Optimisation of clotrimazole emulgel using essential oils of *Citrus sinensis* and *Zingiber officinale* as permeation enhancers

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ABSTRACT

Background: Current trends in emugel formulations are devoid of utilization of natural permeation enhancers which will increase the amount of clotrimazole that is available at the site of infection for treatment of vaginal candidiasis.

Objective: The aim of this study is to develop clotrimazole emulgel using Carbopol® Ultrez 21 and Carbopol 940® and two natural permeation enhancers essential oils from the peel of *Citrus sinensis* and *Zingiber officinale*.

Method: Eight clotrimazole emulgel formulations were prepared according to a 2^3 factorial design using qualitative factors and levels such as gelling agent type, natural permeation enhancers concentrations and emulsifying agent concentration. The formulated emugels underwent rheological evaluation, spreading coefficient, bioadhesive strength measurement. Release kinetics, microbiological assay and stability of the formulations were studied. *In vivo* release studies using cellulose based Ciprophan® membrane and *ex vivo* via rat skin permeation studies were carried out to evaluate the impact of the essential oils on the release of clotrimazole.

Results: The increase in polymer concentration from 1% to 2% led to a corresponding increase in viscosity and spreadability of the emugel formulation. Drug release from the clotrimazole emulgel formulations followed Hixon Crowell model best because it had correlation coefficient of 0.8464 – 0.9259 through membrane and 0.9245-0.9652 through rat skin. Correlation coefficient ranging from 0.9924 to 0.9992 means an excellent model fit.

Conclusion: Utilization of *Zingiber officinale essential oil* in the emugel formulation at 3% w/w while eliciting synergistic antifungal activity with clotimazole, gave an enhanced release of clotimazole *in vivo* and *ex vivo* with flux 131.21±0.19 mg/cm^2/h and 22.01±0.66mg/cm^2/h respectively compared to the innovator brand 89.63±0.12 mg/cm^2/h and 13.99±0.16mg/cm^2/h respectively.

Key words: Emugels, Alkyl acrylate cross polymer, *Citrus sinensis* , *Zingiber officinale ,* Permeation enhancers

Optimisation du clotrimazole émulgel en utilisant des huiles essentielles de *Citrus sinensis* et *Zingiber officinale* comme améliorateurs de pénétration

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RÉSUMÉ

Contexte : Les tendances actuelles dans les formulations d'émulgel sont dépourvues d'utilisation d'activateurs de pénétration naturelle qui augmenteront la quantité de clotrimazole disponible sur le site de l'infection pour le traitement de la candidose vaginale.

Objectif : Le but de cette étude est de développer le clotrimazole émulgel en utilisant Carbopol® Ultrez 21 et Carbopol 940® et deux huiles essentielles amélioratrices de pénétration de la peau de *Citrus sinensis* et de *Zingiber officinale*.

Méthode : Huit formulations de clotrimazole émulgel ont été préparées selon un plan factoriel 23 en utilisant des facteurs qualitatifs et des niveaux tels que le type d'agent gélifiant, les concentrations d'agents améliorant la pénétration naturelle et la concentration en émulsifiant. Les émulgels formulés ont subi une évaluation rhéologique, un coefficient d'étalement, une mesure de la résistance bioadhésive. La cinétique de libération, le dosage microbiologique et la stabilité des formulations ont été étudiés. Des études de libération in vivo utilisant la membrane Ciprophan® à base de cellulose et ex vivo par des études de pénétration cutanée chez le rat ont été réalisées pour évaluer l'impact des huiles essentielles sur la libération du clotrimazole.

Résultats : L'augmentation de la concentration de polymère de 1% à 2% a conduit à une augmentation correspondante de la viscosité et de la capacité d'étalement de la formulation d'émulgel. La libération de médicament à partir des formulations de clotrimazole emulgel a suivi le modèle de Hixon Crowell mieux parce qu'il avait un coefficient de corrélation de 0,8464 - 0,9259 à travers la membrane et 0,9245 - 0,9652 à travers la peau de rat. Le coefficient de corrélation allant de 0,9924 à 0,9992 signifie un excellent ajustement du modèle.

Conclusion : L'utilisation de l'huile essentielle *Zingiber officinale* dans la formulation d'émulgel à 3% p/p tout en induisant une activité antifongique synergique avec le clotimazole, a donné une libération accrue de clotimazole in vivo et ex vivo avec flux 131,21±0,19 mg/cm2/h et 22,01±0,66 mg/cm2/h respectivement par rapport à la marque innovatrice 89,63±0,12 mg/cm2/h et 13,99±0,16 mg/cm2/h respectivement.

Mots-clés : Emulgels, Polymère croisé d'acrylate d'alkyle, *Citrus sinensis*, *Zingiber officinale*, Améliorateurs de pénétration

INTRODUCTION

Clotrimazole is an antifungal agent commonly used in the treatment of fungal infections such as oral thrush, yeast infections of the skin and vagina, athlete's foot and jock itch and ring worm. [1] Clotrimazole is available in various dosage forms, such as creams and vaginal tablets. Clotrimazole is a lipophilic drug and normally presented as a cream formulation.

Gel formulations generally provide faster drug release as compared to ointments and creams. [2] They can also be used to control drug release and serve as protectants of medicaments from hostile environment. However there is a limitation in the delivery of hydrophobic drugs, hence the formulation of emulgels that allows these hydrophobic drugs to enjoy the unique properties of gels.[3]

Emulgel-based delivery systems present numerous advantages over conventional emulsion-based delivery systems, this include high physical stability, being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water-soluble, longer shelf life, bio friendly, transparent and pleasing appearance. Emulsions possess a certain degree of elegance and are easily washed off whenever desired and also have a high ability to penetrate the skin.[4] These biphasic formulations contain a non-polar liquid with a semi solid polar liquid external phase. The interfacial regions can be used to encapsulate hydrophilic, lipophilic and amphiphilic substances with novel rheological properties or controlled release properties. Both lipophilic and hydrophilic drugs have been incorporated into emulgels designed for topical delivery, such as mefenamic acid, itraconazonle and diclofenac .

The aim of this work is to develop an emulgel formulation of clotrimazole via a 2^3 factorial design using qualitative factors and levels such as gelling agent type, natural permeation enhancers concentrations and emulsifying agent concentration to increase and improve the absorption of clotrimazole. This will ensure better delivery of clotrimazole via percutaneous surface promoting the drug flux and penetrates through skin to decrease the barrier resistance. [5,6]

MATERIALS AND METHODS

Materials

Clotrimazole was obtained as a gift sample from Drugfield Pharmaceutical Ltd, Ogun state, Nigeria. Carbopol 940 was obtained from Loba® chemicals, Mumbai. Carbopol ultrez was a gift sample from Metcem® Limited, India. The rhizomes of *Zingiber officinale* was obtained from Ibadan, Oyo State, Nigeria, during the dry season in the month of March. It was identified and confirmed at the herbarium of the Faculty of Botany, University of Lagos, Lagos, Nigeria. The epicarp of *Citrus sinensis* was obtained from the ripe fruit of *Citrus sinensis* in Ipaja, Lagos State. All other chemicals used were of analytical grade and were used without any further chemical modification.

Experimental design

Eight clotrimazole emulgel formulations were prepared according to a 2^3 factorial design using qualitative factors and levels such as gelling agent type, natural permeation enhancers concentrations and emulsifying agent concentration.

Table 1. Factors and Levels for the 2^3 factorial design

	Factors	Levels
A	Gelling agent type	Carbopol Ultrez Carbopol 940
B	Natural enhancer concentration	Citrus oil Ginger oil
C	Emulsifying agent concentration	Span 20 Tween 20

Preparation of emulgel

Extraction and characterization of essential oils

The epicarp of the *Citrus sinensis* were obtained and were weighed on the Brookfield measuring scale in batches. 500g of the weighed epicarp was then loaded in the Clavenger apparatus for extraction of the oil. The oil was distilled from the clavenger and collected fresh and used for the preparation of the emulgels. The rhizomes of ginger were pulverized into smaller pieces prior to insertion into the clavenger apparatus and process repeated as above.

Identification of constituents of essential oils by Gas Chromatography-Mass Spectrophotometry (GC-MS)

The oils obtained from distillation were analysed by GC-MS. The procedure was carried out using a 7890A GC system (Agilent Technologies) equipped with a mass selective detector (MSD) 5975C (Agilent Technologies), injector series model 7683B and HP-5MS capillary column (30 m x 0.320 mm, 0.25 µm film thickness). The temperature of the column was maintained at 35^{0}C for 1 min. It was then raised at the rate of 10^{o}C per min for a hold time of 3 min. The temperature of the injection port was maintained at 220^{o}C and that of the detector at 250^{o}C for 3 min hold time, Helium was the carrier gas. The following parameters were maintained: Pressure= 112.0 kPa, Total flow= 32.7 ml/min, Column flow =1.90 ml/min, Linear velocity =50 cm/sec [7]. The chromatographic effluent was then analyzed by the MSD.

Formulation of emulgel

The composition of Clotrimazole emulgel formulations is shown in Table 2. The gel in formulations was prepared by dispersing Carbopol® in purified water with constant stirring at a moderate speed using mechanical shaker; then the pH was adjusted to 6 to 6.5 using triethanolamine (TEA). The oil phase of the emulsion was prepared by dissolving Span 20 in light liquid paraffin while the aqueous phase was prepared by dissolving Tween 20 in purified water.

Methyl and propyl parabens were dissolved in propylene glycol whereas clotrimazole was dissolved in ethanol, and both solutions were mixed with the aqueous phase. Citrus oil and *ginger* oil were mixed in oil phase. Both the oily and aqueous phases were separately heated to 70–80 °C, then the oily phase was added to the aqueous phase with continuous stirring until it got cooled to room temperature. The obtained emulsion was mixed with the gel in 1:1 ratio with gentle

Table 2. Quantitative composition of the different clotrimazole emulgel formulations (% w/v)

Ingredient	E1	E2	E3	E4	E5	E6	E7	E8
Clotrimazole (g)	1	1	1	1	1	1	1	1
Carbopol 940(g)	1	1	-	-	2	2	-	-
Carbopol Ultrez (g)	-	-	1	1	-	-	2	2
Ethanol (mls)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Liquid Paraffin (mls)	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5
Propylene Glycol (mls)	25	25	25	25	25	25	25	25
Tween 20 (mls)	2.5	2.5	5	5	7.5	7.5	10	10
Span 20 (mls)	4	4	8	8	16	16	16	16
Oi l of *citrus sinensis* (mls)	1.5	-	1.5	-	3	-	3	-
Oil of *zingiber officinale* (mls)	-	1.5	-	1.5	-	3	-	3
Methyl Paraben (mls)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Propyl Paraben (mls)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Purified water to (mls)	100	10 0	100	100	100	100	100	100

EVALUATION OF EMULGEL

Physical examination

The prepared emulgel formulations were inspected visually for their color, homogeneity, appearance, phase separation and consistency.[9]

Rheological study

The viscosity of the formulated batches was determined using a cone and plate viscometer with spindle 7 (Brookfield Engineering Laboratories). The assembly was connected to a thermometer which ensured that the temperature was between 25 °C and 27°C. The formulation whose viscosity was to be determined was added to a beaker covered with thermostatic jacket. The spindle was allowed to move freely into the emulgel and the readings were noted. [10] The viscometer was set at different speeds (10-60 rpm).

Skin irritation test (patch test)

A set of 8 rats was used in the study. The emulgel was applied on the properly shaven skin of rat. Undesirable skin changes, i.e., change in color, change in skin morphology were checked for a period of 24 hours. [11] The Lagos University Teaching Hospital Research Ethics Committee of the College of Medicine University of Lagos provided ethical approval for the study (CM/HREC/02/16/002). All procedure was in compliance with the American Psychological Association guidelines for ethical conduct in care and use of non-human animal in research. [12]

Bioadhesive strength measurement

The modified method was used for the measurement of bioadhesive strength. The apparatus consists of two arm balance. Both the ends are tied to glass plates using strings. One side contains two glass plates. Other side contains single glass plate for keeping weight. The right and left pans were balanced by adding extra weight on the left hand pan. The balance was kept in this position for five minutes.

1g of accurately weighed emulgel was placed between these two slides containing hairless fresh rat skin pieces, and extra weight from the left pan was removed to sandwich the two pieces of glass and some pressure was applied to remove the presence of air. The balance was kept in this position for 5 min. Weight was added slowly at 200 mg/min to the left hand pan until the two glass slides got detached from each other. The weight (gram force) required to detach the emulgel from the glass surface gave the measure of bioadhesive strength. [13] The bioadhesive strength is calculated by using Equation 1

Bioadhesive strength = *weight required {in g} /area (cm^2)* Equation 1

In vitro and *Ex vivo* permeation studies

In vitro release studies was carried out with a cellulose based ciprophan membrane with thickness of 10µm (obtained from regenerated cellulose) from Medicell (London, UK) with a pore size of 0.45µm and the *ex vivo* permeation studies was carried out using skin obtained from male wistar rats (skin thickness 0.45- 0.8 mm). The membranes were mounted on modified Franz diffusion cells with diffusion area of 3.71cm^2. The receptor compartment contained 30mls phosphate buffer (pH of 7.4 at 37.1°C ± 0.2°C). 1g of each hydrogel formulation was applied on the skin surface in the donor compartment area with the stratum corneum facing downwards in the donor compartment. An aliquot of 1 ml was withdrawn at predetermined time intervals (15min, 30min, 1h, 2h, 4h, 8h and 12h) and replaced with equal volume of fresh media. The samples were analysed using a UV/Visible spectrophotometer (UV-Vis 2600 Shimadzu Analytical and measuring instruments). The drug release kinetics and mechanism was evaluated by fitting the cumulative release data into models representing zero order, First order, Higuchi model and Peppas model.[14]

Microbiological assay

One ml (1ml) of *Candida albicans* with optical density 0.5 corresponding to 1×10^6 cfu/ml was inoculated into liquefied potato dextrose agar medium, this was poured into petri dishes. Upon complete solidification via aseptic transfer, 6mm holes were made using a cork borer and formulations were introduced into it. The plates were incubated at 25°C for 72hrs and the zone of inhibition was measured. The activity of the emugel formulations was compared with a marketed clotrimazole formulation. Minimum inhibitory concentration (MIC) of the emugel formulations were determined after 48hrs. The results were given as mean ± SD and $P < 0.05$ was considered as being statistically significant.

RESULTS

Essential oil yield and composition

The essential oil yield from *zingiber officinale* was 3.5% w/w while the essential oil yield from *citrus sinensis* was 1.2% w/w from the peels. The major constituent of the ginger oil was zingibererene constituting 30.1% of identifiable volatile oil content (Table 3) this is in

consonanace with other studies. [7] Zingiberene has been shown to have antibacterial and antifungal properties. [15] Limonene and β-myrcene constituting 71.05 and 4.21% respectively were the major constituents of the essential oil obtained from the peels of *citrus sinensis* (Table 4). These constituents have been found to have antioxidant properties and antimicrobial activity. [15]

Table 3. GC-MS profile of the chemical composition of peels of *Zingiber officinale*

Compound	% Composition	RI	Identification
Zingiberene	30.1	1487	MS, R I
ar - curcumene	10.15	1472	MS, RI
β-sequiphellandrene	9.8	1515	MS, RI
Geranial	9.7	1255	MS, RI
β-bisabolene	6.29	1504	MS, RI
α-bergamotene	3.55	1436	MS, RI
Citronella	1.3	1125	MS, RI
Limonene	0.81	1030	MS, RI
Trace	15.99		MS

Table 4. GC-MS profile of the chemical composition of peels of *Citrus sinensis*

Compound	% Composition	RI	Identification
Limonene	71.05	1030	MS, RI
β-myrcene	4.21	990	MS, RI
Decanal	5.77	1205	MS, RI
Citronellol	1.10	1225	MS, RI
γ-eudesmol	1.05	1621	MS, RI
Trace	15.11		MS

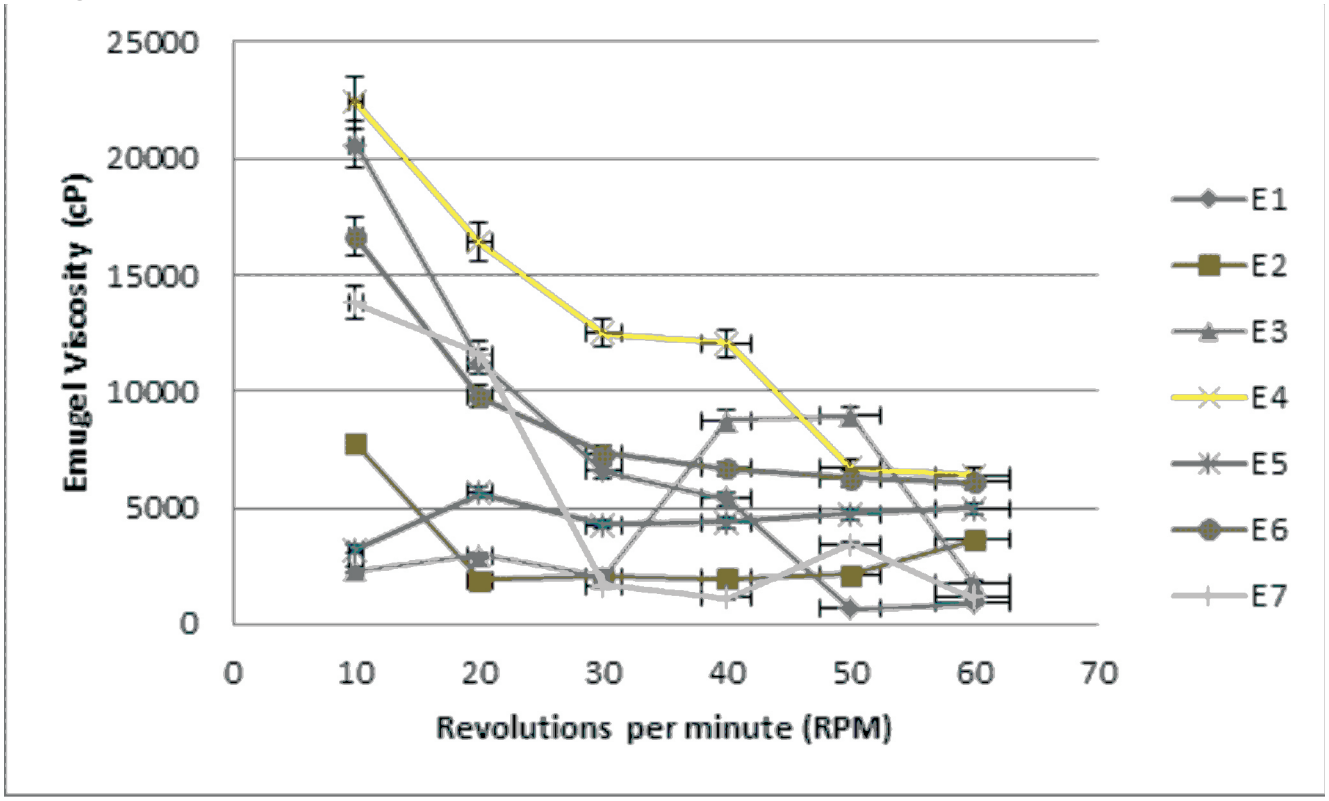
Physical examination

The prepared clotrimazole emulgel formulations were white, semi solid, opaque preparations with a good to excellent homogeneity with no phase separation. However, the use of carbopol ultrez gave formulations with excellent homogeneity. They were easily spreadable with acceptable bioadhesion and fair mechanical properties. The pH values of all the prepared formulations ranged from 6.35 to 6.58, which is considered acceptable to avoid the risk of irritation upon application to the skin. [16]

Skin irritation

A change in colour of the skin, and rashes were not observed on the skin of the rats after observation for twenty four hours, hence product did induce any allergic reaction.

Rheological studies



Figures 1. Plot of shear stress against emugel viscosity

The increase in polymer concentration from 1% to 2% led to a corresponding increase in viscosity as shown in Fig 1. It was seen that E1, E2, E3 and E4 gave lower viscosity readings than E5, E6, and E7. This can be due to the fact that they have a lower concentration of gelling agent than the latter four formulations. Generally, the formulations prepared using carbopol ultrez gave a lower viscosity reading than those prepared with carbopol 940. The essential oils, *zingiber officinale* and *citrus sinensis*, also influenced the viscocity of the emulgels. *Citrus sinensis* is described as a light oil and therefore will not particularly impact on the viscosity of the emulgel. *Zingiber officinale*, which contains zingiberene as its main constituent lead to an increase in the viscosity in E2, E4 and E6. With increasing RPM, it was seen that there was an increase in the viscosity of the system. This could be due to the elastic soft-gelation period at a temperature of 25°C at which the experiment was carried out. [17] Increase in rpm generally led to decrease in viscosity of the emugels.

Bioadhesive strength test

The bioadhesive strength test for the various emulgel formulations is represented in Figure 2. It is seen that the bioadhesive strength of the emulgels was dependent on the concentration of the gelling agents used. The increase in polymer concentration from 1% to 2% led to an increase in the weight required to detach the skin from the apparatus. The properties like polymer chain flexibility, ability to form hydrogen bonds and/or the extent of swelling of the polymers influence the bioadhesive strength of the emulgel formulations. The readings for the bioadhesive strength are seen in Figure 2. E1, E6 and E8 required the most weight to detach the skin. Increased polymer concentration in E6 and E8 as the use of oil of *zingiber officinale* as permeation enhancer may have accounted for the increased bio-adhesive strength of these formulations. Increased skin retention of the formulation is expected as all formulations as they all showed appreciable bio-adhesion.

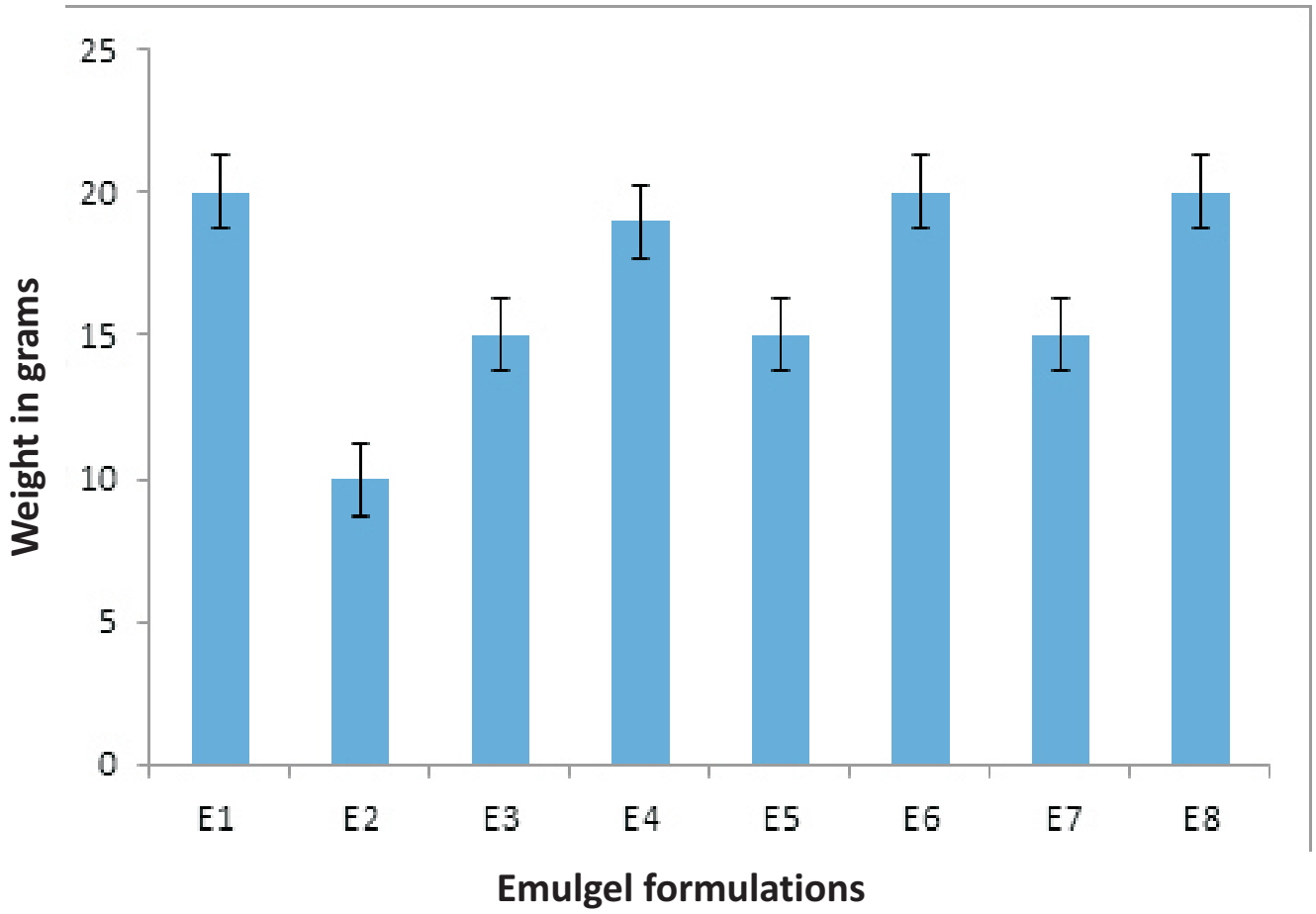


Figure 2. Bio-adhesive strength of formulations E1-E8

Stability studies

The formulations (E1 –E8) were stored in a stability humidity oven for a period of three months at temperature and humidity values of 25°C and 60 RH, 30°C and 65 RH, and 40°C and 75RH respectively. Accelerated stability studies were carried out on the eight formulations to analyse them for their appearance, viscosity and antifungal activity. It was seen that after three months of testing, there were no statistically significant changes in the physical appearance and drug content of the varying emugel formulations as shown in Table 5

Table 5. Accelerated stability testing on the Clotrimazole emugel formulations ($p \leq 0.05$)

Time (Duration)	Formulation	pH	Dynamic Viscosity mPas at 40rpm	Drug content	Antifungal activity (MIC) mg/ml	Physical appearance
DAY 0	E1	6.35 ±0.04	5385 ±3.11	101.2 ± 0.23	2.00	WS/NPS
	E2	6.58 ±0.05	4999 ±9.32	100.0 ±0.11	2.00	WS/NPS
	E3	6.49 ±0.09	8745 ± 5.01	100.5 ± 0.56	2.08	WS/NPS
	E4	6.42 ±0.08	12075 ± 12.09	99.9 ± 0.53	2.11	WS/NPS
	E5	6.53 ±0.1	4365 ± 10.12	100.1 ±0.23	2.01	WS/NPS
	E6	6.57 ±0.06	1158 ±1.11	99.9±0.91	1.91	WS/NPS
	E7	6.49 ± 0.1	1155 ± 5.77	100.1 ± 0.04	1.89	WS/NPS
	E8	6.58 ± 0.32	4882 ± 12.03	100.0 ± 0.11	1.95	WS/NPS
DAY 10	E1	6.35 ±0.12	5386 ±3.51	101.2 ± 0.23	2.00	WS/NPS
	E2	6.58 ±0.04	5000 ± 8.12	100.0 ±0.11	2.00	WS/NPS
	E3	6.49 ±0.10	8749 ± 5.01	100.5 ± 0.56	2.08	WS/NPS
	E4	6.42 ±0.11	12070 ± 11.11	99.9 ± 0.53	2.11	WS/NPS
	E5	6.53 ±0.09	4367 ± 9.55	100.1 ±0.23	2.01	WS/NPS
	E6	6.57 ±0.10	1160 ± 13.09	99.9±0.91	1.91	WS/NPS
	E7	6.49 ± 0.09	1160 ± 5.99	100.1 ± 0.04	1.92	WS/NPS
	E8	6.58 ± 0.21	4890 ± 10.73	100.0 ± 0.11	1.96	WS/NPS
DAY 15	E1	6.35 ±0.12	5386 ±3.51	101.2 ± 0.23	2.00	WS/NPS
	E2	6.58 ±0.04	5000 ± 8.12	100.0 ±0.11	2.00	WS/NPS
	E3	6.49 ±0.10	8749 ± 5.01	100.5 ± 0.56	2.08	WS/NPS
	E4	6.42 ±0.11	12070 ± 11.11	99.9 ± 0.53	2.11	WS/NPS
	E5	6.53 ± 0.09	4367 ± 9.55	100.1 ±0.23	2.01	WS/NPS
	E6	6.57 ±0.10	1160 ± 13.09	99.9±0.91	1.91	WS/NPS
	E7	6.49 ± 0.09	1160 ± 5.99	100.1 ± 0.04	1.92	WS/NPS
	E8	6.58 ± 0.21	4890 ± 10.73	100.0 ± 0.11	1.96	WS/NPS
DAY 30	E1	6.35 ±0.12	5386 ±3.51	101.2 ± 0.23	2.00	WS/NPS
	E2	6.58 ±0.04	5000 ± 8.12	100.0 ±0.11	2.00	WS/NPS
	E3	6.49 ±0.10	8749 ± 5.01	100.5 ± 0.56	2.08	WS/NPS
	E4	6.42 ±0.11	12070 ± 11.11	99.9 ± 0.53	2.11	WS/NPS
	E5	6.53 ±0.09	4367 ± 9.55	100.1 ±0.23	2.01	WS/NPS
	E6	6.57 ±0.10	1160 ± 13.09	99.9±0.91	1.99	WS/NPS
	E7	6.49 ± 0.09	1160 ± 5.99	100.1 ± 0.04	1.99	WS/NPS
	E8	6.58 ± 0.21	4890 ± 10.73	100.0 ± 0.11	2.01	WS/NPS
DAY 90	E1	6.35 ±0.19	5386 ±3.58	100.0 ± 0.03	2.00	WS/NPS
	E2	6.58 ±0.14	5000 ± 8.82	100.0 ±0.11	2.00	WS/NPS
	E3	6.49 ±0.13	8749 ± 5.81	100.0 ± 0.6	2.08	WS/NPS
	E4	6.42 ±0.13	12075 ± 10.11	100 .0 ± 0.3	2.11	WS/NPS
	E5	6.53 ±0.89	4369 ± 10.01	99.9 ±0.83	2.01	WS/NPS
	E6	6.57 ±0.15	1169 ± 12.08	99.9±0.61	1.99	WS/NPS
	E7	6.49 ± 0.1	1162 ± 5.89	99.9 ± 0.14	1.99	WS/NPS
	E8	6.58 ± 0.21	4885 ± 10.03	100.0 ± 0.11	2.01	WS/NPS

WS/NPS…White semisolid cream with No Phase separation

Antimicrobial activities

Table 6. Inhibitory effect of clotrimazole emulgel formulations against *Candida albicans*

Emugel Formulation (1ml/ml clotrimazole)	Zone of inhibition (mm)	(MIC) mg/ml
E1	12.94 ±0.93	2.00
E2	11.98± 0.73	2.00
E3	12.98 ±0.67	2.08
E4	13.08± 0.55	2.11
E5	15.34 ±0.76	2.01
E6	17.78 ±0.23	1.91
E7	14.89 ±0.66	1.89
E8	17.09± 0.89	1.95
Clotrimazole cream	11.29 ± 0.72	1.55
Oil of *citrus sinensis*	15.99 ± 0.13	1.55
Oil of *zingiber officinale*	18.01± 0.02	2.20
One way ANOVA	F= 62.78	
	P < 0.001	

The formulation E1 –E8 were tested for their antimicrobial activities on *Candida albicans* and compared against a standard formulation. The use of control plates that contained plain emulgels showed that emulgel bases had no antimicrobial activity toward the tested *Candida albicans* strain. The antifungal activity of clotrimazole in all different emulgel formulations and the commercially available cream was marked by the presence of zones of inhibition. The essential oils present can also have contributed to the increased antifungal properties of the emulgel formulations via increased transdermal penetration through the stratum corneum. [18, 19]. The oils of *zingiber officinale* and *citrus sinensis* have antioxidant and antimicrobial activities and this will have a synergistic activity with clotrimazole. The oil of *citrus sinensis* has been shown to have a considerable inhibitory action against *candida albicans* in particular. [20] The essential oils used in the formulation do not only act as penetration enhancers but they also inhibited fungal and bacterial growth (Table 6).

***In vitro* and *Ex vivo* permeation studies**

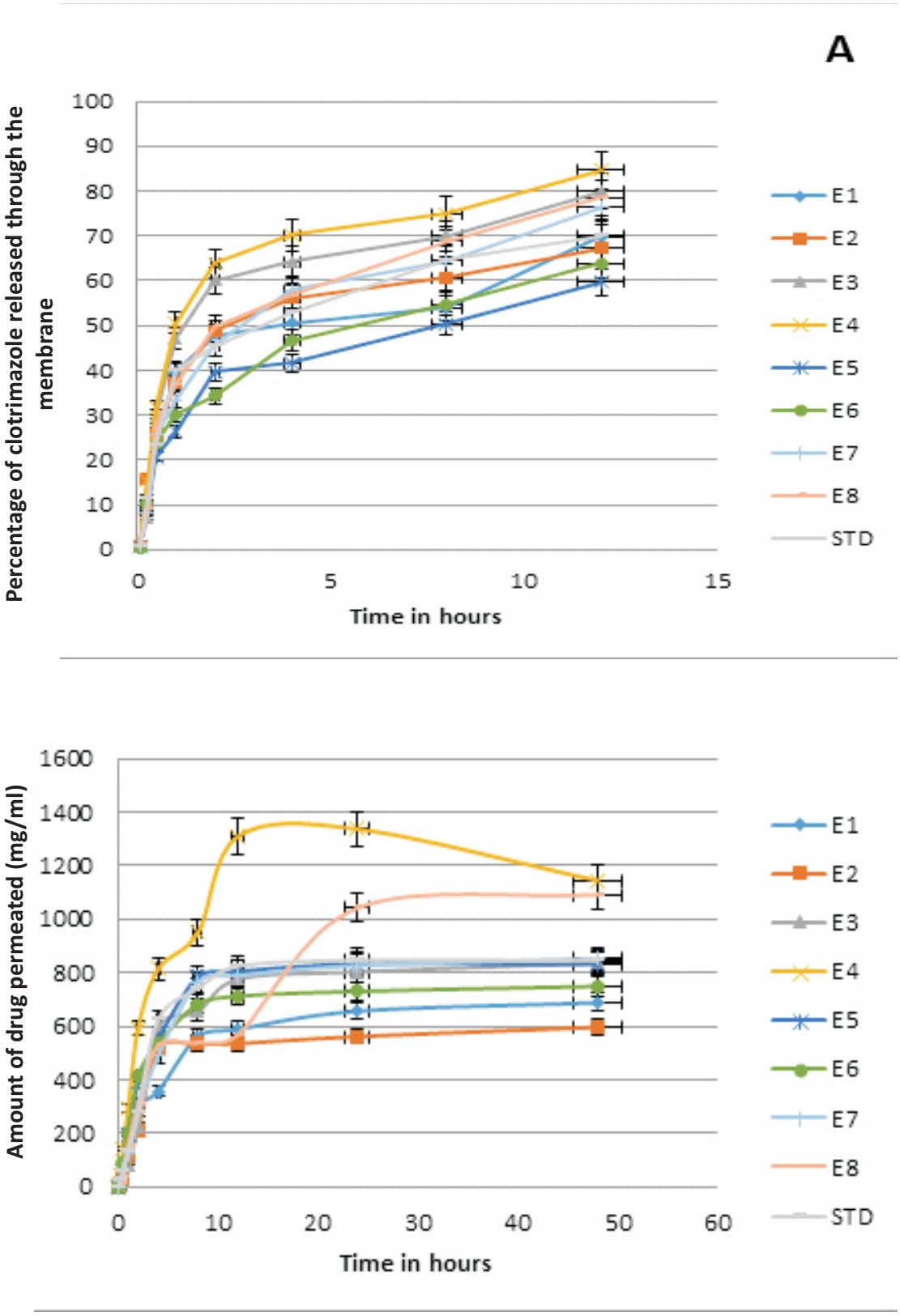


Figure 3. A) *In vitro* release of clotrimazole through the membrane against time in hours; B) *Ex vivo* release of clotrimazole against time in hours

The release of the clotrimazole from the emulgel formulations were higher than the release from the commercial product (Figure 3A). The release of the drug from the emulgel formulations could be ranked in the following ascending order E5< E6 < E2< E1< E7< E8< E3< E4 and the amount of corresponding drug release after 12 hours were 59.7%, 63.8%, 67.3%, 69.8%, 76.32%, 78.5%, 79.9% and 84.7% respectively as shown in Figure 3A

Formulation E4 exhibited the greatest drug release. The formulated emugels had acceptable gel index with the mechanism of release being mainly via Hixon-crowel model R^2= 0.97 in E8 (Table 4)

Table 4. Flux of clotrimazole *in vitro* and *ex vivo* and Kinetic modelling data from the release study

Emugel Formulation	Flux ($mg/cm^2/hr$) *(In vitro)*	Flux ($mg/cm^2/hr$) *(Ex vivo)*	Gel index (G1)	Kinetic modelling *(Ex vivo)*			
				First order (Kf)	Higuchi model (Kh)	Korsmeyer Peppas (Kp)	Hixon-Crowel model (Khc)
E1	84.48±0.21	13.44±0.33	1.32	0.6049	0.821	0.5921	0.9153
E 2	94.56±0.33	15.62±0.21	1.46	0.4811	0.714	0.5921	0.9116
E3	63.73±0.09	14.99±0.09	1.39	0.5702	0.7857	0.5745	0.934
E4	91.67±0.11	16.19±0.08	1.36	0.4765	0.729	0.4975	0.9294
E5	61.01±0.24	15.08±0.17	1.95	0.506	0.7461	0.5569	0.9145
E6	133.5±0.22	23.16±0.41	1.97	0.4934	0.7288	0.5187	0.8908
E7	99.2±0.12	20.01±0.63	1.96	0.5688	0.7945	0.63	0.9421
E8	131.21±0.19	22.01±0.66	1.90	0.6337	0.9287	0.6568	0.9652
Standard (cream)	89.63±0.12	13.99±0.16		0.5229	0.7567	0.6185	0.9542

DISCUSSION

The aim of this work was to develop an emulgel formulation of clotrimazole via a 2^3 factorial design using qualitative factors and levels such as gelling agent type, natural permeation enhancers concentrations and emulsifying agent concentration to increase and improve the absorption of clotrimazole. There were lower drug release from formulations E5 and E6 which were carbopol 940 and ultrez based at a 2% concentration. 1% carbopol dispersion had lower viscosity measurement than 2%. The increase in viscosity of the medium might have inhibited the release of the drug from the base due to the entrapment of the drug in the gel network structure. [2,5] Though E1 and E3 had the same amount of citrus oil incorporated, the drug release in the latter was greater. This release was due to the viscosity of the emugel and its ability to restrict the release of the drug. The addition of more permeation enhancement agent to the 2% formulations did not improve drug release owing to a possibility that drug release was controlled by the penetrating networks of the gel system. Generally formulations with ginger oil as permeation enhancer showed better net drug release as compared to that of citrus oil.

Synthetic membranes are composed of thin sheets of polymeric macromolecules that can control the passage of components through them. Generally, synthetic membranes used in drug diffusion studies have one of two functions: skin simulation or quality control. [21] Synthetic membranes for skin simulation, such as the silicone-based membranes polydimethylsiloxane and Carbosil, are generally hydrophobic and rate limiting, imitating the stratum corneum. In contrast, synthetic membranes for quality control, such as cellulose esters and polysulfone, are required to act as a support rather than a barrier. The amount of drug permeated through the Cuprophan synthetic membrane was higher than that diffused through the rat skin (Figure 3B) during the *in vitro* study and calculation of flux. E4 had the highest amount of drug diffused and permeated through both membranes. These higher permeations of clotrimazole through the synthetic membrane can probably be related to physical and chemical properties of pharmaceutical additives (permeation enhancers and increased emulsifying agent concentration) in the topical formulation which are designed for use on human skin. Cuprophan membrane mimics the human skin and therefore would produce higher values than the rat skin used. The rat skin has fatty layers and contains transporters that are present in different layers of the skin. This will allow for lower permeation.

The thermodynamic activity during the *in vitro* study was constant; therefore the drug diffusion was linear as seen with the results obtained during drug diffusion through the rat skin. The amount of drug permeating the skin or a membrane at a given time i.e. flux was obtained from the slope values plotted for amount diffused per unit area against time. Flux of E4 determined through synthetic membrane and rat skin was higher than the other formulations and the standard (91.67±0.11µg/cm^2 for amount permeated through the membrane and 16.19±0.08µg/cm2 for amount diffused through the rat skin). The incorporation of the drug in an emulgel formulation and the use of essential oils as permeation enhancer can be said to be responsible for enhancement of flux. [21]

The unique pharmacokinetic properties of clotrimazole ensures that the major fraction of an applied dose remains in the stratum corneum, leading to reduced therapeutic concentrations [22] however incorporation of adequate permeation enhancers ensures that the drug is taken through the surface and into the inner epidermis where its enhanced antifungal effect can be elucidated. Drug release from the clotrimazole emulgel formulations followed Hixon Crowell model best because it had correlation coefficient of 0.8464 – 0.9259 through membrane and 0.9245-0.9652 through rat skin. Correlation coefficient ranging from 0.9924 to 0.9992 means an excellent model fit. It is thus assumed that the release rate is limiting by the drug particles dissolution rate and by the diffusion that might occur through the polymeric matrix of the emugel. This model is used to describe the release profile keeping in mind the diminishing surface of the drug particles drug dissolution.

This study incorporated *in vitro* and *ex vivo* models which may not fully represent what happens in vivo, hence the need for studies involving animals and humans.

CONCLUSION

The increase in polymer concentration from 1% to 2% led to a corresponding increase in viscosity and spreadability of the emugel formulation. Utilization of *Zingiber officinale essential oil* in the emugel formulation at 3% w/w while eliciting synergistic antifungal activity with clotimazole, gave an enhanced release of clotimazole *in vivo* and *ex vivo* with flux 131.21±0.19 mg/cm^2/h and 22.01±0.66mg/cm^2/h respectively compared to the innovator brand 89.63±0.12 mg/cm^2/h and 13.99±0.16mg/cm^2/h

respectively. Emulgel of clotrimazole incorporated with essential oil was found to be promising topical product for the treatment of candidiasis. Further clinical studies can strengthen the use of this formulation for the patients suffering from such conditions.

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