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Research article

Formulation and evaluation of fluconazole emulgels for potential treatment of vaginal candidiasis

Oriyomi Temitayo Ogedengbe, Oluwadamilola Miriam Kolawole *

Department of Pharmaceutics and Pharmaceutical Technology, University of Lagos, Nigeria

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ABSTRACT

Localised treatment of vaginal candidiasis could improve the therapeutic outcomes of patients with vaginal candidiasis as well as reduce drug dosage and dosing interval. The aim of the research project was to develop fluconazole emulgel formulations, and evaluate their physicochemical, drug release, antifungal, safety, and stability profile, for potential treatment of vaginal candidiasis. Xanthan gum and HPMC E5LV-based fluconazole emulgels were prepared using the spontaneous emulsification method and their physicochemical properties, drug content, drug release profile, anti-fungal activity against *Candida albicans*, *in vivo* vaginal biocompatibility and stability profile were evaluated using standard protocols. The fluconazole emulgels exhibited satisfactory properties: pH: 5.2–5.4; spreadability: 1.6–2.5 cm; apparent viscosity: 85–314 cP; zone of inhibition against *Candida albicans*: 22–38 mm; drug content: 91–102 %, and vaginal biocompatibility. All the studied fluconazole emulgels exhibited controlled fluconazole release over 6 h and their drug release kinetics fitted well with Korsmeyer-Peppas model. HPMC-based emulgels exhibited unsatisfactory real-time stability profile. To our knowledge, this is the first report where xanthan gum and HPMC E5LV-based fluconazole emulgels have been studied for possible treatment of vaginal candidiasis. Xanthan gum-based fluconazole emulgels are promising drug formulations that could reduce the drug dosage and dosing frequency. In addition, they could serve as alternative dosage forms to Flucos® gel.

1. Introduction

Vaginal candidiasis (vaginitis) is a fungal yeast infection, majorly caused by *Candida albicans,* and it is characterized by vaginal itch, painful sexual intercourse, dysuria, and abnormal vaginal discharge [1]. It affects women in their reproductive years and 70 % of women have this disease during their lifetimes [2]. Also, vaginal candidiasis is one of the opportunistic infections that affect HIV positive women worldwide, and the disease is more prevalent and recurrent in HIV-infected women than in healthy women [2] Risk factors include prolonged antibiotic and oral contraceptive usage; pregnancy; hormone replacement therapy; uncontrolled diabetes mellitus; immunosuppressive therapy, and HIV infection [3].

Fluconazole, a triazole antifungal agent, is recommended for the treatment of superficial and invasive vaginal candidiasis, and it exerts fungicidal activity by binding to fungal cytochrome P-450 to disrupt fungal cell membranes, resulting in cell death [4]. The drug is commercially available as oral tablets/capsules, intravenous injection, as well as vaginal creams and gels [1] Following oral or systemic administration of fluconazole formulations, fluconazole exhibits high volume of distribution, resulting in adverse effects such

* Corresponding author.
E-mail addresses: aclofleur@gmail.com (O.T. Ogedengbe), omkolawole@unilag.edu.ng (O.M. Kolawole).

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as nausea, vomiting, bloating and abdominal discomfort, and limits patient compliance to treatment regimen [1]. In addition, complicated cases of vaginal candidiasis in immunocompromised patients, including recurrent forms of the infection require prolonged duration of treatment for up to 6 months [1].

Local fluconazole delivery to the vagina could overcome the challenges of oral and systemic fluconazole therapy by enhancing vaginal drug bioavailability; reduce drug dosage and dosing frequency as well as improve the therapeutic outcomes of patients with vaginal candidiasis. However, conventional vaginal drug products have short-lived therapeutic action because they are formulated using non-mucoadhesive materials, and cervicovaginal fluid readily washes off, the loaded drug, necessitating frequent vaginal application of the drug formulation for effective therapy [5].

Emulgels comprises of emulsions incorporated into mucoadhesive hydrogel base. Over the last two decades, they have been investigated for topical drug delivery because they combine the benefits of traditional emulsions and gels, facilitating the solubilization of both hydrophilic and hydrophobic drugs as well as controlled release of the loaded drugs via the degradation or erosion of the hydrogel matrix [6]. In addition, they are thixotropic, greaseless, spreadable, washable, emollient, non-staining, long shelf-life, and agreeable appearance [6]. A wide range of anti-infective agents have been formulated as topical emulgels and they include ofloxacin [7], clotrimazole [8], clarithromycin [9], azithromycin [10]; cefpodoxime [11] and erythromycin [12].

Plant-based essential oils such as clove oil and cinnamon oil have been explored as penetration enhancers for the preparation of topical formulations [13,14]. Khullar and coworkers reported that topical mefenamic emulgels formulated using clove oil demonstrated a superior drug permeation enhancing effect relative to menthal oil-based emulgels [13]. In a latter study, Hosny et al. (2021) revealed that cinnamon oil incorporated into hydroxypropyl cellulose nanoemulgels improved its antimicrobial and analgesic activity, for potential treatment of oral infections [15].

Studies have shown that the polymeric gel component of emulgels could dictate their physicochemical and biological properties. For instance, xanthan gum-based indomethacin emulgels exhibited superior physicochemical and anti-inflammatory properties relative to Carbopol 934-based samples [16]. Also, HPMC 2910-based fluconazole emulgels displayed superior physicochemical and antifungal activity relative to Carbopol 940 and Pluronic based fluconazole emulgels [17]. In addition, Carbopol 940-based ofloxacin emulgels showed improved physicochemical and antibacterial activity in comparison to HPMC K100 M-based samples [7]. Furthermore, HPMC K4M-based emulgels exhibited superior controlled drug release and antibacterial activity relative to Carbopol 934 and xanthan gum-based samples [11]. Nevertheless, the reported *in vitro* and *in vivo* performance of emulgels may also be dependent on the nature of the therapeutic agent incorporated in the emulgels.

Various therapeutic emulgels have been commercialized, which include Voltaren® (diclofenac sodium), Miconaz-H (miconazole nitrate), Pernox® (benzoyl peroxide), and Clinagel® (clindamycin phosphate) [18]. However, fluconazole emulgels have not been successfully translated to the clinics.

Previously reported fluconazole emulgels were studied for potential treatment of skin fungal infection, thus they were formulated to have a pH of 5–6.6 and their drug release profiles were evaluated at pH 5.5 [17], which does not simulate vaginal environment. Also, the formulations did not contain permeation enhancers that may be beneficial for the treatment of invasive and recurrent vaginal candidiasis. Development of fluconazole emulgels that could penetrate underlying vaginal tissues to treat invasive and recurrent vaginal fungal infections is desirable.

To our knowledge, palm olein-based fluconazole emulgels have never been studied for the potential treatment of vaginal candidiasis. The aim of the research project is to prepare fluconazole emulgel formulations, and evaluate their physicochemical, drug release, antifungal, vaginal biocompatibility and stability profile, for potential treatment of vaginal candidiasis. The influence of the type of polymeric gelling agent and permeation enhancer, on the properties of the formulations was also investigated.

In this study, we developed fluconazole emulgels and evaluated their physicochemical properties, drug content, drug release profile, antifungal activity, vaginal biocompatibility and stability in order to establish their suitability as drug formulations to treat vaginal candidiasis.

2. Materials and methods

2.1. Materials

Fluconazole (Macklin, China), HPMC E5 LV, methyl paraben, propyl paraben, hydrochloric acid, potassium dihydrogen phosphate (Loba Chemie, India), xanthan gum (Titan Biotech, India), polyethylene sorbitan monooleate (tween 80), clove oil (Molychem, India), sorbitan monolaurate (span 20) (Acros Organics, USA), cinnamon leaf oil (Deo Organics, Sri Lanka), palm olein (raffles oil, Nigeria), methanol (BDH, UK), Strat-M™ membrane, 0.45 μm (Millipore, UK), filter units (0.45 μm), *Candida albicans* (clinical isolate grown at 37 °C for 24 h on Sabouraud dextrose agar), and deionised water.

2.2. Ethical approval of animal experimentation

Ethical approval was obtained in order to carry out the *in vivo* vaginal biocompatibility studies of the developed fluconazole emulgels on female "Priceless" rats (125–138 g; 10–12 weeks) procured from Lagos, Nigeria (CMUL/ACUREC/05/23/1208). The work was carried out in compliance with the regulations of the College of Medicine, University of Lagos Animal Care and Use Research Ethics Committee, Lagos, Nigeria and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines [19].

2.3. Formulation of blank and fluconazole containing emulgels

Eight blank emulgels (Table 1) were prepared by incorporating HPMC or xanthan gum gel base with the blank emulsions, using a previously reported spontaneous emulsification technique [16]. Then, four optimised fluconazole emulgels (FLU-F2, FLU-F4, FLU-F6 and FLU-F8) containing 0.5 % of fluconazole were formulated using blank emulgels with acceptable physicochemical properties.

Briefly, HPMC gel (50 g) was prepared by dispersing predetermined amount of HPMC powder in the calculated amount of distilled water maintained at 80 °C and the dispersion was stirred for 10 min, and stored for 2 h at room temperature to ensure gel hydration. Also, xanthan gum gel base (50 g) was prepared using similar procedure described for HPMC gel base [6,16]. Then, the blank emulsions were prepared using predetermined amounts of the aqueous and oily constituents, and the emulsion was incorporated into the gel base (1:1) (Table 1).

The most promising blank formulations were selected for further development. With the fluconazole emulgels, blank emulsions were prepared and fluconazole was added to the emulsion when it has cooled to 40 °C. Then, the obtained fluconazole emulsion was incorporated into the gel base (mass ratio of 1:1) to generate the fluconazole emulgels. Afterwards, the blank or fluconazole emulgels were packed in wide mouthed cream jar and they were protected from light and moisture.

2.4. Evaluation of emulgels

2.4.1. Organoleptic evaluation

The organoleptic properties of the emulgels such as colour, consistency, and homogeneity were evaluated by visual examination.

2.4.2. pH measurements

The pH of emulgel samples was determined using a digital pH meter.

2.4.3. Conductivity determination

The conductivity of the emulgels was determined at 25 °C using the Jenway 4510 Conductivity Meter (Jenway, UK). The electrode was calibrated using gas-free distilled water prior to sample analysis.

2.4.4. Rheological evaluation

The viscosities of different emulgel samples were evaluated at 25 °C using a viscometer coupled with spindle 2 (NDJ-8T, Mesulab Instruments, China). Briefly, the emulgels were secured into the sample holder and the spindle was lowered into the centre of the emulgels and the machine rotated at different speeds (6, 15, 30, and 60 rpm), in order to determine the shear-dependent viscosities of the formulations.

2.4.5. Spreadability

The emulgels formulation (1 g) were sandwiched between two glass slides, and the 20 g-weight was placed over the upper glass slide until no further spreading was evident. The difference in the spread circle diameter before and after application of the 20 g-weight was recorded, which depicted the spreadability of the emulgels [20].

2.4.6. Drug content

The drug contents of the fluconazole emulgels were evaluated using a previously reported method (equation (1)) [21]. Briefly, the emulgels samples (1 g) which was expected to contain 5 mg of fluconazole, was put in a beaker and 80 mL of methanol was added to the drug and warmed up at 50 °C for 30 min. Then, the emulgel methanolic solution was filtered into 100 mL-volumetric flask and the resultant drug solution was made up to volume using methanol. Afterwards, the sample was agitated in an ultrasonic bath for 5 min to facilitate complete drug dissolution. The absorbance of the prepared solution was measured at the wavelength of maximum absorption

Table 1
Composition of the blank and fluconazole emulgels.

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	FLU-F2	FLU-F4	FLU-F6	FLU-F8
Fluconazole (%)	–	–	–	–	–	–	–	–	0.5	0.5	0.5	0.5
HPMC E5 LV (% w/w)	–	–	–	–	15	20	15	20	–	–	20	20
Xanthan gum (% w/w)	1.5	3	1.5	3	–	–	–	–	3	3	–	–
Palm olein (% v/w)	5	5	5	5	5	5	5	5	5	5	5	5
Clove oil (% w/w)	5	5	–	–	5	5	–	–	5	–	5	–
Cinnamon oil (% v/w)	–	–	5	5	–	–	5	5	–	5	–	5
Span 20 (%w/w)	4	4	4	4	4	4	4	4	4	4	4	4
Tween 80 (% v/w)	4	4	4	4	4	4	4	4	4	4	4	4
Methyl paraben (% w/w)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben (% w/w)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Key: F1 = 1.5% xanthan/clove oil; F2 = 3% xanthan/cinnamon oil; F3 = 1.5% xanthan/clove oil; F4 = 3% xanthan/cinnamon oil; F5 = 15%HPMC/clove oil; F6 = 20% HPMC/clove oil F7 = 15%HPMC/cinnamon oil; F8 = 20% HPMC/cinnamon oil-based emulgels; FLU-F2, FLU-F4, FLU-F6 and FLU-F8 are optimised fluconazole emulgels.

for the drug solution (210 nm) using UV–visible spectrophotometer. In addition, pure drug sample (5 mg) was diluted by a factor of 100 using methanol and its absorbance value was determined.

The percentage drug content was calculated as follows:

$$\%\ drug\ content = Absorbance\ of \frac{emulgels}{pure\ drug}\ x\ 100\ \% \quad (1)$$

According to USP, drug content was satisfactory if 85 %–115 % of drug was detected in the emulgels formulations [22].

2.4.7. In vitro drug permeation studies

The drug release medium was prepared by dissolving 6.81 g of potassium dihydrogen phosphate in 800 mL deionised water; stirred for 30 min and made up to 1 L solution in a volumetric flask using deionised water, and stirred for a further 10 min. Then, the buffer solution was mixed with methanol (1:1) and the pH of the drug release medium was adjusted to pH 4.2 using 0.1 M HCl solution.

The drug release studies of fluconazole emulgels were carried out using the Franz diffusion cells that comprises of a donor and receptor compartment. A suitably sized pre-hydrated cellulose membrane (0.45 μm) was mounted between the donor and receptor medium. The receptor compartment was filled with phosphate buffer (pH 4.2), and the release medium was constantly stirred using a magnetic stirrer and the whole assembly was placed on a magnetic stirrer maintained at a temperature of 37 °C. The formulation (1 g) containing 5 mg of the drug was applied homogenously into the donor compartment. Aliquots (2 mL) was withdrawn from the receptor compartment at predetermined time intervals over 6 h and immediately replaced with an equal amount of fresh prewarmed buffer solution to maintain sink conditions. Withdrawn samples were assayed for drug content spectrophotometrically at the wavelength of 212 nm using a validated UV method, with validation report presented in Table S2. Standard solutions of fluconazole in phosphate buffer/methanol mixture, pH 4.2 (1:1) (5 μg/mL to 100 μg/mL), were used to construct standard calibration curve for fluconazole quantification.

2.4.8. Drug release kinetics

The mechanism of drug release from the emulgels was studied by fitting the drug release data against zero, first order, Higuchi square root, and Korsemeyer-Peppas equation, and the most appropriate model that fitted with the drug release data was chosen [23].

2.4.9. Antifungal activity of the optimised fluconazole emulgel samples against C. albicans

The antifungal activity of fluconazole emulgels was studied using the agar ditch plate technique and the zones of fungal inhibition was calculated as shown in equation (2) [24]. Briefly, the emulgels (1 g) was poured into a ditch cut in the Sabouraud agar plate and freshly prepared *Candida albicans* culture loops were streaked across the agar at a right angle from the ditch to the edges of the plates. The plates were incubated for 24 h at 37 °C. The control plates contained plain emulgels.

The percent antifungal growth inhibition was evaluated as follows:

$$\%\ inhibition = \frac{L2}{L1} x\ 100 \quad (2)$$

Where L1 = Total length of the streaked culture while L2 = Length of inhibition.

2.4.10. Vaginal biocompatibility studies

A total of 15 rats were used for the experiment, with average weight of 0.13 g. Animals were housed in propylene cages with free access to standard laboratory diet and water. The rats were acclimatized for at least 24 h before experimentation. Five groups of female rats consisting of three rats per group were used for the vaginal irritation test. Group 1 was the untreated rat group while Groups 2–5 represented treatment group for the four types of fluconazole emulgels investigated. The fluconazole emulgels (100 mg containing 0.5 mg fluconazole per rat) were applied to the vaginal mucosa every 24 h over 3 days, and the development of erythema and edema was monitored for 72 h. The sensitivity of the rats to the emulgel formulation was graded as 0 (no reaction), 1 (slightly patchy erythema), 2 (patchy erythema) and 3 (severe erythema with or without edema) [16].

2.4.11. Accelerated stability studies: centrifugal analysis

Centrifugal analysis was carried out according to a previously reported method [20]. The test was carried out to evaluate the stability profile of the optimised fluconazole emulgels. The various emulgels (5 g) were transferred into centrifuge vials and secured in the Eppendorf Centrifuge 5810 (Sigma-Aldrich, UK), and the equipment was run at 4000 rpm for 10 min over 2 runs. The creaming index of the samples (equation (3)) was evaluated as detailed below:

$$\text{Creaming index } (\%) = \text{HS}/\text{HE x } 100 \quad (3)$$

Where HS is the height of the cream layer after centrifugal analysis, and HE is the total height of the emulgel formulation before centrifugation. The smaller the value of creaming index, the more stable is the emulgel.

2.4.12. Real-time stability studies at 25 °C/65 % RH and 40 °C/75 % RH

The stability profiles of four optimised fluconazole emulgels were studied for 3 months. The samples (10 g each), were packed in air-tight containers and the products were maintained at 25 °C/65 % RH and 40 °C/75 % RH, and assessed monthly for colour,

consistency, pH and any sign of phase separation.

2.4.13. Statistical analysis

The evaluated parameters were presented as mean ± standard deviation. One-Way ANOVA/Bonferroni post-hoc test was used to evaluate statistical differences between data sets, with $p < 0.05$, indicating significance in statistical differences.

3. Results/discussion

Vaginal candidiasis is a common fungal vaginal infection that affects females during their lifetime. HIV-positive women are particularly burdened with this disease due to their immunocompromised health condition [2]. Topical drug formulations may be preferred to oral drug products because they could offer effective disease treatment at reduced drug dosage and dosing frequency as well as eliminate systemic side-effects.

Though, fluconazole is a BCS Class II drug with satisfactory skin permeability [4], the incorporation of permeation enhancers into fluconazole emulgels may be beneficial to treat invasive and recurrent vaginal candidiasis, especially in immunocompromised patients. To our knowledge, fluconazole emulgels have never been investigated for potential vaginal application. Thus, the aim of this research was to develop fluconazole emulgels and evaluate their physicochemical properties, drug content, drug release, antifungal activity, biocompatibility, and stability profile, for potential treatment of vaginal candidiasis.

Palm olein is the liquid part of fractionated palm oil that contains higher levels of oleic acid (39–45 %) and linoleic acid (10–13 %) in comparison to palm oil [25]. Moreover, it is rich in antioxidants, carotenoids, vitamin E and phospholipids that provide excellent skin nourishment. Thus, palm olein could be used to formulate topical products [26,27]Thus, we have chosen palm olein as major oily phase.

3.1. Organoleptic properties of blank and fluconazole emulgels

All the studied blank and fluconazole emulgels displayed satisfactory organoleptic properties as they were creamy in colour; homogenous, smooth and free of grittiness. There were no remarkable differences in the organoleptic properties of the emulgels after drug incorporation.

3.2. Physicochemical properties of blank and fluconazole emulgels

A healthy vaginal pH ranges from 3.8 to 5, which prevents bacterial and fungal vaginal infections [28]. Moreover, neutral and basic formulations could induce vaginal irritation [28]. Thus, it is desirable that drug formulations intended for vaginal application exhibit satisfactory pH between 5 and 5.5. The need for an increased concentration of HPMC to formulate optimised fluconazole emulgels may be due to the grade of HPMC used for the work (HPMC E5LV). In addition, HPMC exhibits greater hygroscopicity than xanthan gum, necessitating higher concentration of the HPMC to achieve suitable physicochemical properties [17].

The pH values of the blank optimised emulgels ranged from 5.5 to 6.4 and the incorporation of fluconazole into the emulgels improved their acidity, with the fluconazole emulgels exhibiting acceptable pH values from 5.2 to 5.4. In addition, the blank and fluconazole containing xanthan based emulgels were more acidic than HPMC-based samples, suggesting that the type of polymer used to formulate emulgels could influence the pH of the formulation. Xanthan gum and HPMC-based emulgels prepared using 3 % and 20 % of the polymer, respectively, exhibited improved acidity in comparison to those formulated using 1.5 % xanthan gum and 15 % of HPMC (Table 2).

Table 2
Physicochemical properties of blank and fluconazole emulgels.

Sample	pH	Conductivity (μS)	Spreadability (cm)	Viscosity (cP)			
				6 rpm	12 rpm	30 rpm	60 rpm
F1	5.8 ± 0.1	281 ± 6	1.9 ± 0.2	917 ± 10	626 ± 2	253 ± 1	179 ± 1
F2	5.5 ± 0.1	549 ± 7	0.4 ± 0.1	1430 ± 29	1051 ± 19	529 ± 5	340 ± 6
FLU-F2	5.3 ± 0.1	328 ± 5	1.6 ± 0.1	1139 ± 27	737 ± 13	304 ± 3	209 ± 3
F3	5.5 ± 0.1	394 ± 4	2.3 ± 0.1	948 ± 10	334 ± 5	128 ± 9	98 ± 2
F4	5.5 ± 0.1	474 ± 2	1.2 ± 0.1	2053 ± 5	813 ± 9	375 ± 7	217 ± 3
FLU-F4	5.4 ± 0.1	286 ± 3	1.7 ± 0.1	552 ± 13	247 ± 12	110 ± 2	92 ± 7
F5	6.8 ± 0.1	463 ± 2	2.5 ± 0.2	442 ± 9	260 ± 8	98 ± 4	80 ± 1
F6	6.4 ± 0.1	274 ± 5	2.3 ± 0.1	458 ± 7	338 ± 2	127 ± 3	146 ± 4
FLU-F6	5.2 ± 0.1	339 ± 6	3.1 ± 0.1	358 ± 22	180 ± 12	85 ± 3	48 ± 2
F7	6.3 ± 0.1	392 ± 6	2.3 ± 0.2	366 ± 4	174 ± 4	128 ± 2	27 ± 1
F8	5.9 ± 0.1	296 ± 3	1.6 ± 0.2	1239 ± 9	848 ± 10	573 ± 5	450 ± 4
FLU-F8	5.2 ± 0.1	388 ± 9	2.5 ± 0.2	698 ± 30	445 ± 11	314 ± 12	294 ± 9

Key: F1 = 1.5% xanthan gum/clove oil; F2 = 3% xanthan gum/clove oil; F3 = 1.5% xanthan gum/cinnamon oil; F4 = 3% xanthan gum/cinnamon oil; F5 = 15% HPMC/clove oil; F6 = 20%HPMC/clove oil; F7 = 15%HPMC/cinnamon oil; F8 = 20% HPMC/cinnamon oil-based emulgels; FLU-F2, FLU-F4, FLU-F6 and FLU-F8 are optimised fluconazole emulgels.

The rheological profile of polymer may be associated with variation in their shape and degree of crystallites; liquid phase adsorption and ordering within their hydrated three-dimensional gel network [17]. The viscosity of emulgels at 30–40 rpm depicts their apparent viscosity and this parameter is related to their rheological properties such as spreadability and consistency of the formulation [20]. However, the viscosity of the emulgels must be moderate to improve their spreadability and also prevent their premature elimination from the vaginal cavity.

The blank emulgels are presented in order of increasing viscosity at 30 rpm:15% HPMC/clove oil: 98 ± 4 cP < 20% HPMC/clove oil: 127 ± 3 cP < 15% HPMC/cinnamon oil: 128 ± 2 cP < 1.5 % xanthan gum/cinnamon oil: 128 ± 9 cP < 1.5 % xanthan gum/clove oil: 253 ± 1 cP < 3 % xanthan gum/cinnamon oil: 375 ± 7 cP < 3% xanthan gum/clove oil: 529 ± 5 cP < 20 % HPMC/cinnamon oil: 573 ± 5 cP), revealing that higher concentrations of polymers may be required to improve the viscosity of emulgels. Interestingly, 3% xanthan gum/clove oil-based samples were more viscous than those containing 3% xanthan gum and cinnamon oil (529 ± 5 cP versus 375 ± 7 cP). On the other hand, 20% HPMC/cinnamon oil-based emulgels exhibited greater viscosity values than 20% HPMC/clove oil-based samples (573 ± 5 cP versus 127 ± 3 cP), suggesting that the viscosities of emulgels were more dependent on the type of polymer than the type of penetration enhancer.

The blank and fluconazole emulgels exhibited shear thinning behaviour when the formulations were subjected to shear stress ranging from 6 rpm to 60 rpm because the viscosity of the emulgels decreased with increasing shear rate (Fig. 1). This finding may be due to the fact that application of shear stress to emulgels resulted in the disorientation of polymeric molecules, resulting in the alignment of these molecules in the direction of product flow, reducing the internal resistance of the emulgels and decreasing their viscosity [17]. Also, there was significant statistical differences in the viscosity of the emulgels after incorporation of the drug ($p < 0.05$). In addition, the viscosity of the emulgels at 6 rpm was statistically higher than those recorded at 60 rpm ($p < 0.05$). This property is desirable for formulations intended for topical application because it improves their spreadability and ease of removal from containers.

Incorporation of fluconazole into emulgels reduced the intrinsic viscosities of the emulgels (Fig. 1): 3% xanthan/clove oil-based samples (529 cP versus 304 cP), 3% xanthan/cinnamon oil (375 cP versus 110 cP), 20% HPMC/clove oil (127 cP versus 85 cP), and 20% HPMC/cinnamon oil (573 cP versus 314 cP).

The spreadability of emulgels depict their ability to spread readily after application to the affected body part. Moreover, the drug bioavailability of an emulgel has a good correlation with their spreadable nature [11], and the spreadability of the emulgels is inversely related to the intrinsic viscosities of the emulgels. For instance, 3% xanthan gum/clove oil-based blank emulgels with greater viscosity value (529 cP) than 1.5% xanthan gum/clove oil-based emulgels (253 cP) displayed limited spreadability in comparison to similar formulation containing lower polymer concentration (0.4 cm versus 1.9 cm). In addition, HPMC/cinnamon oil-based emulgels exhibited greater viscosity values than those formulated using clove oil (313 cP versus 85 cP) as well as diminished spreadability in comparison to HPMC/clove oil-based samples (2.5 cm versus 3.1 cm). These findings are in good agreement with earlier studies on clarithromycin emulgels, where Carbopol 940 based emulgels prepared using 1.3% polymer exhibited the greatest viscosity values and least spreading coefficient [9]. Interestingly, 20% HPMC/clove oil-based emulgels with the greatest intrinsic viscosity value (573 cP) exhibited satisfactory spreadability of 1.6 cm. This finding is in good agreement with earlier studies by Ramalingam et al., where HPMC K4M based cefpodoxime emulgels with the highest viscosity values (6000 cP) exhibited satisfactory spreadability (38 gcm/sec) [11].

Typically, increase in the conductivity values of emulgels translates to improved formulation stability [29]. Fluconazole incorporation into xanthan based emulgels reduced their conductivity. The reduced conductivity values of fluconazole emulgels could be due to the swelling properties of xanthan gum, which reduces the available water content, resulting in reduced conductivity of the emulgel formulation [29]. Despite the relatively low conductivity values of xanthan gum-based emulgels, their stability was not compromised. This finding may be due to their viscosity-enhancing properties which stabilised the emulgel. On the other hand, the

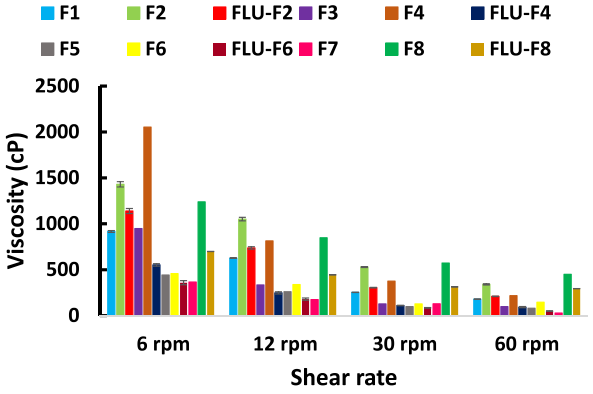

Fig. 1. Shear-dependent viscosity profile of blank and fluconazole emulgels studied at 25 °C from 6 rpm to 60 rpm, viscosity values was expressed as mean ± SD; n = 3; F1 = 1.5% xanthan gum/clove oil; F2 = 3% xanthan gum/clove oil; F3 = 1.5% xanthan gum/cinnamon oil; F4 = 3% xanthan gum/cinnamon oil; F5 = 15% HPMC/clove oil; F6 = 20%HPMC/clove oil; F7 = 15%HPMC/cinnamon oil; F8 = 20% HPMC/cinnamon oil; FLU-F2, FLU-F4, FLU-F6 and FLU-F8 are optimised fluconazole emulgels.

conductivity of HPMC based emulgels was increased with incorporation of fluconazole into the emulgels. This finding may be due to the hygroscopic nature of HPMC that attracts water molecules, resulting in improved conductivity of the sample since aqueous environment results in improved conductivity of the drug formulation [29]. This finding has revealed that conductivity of emulgel formulations may be inversely related to their stability.

3.3. Drug contents of fluconazole emulgels

The drug contents of fluconazole emulgels are presented in order of increasing drug contents: FLU-F2 (90.9 ± 1.3 %) < FLU-F6 (99.4 ± 0.6 %) < FLU-F4 (99.9 ± 1.4 %) < FLU-F8 (102.2 ± 0.8 %). The HPMC-based emulgels exhibited greater drug content (99–102 %) than xanthan-based emulgels (91–100 %). Since clove oil has greater fluconazole solubilization potential than cinnamon oil (165 mg/mL versus 85 mg/mL) [30], it was anticipated that clove oil-based emulgels would exhibit improved drug content in comparison to cinnamon oil-based sample as clove oil could facilitate improved dissolution and release of fluconazole. Surprisingly, cinnamon oil-based emulgels exhibited greater drug content than clove oil-based formulations. Nevertheless, all the studied fluconazole emulgels displayed satisfactory drug content according to USP specification that recommends that drug content should be between 85 % and 115 % [22].

3.4. Drug release data

3.4.1. Standard calibration curve for fluconazole quantification

The wavelength of maximum absorption for fluconazole in phosphate buffer pH 4.2/methanol (1:1), pH 4 was 212 nm. The standard calibration curve of pure fluconazole was linear, obeying Beer-Lambert's Law and revealing that the concentrations of fluconazole (5–100 μg/mL) chosen to plot the calibration graph was suitable. The regression coefficient (r^2), slope and intercept of the graph were 0.99, 0.0079 and 0.1623, respectively (Fig. S1).

3.4.2. Drug release profiles of fluconazole emulgels

The amount of drug released from xanthan- and HPMC-based emulgels after 6 h (Fig. 2) was comparable [(99–100 %) versus (94–97%)]. However, xanthan-based emulgels exhibited improved controlled drug release in comparison to the HPMC-based emulgels. The drug flux across Strat-M™ membrane after 6 h are presented in increasing order: FLU-F6 (2658 ± 24 μg/cm^2) < FLU-F8 (2734 ± 19 μg/cm^2) < FLU-F4 (2790 ± 12 μg/cm^2) < FLU-F2 (2824 ± 3 μg/cm^2), revealing that xanthan-based emulgels exhibited greater drug flux than HPMC-based emulgels ($p < 0.05$).

There was good correlation between the fluconazole drug release rate and the drug flux across the simulant vaginal membrane, for the studied fluconazole emulgels. These findings are not in good agreement with findings by Ramalingam et al. (2022), that reported that HPMCK4M-based emulgels displayed superior controlled cefpodoxime release and antibacterial activity relative to Carbopol 934 and xanthan gum-based emulgels. Nevertheless, different HPMC grades were used in both studies (HPMC K4M versus HPMC E5 LV), which might have contributed to the differences in their drug release pattern.

3.4.3. Drug release kinetic profile

The regression analysis of fluconazole release data based on best curve-fitting method for four different fluconazole emulgels (Table 3) revealed that all the studied fluconazole emulgels fitted well with Korsmeyer-Peppas drug release kinetic model.

This finding suggested that the release of fluconazole from the emulgels was induced by the swelling and dissolution of the polymeric gel base as well as diffusion and dissolution of the drug. This finding was in good agreement with earlier studies where xanthan gum and Carbopol 934-based indomethacin emulgels fitted well with Korsemeyer-Peppas model [16]The Korsmeyer-Peppas associated diffusional exponent (n) was calculated to ascertain the specific drug release pattern of the fluconazole emulgels. All the studied emulgels exhibited non-Fickian Supercase II drug release profile because their calculated n values were greater than 1. These results suggested that controlled drug release from fluconazole emulgels may result from both solvent diffusion and relaxation of

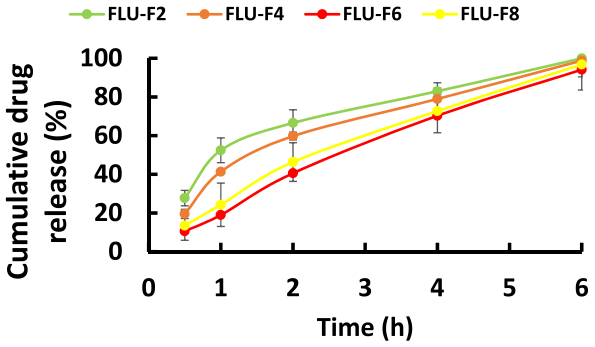

Fig. 2. Drug release profile of xanthan and HPMC-based fluconazole emulgels over 6 h; with study carried out at 37 °C.

Table 3
Fluconazole release kinetics of the emulgels.

Sample	Zero-order		First-order		Higuchi		Korsemeyer-Peppas		
	r^2	K1	r^2	K2	r^2	K3	r^2	K4	n
FLU-F2	0.9034	34.61	0.7612	1.5571	0.962	8.0253	0.9957	1.7169	3.9534
FLU-F4	0.9345	24.151	0.7751	1.4268	0.9825	−5.5953	0.9965	1.6214	3.7334
FLU-F6	0.9892	5.62	0.8749	1.1099	0.9955	−27.318	0.9909	1.3029	3.0000
FLU-F8	0.9843	10.381	0.866	1.2201	0.9981	−22.066	0.9924	1.4039	3.2327

Note: r^2 is the regression coefficient while k1, k2, k3 and k4 are constants associated with zero order, first order, Higuchi and Korsmeyer-Peppas drug release kinetic models, respectively.

polymer chain [31].

3.5. Anti-fungal profiles of fluconazole emulgels

Interestingly, blank and fluconazole exhibited antifungal activity, which may due to the presence of clove oil and cinnamon oil in the blank emulgels that has been reported to exert antimicrobial action [32]. The zones of *Candida* inhibition for the studied blank emulgels are: F2 (19 ± 1 mm) < F6 (22 ± 1 mm) < F4 (25 ± 1 mm) < F8 (32 ± 1 mm). In addition, the anti-fungal activities of the fluconazole emulgels are presented in order of increasing antifungal activity: FLU-F2 (22 ± 1 mm) < FLU-F6 (29 ± 1 mm) < FLU-F4 (31 ± 1 mm) < FLU-F8 (38 ± 1 mm). HPMC/cinnamon oil-based emulgels (FLU-F8) exhibited the greatest zone of fungal inhibition (38 ± 1 mm) while xanthan gum/clove oil-based fluconazole emulgels as well as blank HPMC/clove oil based emulgels displayed the least zones of inhibition (22 ± 1 mm). Nevertheless, all the studied fluconazole emulgels displayed satisfactory zones of inhibitions (22–38 mm). In addition, xanthan gum/clove oil-based fluconazole emulgels exhibited similar antifungal profile with that of Nizoral® cream (ketoconazole) which has a zone of *Candida* inhibition of 22 mm [17]. In addition, xanthan gum/cinnamon oil-based emulgels (FLU-F4) exhibited comparable zone of fungal inhibition with the best fluconazole formulation reported by El-Rahman and coworkers (31 mm versus 32 mm) [17]. The antifungal profile of the studied fluconazole emulgels correlated well with their drug content values as FLU-F2 with the least drug content exhibited the least zone of fungal inhibition.

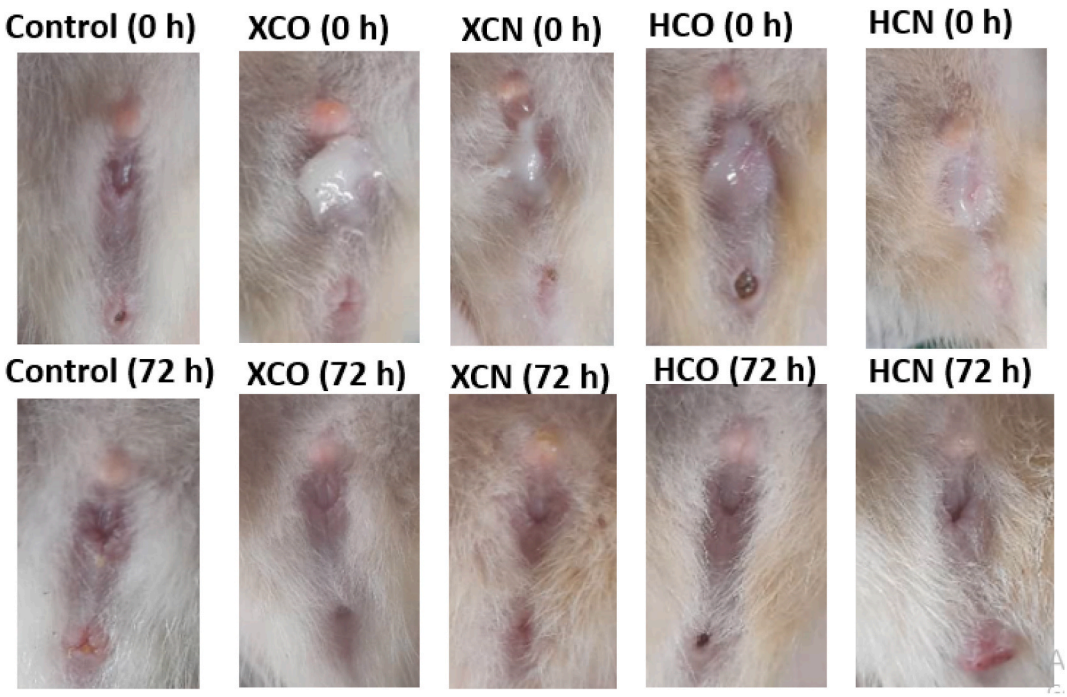

Fig. 3. Images obtained after application of fluconazole emulgels (XCO (xanthan gum/clove oil), XCN (xanthan gum/cinnamon oil), HCO (hydroxypropyl methylcellulose/clove oil) and HCN (hydroxypropyl methylcellulose/cinnamon oil) to the rat vagina; rats were treated every 24 h over 72 h to investigate acute and chronic toxicity of the emulgels; n = 3; untreated rat groups served as the negative control.

3.6. In vivo vaginal biocompatibility profile of the fluconazole emulgels

Based on the murine vaginal irritation test (Fig. 3), it was observed that all the tested emulgels scored zero as they were well tolerated by the female rats. In addition, acute and chronic toxic effect was not inflicted on the female rats as allergic symptoms like erythema and/or edema were not evident within 24 h of applying the emulgels as well as after 72 h of administering a total of three fluconazole emulgel doses, suggesting that the fluconazole emulgels were biocompatible.

3.7. Accelerated stability data

Centrifugal test is an established technique used to evaluate the accelerated stability profile of emulsions and emulgels. According to Stoke's equation, the velocity of creaming is indirectly proportional to the viscosity of the dispersion medium [33]. Emulsifiers or polymers that increase the viscosity of the continuous medium could improve the overall stability of the emulgels [34]. Nevertheless, polymers differ in terms of their stabilization potential. Xanthan gum-based emulgels exhibited improved accelerated stability profile in comparison with HPMC-based emulgels (creaming index: 3–4 % versus 28–31 %) (Table 4; Fig. S2). This finding may be due to the varying molecular weights of xanthan gum and HPMC.

3.8. Real time stability data

The Real-time stability profiles of the optimised fluconazole emulgels are presented in Table 5.

There was good correlation between the accelerated and real-time stability data obtained for the studied emulgels. Xanthan-based emulgels were more stable than HPMC based emulgels based on both accelerated and real-time stability data. This finding may be due to the hygroscopic nature of HPMC, which results in its reduced stability. These findings are in good agreement with previous studies where xanthan gum-based indomethacin emulgels exhibited improved anti-inflammatory, skin permeation and stability profile compared with Carbopol 934-based samples [16].

4. Conclusions

Four types of fluconazole emulgels were successfully developed and characterised. The type of polymer used to formulate fluconazole emulgels had a greater influence on their physicochemical, drug release, anti-fungal and stability profile, than the type of penetration enhancers. The drug permeation efficiency of the penetration enhancers was dependent on the type of polymeric gel base. All the studied emulgel formulations exhibited acceptable drug content, drug release, anti-fungal and vaginal biocompatibility. Despite the fact that HPMC/clove oil-based emulgels (FLU-F8) exhibited the greatest zone of *Candida* inhibition (38 mm), they were not chosen as the most promising formulation due to their real-time instability at ambient and elevated temperatures.

Optimised xanthan gum-based emulgels (FLU-F2 and FLU-F4) were the most promising emulgels and they could help to reduce the drug dosage and dosing frequency required for efficient therapy. In addition, they could serve as alternative topical fluconazole products to the marketed Flucos® gel (fluconazole 0.5 % w/w), for the potential treatment of vaginal candidiasis. Future work will evaluate the *in vivo* antifungal activity of xanthan gum-based fluconazole emulgels in order to confirm their feasibility of being translated from the research laboratory to the clinics.

Ethics statement

The work was carried out in compliance with the regulations of the College of Medicine, University of Lagos Animal Care and Use Research Ethics Committee, Lagos, Nigeria (CMUL/ACUREC/05/23/1208) and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. The actions taken in order to comply with ARRIVE checklist are presented in Table S1.

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Data availability

Data will be made available on reasonable request from the corresponding author.

Table 4
Centrifugal profile of fluconazole emulgels carried out at 4000 rpm for 10 min over two cycles.

Sample	Phase separation (cm)	Creaming index (%)
FLU-F2	0.13 ± 0.06	3 ± 1
FLU-F4	0.17 ± 0.06	4 ± 1
FLU-F6	1.13 ± 0.06	28 ± 1
FLU-F8	1.23 ± 0.06	31 ± 1

Table 5
Real-time Stability profile of studied formulations stored at 25 ± 2 °C and 40 ± 2 °C for 90 days.

Days	Storage at 25 ± 2 °C				Storage at 40 ± 2 °C			
	FLU-F2	**FLU-F4**	**FLU-F6**	**FLU-F8**	**FLU-F2**	**FLU-F4**	**FLU-F6**	**FLU-F8**
0	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–
15	–	–	–	–	–	–	–	–
30	–	–	–	–	–	–	–	–
60	–	–	–	–	–	–	–	–
90	–	–	-+	–	–	–	-+	-+

– = absence of phase separation after predetermined time period.
-+ = partial phase separation after predetermined time period.

CRediT authorship contribution statement

Oriyomi Temitayo Ogedengbe: Writing – original draft, Resources, Methodology, Investigation, Funding acquisition. **Oluwadamilola Miriam Kolawole:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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