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ORIGINAL ARTICLE

Optimized formulation for topical administration of clotrimazole using Pemulen polymeric emulsifier

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Abstract

Background: Emulgel topical formulation is a vehicle of potential for topical delivery of antifungal drugs.

Methods: The imidazole derivative antifungal drug, clotrimazole (CZ), was formulated into emulgels using two grades of hydrophobically modified co-polymers of acrylic acid, namely Pemulen TR1 and TR2. The prepared emulgels were evaluated for their rheological properties, short- and long-term stability, *in vitro* release at 37°C. Microbiological evaluation of the formula showed that optimum stability and release was carried out to measure its antifungal activity.

Results: All formulae showed non-Newtonian shear thinning behavior with little thixotropy or antithixotropy. Five of the prepared formulae showed good physical stability under different treatment conditions. Isopropyl myristate (IPM) emulgels exhibited higher rate of CZ release than either jojoba oil (JB) or liquid paraffin-based emulgels. A selected formula containing JB together with a combination of Pemulen TR1 and TR2 showed excellent stability as well as high rate of CZ release. Microbiological evaluation of the selected formula containing similar amount of CZ revealed 1.2-folds increase in the antifungal activity compared to commercially available formulation.

Conclusion: Emulgel dosage form based on Pemulen polymeric emulsifier and JB is a promising vehicle for topical delivery of CZ and further *in vivo* animal studies are recommended.

Keywords: Clotrimazole; emulgel; jojoba oil; isopropyl myristate; heavy liquid paraffin; polymeric emulsifiers; Pemulen; physical stability; in vitro release; microbiology

Introduction

For skin care and topical treatment of dermatological disease, a variety of vehicles ranging from solids to semisolids and liquid preparation are available to clinicians and patients. Within the major group of semisolid preparations, the use of emulgels has expanded both in cosmetics and pharmaceuticals (Provost et al., 1998).

Emulgels are oil-in-water systems containing a gum solution as a thickened aqueous outer phase. They are sometimes called 'o/w emulsion gels or cream gels'. These systems exhibit jelly-like consistency and homogenous behavior. Their wide utilization as pharmaceutical dosage form comes from the wide utilization of emulsion systems particularly for dermatological formulae (Marquardt and Sucker, 1998). Emulsion gels are gaining importance due to many reasons: they have better application property in comparison to classical formulation such as creams and ointments, they have faster and more complete release of the drug from the vehicle to the skin i.e., higher efficacy (c.f., creams, ointments). Furthermore, they are convenient to apply on hairy skin due to absence of greasiness and lack of residues upon application. They permit the incorporation of both aqueous and oleaginous ingredients, so poorly water-soluble drugs like antifungal agents are easily incorporated in such type of vehicles through the

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proper choice of the oily phase. Finally, their rheological properties can be controlled easily by the proper choice of the gelling agent as well as the oily ingredient.

Emulgel is composed of ordinary components of o/w emulsion in addition to gelling agent. In this study jojoba oil (JB) is used as the oily phase. JB is not a triglyceride. It is a liquid unsaturated wax composed of esters of long carbon chain fatty acids (C_{20} to C_{22}) and long carbon chains unsaturated alcohols (C_{20}, C_{22}). More than 60% of JB esters contain *cis*-11-eicosenoic (jojobenoic) acid (C_{20}) (Miwa, 1984). The unsaturated fatty acid content is the reason for the liquid nature of JB. JB is characterized by its resistance to oxidation, ability to withstand high temperatures and pressures without breakage, and low rancidity potential. This high stability makes JB a useful vehicle for cosmetic applications (Bagby, 1988).

Pemulens are hydrophobically modified co-polymers of acrylic acid (acrylates/C_{10}-C_{30} alkyl acrylates) and cross-linked with allyl pentaerythritol (Figure 1). It could act both as a primary emulsifier and viscosity-enhancing agent (Hemker 1990). The lipophilic portion of Pemulen adsorbs at the oil–water interface and the hydrophilic portion swells in the water forming a gel network around oil droplets to provide exceptional emulsion stability to a broad range of oils (Lochead et al., 1986; Hemker, 1990; Bremecker et al., 1992; Lochead, 1992). Pemulens are novel oil-in-water (o/w) emulsifiers that provide numerous benefits to emulsions prepared with them including low usage level, low irritancy, simple preparation procedures, and fast release of the oil phase. This rapid release is mainly due to the deswelling of the acrylic hydrophilic portion of the Pemulen emulsifier hydrogel upon contact with the salts commonly present on skin surface. Interestingly, Pemulen could also control the release of drugs having high log *P* values (Wahlgren et al., 2009). There are two types of Pemulens: TR1 and TR2 and the main difference between them is the more hydrophobic modifications of TR2 type and therefore it is able to emulsify a greater amount of the oily phase.

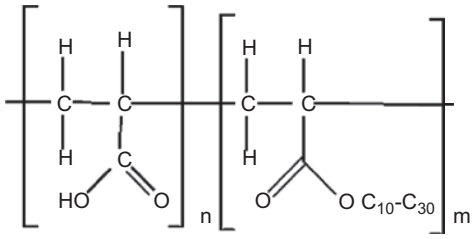

Figure 1. Structure of Pemulen emulsifier.

The present study aims at optimally formulate clotrimazole (CZ) into emulgel dosage form using JB, isopropyl myristate (IPM), and liquid paraffin (LP) as the oily phases, and Pemulen (TR1, TR2, or combination) as emulsifier. To achieve this goal, we studied the influence of vehicle composition on the rheological properties, stability, and the *in vitro* drug release. The antifungal activity of selected CZ containing formulation against *Candida albicans* has been evaluated and compared to commercial formulation Canesten.

Materials and methods

Materials

CZ was kindly provided by the Arab Drug Company (Cairo, Egypt). JB was purchased from Egyptian Natural Oil Company (Cairo, Egypt). Pemulen TR1 and Pemulen TR2 were kindly supplied by Noveon Inc. (Cleveland, OH). IPM and cellulose membrane (M.Wt. cutoff 12,000–14,000) were supplied from Sigma Chemical Company (Saint Louis, MO). Heavy LP, propylene glycol, dimethyl formamide (DMF) (analytical grade), sodium chloride, potassium mono-hydrogen phosphate, di-potassium hydrogen phosphate, and hydrochloric acid (HCl) were purchased from El Nasr Pharmaceutical Chemicals (Cairo, Egypt). Triethanolamine (TEA) (pharmaceutical grade) was supplied from Morgan Chemicals Ind. Co. (Cairo, Egypt). Canesten cream B.N 2346109 is supplied from Alexandria Pharmaceutical Company (Alexandria, Egypt). *C. albicans* ATCC No 60193 standard strain was kindly provided by the Department of Microbiology, Ain Shams University. Sabouraud dextrose agar and sabouraud dextrose broth were purchased from Oxoid Limited (Hampshire, England).

Table 1. Composition and codes of IPM and JB Pemulen emulgels.

	Formula's code								
	Percentage (w/w) of the components								
Components	TM1/TJ1	TM2/TJ2	TM3/TJ3	TM4/TJ4	TM5/TJ5	TM6/TJ6	TM7/TJ7	TM8/TJ8	TM9/TJ9
IPM/JB	30	30	30	30	30	30	30	30	30
TEA	QS	QS	QS	QS	QS	QS	QS	QS	QS
Pemulen TR1	0.2	0.3	0.4	—	—	—	0.1	0.15	0.2
Pemulen TR2	—	—	—	0.1	0.2	0.3	0.1	0.15	0.2
Propylene glycol	5	5	5	5	5	5	5	5	5
Distilled water	63.8	63.7	63.6	63.9	63.8	63.7	63.8	63.7	63.6
CZ	1	1	1	1	1	1	1	1	1

J is assigned to JB emulgels; M is assigned to IPM emulgels; QS: quantity sufficient to adjust the pH to 5.5–6 using TEA.

Preparation of Pemulen emulgels

The assigned codes and detailed composition for the prepared formulae are given in Tables 1 and 2. All Pemulen emulgel samples were prepared by the indirect method, briefly, Pemulen TR1 and/or TR2 were dispersed in the calculated amount of the lipophilic phase containing CZ using thermostatically controlled heater with magnetic stirrer (Thermolyne Corporation, Dubuque, IA). The prepared lipophilic phase is then mixed using the over head mixer (Heidolph, Kelheim, Germany) for 1 min until a smooth dispersion was attained. The aqueous phase containing water and propylene glycol was slowly added and the whole mixture was mixed using the over head mixer for 10 min at 2000 rpm. The final pH of the preparation is adjusted between 5.5 and 6 by mixing with TEA (Bremecker et al., 1992; Milic-Askrabic et al., 1998; Simovic et al., 1999). The emulgels were left overnight at 20°C for equilibration.

Evaluation of the prepared emulgels

Rheological properties

The rheological properties were determined using rotational Brookfield viscometer of cone and plate structure (model programmable DV2; Brookfield, Middleboro, MA) (Gasperlin et al., 2000; Jiménez Soriano et al., 2001; Contreras and Sanchez, 2002). About 0.5 g of the formula to be tested was applied to the plate and left to attain an equilibrium condition, measurements were made at 20°C at shear rate ranging from 0.1 to 2 sec^{-1} corresponding to 0.05 to 1 rpm with 10 sec between two successive speeds and then in a descending order. The hysteresis loop between the upward and downward curve was studied. The flow index was determined by linear regression of the logarithmic form of equation (1) (Bourret et al., 1994; Jiménez Soriano et al., 2001).

$$\tau = k\,\gamma^{n} \tag{1}$$

where τ is the shear stress, γ is the shear rate, k is the consistency index, and n is the flow index.

$n = 1$ when the flow is Newtonian, if $n > 1$ or $n < 1$ indicates shear thickening or shear thinning, respectively. Also apparent viscosity at 0.8 sec^{-1} was determined from the rheograms.

Stability assessment

Temperature cycle test. The emulgel samples were subjected to two complete temperature cycles, each of 24 h, starting at –4°C (8 h) and 40°C (16 h) (Marquardt and Sucker, 1998).

Centrifugation. The emulgel samples were subjected to centrifugation using the centrifuge (Jencons, East Grinstead, UK) at 300 rpm twice, each for 15 min (Rieger, 1982; Simovic et al., 1998).

Long-term stability assessment. The prepared formulae were shelf stored for 1 year (Harry et al., 2000). Rheological assessment described under 'Rheological properties' was conducted on the samples after exposure to three different treatments *viz*. temperature cycle test, centrifugation, and shelf storage. The flow behavior of the different emulgel bases was studied according to different mathematical models that describe the viscoplastic fluids (Fresno et al., 2002; Krajisnik and Milic, 2003; Gad et al., 2008) namely:

$$\text{Bingham } (\sigma = \sigma_0 + \eta\gamma) \tag{2}$$

$$\text{Power's law } (\sigma = k\gamma^{n}) \tag{3}$$

$$\text{Casson's model } (\sigma^{1/2} = \sigma_0^{1/2} + \eta\gamma^{1/2}) \tag{4}$$

where σ = shearing stress, σ_0 = yield value, η = viscosity, γ = shearing rate, k = consistency index, and n = flow index. Curve fitting was carried out using GraphPad Prism for Windows, Version 5.0 (GraphPad Software Inc., La Jolla, CA).

In vitro drug release

This study was carried out using the modified USP dissolution apparatus (Pharma Test, type PTW 2; Pharma Test, Germany). Samples, each of 2 g of the preparation, were spread on a cellophane membrane previously soaked for overnight in the receptor medium. The loaded membrane was firmly stretched over the edge of a glass cup of 2.59 cm diameter. The cup was then immersed in the dissolution vessel which contained 100 mL of the

Table 2. Composition and codes of LP Pemulen emulgels.

	Formula's code								
	Percentage (w/w) of the components								
Components	TP1	TP2	TP3	TP4	TP5	TP6	TP7	TP8	TP9
LP	40	40	40	40	40	40	40	40	40
TEA	QS	QS	QS	QS	QS	QS	QS	QS	QS
Pemulen TR1	0.2	0.3	0.4	—	—	—	0.1	0.15	0.2
Pemulen TR2	—	—	—	0.1	0.2	0.3	0.1	0.15	0.2
Propylene glycol	5	5	5	5	5	5	5	5	5
Distilled water	53.8	53.7	53.6	53.9	53.8	53.7	53.8	53.7	53.6
CZ	1	1	1	1	1	1	1	1	1

QS: quantity sufficient to adjust the pH to 5.5–6 using TEA.

release medium (25% v/v DMF in 0.02 N HCl) previously warmed and maintained at 37 ± 0.5°C. Agitation was affected by paddle at 50 rpm and aliquots each of 5 mL were withdrawn from the release medium at different time intervals. Withdrawn samples were replaced by equal volumes of fresh release medium. The samples were assayed spectrophotometrically at λ_{max} 264.5 nm using ultraviolet spectrophotometer (UV1601; Shimadzu, Kyoto, Japan). Experiments were carried out in triplicates, the results were averaged and blank experiments were carried using plain bases. From the obtained results, the effect of gelling agent type and concentration as well as the oil type was studied.

Kinetics of drug release. The data obtained from the *in vitro* release experiments were analyzed according to the following equations:

$$\text{zero order } (C_t = C_0 - k_t) \quad (5)$$

$$\text{first order } (\ln C_t = \ln C_0 - k_t) \quad (6)$$

$$\text{Higuchi diffusion model (Higuchi, 1962)} \quad (Q/A = 2C_0 (D/\pi)^{1/2} t^{1/2}) \quad (7)$$

where C_t is the drug concentration at time (t), C_0 is the drug concentration at time zero, k is the release rate constant, t is the time, Q is the cumulative amount of drug released at time (t), A is the surface area, and D is the diffusion coefficient of the drug. Percentage coefficient of variation in each case was calculated.

In vitro antimycotic activity of selected CZ preparations

The microbiological evaluation of selected CZ preparation (formula that showed optimum stability and release) and the commercial preparation Canesten cream was carried out according to Ibrahim et al. (1991): a single well-isolated colony of *C. albicans* ATCC No 60193, which is grown and maintained on sabouraud dextrose agar slants at 4°C and subcultured monthly, was picked and inoculated into a tube containing 10 mL of sterile sabouraud dextrose broth (sterilized by autoclaving at 121°C for 15 min). The broth was incubated at 35°C for 24 h. After incubation, the resulting growth was centrifuged, washed with phosphate buffer saline (PBS), and re-suspended in fresh PBS to turbidity equivalent to 0.5 McFarland. An inoculum of 1.5 mL of the above suspension was transferred to sterile Petri dish (20 cm in diameter), then 30 mL of molten sabouraud dextrose agar (sterilized by autoclaving at 121°C for 15 min) was added to the inoculum and mixed well and left to solidify.

Stainless steel cylinders (11 mm internal diameter) were sterilized using hot air oven at 180°C for 1 h. Each cylinder was aseptically filled with accurately weighed 250 mg of the selected tested formulae, and put on the surface of inoculated sabouraud dextrose agar plates. The plate was incubated aerobically at 37°C for 24 h. After incubation, the inhibition zone diameter was measured using a ruler. The extent of release (zone of inhibition) was measured by calculating the mean of four readings. The test was applied also for non-medicated bases. The experiment was done in triplicates.

Statistical analysis

Statistical significance of difference was tested either using Student's *t*-test or one-way analysis of variance test (Sigma plot for Windows, Version 11.0; Systat Software Inc.). The level of significance was set at $\alpha = 0.05$.

Results and discussion

Preparation of Pemulen emulgels

The indirect method used in the preparation of CZ emulgels gave a relatively stable emulsion with all oils (Robin et al., 1999). Most of the emulgels were successfully prepared except some LP formulae, namely TP1, TP2, TP3, and TP9. This may be explained by the inability of Pemulen TR1 as emulsifying agent in formulae TP1, TP2, and TP3 to accommodate the large percentage of the oily phase incorporated (40% w/w). In formula TP9, the total amount of polymeric emulsifier used was 0.4%, which could be incompatible with the large percentage of oil used (40%). In general, modest amounts of Pemulen are used to emulsify the oil, i.e., more Pemulen is not necessarily better. As the amount of oil increases, the amount of Pemulen required for its successful emulsification tends to be reduced.

Rheological properties

Most fresh emulgel samples exhibited non-Newtonian shear thinning (pseudo)plastic flow with weak thixotropic or antithixotropic behavior (Figure 2) (only emulgels that showed consistent rheological model in the stability study are shown).

As seen in Table 3, with the exception of formula TM3, all samples showed pseudoplastic behavior as evidenced by a flow index (n) less than 1, whereas, formula TM3 showed a shear thickening behavior which is obvious from its value of n (1.259) which is greater than one. This behavior could be explained by the mechanical entanglement of the polymer chains by shearing. The higher the shearing rate the more the polymer chains may prevent the relative motion between the molecules. This observation is more pronounced with branched polymer and could be facilitated by the presence of IPM as an oily phase.

Emulsifier type and concentration affected the pseudoplasticity of the formulation. In most cases, systems prepared with the less hydrophobic Pemulen type TR1 have higher flow index than those of more hydrophobic Pemulen TR2. Some formulations showed a direct correlation between the polymer concentration and the flow index, i.e., by increasing the polymer concentration the flow index increases. For example, increasing the TR2 concentration in formulae TM4 to TM6 increases the flow index from 0.23 to 0.42. These results are in agreement

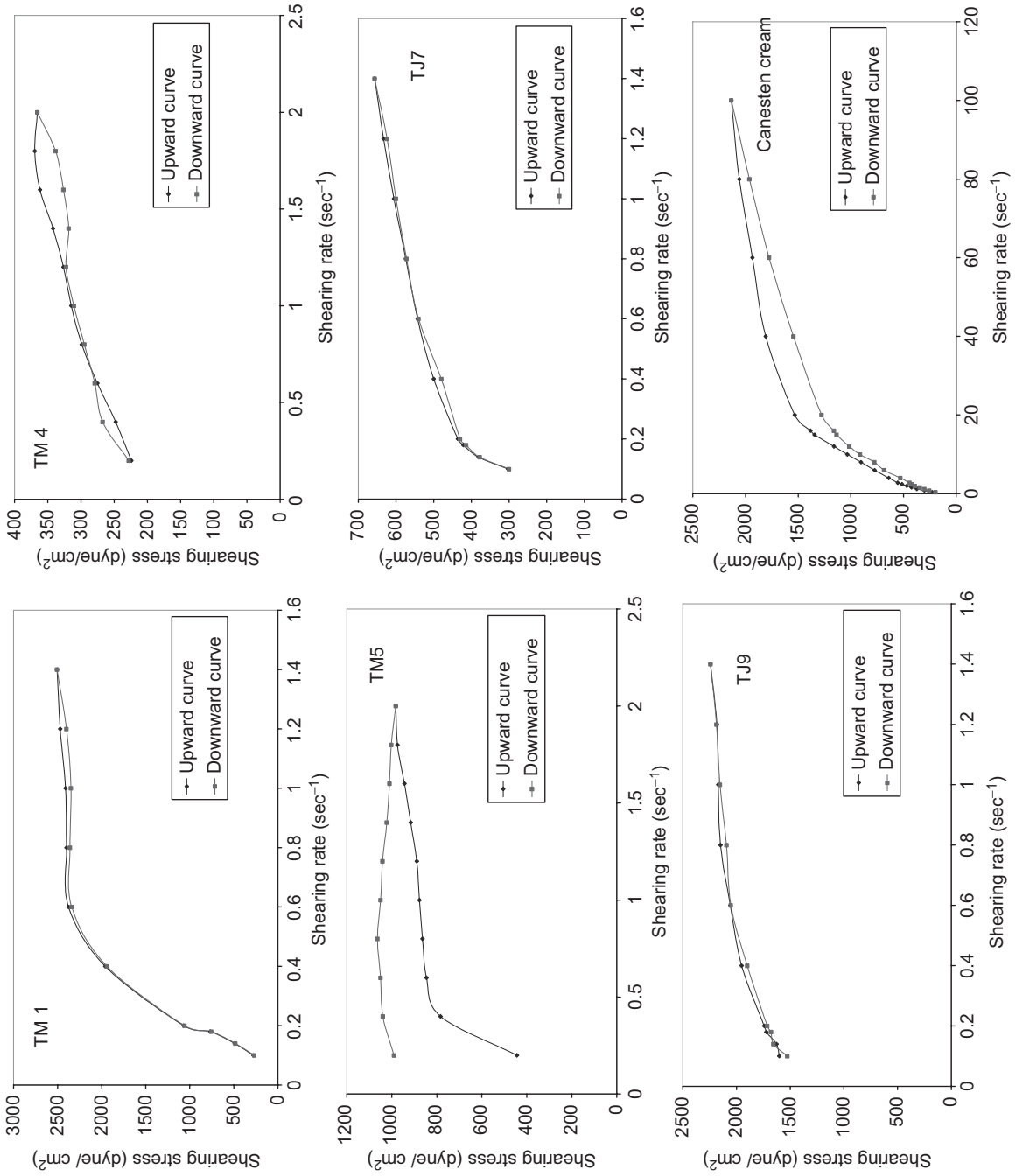

Figure 2. Flow curve for selected emulgel samples TM1, TM4, TM5, TJ7, TJ9, and Canesten cream.

Table 3. Flow index and viscosity at 0.8 sec^{-1} for different Pemulen emulgel formulae.

Formula's code	Flow index	Viscosity (cp)*
TM1	0.7731	300,000
TM2	0.9265	382,000
TM3	1.2590	484,000
TM4	0.2326	37,354
TM5	0.2821	108,000
TM6	0.4201	132,000
TM7	0.5919	187,000
TM8	0.5809	240,000
TM9	0.8019	254,000
TJ1	0.5979	121,000
TJ2	0.4412	132,000
TJ3	0.3607	134,000
TJ4	0.2995	25,558
TJ5	0.2069	32,931
TJ6	0.2673	34,405
TJ7	0.2534	71,759
TJ8	0.3391	73,725
TJ9	0.1349	94,325
TP4	0.4513	16,711
TP5	0.3364	31,456
TP6	0.2961	33,422
TP7	0.2991	57,997
TP8	0.3149	66,353
Canesten	0.4314	26,541

*Viscosity measured at 0.8 sec^{-1} at 20°C.

with those obtained by Dolz et al. (1998) who studied the influence of Carbopol 940 concentration on the flow behavior hydrogels and found that an increase in the flow index occurs with increasing the concentration and agitation time.

Interaction of Pemulen emulsifier with the oil phase has been shown to be of great importance in controlling the flow behavior of the studied emulgel systems as well. Emulgel systems prepared by JB as the oil phase displayed relatively higher pseudoplasticity as evidenced by its low mean flow indices which are 0.4666, 0.2579, and 0.2425 with all polymer used TR1, TR2, and a combination of TR1 and TR2, respectively. These values were compared to 0.9862, 0.316, and 0.6582 as well as 0.3613 and 0.3070 when the oily phase was IPM or LP, respectively.

The flow index of the commercial preparation Canesten was 0.431; this value is in the same range as those of the prepared formulae.

The apparent viscosity values at 0.8 sec^{-1} and 20°C for different emulgel bases are shown in Table 3. The apparent viscosity at this low shear rate is an indicator of spreadability upon topical application (Adeyeye et al., 2002). The apparent viscosity ranged from 484,000 to 16,711 cp with the highest value for sample TM3 (IPM emulgel containing 0.4% Pemulen TR1) and the lowest value for sample TP4 (LP emulgel containing 0.1% Pemulen TR2). A direct relationship exists between Pemulen concentration and the formulation viscosity, e.g., increasing Pemulen concentration from 0.2 to 0.4% in TM1 to TM3 increased the apparent viscosity from 300,000 to 484,000 cp. Emulgels containing Pemulen TR1 showed higher viscosity than those containing Pemulen TR2, whereas those containing combination are of intermediate viscosity. This is clearly evident by comparing the mean viscosity values for IPM emulgels with different polymers; it exhibited a mean viscosity of 388,667, 92,451, and 227,000 cp with Pemulen TR1, Pemulen TR2, and a combination of both, respectively. The previous finding could be attributed to the higher hydrophilicity, hydration as well as capability of water immobilization of Pemulen TR1. Similarly, the nature of the oily phase affected the viscosity of the preparation. IPM emulgels exhibited higher viscosities than other studied oils, whereas LP emulgels exhibited the lowest viscosities. Using Pemulen TR2 as gelling agent, LP showed a mean viscosity of 27,196 cp, whereas IPM showed a mean viscosity of 92,451 cp. This could be explained by the interference of the oil with the polymer–water interaction. It is suggested that the lipophilic nature of paraffin oil interfered with the interaction of Pemulen emulsifier molecules with the aqueous phase (responsible for the three dimensional build up) resulting in lower degree of hydration and lower viscosity results.

The apparent viscosity of the commercial formulation Canesten at 0.8 sec^{-1} was 26,541 cp, which is within the same range of the studied formulations.

Stability assessment

The effect of accelerated stability testing conditions (temperature cycle test and centrifugation) and long-term storage on the shelf for 1 year on each of color change, phase separation and rheological properties of different formulae was assessed. Pemulen emulgels showed no color change, signs of phase separation, or drop in consistency during storage.

Fitting of the ascending curve of the rheograms of the fresh and treated samples to different mathematical models (Bingham, Power's law, and Casson) revealed that formulae TM1, TM4, TM5, TJ7, and TJ9 showed consistent model (Power's law) under different treatments.

Figures 3 and 4 illustrate the fitted parameters (K and n) for the selected formulae under different treatments. It is noteworthy, although formula TM1 contained a relatively small amount of Pemulen TR1, yet it showed stable properties. Similarly, although formula TJ9 contained a higher amount of Pemulen emulsifier compared to formula TJ7, it showed less stability. This is in agreement with the results obtained by Miller and Loffler (2001) who stated that greater amount of emulsifier did not always lead to a more stable system. Formula TJ7 showed the best stability as it followed a consistent model (Power's law) and exhibited non-significant change in the consistency index under different treatment conditions ($P > 0.05$). According to this finding, it could be concluded that both the percentage of emulsifier and combination of hydrophilic and hydrophobic polymers play an important role in the stability of the prepared emulgel system. Similar results were obtained by Qui et al. (1998) who formulated

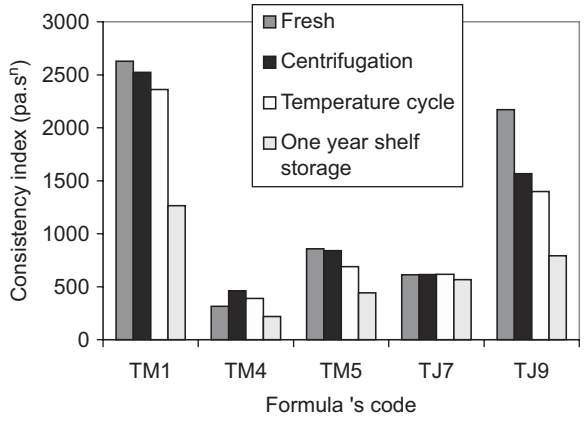

Figure 3. Consistency index (K) for some selected Pemulen formulae.

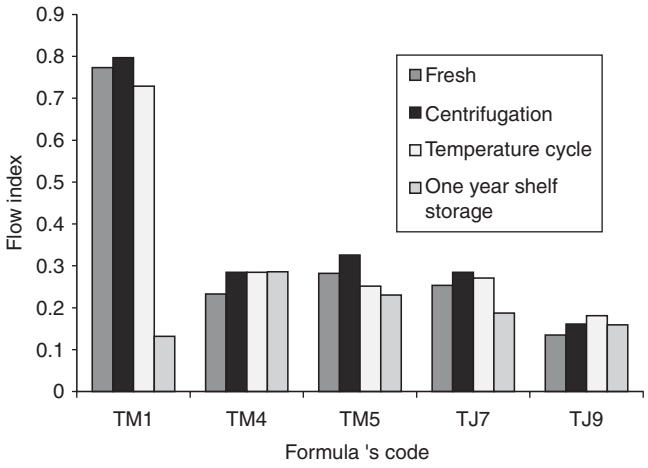

Figure 4. Flow index (n) for some selected Pemulen formulae.

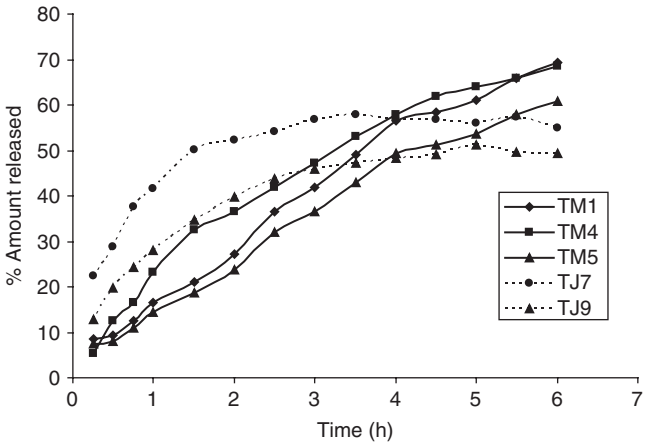

Figure 5. Release profile of formulations (TM1, TM4, TM5, TJ7, TJ9).

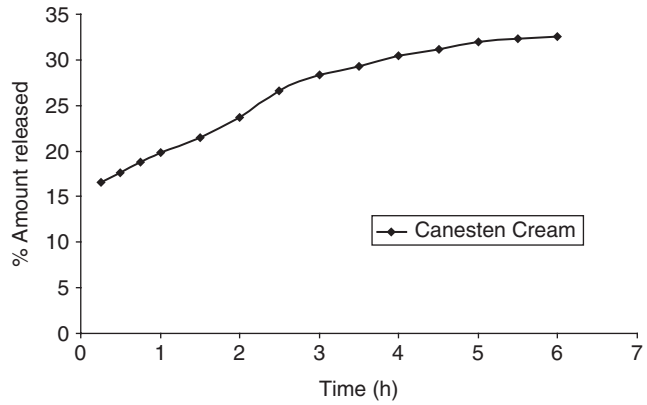

Figure 6. Release profile of commercial formulation Canesten cream.

a topical insect repellant emulsion gel formula based on a combination of Carbopol 940 NF and Pemulen TR2 and found that it was chemically and physically stable during 4-month accelerated stability test at 45°C.

It is clear from the stability data shown in Figures 3 and 4 that formula (TM4) showed an increase in both consistency and flow indices after different treatments. The lowering of both indices could be due to the relaxation of the polymer chains and coalescence of the oil droplets (Jiao and Burgess, 2003).

In vitro drug release

The *in vitro* release of CZ from different emulgel bases and commercial formulation at 37°C was investigated (Figures 5 and 6) (only the release profile of the formulations that showed consistent model in the stability study are shown). The release from systems prepared with IPM (TM1, TM4, TM5) as oily phase proceeded at first by lower rate compared with the corresponding systems prepared with JB (TJ7, TJ9) (Figure 5). However, after the elapse of the first 3 h the drug release from IPM emulgel systems started to exhibit higher rates, whereas the release from systems containing JB displayed a plateau phase after the same period. The initial rapid release rate from studied systems could be explained by the development of an equilibrium condition due to contact of the release medium and the electrolyte sensitive gel. The electrolyte causes the gel structure to collapse with a resultant liquefaction of the preparation. This enhances the diffusion of the drug, a situation similar to release from a drug solution (Bremecker et al., 1992). On the other hand, the release profile from the commercial preparation Canesten proceeded slowly and only 32.5% of its CZ content was released within 6 h. Surprisingly, the release from that cream was lower than from the emulgels prepared with either JB or IPM (Figure 6).

The release data were analyzed according to zero-order, first-order, diffusion-controlled release mechanism. The results obtained are presented in Table 4. All the studied formulations followed the first-order release mechanism ($r^2 > 0.97$). The fitted parameter K_1 has been used to compare the rate of CZ release between different studied formulations. The effects of Pemulen concentration, Pemulen type as well as oil type were also studied.

The effect of Pemulen concentration and type

For IPM emulgels, increasing the concentration of Pemulen TR1 resulted in a decrease in rate of CZ release.

Table 4. Kinetic data of CZ release from different Pemulen emulgel bases.

Formula's code	Zero order		First order			Higuchi diffusion model		
	c.v.%	K_0 (mg/h)	c.v.%	K_1 (h^{-1})	$t_{1/2}$ (h)	c.v.%	KH ($\times 10^2$ mg/cm^2 $h^{0.5}$)	Release order
TM1	4.50	2.27	1.43	0.20	3.51	8.96	13.60	First order
TM2	3.00	2.10	1.17	0.16	4.23	10.88	13.95	First order
TM3	3.79	1.88	1.34	0.14	4.82	10.41	12.33	First order
TM4	8.20	2.15	1.41	0.19	3.61	3.46	12.64	First order
TM5	4.04	2.12	1.18	0.17	3.98	8.96	13.14	First order
TM6	4.52	1.93	1.30	0.15	4.63	8.97	12.47	First order
TM7	3.30	2.24	1.44	0.18	3.79	10.88	14.41	First order
TM8	3.98	1.95	1.43	0.15	4.58	10.41	12.53	First order
TM9	3.70	1.85	1.30	0.14	4.95	10.41	12.22	First order
TJ1	10.32	1.37	3.53	0.11	6.34	46.77	8.26	First order
TJ2	9.44	0.85	3.44	0.07	10.06	75.28	4.84	First order
TJ3	8.08	0.87	2.81	0.07	10.38	69.01	5.22	First order
TJ4	13.42	1.24	4.76	0.10	7.07	44.94	7.53	First order
TJ5	10.98	1.07	3.86	0.08	8.54	52.43	6.63	First order
TJ6	8.78	0.90	3.07	0.07	10.56	61.03	5.70	First order
TJ7	14.30	0.95	5.35	0.09	8.06	70.69	5.05	First order
TJ8	11.57	1.07	4.10	0.09	7.69	64.06	5.99	First order
TJ9	9.44	1.19	3.19	0.09	7.40	54.60	7.10	First order
TP4	8.89	1.31	2.92	0.10	6.88	46.07	8.13	First order
TP5	8.22	1.25	2.70	0.09	7.41	46.50	7.94	First order
TP6	6.81	1.10	2.25	0.08	8.96	47.53	7.44	First order
TP7	11.23	1.49	3.74	0.12	5.58	44.73	8.64	First order
TP8	10.27	1.44	3.36	0.12	5.93	45.09	8.52	First order

This finding is obvious by comparing the release rate constant K_1 of formula TM1 and TM3. Formula TM1 with low TR1 level showed higher release rate (0.2 h^{-1}) compared to formula TM3 (0.14 h^{-1}). Similarly, increasing the Pemulen TR2 concentration from 0.1 to 0.3% in formulae TM4 and TM6 decreased the release rate constant from 0.19 to 0.15 h^{-1}. This may be attributed to the low viscosity of the formulations containing low concentrations of Pemulen. The other two oils exhibited similar behavior as IPM. Interestingly, polymer type also affected the rate of CZ release. In general, formulations containing Pemulen TR1 released CZ faster than those containing TR2. By comparing formula TM1 to TM5 where both have similar concentration of Pemulen but of different type. The viscosity of formula TM1 (300,000 cp) is found to be higher than that of formula TM5 (108,000 cp), yet the rate of drug release from TM1 (0.2 h^{-1}) was greater than that of TM5 (0.17 h^{-1}). The higher hydrophobicity of TM5 compared to TM1 due to its content of Pemulen TR2 (more hydrophobically modified acrylic acid polymer) could be the possible reason for slowing down the release.

The effect of oil type

Comparing K_1 for formula TJ1 (JB emulgel) to formula TM1 (IPM emulgel) (Table 4), it could be noted that formula TM1 has a higher release rate constant (0.2 h^{-1}), whereas, formula TJ1 has a lower release rate constant (0.11 h^{-1}). Similar results are obtained upon comparing K_1 values for formulae containing Pemulen TR2, namely: e.g., formulae TM4, TJ4, and TP4, as they were 0.19, 0.10, and 0.09, respectively. Therefore, it is obvious that IPM showed higher rate of drug release than either JB or LP. This lower release rate of CZ from either LP or JB emulgel systems could be explained by the relatively higher lipophilicity of either LP or JB compared to that of IPM. This higher lipophilicity of the oil phase results in higher affinity of the lipophilic drug, in this case CZ, and hence decreased release from the oil phase is expected.

All the previous results showed that formula TJ7 has the highest stability and moderate release; therefore further microbiological evaluation for TJ7 will be carried out.

In vitro antimycotic activity of selected CZ preparations

The effect of base composition on antimycotic activity of CZ against *C. albicans* using agar diffusion method was investigated. Table 5 illustrates the extent of drug release (inhibition zone diameter) from the tested bases, the antimycotic activity of formula TJ7 was noticed to be statistically significant ($P < 0.05$) compared to the commercial preparation Canesten cream. This may be explained by the greater solubility of CZ in the TJ7 emulgel (possibly due to high water content of JB) in comparison to the commercial preparation, and hence greater partitioning of CZ at the boundary between the diffusion medium and the preparation. Also the higher release rate of CZ from TJ7 compared to the commercial formulation could be another reason for this increased antifungal activity.

Table 5. Inhibition zones produced by *in vitro* antimycotic activity of 1% CZ in different formulations using cylinder plate method and *Candida albicans* as test organism.

Formula	*Mean zone of inhibition (cm)
TJ7	2.73 ± 0.06
Canesten® cream	2.34 ± 0.06

*Mean value ± SD, $n = 3$.

Conclusion

Most emulgel samples exhibited non-Newtonian shear thinning (pseudo)plastic behavior with weak thixotropy/antithixotropy in which, the apparent viscosity at 0.8 sec^{-1} was dependent on the type and concentration of the polymer used, as well as on the type of the oily phase. At the same time, formula TJ7 showed consistent rheological model and the least change in both consistency and flow indices. The *in vitro* release showed variation in the rate of released CZ. Analysis of the drug release data showed an inverse correlation between the concentration of gelling agent and the rate of drug released. The polymer type also affected the rate of drug release as preparation containing Pemulen TR1 released CZ faster than those containing Pemulen TR2. Variation in the oil type also had an influence on the *in vitro* drug release. As IPM showed the highest drug release rate compared to the other used oils. Formula TJ7 is selected for further microbiological evaluation as it has high stability and moderate release properties. Comparing the antifungal activity of formula TJ7 and the commercial preparation Canesten cream against a standard strain of *C. albicans* revealed a greater antifungal activity of formula TJ7. Further *in vivo* animal studies as well as *in vitro* permeation and the skin retention studies, using hairless rat skin to quantify the amount of CZ permeated through the skin, to calculate the local accumulation efficiency of the obtained promising stable formulation is recommended.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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