 1. Study of the shear stress effect on vaginal mucosa. Vaginal tissue fixation to the paddle of the device (A). Image of one batch previous immersion in the PBS medium (B). Image of one batch during the experiment (C). Detailed image of baskets (D).

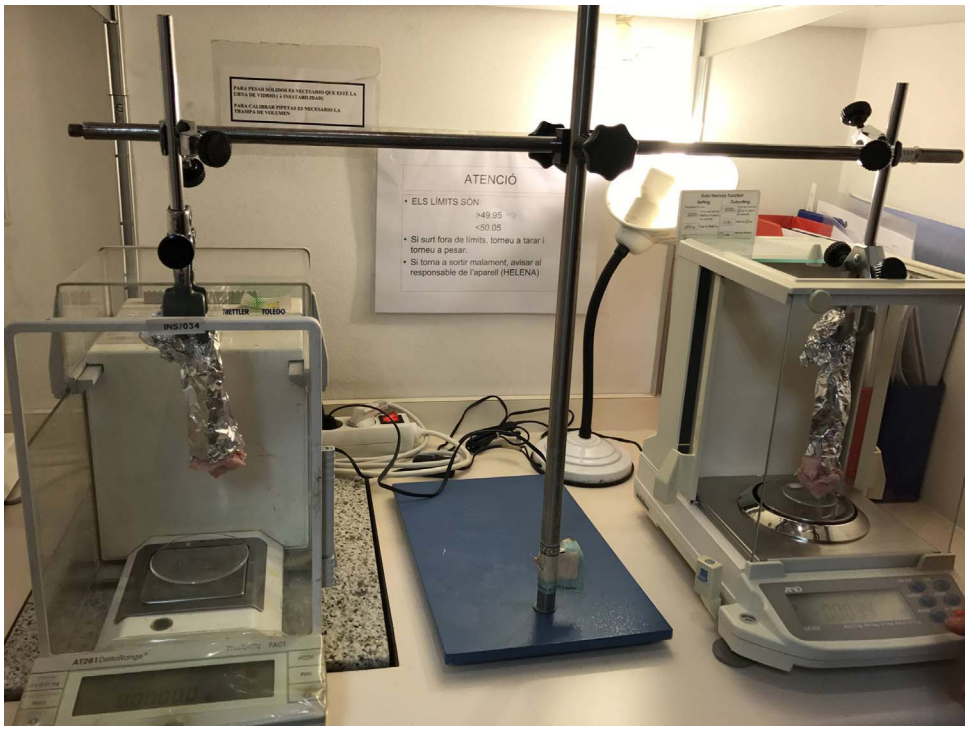

Fig. 2. Image of the retention time study apparatus.

repeated 10 times under the same conditions.

2.7. Stability studies

The semisolid formulation was studied as regards its physical and microbiological stability. 2 g samples were stored in amber glass vials at three different temperature conditions (4, 30 and 40 °C) for 3 months.

2.7.1. Physical stability

Samples were inspected for physical parameters such as appearance in terms of color, odor and visual consistency at different time points: 0, 7, 14, 30, 60 and 90 d.

The pH value was measured by immersing the probe directly into the sample using a Crison 501 digital pH/mV-meter (Crison Instruments, Barcelona, Spain) with the electrode for viscous samples. Specification was set at below 7 with no change allowed during the study duration.

2.7.2. Microbiological stability

The European Pharmacopoeia monograph for microbiological quality of non-sterile pharmaceutical preparations was used as a reference for the study of the microbial content (Council of Europe, 2010). Accordingly, specifications for total aerobic microbial count (TAMC) and total combined yeasts/molds count (TYMC) were established as $< 10^2$ colony-forming units (CFU)/g and $< 10^1$ CFU/g, respectively. Additionally, the absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* was investigated in accordance with requirements of products intended for vaginal use.

For the aerobic microbial count, samples of the semisolid formulation were spread by triplicate onto tryptone soy agar plates (TSA; CM0131B, Oxoid Ltd, Basingstoke, UK). Plates were incubated for 1 week at 37 ± 2 °C in order to count the number of CFU. For the yeast and molds count, in a similar way, samples were spread onto Sabouraud agar plates with chloramphenicol and incubated at 30 ± 2 °C for 1 week.

To confirm the absence of the specified microorganisms (pathogenic microorganisms), 4 mL of isopropyl myristate were added to 1 g samples. The suspension was then transferred to bottles containing 100 mL of tryptone soya broth (TSB; CM0129B, Oxoid Ltd) and incubated at 37 ± 2 °C for 72 h to potentiate bacterial/yeast growth. After 72 h, different selected mediums were inoculated to confirm the absence of microorganism growth. Selected mediums were mannitol salt agar to study potential *S. aureus* growth (MSA; CM0085, Oxoid Ltd); and for *Pseudomonas* ssp. detection pseudomonas agar base (CM0559, Oxoid Ltd) with CFC selective agar supplement (SR0103E, Oxoid Ltd); and Saboraud agar with chloramphenicol for yeast detection (CM0041B, Oxoid Ltd). Finally, to confirm the method suitability and discard possible inhibition growth caused either by the formulation or the isopropyl myristate, some sample aliquots were inoculated with a < 100 CFU microbiological load of the specified microorganisms by using the following strains: *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 9027 and *C. albicans* ATCC 10231 (American Type Culture Collection, Manassas, VA, USA).

2.8. In vitro release assays

2.8.1. Membrane selection

The membrane selection was accomplished by the evaluation in triplicate of two types of 45 mm in diameter and 0.45 µm in pore size artificial membranes; nylon (Waters Corporation, Milford, MA, USA) and polysulfone (Pall Corporation, Gelman Sciences, Ann Arbor, MI, USA). Vertical Franz diffusion cells (FDC 400, Crown Glass, Somerville, NY, USA) with an effective diffusional area of 2.54 cm^2 and 12 mL capacity receptor chamber were utilized.

Phosphate buffered saline (PBS, pH = 7.4) was utilized as receptor medium. This medium was continuously stirred by a small magnetic bar (600 rpm) and thermostated at 37 ± 1 °C assuring sink conditions.

2.8.2. Release assay

Drug release studies were carried out by placing 100 mg of sample on the donor compartment of each cell and 0.3 mL aliquots were withdrawn from the receptor compartment via syringe at predefined time points for 6 h. The extracted volume was immediately replaced by an equivalent volume of PBS. The whole experiment was performed with six cells and temperature maintained at 37 ± 1 °C.

2.9. Ex vivo permeation studies

The *ex vivo* drug permeation study was performed in triplicate with Franz-type vertical cells (Vidra Foc, Barcelona, Spain). Porcine vaginal mucosa pieces were placed in the membrane holders between the donor (1.5 mL) and the receptor (6 mL) compartments; providing a diffusion area of 0.636 cm^2. The receptor compartment was filled in with PBS pH = 7.4 and kept under stirring at 600 rpm. Cell temperature was controlled at 37 ± 1 °C by a circulating water bath and sink conditions were guaranteed. 100 mg samples were placed on the donor compartment and 300 µL aliquots were withdrawn from the receptor compartment for a maximum of 6 h and replaced by the same volume of receptor medium.

2.10. Ex vivo recovery and extraction assays

When permeation test was finished, tissue membranes were dismantled and cleaned with gauze soaked in 0.05% solution of sodium dodecyl sulfate and washed in distilled water. DOX retained in the vaginal mucosa was extracted using methanol-water (70:30) extraction medium during 20 min under sonication in an ultrasound bath. Three weighed porcine vaginal mucosae onto which a known DOX amount had been previously added were used as reference to determine the extractive potential of the technique.

2.10. DOX quantification

A validated high-performance liquid-chromatography (HPLC) method was used to quantify DOX from the release and permeation studies (Sanz et al., 2015b). The HPLC system consisted of a Waters Alliance 2695 with a Waters 2996 photodiode array detector set a 208 nm (Waters Co., Milford, MA, USA). A C18 3.5 µm, 4.6 × 100 mm, column was employed (SunFire, Waters Co., Milford, Ma, USA). The mobile phase consisted of methanol:ammonium acetate buffer (0.05M, pH = 6): water (72:18:10, v/v/v) under isocratic elution at the flow rate of 0.7 mL/min. The injection volume was 30 µL. All tests were performed at room temperature.

2.11. Data & statistical analysis

Data obtained from the release study was plotted as a function of time and fitted to different kinetic equations to discern which model best describes the release pattern of the formulation:

$$\text{Zero order: } \%Rt/\%R\infty = k \times t \quad (1)$$

$$\text{First order: } \%Rt/\%R\infty = 1 - e^{-k \times t} \quad (2)$$

$$\text{Hyperbola: } \%Rt/\%R\infty = R\infty \times {}^{t}\!/\!_{(k+t)} \quad (3)$$

$$\text{Higuchi: } \%Rt/\%R\infty = k \times t^{1/2} \quad (4)$$

$$\text{Modified Higuchi: } \%Rt/\%R\infty = k \times t^{1/2} + p \quad (5)$$

Where $\%R_t$ is the drug released (%) at time t, $\%R_\infty$ is the total drug released (%), $\%Rt/\%R_\infty$ is the fraction of drug released at time *t*, *k* is the release rate constant. The best fit was estimated by the Prism® software v. 5.01 (GraphPad Softwre Inc., San Diego, CA, USA) and according to the AIC (Akaike's Information Criterion) value.

For the *ex vivo* study, the cumulative amount of DOX (Q_t) permeated through porcine vaginal mucosa was estimated at each time point from the concentration of DOX present in the receptor medium and it was plotted as a function of time. Permeation parameters as flux (*J*, µg/h/cm^2) and lag time (T_l, h) were calculated at steady state by linear regression analysis using the Prism® software v. 5. The permeability coefficient (*Kp*, cm/h) was estimated by dividing the flux by the initial drug concentration (C_o, mg/mL) in the donor compartment. Additionally, the partition parameter (P_1) and the diffusion parameter (P_2) were established from the estimated K_p and T_l values. Finally, the potential DOX concentration to be achieved at systemic level after vaginal application was predicted as the theoretical human plasmatic steady-state concentration (Css, µg/mL) from the following equation:

$$Css = S \times {}^{J}\!/\!_{Clp} \quad (6)$$

Where *J* is the flux estimated with the permeation study, *S* is the hypothetical area of application in the vagina mucosa and *Clp* is the plasma clearance in humans.

The results within this study are given as the mean ± SD unless stated otherwise. Statistical analysis was performed with Prism® software v. 5.01. Results were analyzed by ANOVA one-way analysis of variance ($p < 0.05$).

3. Results and discussion

3.1. Semisolid formulation

The selected vehicle is an excipient for buccal administration composed of the anionic polymer sodium carboxymethyl cellulose (NaCMC) a hydrophilic gelling product and a fatty base. NaCMC is considered a polymer very suitable to elaborate mucoadhesive devices because of its properties such as film-forming ability, biodegradability, swellability, non-toxicity and mucoadhesivity (Perioli et al., 2009). Additionally, menthol and transcutol® were selected as penetration enhancers in order to promote drug release, drug mucosa penetration and drug retention at mucosal level in the same way as they did for a previously studied buccal formulation (Sanz et al., 2017). The semisolid formulation presented white color; it was translucent with a slight minty flavor.

3.2. Rheological characterization

Fig. 3 depicts the steady-state rheological measurements as a function of the shear rate. A non-linear relationship was observed between the shear stress and the shear rate. Besides, the viscosity decreased as the shear rate increased from 0 to 100 s^{-1}. This was indicative of the shear-thinning action. Thus, the formulation presented a non-Newtonian pseudoplastic behavior. Additionally, the hysteresis loop formed by the upward flow curve and the downward one suggested a thixotropic response. Both characteristics are desirable in topical formulations since the pseudoplastic flow allows the formation of a consistent film covering the application area whereas thixotropy helps to maintain the suspending components' stability and it may have a positive effect on drug release by facilitating its diffusion through the structural disarrangement (Acosta et al., 2015). Viscosity mean value at 100 s^{-1} was estimated at 0.445 ± 0.020 Pa/s whereas thixotropy was of 447 Pa/s.

Rheological oscillatory tests were performed to assess the viscoelasticity of the formulation. In vaginal drug delivery, viscoelasticity plays an important role to assure the proper viscous-elastic balance to facilitate easy application, adequate spreading inside the vaginal cavity

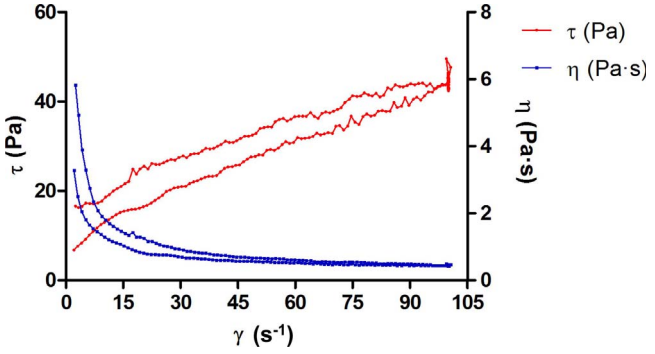

Fig. 3. Viscosity curves (blue line) and flow curves (red line) of the DOX mucoadhesive formulation.

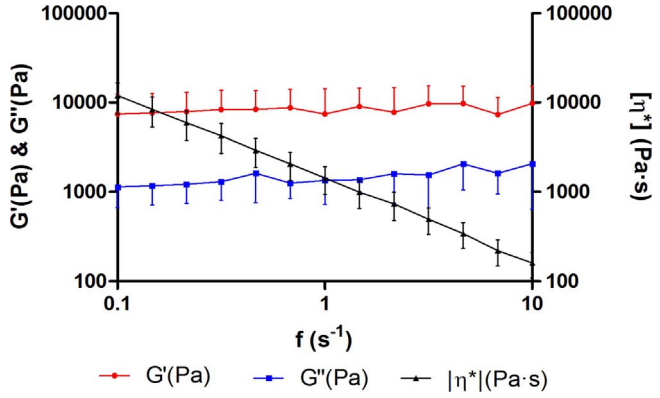

Fig. 4. Storage modulus (G'), loss modulus (G'') and complex viscosity (η*) obtained with the frequency sweep test of the DOX mucoadhesive formulation.

and increased drug residence by better withstanding *in vivo* stresses. A vaginal product with low viscosity (loss tangent (ratio G''/G'): tan (δ) > 1) would be easily spread within the vagina promoting the vaginal epithelium contact. However, its retention in the vaginal cavity would not be expected to last long due to its inability to resist the *in vivo* stresses and dilution with vaginal fluids, causing its leakage from the vagina. Oppositely, a highly elastic product (tan(δ) < < 1) would offer a higher residence time due to its greater resistance to dilution and *in vivo* stresses. However, its application and proper intravaginal spreading would be compromised (Yu et al., 2011).

As per the oscillatory stress sweep results (data not shown), a constant shear stress of 10 Pa and a 0.5 mm plate gap were selected for the frequency sweep test. Fig. 4 shows the results of the frequency sweep test (dynamic test). The storage modulus (G') was significantly higher than the loss modulus (G'') indicating the prevalence of the elastic component over the viscous one (tan(δ) < 1) which also suggested a proper balance for vaginal application.

A vaginal semisolid formulation is usually applied using a vaginal applicator from which the product is extruded. Once applied, the drug is expected to spread through the vaginal cavity, covering the mucosa epithelium and remaining the necessary time to enable its protective action and/or to allow for the release of the drug substance.

The observed pseudoplastic, tyxothropic and predominant elastic behavior of the test formulation can positively influence its application, spreadability and retention on the vaginal cavity. In particular, pseudoplasticity confers to the product a strong decrease in viscosity whenever the shear grows, allowing for the product to be expelled through an applicator with a lower energy input at the same flow velocity.

It was deemed necessary to study its syringeability, extensibility and mucoadhesive properties. The mean syringeability value for the proposed formulation was 39.02 ± 5.3 mJ. This low result implies that the product was easily discharged from the applicator, since values below 380 mJ are considered suitable for injectable administration by syringe (Campaña-Seoane et al., 2014). Besides, it should also be considered that the vaginal applicators consist of a hollow tube which open lumen is much bigger than the one in syringes. So, in clinical practice, the work to expel the formulation should be lower. Additionally, as soon as applied, the formulation should spread throughout the vaginal mucosa. The spreadability results were fitted to mathematical models in an attempt to predict its behavior and compare with other results. Hyperbola (two site binding) model was the model with the best adjustment quality. The goodness of the fitting was confirmed by a coefficient of determination (R^2) of 0.9956.

The results of the extensibility study are presented in Table 1. As expected, extensibility values got bigger with increasing loaded weight. Our reported results are within the range of extensibility measurements found for other vaginal semisolid products (Campaña-Seoane et al., 2014).

Table 1
Results of the extensibility test. Values are given as mean ± SD (n = 3).

Loaded weight	20 g	50 g	100 g	200 g	300 g
Surface (cm^2)	6.7 ± 0.5	8.0 ± 0.3	9.7 ± 0.3	11.3 ± 0.3	12.8 ± 0.4

3.3. Mucoadhesive properties

To overcome the potential lack of tissue retention, low spreadability, leakage and self-cleaning action of the vaginal tract, vaginal formulations should be deeply studied during their development as regards their rheological and bioadhesive properties.

Bioadhesion concerns the attachment of macromolecules to a biological tissue. A particular case of bioadhesion is mucoadhesion and it occurs whenever the attachment is with mucus glycoproteins or mucosal membranes (Palmeira-de-Oliveira et al., 2015). Thus, in the particular case of vaginal drug delivery, we may refer to either bioadhesion or, more specifically, mucoadhesive capacity of the product to be retained at mucosal level. Satisfactory mucoadhesion to the vaginal tissue should allow the intimate and prolonged contact between the drug and the mucosa epithelium avoiding uncomfortable formulation leakage outside the vagina. Mucoadhesion plays an important role in increasing the residence time at the vaginal cavity (Rençber et al., 2017). Besides, as the proposed formulation is pseudoplastic, it is expected that the likelihood of leakage and spreadability may be increased after dilution with vaginal fluids (Yu et al., 2011). Thus, the mucoadhesive properties of the proposed formulation have been sensitively studied with and without the presence of SVF.

There is not a unique standard method worldwide accepted for the characterization of the bioadhesive properties of formulations when applied onto mucosa. The most commonly methods used to determine bioadhesion are those measuring tensile force, shear stress strength and residence time (Caramella et al., 2015).

Thus, in the present work, a couple of simple tests assessing the effect of shear stress and retention time plus some more complex for measuring attachment and detachment forces (tensile force) were selected for the mucoadhesion study. While the study of the detachment force is the method most commonly used (Bassi da Silva et al., 2017), simple tests are easy to operate and provide a preliminary insight into the bioadhesive properties of formulations under development without the need to employ more sophisticated equipment. Additionally, those simpler tests also allow studying the influence of vaginal fluids, making possible to promptly discard formulations significantly affected by them.

The binding of the semisolid formulation to the vaginal mucosa and its cohesiveness was initially assessed with the paddle dissolution apparatus (Fig. 1). No differences in the vaginal retention of the tested product were observed under the stirring conditions for the first 5 h of the experiment as the samples remained attached to the porcine mucosa showing similar initial adhesiveness. Mucoadhesiveness was considered similar for all samples despite the use of SVF in half of the tested mucosae. The formulation detachment occurred after 5 h in all samples. The technique proposed by the authors was easy to use and provided consistent mucoadhesive information with a simple method and common laboratory equipment.

Effect of gravity is biologically relevant for the retention of a drug formulation inside the vaginal cavity. Postural changes may affect the confinement of the inserted medication as the inclination of the vaginal axis varies significantly with posture (Kieweg et al., 2004). On this sense, potential formulation leakage due to gravity was studied using vertically hanged mucosae (Fig. 2). The study also considered the potential increase in leakage by dilution with vaginal fluid as pseudoplastic formulations are expected to increase their spreading capacity

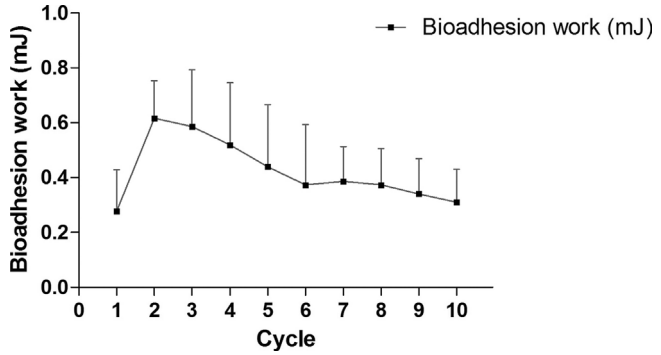

Fig. 5. Differences on the work of mucoadhesion in 10 consecutive cycles in the presence of SVF.

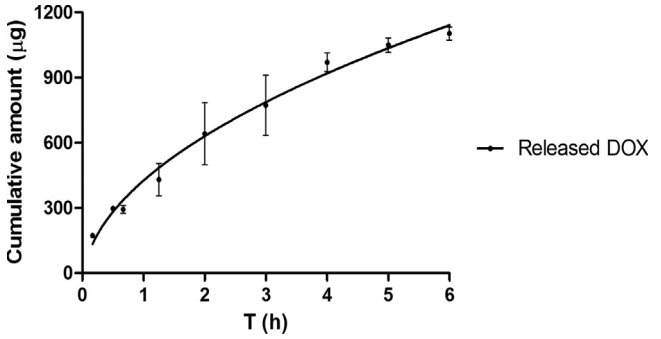

Fig. 6. Released cumulative amount of DOX from the semisolid formulation and its modelistic approach (modified Higuchi model) at 37 °C. Results are given as mean ± SD (n = 6).

following dilution with fluids. The semisolid formulation was retained in the suspended vagina for the entire observational period. No single amount of cream was measured by the balances underneath the suspended vaginas for any of the replicates nor differences were observed between the vaginas containing SVF and those without it. Thus, the proposed formulation showed good retention under the experimental conditions.

Finally, measurements using the texture analyzer device confirmed the considerable bioadhesive properties of the formulation. In the absence of SVF, the maximum force and the bioadhesion work were determined at 1.46 ± 0.26 N and 0.69 ± 0.33 mJ, respectively. Thus, the bioadhesive characteristics of the tested samples were considered appropriate as the observed mean bioadhesion work was higher than those of reported commercially available vaginal formulations tested by the same methodology (Crinone®, 0.50 mJ; Zidoval®, 0.40 mJ) (Campaña-Seoane et al., 2014). Similarly, the study of bioadhesive properties in presence of SVF confirmed the suitability of the formulation. The work of adhesion was not significantly modified which each repetition cycle (see Fig. 5), confirming that dilution in SVF does not imply a loss of mucoadhesive capacity of the tested product.

3.4. Stability studies

No differences were observed for any of the investigated parameters on samples throughout the entire study despite storage at different temperature conditions. Only samples stored at 40 °C showed a slightly different consistency at visual level. Thus, the formulation was considered stable when stored for 3 months at < 30 °C.

The pH measurements gave a result of ~ 6 for all studied samples at all temperature conditions and time points, confirming the physical stability of the formulations. This pH value was considered suitable for vaginal application. The physiological vaginal pH of women of reproductive age is around 3.5 – 4.5, deviating to lower values in the middle of the menstrual cycle and to higher values during menstruation. Formulations with neutral and acidic pH are adequate for vaginal use (Rençber et al., 2017).

All test samples showed same result as regards the microbiological study. Neither bacterial nor yeast/mold growth was observed for any of the plates directly inoculated with the samples. In the same way, no specific growth was observed in the selective media confirming the absence of pathogenic microorganisms such as *S. aureus*, *P. aeruginosa* and *C. albicans*. Suitability of the method was proven by the promotion of the growth of the three pathogenic microorganisms from small inoculates (< 100 CFU) in the tested conditions. Thus, the employed method was not inhibited by the sample itself nor by the isopropyl myristate. Fresh and long-term stored samples at different temperature conditions depicted similar satisfactory results. Thus, it was concluded that the formulation was stable from a microbiological point of view.

3.5. Release studies

Polysulfone membrane was selected as the artificial membrane to be used for the release experiences based on its best results (data not shown). After the 6 h *in vitro* experiment the percentage of DOX released was 22%. This result was considered adequate to further appraise the formulation for *ex vivo* permeation studies and final *in vivo* application as the obtained release percentage is well above the release percentage provided by a similar DOX semisolid formulation proposed by our research team for mucosa application in the oral cavity (Sanz et al., 2017). Fig. 6 shows the drug release pattern from the semisolid formulation. Results were fitted to non-linear regression models and the lower AIC value confirmed that the release kinetics for the drug release fitted best to the modified Higuchi's square root model. AIC was estimated as suitability indicator (Mallandrich et al., 2017). According to the best fitting model (modified Higuchi), the pertinent modelistic biopharmaceutical parameters were estimated for the formulation (k = 493.4 ± 27.8 µg/h$^{0.5}$; p = − 67.25 ± 44.84 µg). Thus, the diffusion process is considered the predominant mechanism of drug release for the tested formulation. The release kinetics described by the Higuchi's model is based on Fick's law direct proportionality between the cumulative amount of released drug and the square root of time (Siepmann and Peppas, 2011).

3.6. Ex vivo permeation and recovery assays

In its final development stage, the potential permeation of DOX formulation was evaluated with the aim to demonstrate whether the therapeutic effect might take place at local level in the vaginal mucosa or at systemic level when applied during clinical practice. Ideally, some degree of permeation and drug retention in the mucosa is necessary without reaching systemic therapeutic levels to avoid antidepressant effect of DOX at central level. Porcine vaginal mucosa presents substantial similarities with human vaginal mucosa and it is the animal model most commonly used for permeation studies (Machado et al., 2015). Thus, porcine vaginal mucosa was selected to test DOX permeation. The estimated permeation kinetic parameters depicted from the *ex vivo* studies are provided in Table 2. Intact barrier properties of the mucosa suitable for permeation studies were confirmed by VTMWL results in *ex vivo* vaginal tissue (median: 25.5 g/h·m^2; min - max: 24.2–28.3 g/h·m^2).

Being developed for local effect, the total amount retained in the mucosa at the end of the permeation assay was also estimated (Table 2). The extraction medium used for the retention estimation was chosen according to previous experiments with DOX and other mucosa tissue (Sanz et al., 2017). However, foreseen potential dissimilarities between different mucosa tissues, the extractive potential of the selected medium was calculated for the concerned tissue (vaginal mucosa). According to the obtained results, the extractive technique was capable

Table 2
Estimated permeation and retention parameters of DOX semisolid formulation. Values are reported as the median (minimum and maximum, n = 3).

Biopharmaceutical parameter	Median	Minimum	Maximum
Retained quantity (μg/cm^2/g mucosa)	642.6	529.5	903.6
Flux/area (μg/h cm^2)	3.57	3.11	22.1
Lag time (h)	3.0	2.3	3.1
Permeability coefficient × 10^{-4} (cm/h)	0.714	0.622	4.42
Partition parameter × 10^{-4} (cm)	13.3	8.65	79.3
Diffusion parameter × 10^{-2} (h^{-1})	5.58	5.37	7.19

of extracting 39% of the DOX amount retained in the porcine vaginal mucosa. In overall, the proposed formulation was able to permeate and retain significant amounts of DOX in the vaginal mucosa. However, the obtained biopharmaceutical parameters suggested that the proposed formulation showed slower and lower permeation capability than a similar formulation studied for a different mucosa tissue with same penetration enhancers (Sanz et al., 2017). Nevertheless, it should be noted that this other formulation was studied on porcine buccal mucosa dermatomed at 500 μm, while the porcine vaginal mucosa was studied on its complete thickness. Additionally, the mean percentage of DOX retained in the vagina mucosa per applied dose did not present statistically differences with the percentage obtained for the oral formulation in buccal mucosa (14% and 17%, respectively; $p < 0.05$). DOX retained in the mucosa may act as a reservoir no only by increasing the local therapeutic effect but also prolonging it.

Finally, in order to assess the potential appearance of systemic side effects combined with the administration of the proposed formulation in clinical practice, equation (6) was employed. Predicted plasmatic concentrations were estimated from the *J* value obtained in the *ex vivo* experiment. A DOX plasmatic clearance of 1000 mL/min was taken as a reference (Sandig et al., 2013) and it was assumed that the drug product would spread over a vaginal surface of 100 cm^2 (Katz et al., 2015). The predicted plasmatic concentration of 6.0 ng/mL (median; range: 5.2 − 37 ng/mL) was well below the therapeutic level for DOX antidepressant effect at systemic level (150 − 250 ng/mL) (Drake et al., 1999). Thus, it is not expected for the vaginal application of the developed DOX formulation to produce any antidepressant side effect nor to cause any other significant systemic side effect related to DOX use.

4. Conclusions

A semi-solid formulation designed for vaginal application should exhibit acceptable rheological and mucoadhesive characteristics to guarantee its easy application and proper retention in the vaginal cavity. The proposed formulation, with its elastic prevalence over the viscous component, its pseudoplastic and thixotropic rheological behavior, its extensibility capability and proper syringeability, is proposed as an appropriate candidate for vaginal use. Additionally, its mucoadhesive properties (even in the presence of SVF) confirm its potential use in clinical practice. Besides, the simple bioadhesive test based on shear stress proposed with this work has been of help to gain first insight into the assessment of the bioadhesive characteristics and their potential changes due to the presence of vaginal fluid or other diluting factors. The proposed method may significantly assist to identify formulations that indicate increased retention within the vaginal cavity. On the other hand, the proposed formulation has rendered promising results as regards drug release, mucosa permeation and drug retention without promoting the drug absorption at systemic level, which minimizes the potential occurrence of systemic adverse events. Thus, the DOX semisolid formulation containing a mucoadhesive excipient plus menthol and transcutol® as permeation enhancers, might be a suitable candidate to fill a gap in the existing therapeutic tools in the vaginal neuropathic pain associated with gynecological surgery and lacerations. However, further *in vivo* testing may be necessary in the future to evaluate analgesic results.

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References

Acosta, N., Sánchez, E., Calderón, L., Cordoba-Diaz, M., Cordoba-Diaz, D., Dom, S., Heras, Á., 2015. Physical stability studies of semi-solid formulations from natural compounds loaded with chitosan microspheres. Mar. Drugs 13, 5901–5919. http://dx.doi.org/10.3390/md13095901.

Amores, S., Domenech, J., Colom, H., Calpena, A.C., Clares, B., Gimeno, Á., Lauroba, J., 2014. An improved cryopreservation method for porcine buccal mucosa in ex vivo drug permeation studies using Franz diffusion cells. Eur. J. Pharm. Sci. 60, 49–54. http://dx.doi.org/10.1016/j.ejps.2014.04.017.

Bassi da Silva, J., Barbosa de Souza Ferreira, S., de Freitas, O., Bruschi, M.L., 2017. A critical review about methodologies for the analysis of mucoadhesive properties of drug delivery systems. Drug Dev. Ind. Pharm. 43, 1053–1070. http://dx.doi.org/10.1080/03639045.2017.1294600.

Campaña-Seoane, M., Peleteiro, A., Laguna, R., Otero-Espinar, F.J., 2014. Bioadhesive emulsions for control release of progesterone resistant to vaginal fluids clearance. Int. J. Pharm. 477, 495–505. http://dx.doi.org/10.1016/j.ijpharm.2014.10.066.

Caramella, C.M., Rossi, S., Ferrari, F., Bonferoni, M.C., Sandri, G., 2015. Mucoadhesive and thermogelling systems for vaginal drug delivery. Adv. Drug Deliv. Rev. 92, 39–52. http://dx.doi.org/10.1016/j.addr.2015.02.001.

Council of Europe, 2010. Microbiological examination of non-sterile products: microbial enumeration tests. 2.6.12. European Pharmacopoeia, sixth ed. Council of Europe, Strasbourg.

das Neves, J., Amaral, M.H., Bahia, M.F., 2008. Performance of an in vitro mucoadhesion testing method for vaginal semisolids: influence of different testing conditions and instrumental parameters. Eur. J. Pharm. Biopharm. 69, 622–632. http://dx.doi.org/10.1016/j.ejpb.2007.12.007.

das Neves, J., Rocha, C.M.R., Gonçalves, M.P., Carrier, R.L., Amiji, M., Bahia, M.F., Sarmento, B., 2012. Interactions of microbicide nanoparticles with a simulated vaginal fluid. Mol. Pharm. 9, 3347–3356. http://dx.doi.org/10.1021/mp300408m.

Drake, L.A., Cohen, L., Gillies, R., Flood, J.G., Riordan, A.T., Phillips, S.B., Stiller, M.J., 1999. Pharmacokinetics of doxepin in subjects with pruritic atopic dermatitis. J. Am. Acad. Dermatol. 41, 209–214. http://dx.doi.org/10.1016/S0190-9622(99)70051-4.

Katz, D.F., Yuan, A., Gao, Y., 2015. Vaginal drug distribution modeling. Adv. Drug Deliv. Rev. 92, 2–13. http://dx.doi.org/10.1016/j.addr.2015.04.017.

Kieweg, S.L., Geonnotti, A.R., Katz, D.F., 2004. Gravity-induced coating flows of vaginal gel formulations: in vitro experimental analysis. J. Pharm. Sci. 93, 2941–2952. http://dx.doi.org/10.1002/jps.20194.

Leenstra, J.L., Miller, R.C., Qin, R., Martenson, J.A., Dornfeld, K.J., Bearden, J.D., Puri, D.R., Stella, P.J., Mazurczak, M.A., Klish, M.D., Novotny, P.J., Foote, R.L., Loprinzi, C.L., 2014. Doxepin rinse versus placebo in the treatment of acute oral mucositis pain in patients receiving head and neck radiotherapy with or without chemotherapy: a phase III, randomized, double-blind trial (NCCTG-N09C6 [Alliance]). J. Clin. Oncol. 32, 1571–1577. http://dx.doi.org/10.1200/JCO.2013.53.2630.

Machado, R.M., Palmeira-de-Oliveira, A., Gaspar, C., Martinez-de-Oliveira, J., Palmeira-de-Oliveira, R., 2015. Studies and methodologies on vaginal drug permeation. Adv. Drug Deliv. Rev. 92, 14–26. http://dx.doi.org/10.1016/j.addr.2015.02.003.

Mallandrich, M., Fernández-Campos, F., Clares, B., Halbaut, L., Alonso, C., Coderch, L., Garduño-Ramírez, M.L., Andrade, B., Del Pozo, A., Lane, M.E., Calpena, A.C., 2017. Developing transdermal applications of ketorolac tromethamine entrapped in stimuli sensitive block copolymer hydrogels. Pharm. Res. 34, 1728–1740. http://dx.doi.org/10.1007/s11095-017-2181-8.

Nowak, J., Laffleur, F., Bernkop-Schnürch, A., 2015. Preactivated hyaluronic acid: a potential mucoadhesive polymer for vaginal delivery. Int. J. Pharm. 478, 383–389.

Ochoa Andrade, A., Parente, M.E., Ares, G., 2014. Screening of mucoadhesive vaginal gel formulations. Brazilian J. Pharm. Sci. 50, 931–942. http://dx.doi.org/10.1590/S1984-82502014000400029.

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

Palmeira-de-Oliveira, R., Palmeira-de-Oliveira, A., Martinez-de-Oliveira, J., 2015. New strategies for local treatment of vaginal infections. Adv. Drug Deliv. Rev. 92, 105–122. http://dx.doi.org/10.1016/j.addr.2015.06.008.

Perioli, L., Ambrogi, V., Venezia, L., Giovagnoli, S., Pagano, C., Rossi, C., 2009. Formulation studies of benzydamine mucoadhesive formulations for vaginal administration. Drug Dev. Ind. Pharm. 35, 769–779. http://dx.doi.org/10.1080/

03639040802592435.
Rençber, S., Karavana, S.Y., Şenyiğit, Z.A., Eraç, B., Limoncu, M.H., Baloğlu, E., 2017. Mucoadhesive in situ gel formulation for vaginal delivery of clotrimazole: formulation, preparation, and in vitro/in vivo evaluation. Pharm. Dev. Technol. 22, 551–561. http://dx.doi.org/10.3109/10837450.2016.1163385.
Sandig, A.G., Campmany, A.C., Campos, F.F., Villena, M.J., Naveros, B.C., 2013. Transdermal delivery of imipramine and doxepin from newly oil-in-water nanoemulsions for an analgesic and anti-allodynic activity: development, characterization and in vivo evaluation. Colloids Surf. B. Biointerfaces 103, 558–565. http://dx.doi.org/10.1016/j.colsurfb.2012.10.061.
Sanz, R., Calpena, A.C., Mallandrich, M., Clares, B., 2015a. Enhancing topical analgesic administration: review and prospect for transdermal and transbuccal drug delivery systems. Curr. Pharm. Des. 21, 2867–2882. http://dx.doi.org/10.2174/1381612821666150428145627.
Sanz, R., Clares, B., Mallandrich, M., Casals, I., Bellido, D., Calpena, A.C., 2015b. Validation of doxepin quantitative determination methods for their application to in vitro, ex vivo and in vivo studie. Curr. Pharm. Anal. 11, 269–277. http://dx.doi.org/10.2174/1573412911666150410003357.
Sanz, R., Calpena, A.C., Mallandrich, M., Gimeno, Á., Halbaut, L., Clares, B., 2017. Development of a buccal doxepin platform for pain in oral mucositis derived from head and neck cancer treatment. Eur. J. Pharm. Biopharm. 117, 203–211. http://dx.doi.org/10.1016/j.ejpb.2017.04.019.
Siepmann, J., Peppas, N.A., 2011. Higuchi equation: derivation, applications, use and misuse. Int. J. Pharm. 418, 6–12. http://dx.doi.org/10.1016/j.ijpharm.2011.03.051.
Steege, J.F., Zolnoun, D.A., 2009. Evaluation and treatment of dyspareunia. Obstet. Gynecol. 113, 1124–1136. http://dx.doi.org/10.1097/AOG.0b013e3181a1ba2a.
Thompson, I.O.C., Van der Bijl, P., Van Wyk, C.W., Van Eyk, A.D., 2001. A comparative light-microscopic, electron-microscopic and chemical study of human vaginal and buccal epithelium. Arch. Oral Biol. 46, 1091–1098. http://dx.doi.org/10.1016/S0003-9969(01)00082-6.
Van Eyk, A.D., Van Der Bijl, P., 2004. Comparative permeability of various chemical markers through human vaginal and buccal mucosa as well as porcine buccal and mouth floor mucosa. Arch. Oral Biol. 49, 387–392. http://dx.doi.org/10.1016/j.archoralbio.2003.12.002.
Vanić, Ž., Škalko-Basnet, N., 2013. Nanopharmaceuticals for improved topical vaginal therapy: can they deliver? Eur. J. Pharm. Sci. 50, 29–41. http://dx.doi.org/10.1016/j.ejps.2013.04.035.
Vermani, K., Garg, S.D., Zaneveld, L.J., 2002. Assemblies for in vitro measurement of bioadhesive strength and retention characteristics in simulated vaginal environment. Drug Dev. Ind. Pharm. 28, 1133–1146. http://dx.doi.org/10.1081/DDC-120014580.
Yu, T., Malcolm, K., Woolfson, D., Jones, D.S., Andrews, G.P., 2011. Vaginal gel drug delivery systems: understanding rheological characteristics and performance. Expert Opin. Drug Deliv. 8, 1309–1322. http://dx.doi.org/10.1517/17425247.2011.600119.