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Design and development of Amphotericin-B loaded transungual drug delivery system for the effective management of onychomycosis

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ABSTRACT: The present research aims to formulate and evaluate the emulgel containing different penetration enhancers for the transungual delivery of Amphotericin B (AmB). Amphotericin B is a widely used antifungal agent in treating infections caused by *Candida*, *Aspergillus*, and *Fusarium,* which are also responsible organisms for onychomycosis. The emulsions were prepared using the fusion method of oil, surfactant, co-surfactant, and distilled water. The formulated emulsions were homogenized with a high-speed homogenizer to reduce globule size and incorporated into the gel base of different concentrations. The formulation undergoes physical evaluations, as well as spreadability, extrudability, washability, viscosity, and *in-vitro* drug release studies. EG5 was selected as an optimized Formulation from the drug release study as it showed a better release profile. Different penetration enhancers were added and further evaluated in the optimized formulation for parameters like *in-vitro* drug release, drug uptake, and stability study. Physical characteristics, spreadability, extrudability, washability, and viscosity of all the prepared emulgels were acceptable. Optimized formulation EG-5SS showed better drug release, uptake, and oil binding capacity. All the studies concluded that the EG-5SS was the best formulation and sodium sulfide could be used as a potential permeation enhancer.

KEYWORDS: Emulgel; transungual; penetration enhancers; candida; onychomycosis.

1. INTRODUCTION

Onychomycosis is a common nail disease that affects almost 5.5% of the world's population and represents 50% of all occurrences of nail infections [1, 2]. Onychomycosis of the fingernails can result in irritation, pain, and the loss or impairment of tactile functions. Toenail dystrophy may impede gait, physical activity, and shoe fitting. Furthermore, onychomycosis elicits adverse physical and psychosocial consequences [2]. Onychomycosis may be affected by journey frequency, profession, environment, and aging. An elevated incidence can be attributed to several factors, including an aging population, HIV infection or immunosuppressive medication, active engagement in sports, the use of commercial swimming pools, and the use of occlusive footwear [1-3]. Toenails are damaged seven times more often than fingernails because they grow three times more slowly [3]. Indian patients are also more likely to get onychomycosis if they walk barefoot, wear shoes that don't fit properly, bite their nails (onychophagia), or work with chemicals [4]. Dermatophytes are often thought to cause onychomycosis (about 90% of the time in toenails and 50% in fingernails). *Tinea unguium* is the name for dermatophytes that get into the nail plate. The most common causing agent is *Trichophyton rubrum* (*T. rubrum*); the next most common is *T. mentagrophytes* [1, 2]. It was thought that yeasts were just pests, but it is becoming more apparent that they can cause skin diseases. *Candida albicans* cause 70% of cases.

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With a broad spectrum of action and excellent therapeutic efficacy, AmB is an antifungal (and antiparasitic) drug that exhibits a crucial fungicidal impact [5, 6]. However, this molecule's large molecular weight may make it more challenging to get through the nail plate. Since its principal mechanism of action includes the direct pharmacological interaction with the fungal cell wall via ergosterol, the predominant sterol component present in the fungal cell membrane, AmB is employed as an immediate-acting fungicide compared to other antifungals. The influx of water, ions, and low-molecular-weight polar proteins is caused by the pores formed due to this interaction. Thus, the pathogen's survival ability diminishes [7, 8].

Additionally, as the literature results have already been documented, this drug exhibits extraordinary antifungal activity in the clinical practice of onychomycosis [9]. A lipophilic drug, amphotericin B inserts into lipid bilayers by binding to sterols [10]. Amphotericin B should be especially well-suited for lipid-based delivery methods based on these features. The diseased nail is painful to remove surgically or chemically. Therefore, the only available therapeutic options are topical or systemic drug administration [11, 12].

Drug interactions and side effects are two barriers to systemic therapy [13, 14]. While systemic treatment has a high success rate, aged and immunocompromised people may experience problems. Moreover, there are significant drawbacks to long-term systemic antifungal usage. Topical delivery also has difficulties with inadequate nail penetration [15–17]. Low or insufficient drug penetration on and across the highly keratinized nail barrier, made of keratin with strong disulfide bonds, dramatically hinders the delivery of a therapeutically active composition via the transungual route [18].

Emulgels are a more reliable and superior delivery method for lipophilic or water-insoluble drug substances. These emulsions, either water-in-oil or oil-in-water, are mixed with a gelling agent to enhance the viscosity of the ultimate formulation, which helps the formulation adhere to the applied surface for a long time to support drug permeation [19–21]. Thixotropic, greaseless, readily spreadable, easily removable, emollient, non-staining, long-lasting, translucent, and aesthetically pleasant emulgels exhibit several advantages [22, 23]. The formulation of the emulgel loaded with AMB, Coconut Oil, Tween 20, Span 20, Propylene Glycol, Liquid Paraffin, Carbopol, and Distilled water were used.

Taking into account the aforementioned benefits and drawbacks of AmB, the main objective of this research was to produce an emulgel that contained AmB for topical management of onychomycosis. Two penetration enhancers, sodium sulfide and urea, were employed further to increase AmB's penetration through the keratinized nail plate.The penetration enhancers could be able to improve the permeation of the drug through the breaking of α-keratin disulfide bonds present in the nail plate or by hydrating the nail plate to form a porous surface to support drug permeation.

2. RESULT AND DISCUSSIONS

2.1. Physical appearance

The physical observation of prepared AmB emulgel formulations was done. The color of the formulations was yellowish, and they showed good consistency. In the homogeneity study, it was observed that no gritty particles were present in the prepared formulations; this may be because of the use of a high-speed homogenizer. The observed results are shown in Table 1. All the emulgels showed good washability.

2.2. pH

The pH values of the prepared emulgels, which were recorded in Table 1, ranged from 5.6 to 6.2. Because the measured pH values were so close to those of skin and nails, the formulations were deemed to be non-irritating when applied [24].

2.3. Viscosity

The viscosity tests were done using a rotational viscometer (Labman-Viscometer, LMDV-60), and all the formulations showed shear-thinning properties. All the experiments were done at a speed of 100 rpm for 10 minutes at room temperature. The recorded viscosity of emulgels was in the range of 4241 to 8264 cps. Each emulgel exhibited an ideal viscosity, confirming the adhesive action of the produced emulgels. This behavior is crucial for transungual delivery since it enhances the adhesion of the formulation to the nail plate. Table 1 displays the observed outcomes.

Table 1. Evaluation parameters and results

Parameters	EG-1	EG-2	EG-3	EG-4	EG-5	EG-6
Drug Content (%)	94.6	94.8	95.3	96.1	95.9	94.9
Color	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
pH	5.8	6.2	5.6	5.8	5.9	6.1
Viscosity (cps)	8264	7461	6812	5916	5192	4241
Spreadability	*	*	**	**	***	***
Extrudability	*	*	**	**	***	***
Homogeneity	*	*	*	*	*	*
Washability	***	***	***	***	***	***

Optimized formulations			
Parameters	**EG-5**	**EG-5 SS**	**EG-5 U**
Oil binding	97.44±0.43	98.54 ±0.56	98.11 ±0.35
Drug uptake (µg/mm²)	32.24	46.91	39.12

* Satisfactory; ** Good; *** Excellent

2.4. Spreadability

The term "spreadability" refers to how easily a substance spreads when applied to an affected area. From the perspective of patient compliance, the spreadability of topical formulation is a vital parameter for consistent and easy application. The AmB emulgel is efficiently spreadable with little shear stress, according to recorded spreadability data. Table 1 displays the spreadability of prepared formulations.

2.5. Extrudability

The emulgel formulations were introduced onto aluminum collapsible tubes. The formulation was extruded by pressing the tube, and the formulation's extrudability was noted. The observed extrudability of formulations was found to be good. After the application of mild pressure, more than 80% of emulgels could be extruded from the tube. Results are shown in Table 1.

2.6. Leaching study

This study has been done to evaluate the oil-binding capacity of optimized formulations with the gelling network. The study suggested that optimized emulgels showed satisfactory oil binding capacity. It can be concluded that oil is well-entrapped in the gel matrix. The study indicated that the emulgel has good stability. The observed results are shown in Table 1, Figure 1(c).

2.7. Drug Content:

The % drug content of all the developed emulgels was found to be in the range of 94.6 - 96.1 % (Table 1).

2.8. Microscopic Analysis

The microscopic structure of emulgels relies on the gelator. The black-spotted globules represent the existence of oil globules in the optimized formulation [25]. The oil is present as tiny globules in the optimized emulgel, which confirms the availability of surfactant along with co-surfactant and reduces the globule size of the oil [26]. The white color particle may be part of hydrogel, i.e., Carbopol 934. At high-speed mixing, they are uniformly distributed over the formulation (Figure 2a).

2.9. FTIR analysis

Figure 1 (a) represents the FTIR interaction of the pure drug and formulations. Amphotericin B shows a characteristic peak at a region of 3360 due to the -OH stretching. At the region of 2934.5 cm^{-1}, a peak was found due to the polyene CH stretching. The peak at 1554 cm^{-1} was due to the polyene C=C bond. A bond arises at 1627 cm^{-1} due to the NH_2 in-plane band [27]. There was no extra peak appearing or disappearing in the formulation, revealing that there was no interaction with the used excipient.

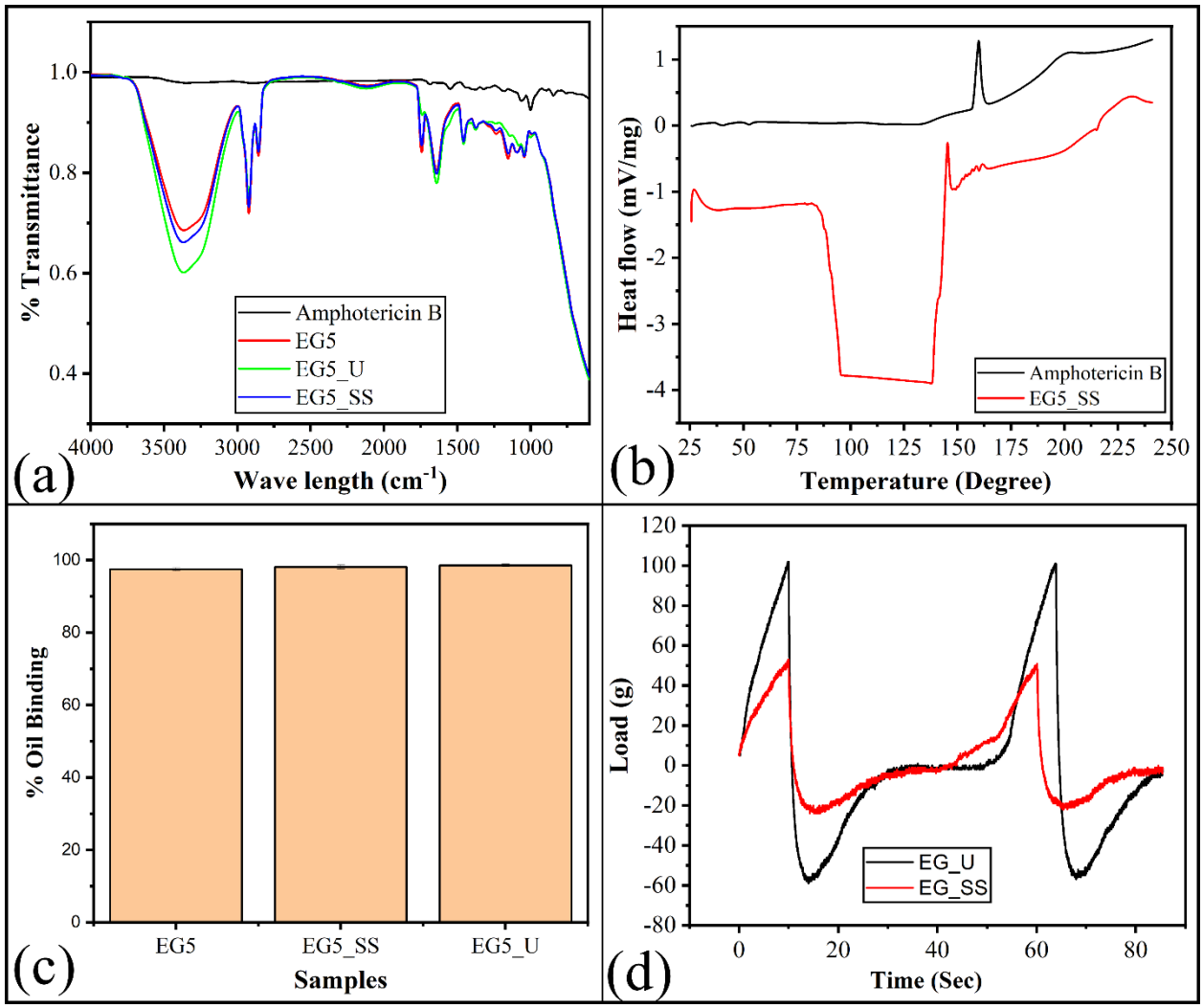

Figure 1. (a) FTIR analysis, (b) DSC thermogram, (c) Oil Binding, (d) Texture Analyser

2.10. DSC

The thermal peak shows a sharp exothermic peak at a region of 160 °C Figure 1(b). The same type of finding was reported by Zu et al. [29]. The presence of a sharp peak suggested that the drug is crystalline in nature. In the prepared emulgel, a broad peak was found, indicating the biphasic nature of the formulation. The same type of observation was found by Giri et al. [28]. In the prepared formulation, no sharp crystalline peak was seen, suggesting that the drug was present in the amorphous form.

2.11. Drug uptake study

The study suggested that the formulation containing a permeation enhancer has shown improved drug uptake by the nail clippings compared to the formulation containing no penetration enhancer. Among all the optimized emulgels, EG-5SS showed maximum drug uptake (Table 1, Figure 3).

2.12. Antifungal activity

The optimized formulation containing sodium sulfide (EG-5SS) showed an optimum zone of inhibition against *candida albicans*. This confirmed that the developed formulation was effective against *candida albicans*, which is a prominent responsible microorganism for onychomycosis.

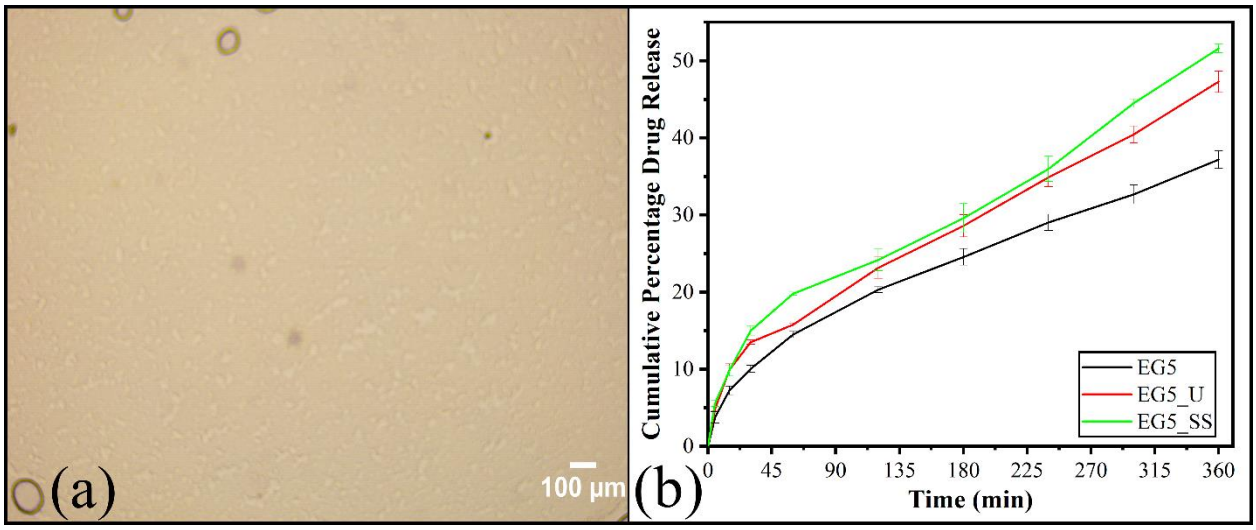

Figure 2. (a) Micrograph of EG5_SS (b) Drug release profile of the prepared formulations

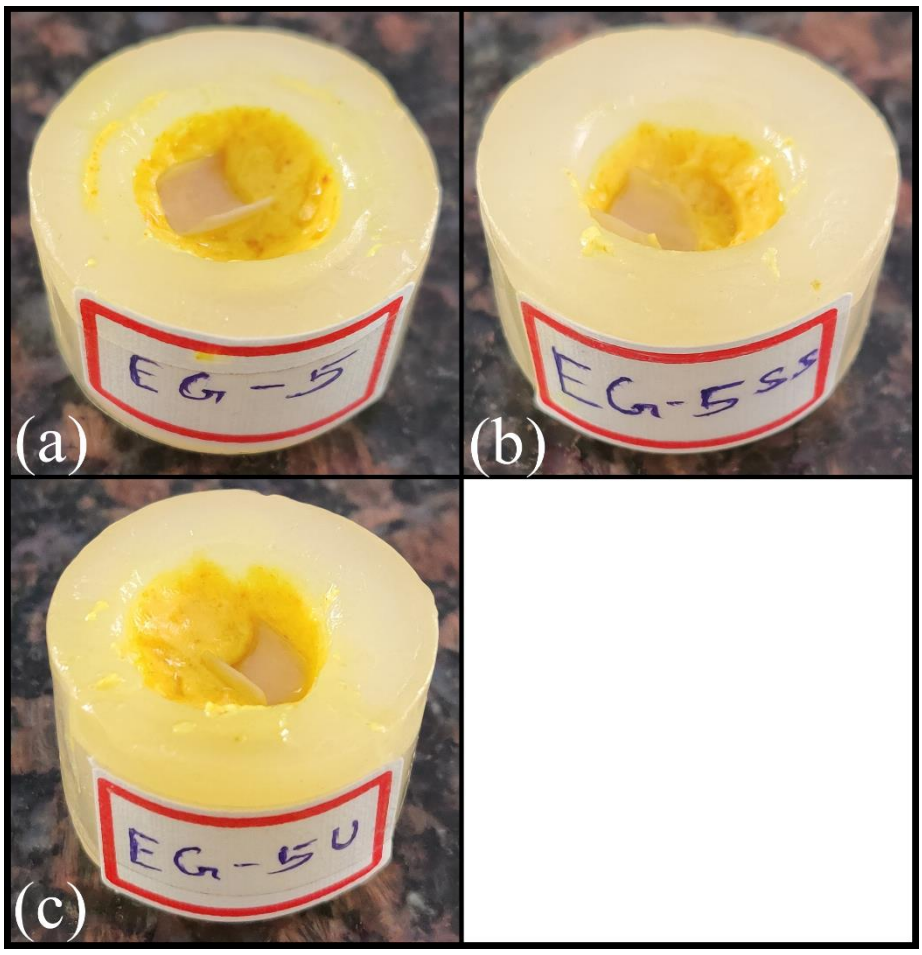

Figure 3. Drug uptake study by the nail clips

2.13. Texture Analysis

The hardness of optimized formulation EG-5U and EG-5SS was determined to be 102 and 53, respectively, Figure 1(d), Table 2. From the analysis, it has been found that EG-5SS showed less cohesiveness. Springiness was related to the elasticity of the sample. It has been found that EG-5U has more elasticity than the EG-5SS. It can be concluded that EG-5SS was found to have less hardness so that it would spread better, and less adhesiveness helped to peel out the sample [28].

Table 2. Texture profile of prepared emulgels

Formulation/ Parameter	Hardness (g)	Adhesive Force (g)	Adhesiveness (mJ)	Cohesiveness	Springiness (mm)
EG5_U	102	59	6.1	1.01	11.56
EG5_SS	53	24	2.9	0.85	9.39

2.14. Drug release study

Amphotericin B is a macrolide polyene antibiotic with broad-spectrum antifungal and antiprotozoal activity. The objective of topical and dermatological dosage forms is to administer drugs in a convenient manner to a localized area, ensuring sustained release and longer adherence [30]. A 6-hour drug release study was conducted to evaluate the release of drugs from the formulations. It has been found that after a 6-hour, the optimized formulation (EG-5) showed a release of 37%. After the incorporation of a penetration enhancer, the release was found to be 47% and 52% in EG-5U and EG-5SS, respectively (Figure 2b, Table 3). It increases the drug release by 1.5 times more than the control formulation (EG-5). For better understanding, the cumulative percentage of drug release was fitted to the KP and PS Models. From the KP model, it was found that the diffusion exponent (n) was more than 0.45, suggesting that release was mainly driven by the non-fickian or anomalous transport [31]. In the PS model, the 'm' value meant that the release exponent and the analysis indicated that all the releases were found to be less than 0.45. So, it can be concluded that the drug release was due to the fickian diffusion. AIC and MSC parameters were found within the range of 3 to 4, suggesting a good fit. The release model co-relation coefficient was found to be more than 0.98, and the PS model shows a better fit than the KP model [32].

Table 3. Release parameter

Formulations/Model Parameter		EG5	EG5_U	EG5_SS
Korsmeyer-Peppas model (KP Model)	Kp	1.6497	1.74516	1.96045
	n	0.52522	0.55158	0.54389
	R^2	0.99884	0.98973	0.98128
	AIC	9.36781	35.2935	42.7357
	MSC	6.11032	3.93744	3.33446
Peppas-Sahlin model (PS Model)	K_1	1.93164	2.16286	2.38805
	K_2	0.04466	0.15673	0.16444
	m	0.45141	0.41443	0.41394
	R^2	0.99922	0.99311	0.99287
	AIC	7.3108	33.3022	42.2413
	MSC	6.31602	4.13658	3.3839

2.15. Stability study

An accelerated stability investigation was carried out with a stability chamber for six months at 40 ± 2°C and 75 ± 5% RH. Samples were examined for phase separation, drug concentration, pH, and physical appearance at various points in time. Based on the observations, it was determined that the aforementioned parameters did not exhibit any changes of that sort (Table 4).

Table 4. Stability parameters of optimized emulgels

PARAMETERS	EG-5	EG-5 SS	EG-5 U
Physical appearance	No change	No change	No change
pH	No change	No change	No change
Drug content	No change	No change	No change
Phase separation	No change	No change	No change

3. CONCLUSION

Different emulgels (EG1, EG2, EG3, EG4, EG5, and EG6) were developed using coconut oil, tween-20, span-20, Propylene Glycol, Liquid Paraffin, carbazole 940, and distilled water. A variety of factors, including drug concentration, pH, viscosity, spreadability, extrudability, in-vitro drug release, DSC, FTIR, and nail uptake study, were assessed with formulated preparations of AmB. All the formulations showed good rheological behavior and showed shear-thinning properties. The optimized formulation EG-5SS showed significantly about 1.5 times more drug release (52%) and drug uptake (46.91µg/mm^2) than the base formulation EG-5. pH of the developed emulgels was found in the range of 5.6 to 6.2, which is near the pH of the nail plate and skin. Hence, it can be considered that it will not irritate the skin near the nail plate after topical application. The result of texture analysis suggested that EG-5SS was found to have less hardness so that it would spread better. From the FTIR analysis, it was found that there was no interaction between the drug and excipients. DSC study suggested that the drug was present in the amorphous form in the optimized emulgel. In the *in-vitro* antifungal effectiveness test, the optimized formulation (EG-5SS) was efficient against *candida albicans*, which is a significant microorganism responsible for onychomycosis. From the stability study, it was found that the prepared formulation was stable, and no deviation from the test parameters was recorded during and after the completion of the study. From the study, it was found that the optimized emulgel with sodium sulfide as a penetration enhancer showed promising results and enhanced drug uptake. So, on the basis of the observed result, it can be concluded that the formulation having sodium sulfide as a penetration enhancer was able to break the α-keratin disulfide bonds present in the nail plate and improve the nail permeation of the drug through the nail plate. So, sodium sulfide can be used as a potential penetration enhancer to enhance the topical permeation of drugs for different formulations.

4. MATERIALS AND METHODS

4.1. Chemicals required

Amphotericin B was a gift sample from Alembic Pharmaceuticals, Vadodara, India. Carbopol 940, Tween 20, Tween 80, and Span 20 were purchased from Sisco Research Laboratories Pvt. Ltd. Maharastra. Liquid Paraffin and Propylene Glycolwas obtained from CDH, Delhi. The coconut oil was purchased from Organic India.

4.2. Method of preparation of Amphotericin-B loaded emulgel

All the ingredients were weighed and separated by the formulation table (Table 5). The Required amount of Carbapol 940 was taken in a beaker containing double distilled water and allowed to soak overnight; a small amount of triethanolamine was added. After the preparation of the gel, an emulsion was developed by using coconut oil as the oil phase, tween 20 and span 20 as surfactant and co-surfactant, respectively. Distilled water was used as an aqueous phase. Tween 20 was mixed with distilled water in a beaker and marked as an aqueous phase. The oil phase was developed in a separate beaker by combining coconut oil with span 20, liquid paraffin, and the drug. Oil and aqueous phases were heated up to 70 to 80 ℃ separately. The oil phase was then added to the aqueous phase and homogenized with a high-speed homogenizer to reduce the particle size and enhance the stability of the emulsion. The emulsion was then allowed to cool at room temperature and added to the formulated gel base with continuous stirring followed by a high-speed homogenizer. Among all the developed emulgel formulations, a drug release study was carried out. Formulation EG5, which demonstrated superior drug release, was chosen as the optimal formulation. This superior drug release could be due to the concentration of the gelling agent (carbapol 940). Two penetration enhancers, sodium sulfide, and urea, were separately added to the EG5, marked as EG-5 U and EG-5 SS, and further assessed (Figure 4).

Table 5. Formulation table

INGREDIENTS	EG1	EG2	EG3	EG4	EG5	EG6
Amphotericin-B (%)	0.3	0.3	0.3	0.3	**0.3**	0.3
Tween 20 (ml)	0.09	0.09	0.09	0.09	**0.09**	0.09
Span 20 (ml)	0.18	0.18	0.18	0.18	**0.18**	0.18
Propylene Glycol (ml)	0.96	0.96	0.96	0.96	**0.96**	0.96
Liquid Paraffin (ml)	1.87	1.87	1.87	1.87	**1.87**	1.87
Coconut Oil (ml)	0.21	0.21	0.21	0.21	**0.21**	0.21
Carbopol-940 (gm)	0.9	0.8	0.7	0.6	**0.5**	0.4
Distilled water (ml)	18	18	18	18	**18**	18

Figure 4. Pictograph of the optimized formulations

4.3. Evaluation of emulgels

4.3.1. Physical appearance

All the ready emulgels were assesed for their physical appearance, including color, consistency, homogeneity, grittiness, and washability. A small amount of emulgel was pressed between fingers to check if any gritty particles were present. A washability test was done under tap water [29, 33].

4.3.2. pH

The pH of developed emulgels was recorded using an electronic pH meter (Electronics India, Model-111) [29, 33].

4.3.3. Viscosity

The prepared emulgel compositions' viscosity was measured using a rotational viscometer. The produced emulgel formulation had been added to the beaker and allowed to settle at 25–30 °C for 30 minutes in order to measure viscosity. After adjusting the spindle so that it doesn't come into contact with the beaker's bottom, spin it for ten minutes at a humble 100 RPM. The measured viscosity was recorded [29, 34].

4.3.4. Spreadability

A self-modified apparatus determined spreadability and was suitably modified in the laboratory. The apparatus contained two glass slides; the lower one was fitted on a wooden block provided by a pulley at one end. 1 gm of developed emulgel was placed on the lower slide fitted on the block. Another glass slide was placed over the emulgel, and 500 gm weight was placed over the slides for 1 minute. Excess of the gel was scrapped off from the edges. On the top glass slide, 50 gm weight was tied and allowed to travel over the stationary lower slide. The time the upper slide took to travel to the end of the fixed slide (7.5 cm) was noted down [35]. Spreadability is calculated by using the following formula:

S = M ×L/T

Where S= spreadability, M= weight tied with the upper slide, L = traveled length (7.5 cm), and T = time is taken to separate the slides from each.

4.3.5. Extrudability

Prepared emulgels were introduced into aluminum collapsible tubes. The required pressure to extrude the formulation from the tubes was measured [35, 36].

4.3.6. Leaching study

A 5 gm sample of each developed formulation was introduced into previously weighted centrifuge tubes, and the weights, including formulation, were noted. A centrifuge (Remi Centrifuge, India) was used to centrifuge the tubes for 15 minutes at 10000 RPM at room temperature. After the centrifugation, the tubes were reversed on Whatman filter paper (#102, 10*10 cm diameter); after 5 and 30 minutes, the weight of the tubes containing the emulgels was recorded [37]. The percentage loss of oils from different tubes was determined using the formula:

$$\%\ Leaching = \frac{(b-a)-(c-a)}{(b-a)} X\ 100$$

Where,

a= the empty centrifuge tube's weight

b= weight of the tube with sample

c= the sample's weight after thirty minutes with tube

4.3.7. Drug Content

Drug concentration in emulgel was measured by UV visible spectrophotometer (LABINDIA, UV-3200). 1 gm of the formulation was taken and dissolved with a specific amount of methanol. The solution was filtered with a syringe filter. Absorbance was measured after suitable dilution at 413 nm in UV/VIS spectrophotometer [23].

Drug entrapment efficiency

The drug entrapment efficiency (DEE) was determined using the following formula:

$$DEE = \frac{Amountofdrugactuallypresent}{Theoreticaldrugload} X\ 100$$

4.3.8. Drug release study

A USP-I dissolution apparatus (Model: DS-8000, LABINDIA, India) was used for the study. Dialysis bags were soaked overnight in a beaker containing normal saline solution. Bags were tied with threads in one end; an accurately weighed 1gm formulation was introduced into the dialysis bag (HiMedia, Mol. Wt. cut off- 10kDa), and the other end of the bag was tied and placed in the USP-I (basket) and submerged in a receptor chamber containing 400 ml of phosphate buffer blended with 2% Tween 80. At scheduled times, 5ml of aliquot was withdrawn, and the same volume was reintroduced to maintain the sink condition. The experiment was conducted at a constant temperature of 37 ± 1°C. The aliquot underwent spectrophotometric evaluation at 413 nm [38].

4.3.9. Microscopic Analysis

The surface morphology of the optimized emulgel (EG-5 SS) was studied with the help of a bright field microscope (Model: *Magnus MX21i LED, Japan*). The formulation (≈ 10mg) was placed on a glass slide, smearing was done, and it was encased with a cover slip. The slide was sited under the microscope, observed under 10X object, and the photograph was captured [39].

4.3.10. Texture Analysis

The optimized emulgels' texture profiles were assessed with a CTX texture analyzer (Ametek Brookfield, United States) with an initial trigger force of 5.0 g. Developed emulgels of 35 g were introduced into different glass beakers. A glass prob having a 35 mm diameter was introduced two times in each formulation at a pre-programmed rate of 1 mm/s and depth up to 10 mm. A 10-second gap was provided between compression cycles. Emulgel's strength, adhesiveness, cohesiveness, and springiness of optimized emulgels were taken from the software (Texture Pro) [28].

4.3.11. *FTIR analysis*

The FTIR analysis of emulgels was done using an Alpha-E ATR-FTIR (Bruker, Germany). Attenuated total reflectance (ATR) mode was used to examine the samples between 4000 and 500 cm^{-1} [40].

4.3.12. *DSC*

The thermal stability of the optimized emulgel was evaluated using a DSC-60 instrument (Shimadzu, Japan, Model-01075). During the study, the flow of nitrogen was kept constant at 50 ml/minute, with a temperature range of 30-250 °C [41].

4.5.13. *Drug uptake study*

Freshly clipped nails were collected from the grooming parlor and washed thoroughly and repeatedly with 0.1 N HCl and acetone. After rinsing with distilled water, they were allowed to dry at room temperature for one day. These nail clippings were weighed and placed (dorsal surface towards the formulation) in wax blocks containing emulgels and kept aside for 24 hours at room temperature. The nails were taken out, wiped with tissue paper, and weighed. The nail clippings were then kept in tubes, and 2 ml of methanol was added and left into them. After three days, methanol extract was analyzed spectrophotometrically at 413 nmto determine the drug content taken by the nail clippings. The % drug uptake/mm^2 was calculated [42].

4.3.14. *Antifungal activity*

The disc diffusion method was applied to determine the antifungal activity of the optimized emulgel. A sterile disc was dipped into the optimized formulation, applied in the middle of the agar plate inoculated with *candida albicans*, and left for three days in the incubator [41].

4.3.15. *Stability study*

A stability study was performed on optimized emulgel. The emulgel was placed in the 15g (Hi-Media) glass container and kept at 40 ± 2 °C with RH 75 ± 5 %. At 0 days, six weeks, three months, and six months, samples were taken out and examined for their physical characteristics, drug content, and pH [43].

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Conflict of interest statement: The authors declare no conflict of interest.

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